PIXE and ICP-MS Analysis of Andrographis Paniculata Medicinal Plant

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Abstract: The concentrations of elements Li, Be, Al, Cl, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Ag, Cd, Ba, Pb and U in *AndrographisPaniculata*medicinal plant used in the treatment of Diabetes Mellitus were determined by using Particle Induced X-ray Emission (PIXE) and Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) techniques. This plant was collected from four different geographical locations in Andhra Pradesh, India in order to assess the regional variation of elemental concentrations. Appreciable levels of K, Ca, Cr, Mn, Cu and Zn determined in this plant can be correlated to the antidiabetic property of *AndrographisPaniculata* since these elements are known to regulate and potentiate insulin action. Presence of toxic elements As, Cd and Pb necessitates the adoption of precautionary measures while prescribing dosage of the herbal medicine prepared from this plant for the treatment diabetes mellitus.
Keywords:PIXE, ICP-MS, Trace elements, Medicinal Plants.

1. INTRODUCTION

A plethora of well known analytical techniques with remarkable sensitivities and detection limits have made it possible to analyze a wide variety of samples for their elemental content from every conceivable field of scientific or technical interest. Particle Induced X-ray Emission (PIXE) is a relatively simple yet powerful analytical technique used for the multi-elemental analysis of a wide range of samples [1]-[5]. This technique serves as an excellent tool for elemental analysis, particularly trace elemental analysis because of its capability to detect simultaneously several elements present at very low concentrations and the ease of sample preparation and analysis. Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) is another reliable and effective technique used to quantify several elements with 1pg/ml sensitivity in a variety of samples [6]-[8]. In this method, initially the samples are ionized with inductively coupled plasma. Consequently a mass spectrometer is used to separate and quantify those ions. ICP-MS allows determination of elements with atomic mass from 7 to 250. This encompasses Li to U. This technique has the capability to scan all the elements simultaneously. The other advantage of using ICP-MS lies in the fact that it allows isotopic speciation.

Since times immemorial, in every culture throughout the world herbal drugs constituting medicinal plant preparations have been used for healing purposes and maintaining good health. The earliest recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The herbal medicines have been derived from rich traditions of ancient civilizations and scientific heritage.Initially with the onset of industrial revolution and the upsurge of allopathic medicine,herbal drugs suffered a setback. Concerns over the iatrogenic effects of conventional medicine, advances in phytochemistry and the desire for more self-reliance led to extensive research on identification of plant compounds effective in curing certain diseases.

In recent years, considerable attention is being paid in assessing the therapeutic effect of medicinal plants from their elemental content point of view by using different analytical techniques. In a study on total concentration of selected trace elements in neem powder and in neem tea by ICP-MS, Novotnik et al [9] observed substantial amounts of essential elements and negligible amounts of toxic elements. Mineral elemental concentration of some Chinese medicinal herbs used to improve kidney function was determined by flame atomic absorption spectroscopy [10]. Regional variation in essential and toxic elemental content of *Murrayakoenigii* (curry leaves) used as a spice and medicinal herb was studied by INAA [11].Naga Raju et al [12], [13] determined elemental concentration in the medicinal plants used in the treatment of diabetes and cancer using PIXE.

In the present study, PIXE and ICP-MS techniques are used to identify and quantify the elements that are present in the medicinal plant *AndrographisPaniculata,* which iswidely used in the treatment of diabetes mellitus (DM). An attempt has also been made to study the variation of elemental concentrationwith respect to geographical location.

2. **Materials and Methods**

2.1. Sample Collection and Preparation

Whole plant samples of *AndrographisPaniculata* medicinal plant were collected fromVizianagaram, Visakhapatnam, Vijayawada, and Tadepalligudem regions of Andhra Pradesh, India. In each region 10 samples were chosen from different sites for elemental analysis. Thesamples were washed thoroughly with tap water followed bydoubled distilled water to remove the surface contamination and were then oven dried at 60^{o}C. The dried plant samples were ground to a fine powder by usingan agate mortar.A known quantity of yttrium with known concentration (100 ppm) was added as an internal standard to the samples to check the reliability of the experimental results obtained in the present study.

For ICP-MS analysis, 50 mg of eachpowdered sample was taken into a high-pressure closed Teflon decomposition vessel and1mL of concentrated HNO_3 (Trace metal grade, Fisher Make)was added to it. After 30 minutes of pre-digestion, 4 mL of Milli-Q water was added to 1mL of each acid mixture and the vessels were slowly shaken and sealed. Consequently, decomposition of thesampleswas carried out in a programmed Microwave Accelerated Reaction System (MARS5, SEM, USA).Ten replicate digestions were made for each sample. Finally,the digested samples were transferred to a 25 mL volumetric flask and made up the volume with Milli-Q water. Blanks were prepared in same manner by excluding the sample. For PIXE analysis, a homogeneous mixture of 90 mg of powdered sample with 60 mg of high purity graphite powder was prepared and compressed into a pellet using a pelletizing machine.

2.2.ICP-MS analysis

Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) analysis has been carried using Agilent 7700 series ICP-MS system available at Centre for Study on Bay of Bengal, Andhra University, Visakhapatnam, India.The optimal operating conditions for ICP-MS analysis is given in Table 1.

2.3.PIXE analysis

Present PIXE measurements were carried out by irradiating the samples with 3 MeV proton beam from the 3 MV Pelletron Accelerator facility available at Ion Beam Laboratory, Institute of Physics, Bhubaneswar, India. The detailed procedure for PIXE experiment can be found elsewhere in our earlier works [14],[15]. The obtained PIXE spectra of *AndrographisPaniculata*medicinal plant corresponding to the four different regions are shown in Fig. 1. Guelph PIXE (GUPIXWIN) software package[16] was used to analyze the obtained PIXE spectra and thereby identify and quantify the elements present in the studied samples. The precision and accuracy of the results were checked by analyzingNIST Certified Reference Material – Peachleaves (Sample No.1547) in same experimental conditions.

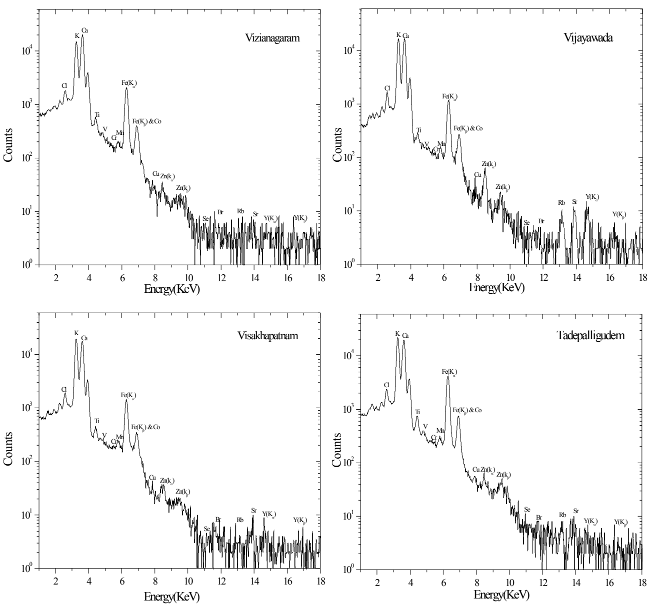

Fig.1. PIXE spectra of AndrographisPaniculata medicinal
plant from four regions.

Table.1. Optimal operating conditionsfor ICP-MS analysis

Parameters with values
Plasma conditions
Rf frequency:27 MHz
Rf power:1 kW
Gas flow rate
Carrier gas: Ar 0.8 L/min
Auxiliary gas:Ar 1.1 L/min
Coolant gas:Ar 15 L/min
Sampling conditions
Sampling depth:7 mm from work coil
Sampling cone: Nickel 1.0 mm orifice diameter
Skimmer cone: Nickel, 0.4 mm orifice diameter
Nebulizer: Cross-flow type
Sampling uptake rate:0.4 ml/min
Data acquisition
Data point: Multi-element hope by peak hopping
Dwell time:3 points/peak
Integration:50 ms/point
Repetition: 1000 times

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3. RESULTS AND DISCUSSION

The results of PIXE measurements for the determination of concentration of elements in *AndrographisPaniculata*medicinal plantcollected from four different regions are presented in Table.2and the ICP-MS results for the concentration of elements are presented in Table 3. From the tables, it is clear that the fifteen different elements namely Cl, K, Ca, Ti, V, Cr, Mn, Fe, Co, Cu, Zn, Se, Br, Rb and Srwere determined using PIXE whereas twenty different elements Li, Be, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se,Rb, Sr, Ag, Cd, Ba, Pb and U were determined using ICP-MS. Among all the determined elementsAl, Cl, K and Ca are found to be most abundant elements followed by Fe. The significant levels of elements present in the analysed medicinal plant, correlate well with the traditional use of this plantin the treatment of diabetes mellitus.

The role of trace elements in various biochemical and physiological processes in humans is well understood. Elements by themselves or as component of enzymes may play a vital role in the development and management of DM as it is a disease of metabolic abnormality. Out of all the elements identified in the studied plant, the elements K, Ca, Cr, Mn, Cu and Zn have insulin potentiating property as they are responsible for the secretion of insulin from the beta cells of the islets of Langerhans.

Table.2. Concentration of elements (μg/g) in *AndrographisPaniculata* medicinal plant from four regions by PIXE.

Elements	Vizianagaram	Visakhapatnam	Vijayawada	Tadepalligudem
Cl(%)	0.822±0.057	1.21±0.068	0.890±0.045	1.48±0.085
K(%)	2.68±0.028	4.51±0.043	3.01±0.029	5.18±0.049
Ca(%)	4.14±0.034	4.82±0.043	3.53±0.030	5.96±0.049
Ti	484±37	418±40	159±26	841±49
V	14.6±2.62	9.75±1.75	11.8±2.59	13.9±2.78
Cr	9.14±1.82	27±5.13	8.22±1.23	18.3±1.57
Mn	87.9±15.4	153±21	85.2±12.6	177±24
Fe(%)	0.311±0.002	0.247±0.003	0.170±0.002	0.593±0.005
Co	5.01±0.901	0.932±0.177	2.62±0.524	1.34±0.281
Cu	18.4±7.60	7±1.47	34.5±6.49	9.20±1.74
Zn	38.5±7.89	49.2±10.2	97.9±8.66	80.4±13.2
Se	1.88±0.282	1.72±0.275	0.696±0.125	1.12±0.201
Br	5.7±1.08	11.8±2.47	11.2±7.91	14±12.9
Rb	20.4±13.9	29.5±15.5	49.2±13.8	43.3±9.09
Sr	121 ±22	92±12	113±20	176±23

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Table.3. Concentration of elements (μg/g) in *AndrographisPaniculata* medicinal plant from four regions by ICP-MS.

Elements	Vizianagaram	Visakhapatnam	Vijayawada	Tadepalligudem
Li	0.710±0.196	ND	ND	1.05±0.30
Be	0.057±0.007	0.062±0.006	0.034±0.005	0.181±0.009
Al(%)	0.261±0.040	0.127±0.013	0.199±0.013	0.483±0.068
V	7.08±0.46	2.61±0.36	3.26±0.45	6.78±0.46
Cr	3.82±0.24	22.4±1.5	3.37±0.40	12.6±0.71
Mn	64.0±3.9	116±9	70.4±5.5	92.2±5.7
Fe(%)	0.297±0.027	0.185±0.023	0.204±0.017	0.460±0.049
Co	3.46±0.15	0.189±0.010	1.94±0.13	0.769±0.032
Ni	2.78±0.36	8.64±0.82	1.51±0.20	2.85±0.30
Cu	17.0±1.1	6.12±0.48	28.2±1.9	7.60±0.58
Zn	31.7±2.3	39.7±2.7	53.6±3.6	52.6±3.4
As	0.281±0.030	0.212±0.025	0.220±0.009	0.421±0.025
Se	0.144±0.046	0.141±0.038	0.072±0.020	0.236±0.044
Rb	19.7±1.2	21.6±1.6	23.5±1.4	39.7±2.6
Sr	91.1±9.9	78.7±9.4	66.2±14.5	136±30
Ag	0.022±0.007	0.103±0.037	0.041±0.019	0.370±0.147
Cd	0.020±0.004	0.082±0.012	0.018±0.003	0.051±0.006
Ba	34.4±0.8	127±5	95.5±2.3	79.8±5.0
Pb	1.67±0.28	1.21±0.17	1.45±0.107	5.23±0.660
U	0.090±0.020	0.021±0.005	0.067±0.015	0.451±0.104

From the obtained results, it is evident that significant levels of zinc are present in the analyzed medicinal plant.Analyses of the sample from Vijayawada region by both techniques yielded highest levels of zinc. It has been reported that zinc plays an important role in the production, storage and regulation of insulin and its deficiency is linked withseveral complications such as hypertension, retinopathy, thrombosis, and increased tissue resistance to insulin action in type 2 DM[17],[18]. Deficiency of intracellular Zn makes the beta cells of pancreas more vulnerable to free radical attack.

Chromium is not just a critical cofactor in insulin action but it is also an active component of glucose tolerance factor, which is required for optimal glucose utilization by the cells [19]. Some studies have shown that by supplementing Cr, blood sugar levels were decreased and also reduced the insulin requirement by the diabetic patients [20]. The highest concentration of Cr in the present study was observed in the samples collected from Visakhapatnam region.Even though very low levels of toxic elements As, Cd and Pb are determined by ICP-MS technique, this necessitates the adoption of precautionary measures while prescribing dosage of the herbal medicine prepared from this plant for the treatment diabetes mellitus.

The variations in the elemental composition of *AndrographisPaniculata*medicinal plantcollected from four different regions can be attributed to several factors like climatic conditions, mineral composition of the soil in which the plant grows, the age of the plant, fertilizers used and environmental pollution levels.

4. Conclusion

In the present study on elemental analysis of *AndrographisPaniculata*medicinal plantcollected from four different regions of Andhra Pradesh, various elements of interest were identified and quantified. Since diabetes mellitus is associated with marked alterations in the concentrations of trace elements, regulation of trace element concentrations is being proposed as a potential strategy for preventing and treating DM.

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