Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 3429-3433
Received: 13-03-2019
Accepted: 15-04-2019

Salma Ibrahim
Department of Physics, Calicut University, Kerala, India

M Aslam
Govt Arts & Science College, Calicut, Kerala, India

MM Musthafa
Department of Physics, Calicut University, Kerala, India

Correspondence
Salma Ibrahim
Department of Physics, Calicut University, Kerala, India

Trace elemental fingerprinting of selected herbs used in Ayurveda using XRF and ICPMS

Salma Ibrahim, M Aslam and MM Musthafa

Abstract
Energy Dispersive X-ray Fluorescence (ED-XRF) spectroscopy and Inductively Coupled Plasma Mass Spectroscopy (ICPMS) are used for qualitative and quantitative determination of major, minor and trace elements in some herbs which are used widely in many Ayurvedic medicines. Concentrations of major elements like Mg, K, Al, Ca, Cl, Fe, Mn, S, P and trace elements like Mn, Cu, Zn, Sr, Rb are determined by XRF while that of Ti, V, Cr, Co, Ni, Cu, Zn, Br, Cd, Hg, Zr are done using ICPMS. Elements which show the same concentration in both of the methods are identified. Combining these two analyses, a comprehensive elemental fingerprint of the samples is produced. The result will serve as a data bank for fingerprinting of herbals and herbal products to ensure quality and authenticity.

Keywords: Trace elements, X-ray fluorescence, herbal medicines, fingerprint

Introduction
Application of energetic beams as a tool for material characterization has been a well-developed area of research. X-ray Fluorescence (XRF) is an important technique for qualitative and quantitative determination of the concentration of elements in different matrices with high detection sensitivities. XRF has been used for elemental analysis in diverse matrices like ancient coins [1], paintings [2], geological [3] and biological samples [4, 5] to quantify major and trace elements. In herbal samples of medicinal importance, even though the correlation between trace elements and their medicinal properties are not well established, trace elements are thought to be responsible for forming active constituents whose deficiency or excess can cause several disorders [6]. Each herb has a typical pattern of absorption and transport of trace elements which forms the basis for herb characterization by the elemental pattern. When the elemental concentration is of sub-ppm range, Integrated Coupled Plasma Mass spectroscopy (ICPMS) is a good candidate for quantification. Even though it is destructive, costly and needs careful sample preparation procedures, it is employed for quantification with better accuracy as the limit of detection of ICPMS is lower compared to XRF.

Literature reveals many studies on elemental analysis of medicinal plants by employing XRF and ICPMS technique. Ekinci *et al*. used EDXRF alone for trace elemental analysis of some selected medicinal plants [7]. Similar studies have been conducted by A. Khuder *et al*. in selected medicinal plants from Syria [8]. Milani *et al*. used ICPMS for the quantitative determination of trace elements in tea and herbal beverages [9]. Similar studies have been conducted by Shirazi *et al*. in the compound formulation of herbals and spices [10]. Ahmed *et al*. reported a comparative study of trace elements using XRF and ICPMS on marine sediments [11]. Queralt *et al*. used EDXRF, TXRF and ICPMS for quantitative determination of essential trace elements in some medicinal herbs and their infusions [12]. However, there is limited data regarding the complete elemental profile of herbs used in the different system of medicines

Owing to the low detection limits, ICPMS complements XRF and a comprehensive elemental profile of major and trace elements can be generated. A typical elemental pattern generated by combining the results of the two techniques will serve as a fingerprint of the herb. The fingerprint of elements generated will serve the data bank of comparison for that herb and can be used in quality control of compound herbal products prepared from the herbs.

The compound herbal formulation produced by different manufacturers differs significantly in their elemental content even though they claim to adopt the same reference for the preparation method which is evident from our previous report [13]. This might be due to adulteration which is a deliberate addition of cheap easily available drug similar or different appearance instead of the required drug. In addition to that, accidental inclusion of heavy metal pollutants like Pb, As, Hg due to the failure of good manufacturing practices is also rising as an issue of concern. The present method of quality control of herbs and herbal formulations use chemical methods,

which include thin layer chromatography, alcohol, and water-soluble percentage, evaluation of P^H [14], etc. Permissible limits of only a limited number of elements are available at present. Moreover, the estimation of elements using chemical methods is time-consuming and prone to contamination. Comprehensive elemental profiles of commonly used herbs of medicinal importance are required. Considering the above facts, a systematic study of study trace the elemental pattern of the following herbs; *Piper nigrum, Piper longum, Zingiber officinale, Elettaria cardamomum and Cinnamomum verum* has been undertaken using XRF and ICPMS.

Sample preparation

Dried samples of the relevant part of the herbs were collected from authentic sources and the same has been certified by Centre for Medicinal Plant Research Kottakkal. The botanical name, common name and the specific part used for the study are given in Table1. The samples were washed in flowing water, then with double distilled water and allowed to dry in a hot air oven at 60 ^{O} C. Dried samples were ground into powder in stainless steel grinder and sieved (ASTM No 80). The samples were then r freeze-dried for 5 hrs at -81^{0}C and then ground well in an agate mortar to ensure homogeneity.

Table 1: Sample details

S. No	Botanical Name	Common name	Parts Commonly used for the particular curative purpose
1	*Piper longum*	Long pepper	Fruit
2	*Piper nigrum*	Black pepper	Fruit
3	*Zingiber officinale*	Ginger	Root tuber
4	*Elettaria cardamomum*	Cardamom	Fruit
5	*Cinnamonum verum*	Cinnamon bark	Stem bark

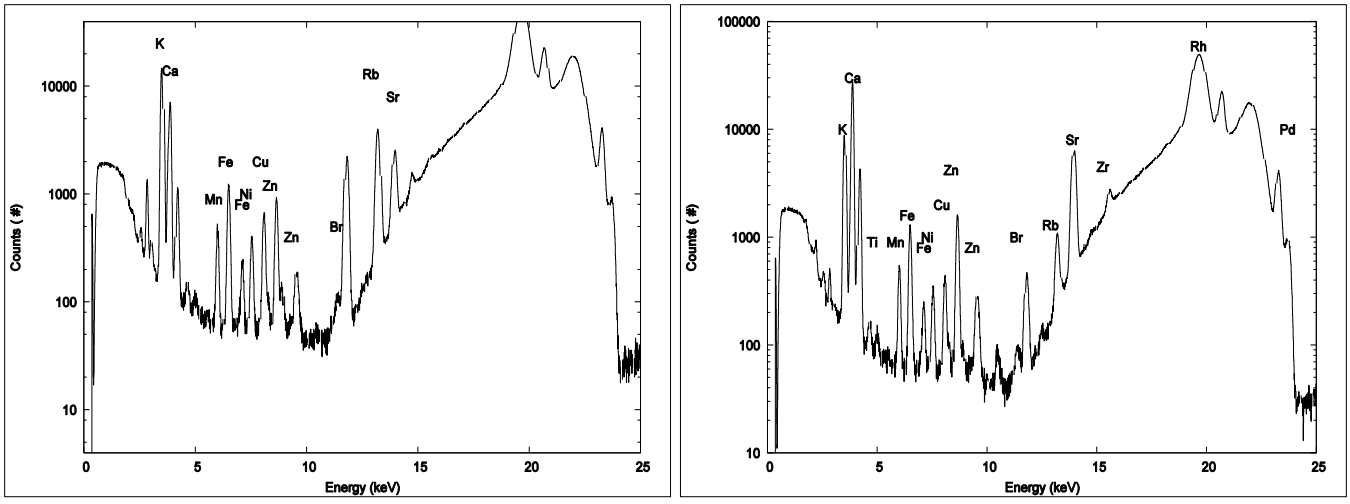
For XRF analysis, 400 mg of sample was weighed and mixed and pressed into pellets of 20 mm diameter by applying a pressure of 10 tonnes. Three replicas for each pellet were also made. These pellets were used as targets in XRF analysis. Samples of NIST Apple leaves were also prepared following the same procedure. Wet digestion method was adopted for ICPMS sample preparation. The powdered sample was digested in Milestone Ethos UP microwave digester. All the chemicals used were of supra pure or trace metal grade. 0.1g of the sample was taken in the digestion vessel and 7 ml of concentrated HNO_3 and a 3ml of H_2O_2 are added to it. After 30 minutes of pre-digestion, the vessel was closed and kept inside the digester at 483K. After completion of digestion, the samples were transferred to a volumetric flask and made up to 50 ml. NIST standards were also digested following the same procedure. Sample blank solution was prepared following the same procedure as above without adding the sample. Instrument calibration was done for all the analyzed elements by mixing the standard solutions in the required proportions.

Experimental details

XRF measurements were performed using XEPOS ED-XRF Spectrometer at CSIF, University of Calicut. The X-ray tube anode is made up of Co/Pd binary alloy and the characteristic X rays acquired using silicon drift detector with an active area 30mm^2. ICPMS is done by using Agilent 7800 mass spectrometer.

Observation and Analysis

The ED XRF spectra of *E. cardamomum* and *C. verum* is shown in Fig. 1a, and Fig. 1b. Kα-1energies of each element is labeled in the plot. The accuracy of the measurements is checked by using NIST Apple leaves. Comparison of obtained elemental concentration with certified values is given in Table 2. It shows that the obtained concentrations are in good agreement with the certified values.



(a) (b)

Fig 1: ED-XRF spectrum of (a) E. cardamomum and (b) C. verum

Quantification of trace elements for NIST SRM1515 done using ICPMS is shown in Table 3 and is compared with the certified values. It is evident that the observed values are in good agreement with certified value for trace elements.

Table 2: Comparison of elemental concentration obtained from XRF with certified value for NIST Apple leaves

Elements	Obtained concentration ($mgkg^{-1}$)	Certified concentration ($mgkg^{-1}$)	Elements	Obtained concentration ($mgkg^{-1}$)	Certified concentration ($mgkg^{-1}$)
Mg	2759.00	2710±120	Mn	52.97	54.1±1.1
Al	282.20	284.5±5.8	Fe	81.49	82.7±2.6
P	1605.00	1593±68	Cu	5.02	5.69±0.13
S	1798.00	1800.00	Zn	11.10	12.45±0.43
Cl	583.60	582±15	Rb	10.02	10.2±1.6
K	16070.00	16080±210	Sr	24.16	25.1±1.1
Ca	15270.00	15250±100	Pb	0.40	0.470±0.02

Table 3: Comparison of elemental concentration obtained from ICPMS with certified value for NIST Apple leaves

Elements	Obtained concentration (mgkg-1)	Certified concentration (mgkg-1)
Cu	5.92±0.02	5.69±0.13
Pb	0.35±0.1	0.470±0.024
Ni	0.72±0.05	0.936±0.094
Zn	12.73±0.09	12.45±0.43
V	0.31 ±0.06	0.254±0.027

Results and Discussion

The XRF spectrum shows well-defined peaks of good intensity for K, Ca, Fe, Mn, Cu and Zn up to 12 keV. The broad peak at high energy side is due to Bremsstrahlung at X-Ray target. Intense peaks for Co $K_{\alpha 1}$ and Pd are observed due to the Co/Pd binary target. The concentration of major and minor elements from XRF are compare plotted in Fig 2a, Fig. 2b. Results show that the major and trace elemental concentration vary widely among different herbs. Mg, Al, P, S, Cl, K, Fe are the major elements of which K and Ca have high abundance in all the samples. Trace element concentration obtained from ICPMS for all the five samples is given in table 3.

Table 3: Trace element concentration obtained from ICPMS for all the five samples is given in table 3.

Elements	*P. longum*	*P. nigrum*	*Z. officinale*	*E. cardamomum*	*C. verum*
Ti	2.41±0.07	3.99±0.07	41.14±0.07	0.9±0.07	5.41±0.07
V	0.04±0.06	0.12±0.06	1.11±0.06	0.06±0.06	0.16±0.06
Cr	0.64±0.04	0.76±0.04	1.90±0.04	9.9±0.04	0.86±0.04
Co	0.04±0.07	0.04±0.07	1.39±0.07	0.1±0.07	0.1±0.07
Ni	1.23±0.05	1.06±0.05	3.03±0.053	0.71±0.053	ND
Cu	10.62±0	11.46±0.0	36.75±0.0	6.58±0.0	6.56±0.0
Zn	29.38±0.9	14.83±0.1	21.86±0.1	44.68±0.1	27.77±0.1
Br	4.84±0.14	19.64±0.14	1.76±0.14	6.16±0.14	3±0.14
Pd	0.03±0.03	0.07±0.03	0.01±0.03	0.04±0.03	0.24±0.03
I	ND	ND	ND	ND	ND
Cd	0.004±0.1	0.01±0.1	0.05±0.1	0.02±0.1	0.09±0.1
Hg	0.02±0.08	0.04±0.08	ND	0.05±0.8	0.01±0.8
Pb	0.34±0.10	0.24±0.10	0.65±0.10	0.8±0.10	0.89±0.10
Zr	0.19±0.05	0.41±0.05	1.53±0.05	0.06±0.05	0.54±0.05

ND: Not detected

In all the samples, K is the major element with highest concentration except for C. verum and E. Cardamomum. P. longum consist of major elements in the order K> Ca > Cl > S > Mg > Al > Fe. In P. nigrum, major elements are in the order K > Ca > Cl > S > P > Mg > Al > Fe. Major elements in Z. officinale, follows the order K > Cl > P > Mg > Ca > Mn >Fe > Al. E. cardamomum has Ca > K > S > Cl > Mg > Mn > Fe > Al as major elements. C. verum has major elements in the order Ca > K > S > Cl > P > Mg > Al > Fe. TE profiles of the herbs shows that Mn concentration is significantly high in Z. officinale and E. cardamomum with values 410 ± 0.31 ppm and 382.17 ± 1.28 ppm while in all other samples it is less than 35 ppm.

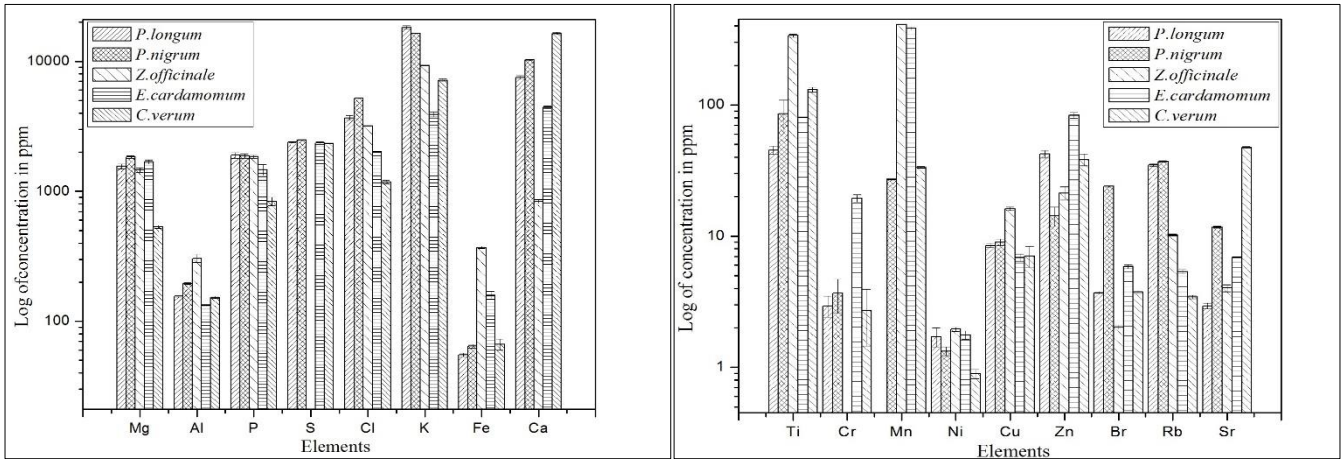


Fig 2: TE profile comparison of the selected herbal samples using XRF

Rb is an important trace element which is highest in *P. nigrum* (937.09 ± 0.5 ppm) and P. longum (34.72 ± 0.07ppm) respectively. Studies reveal that Rb has an effect on improving depression and mood disorders [15]. Rb concentration is found to be the lowest in *C. verum*. Sr is an important trace element in many body functions whose concentration in *C. verum* is significant (47.37 ± 0.6 ppm) when compared to a mean value of 4 ppm in all other samples.

Ti is found to be present with the highest concentration in *Z. officinale* (41.14 ± 0.07 ppm) which is well above the mean value of Ti present in all the samples. This can be due to the contamination coming from the soil. V is less than 1 ppm in all samples with an exception of *Z. officinale* (1.11 ± 0.064 ppm). *Z. officinale* is also found to contain the highest concentration of Ni (3.03 ± 0.053ppm)

Cr concentration in *E. cardamomum* is 9.9 ± 0.04 ppm which is well above the mean value of 2.81 in the selected herbs. Pd, Co has a significantly intense peak in XRF but its concentrations less than 00.3 ppm in ICPMS results for all the samples. This discrepancy is due to the X-rays produced from the X-ray target made of Co/Pd binary alloy. The tube has got a ceramic covering which contributes to a prominent Zr peak in the XRF spectra of all the samples even though Zr is present with an average concentration less than 0.55 ppm.

Zn is highest in *E. cardamomum* whereas its concentration is comparable in *C. verum* and *P. longum*. Br is highest in P. longum (19.64 ± 0.14 ppm) whereas its concentration ranges from 2 to 6 ppm in all other samples. Cd, Hg, As and Pb concentrations are found to be far less than the permitted limit for toxic heavy metals. *P. nigrum*, *P. longum* and *Z. officinale* have been administered together in many Ayurvedic medicines to improve the bioavailability of certain medicines. This is attributed to the improved permeability of intestinal epithelial cells in presence of these drugs [16]. The elemental analysis suggests that Zn and Cu are significant trace elements in the trio. Comparison of the elemental concentration of NIST Apple leaves using XRF and ICPMS for Cu, Pb, Ni, Zn, V shows that the results of the two methods are comparable for these elements.

Conclusion

The concentrations of trace elements in five medicinal herbs used for the treatment of various ailments as well food additives were determined by EDXRF and ICPMS techniques. The concentration of K and Ca is found to of highest percentage in all the samples. Ranges of trace elemental concentrations have been found to vary widely. Presence of Rb in *P. nigrum P. longum* and *Z. officinalis* is significant. Cr content in *E. cardamomum* is exceptional and it needs further studies to ensure the source of Cr in the seeds of *E. cardamomum*. Lead, Arsenic, Cadmium and Mercury are present at a concentration less than 1 ppm in all the samples. It is evident that the result of XRF is well correlated with that of ICPMS. The elemental fingerprint of a particular plant part generated by using the two methods is useful for the development of a systematic method of identification and quality control of herbals and for standardization of herbal formulations.

Acknowledgment

The authors thankfully acknowledge Dr. M Sudarsan, UGC DAE CSR Kolkata for providing freeze drier facility and CSIF, the University of Calicut for providing the EDXRF facility.

References

1. Nguyen The Quynh, Truong Thi An, Tran Duc Thiep. Elemental Analysis of the Ancient Bronze Coins by X-ray Fluorescence Technique Using Simultaneously Radioisotope Source and X-ray Tube. Communications in Physics. 2004; 14(1):50-56.
2. Michael Mantler, Manfred Schreiner. X-Ray Fluorescence Spectrometry in Art and Archaeology. X-Ray Spectrom. 2000; 29:3-17.
3. Hutton JT, Elliott SM. An accurate XRF method for the analysis of geochemical exploration samples for major and trace elements using one glass disc. Chemical Geology. 1980; 29:1-11.
4. Cesareo R, Viezzoli G. Trace element analysis in biological samples by using XRF spectrometry with secondary radiation. Physics in Medicine & Biology. 1983; 28:11.
5. Lue B, Marco P, Edwin A. Hernandez Caraballob. Direct analysis of biological samples by total reflection X-ray fluorescence. Spectrochimica Acta Part B. 2004; 59:1077-090.
6. Osamu Wada. What are Trace Elements? -Their deficiency and excess states. JMAJ. 2004; 47:351.
7. Neslihan Ekinci, Raci Ekinci, Recep Polat, Budak G. Analysis of trace elements in medicinal plants with energy dispersive X-ray fluorescence. Journ. of Radioanalytical and Nucl. Chem. 2004; 260(1):127-131.
8. Khuder A, Sawan M, Karjou J, Razouk K. Determination of trace elements in Syrian medicinal plants and their infusions by energy dispersive X-ray fluorescence and

total reflection X-ray fluorescence Spectrometry. Spectrochimica Acta Part B: Atomic Spectroscopy. 2009; 64(7):721-725.
9. Milani, Raquel F. Evaluation of Direct Analysis for Trace Elements in Tea and Herbal Beverages by ICP-MS. J. Braz. Chem. Soc. 2015; 26(6):1211-1217.
10. Ovais Ullah Shirazi, Muhammad Muzaffar Ali Khan Khattak, Nor Azwani Mohd Shukri. Quantitative analysis of minerals in the selected formulations of spices and herbs using ICP-MS. Progress in Nutrition. 2016; 18(2):161-165.
11. Amel Yousif Ahmed, Md. Pauzi Abdullah, Abdul Khalik Wood, Suhaimi Hamza M, Mohamed Rozali Othman. Determination of Some Trace Elements in Marine Sediment using ICP-MS and XRF (A Comparative Study). Orient. J. Chem. 2013; 29(2):645-653.
12. Queralt I, Ovejero M, Carvalho ML, Marques AF, Llabres JM. Quantitative determination of essential and trace element content of medicinal plants and their infusions by XRF and ICP techniques. X-Ray Spectrom. 2005; 34:213-217.
13. X-ray Fluorescence Fingerprinting of Trace Elements in Ayurvedic Medicine Triphala Churna Salma Ibrahim R. V, Musthafa M. M., Mohamed Aslam P, NUCAR 2019 BARC, 2019.
14. The Ayurvedic Pharmacopoeia of India Part- I Volume I Government of India, Ministry of Health and Family Welfare Department of Ayush
15. Torta RR, Ala G, Cicolin A, Costamagna S, Fiori L, Ravizza L. Rubidium Chloride in the treatment of major treating depression. Minerva Psichiatr. 1993; 334(2):1101-110.
16. Johri RK, Zutshi U. Ayurvedic formulation 'Trikatu' and its constituents. Journal of Ethnopharmacology. 1992; 37:85-91.
17. NIST SRM 1515 Apple leaves. CoA, US Department of Commerce, 2017.