Trace element analysis of some indigenous medicinal plants of Ethiopia by PIXE

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Abstract: **Trace elemental analysis was carried out in three indigenous medicinal plants of Ethiopia using Particle Induced X-ray Emission (PIXE) technique. A 3 MeV proton beam was used to excite the samples and spectra were recorded using a Si(Li) detector. Data analysis was done using Gupix Software. The elements S, Cl, K, Ca, Ti,V, Mn, Fe, Co, Cu, Zn,Ge,Rb and Sr were detected and their concentrations determined.**

Key words: Trace elemental analysis, PIXE, Medicinal plants, Ethiopia.

I. INTRODUCTION

Man has been using several plants and herbs even from pre-historic times to cure various ailments. Plant based drugs are being increasingly used in many African, Asian and Latin American countries to cure various ailments. During the outbreak of swine flu a few years ago, it was reported that a majority of cases were treated with herbal preparations in China. Usage of traditional herbal medicines, which is widely prevalent in developing countries, has spread to industrialized nations also as a complementary way to treat and prevent diseases according to a WHO report [1].

Medicinal plants contain both organic and inorganic constituents. Research work has been carried out extensively on the organic constituents of the medicinal plants [2, 3], while the role of inorganic elements in the medicinal plants has received relatively less attention The chemical constituents present in the plants responsible for their medicinal properties include bases comprising alkaloids, amines, glycosides, etc. Trace elements play an important role in the formation of these compounds. They are intimately involved in the physiological functions and are important co-factors in the production of enzymes. They are also necessary for the maintenance and regulation of cell, gene and membrane functions. The data obtained on concentrations of different elements of the medicinal plants will be useful in deciding the dosage of traditional drugs prepared using these plant materials. Such analysis is also necessary to ensure that they do not contain heavy metals in toxic proportions.

A good number of studies on elemental analysis of medical plants are found in literature [4-12]. Chen et al [4] analyzed 75 samples of various diuretic, phlegm eliminating and stomach invigorating natural drugs by using Neutron Activation Analysis. Abugassa et al [5] estimated trace elements in some medicinal herbs by Neutron Activation Analysis. In a similar study specific parts of several plants (fruits, leaves, stem, bark and roots) often used as medicines in Indian Ayurvadic system have been analyzed by Singh and Garg [6]. Jyothi et al [7] determined concentrations of the trace elements Zn, Cu and Cd in the leaves of some medical plants by differential pulse anodic stripping analysis. Ray et al [8] analyzed the anti-diabetic medicinal plants using EDXRF technique. Nagaraju et al [9] identified Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Br, Rb and Sr in some anti-diabetic medicinal plants using PIXE technique. Nomita Devi et al [10] determined concentrations of light trace elements such as Na, Mg, Al and P by PIGE technique and medium Z elements such as K, Ca, Mn, Fe, Cu, Zn, Rb and Sr by PIXE in some commonly used medicinal plants of north east India. Ekinci et al [11] investigated elemental composition and concentration of some medicinal plants by using EDXRF technique. Gowrishankar et al [12] determined trace elemental concentrations of some commonly used medicinal plants in India for curing ailments ranging from common cold, skin diseases, and dental infections to major disorders like diabetes, hypertension, jaundice, rheumatism, etc.,. Trace-elemental distribution of some anti-cancer and anti-epilepsy medicinal plants has been studied by

our group, Sattar et al [13, 14]. In the present study trace elemental analysis of three indigenous medicinal plants of Ethiopia which are widely used for curing different ailments has been undertaken.

In Ethiopia, *Croton macrostachyus* has folk medicinal uses as purgative and vermifuge, and for treatment of various skin problems, helminthes and venereal diseases [15]. Similarly *Solanum incum* has many medicinal uses based on its analgesic properties[16].Throughout tropical Africa ailments like sore throat, angina, stomach ache, rheumatic pains etc. are treated by *Solanum incum,. Salivia nilotica* is a perennial small shrub that grows in the eastern African highlands from Ethiopia to Zimbabwe. Its leaves are used to treat a disease locally called "Michi" in Amharic, characterized by fever; head ache and sometimes lip sores or ulcers in the nose or face. They are also used to treat inflammatory disorders. Methanol extracts of the leaves of *salivia nilotica were* found to exhibit anti-microbial and anti-oxidant properties in an in vivo study by Sewuye and Asres [17]. This extract could significantly lower carrageen induced paw edema. In another study by Ahfaha et al [18], it was shown that these leaves have not only anti-microbial property but also strong free radical scavenging potential.
These three medicinal plants were chosen for trace elemental analysis using PIXE technique in the present study.

II. MATERIALS AND METHODS

The bark of *Solanum incum* and leaves of *Croton mycrostachyus* and *Salivia nilotica* were collected from plants in their natural habitat in areas surrounding Dilla town in the Gideo zone of Ethiopia. Each plant sample was cleaned, dried, ground and then homogenized in an agate mortar. A quantity of 120mg of each powder sample was mixed with high-purity graphite powder (99.999%) in 3:2 ratio. The purpose of mixing graphite powder is to monitor the beam current. This mixture was compressed using a 10-ton hydraulic press into pellets to be used as targets for the PIXE experiment. In a similar method, thick targets of certified reference material of NIST apple leaves (1515) were prepared and irradiated for quantification and verification of the results. A proton beam of 3MeV energy was employed to excite the samples. The beam current was kept at about 20 nA. An Si(Li) detector was used for the detection of X- rays produced .This work was carried out using the 3MV pelletron facility at the Institute of Physics, Bhubaneswar. The targets were positioned at an angle of 45^0 to the beam direction. The proton beam was collimated to a diameter of 4mm and was made to fall on the target. The detector was kept at an angle of 45^0 to the target position and at an angle of 90^0 to the proton beam direction. The characteristic X – rays emitted from each sample were recorded with a high resolution Si (Li) detector (FWHM 160eV at 5.9KeV energy) which has a sensitive area of 30Sq.mm and provided with a thin Beryllium window of thickness 8μm. The spectra were collected for a sufficiently long time so that good statistical accuracies were maintained. For each sample, the total charge collected and the average beam current were noted. Data analysis was carried out by using Gupix software and trace element concentrations were determined.

Element	NIST-Apple leaves (1515)	
	Measured Value	***Certified Value***
K(%)	1.48 ± 0.05	1.60 ± 0.02
Ca(%)	1.61 ±0.06	1.53 ± 0.02
Mn	48.5 ± 2.4	54 ± 3
Fe	88.1 ± 4.5	83 ± 5
Cu	5.3 ± 0.4	5.6 ± 0.2
Zn	12.9 ± 0.7	12.5 ± 0.03
Se	0.06 ± 0.01	0.05 ± 0.01
Rb	9.3 ± 1.0	10.2 ± 1.5
Pb	0.54 ± 0.08	0.47 ± 0.02

TABLE 1. Comparison of measured values of elemental concentration (ppm) with certified values in NIST-Apple leaves (1515).

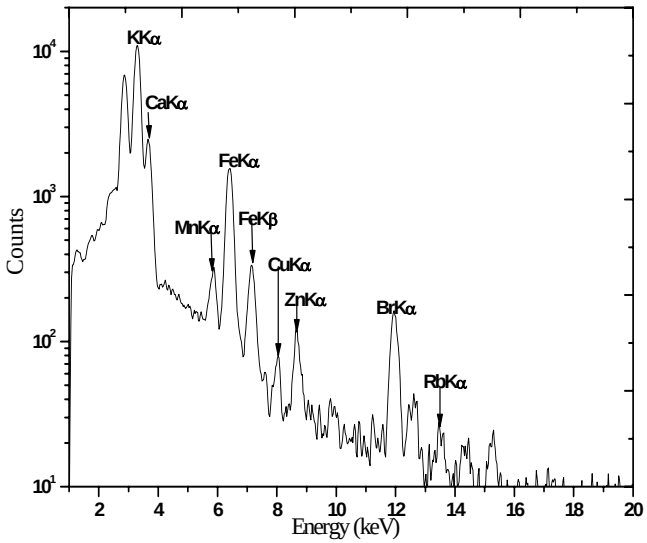

Fig.-1: PIXE Spectrum of NIST-apple leaves (1515)

III. DATA ANALYSIS

The obtained PIXE spectra corresponding to different medicinal plants were analyzed using GUPIX software package [19, 20].This package has provision to convert the X-ray intensities into elemental concentrations using a standardization technique involving parameters such as solid angle, charge collected etc. Fifteen pellets of each sample were subjected to analysis and variations in concentrations from sample to sample were within the standard deviation. To ensure the reliability of this technique in reproducing the concentration of low Z-elements, we have checked the accuracy of the technique by analyzing NIST apple leaves (1515). The obtained results along with the certified values are furnished in Table-1, and the corresponding spectrum in Fig-1.

IV. RESULTS & DISCUSSION

The typical PIXE spectra of the medicinal plants chosen for analysis in the present study are shown in Fig. 2 to 4. The average concentrations (ppm) of the trace elements detected in the samples of various medicinal plants analyzed and corresponding standard deviations for each element are reported in Table-2.

Element	*Solanum incum (Bark)* $n^{\#} = 15$	*Crotonm mycrostachyus (leaves)* $n^{\#} = 15$	*Salivia nilotica (leaves)* $n^{\#} = 15$
S	9428 ± 1185	12985 ± 2132	9944± 1703
Cl	3599 ± 692	2947 ± 391	3073±258
K	5463 ± 671	18998 ± 1223	11030±1313
Ca	31793 ± 2627	8303 ± 1468	18728±1355
Ti	255 ± 96	48.5 ± 8.4	268±98
V	96.15 ± 21.23	9.02 ± 2.8	27.52±6.72
Mn	96.1 ± 16.9	1222 ± 245	149±9
Fe	1731 ± 500	447 ± 75	1243±110
Co	BDL*	31.2 ± 8.4	BDL*
Cu	22.1 ± 5.2	6.15 ± 1.65	17.42±3.39
Zn	45.1 ± 18.2	37.25 ± 5.27	107±11
Ge	21.75 ± 8.65	BDL*	7.2±3.4
Br	BDL*	9.36 ± 1.71	BDL*
Rb	BDL*	10.64 ± 2.43	BDL*
Sr	106 ± 12	21.91 ± 4.73	59.04±6.68

TABLE 2. Average concentrations of the elements in ppm in different samples of each medicinal plant with corresponding standard deviations:

#n: No. of Samples, *BDL: Below Detection Limit.

The elements S,Cl,K,Ca,Ti,V,Mn,Fe, Co,Cu,Zn,Ge,Br,Rb and Sr were detected and their concentrations determined to ppm level.

Certain beneficial characteristics of these elements, in general, which may play a role either directly or indirectly in the therapeutic value of the plant samples, are first presented. Sulphur has a long history of use for a variety of dermatological disorders, as an ingredient of acne ointments [21,22]. Sulphur aids in healing of wounds via keratin and has a history of folklore usage as a remedy of skin rashes[22]. Sulphur containing baths have long history of folk usage as a remedy for skin diseases like psoriasis.

Manganese is an antioxidant nutrient and is essential for the breakdown of fats and cholesterol and also helps in the nourishment of the nerves and brain [23]. It enables the body to utilize vitamin-C, vitamin-B1, biotin as well as choline. It is thought to also help neutralize free radicals.

Zinc is essential to all organisms and it is an important trace element playing a definite role in the metabolism, growth and development. It is an essential component of over 200 enzymes having both catalytic and structural roles. Zinc deficiency is characterized by recurrent infections, lack of immunity [24]. There is clinical evidence to prove that Zn can help in eliminating ulcers and promoting healing of wounds. [25,26]. Zinc is an antioxidant nutrient, necessary for protein synthesis and wound healing. It governs the contractility of muscles, important for blood stability, maintains the body's alkaline balance, helps in normal tissue function and aids in the digestion and metabolism of phosphorus. Zinc oxide is used in the preparation of some skin ointments.

Iron is essential for human body for the production of hemoglobin, in the oxygenation of red blood cells.

Potassium is the principal positively charged ion inside body cells. It plays a major role in maintaining fluid and electrolyte balance and cell integrity. It is also critical to maintaining the heartbeat [27]. Potassium also helps in protein and

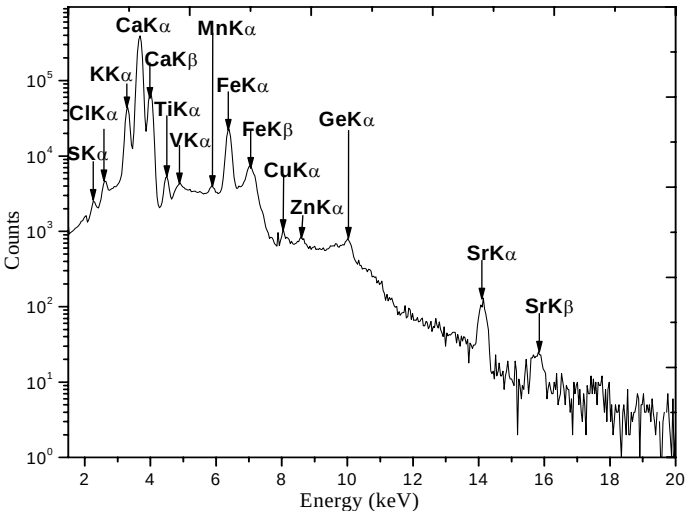

Fig.-2: PIXE spectrum of *Solanum incum* bark

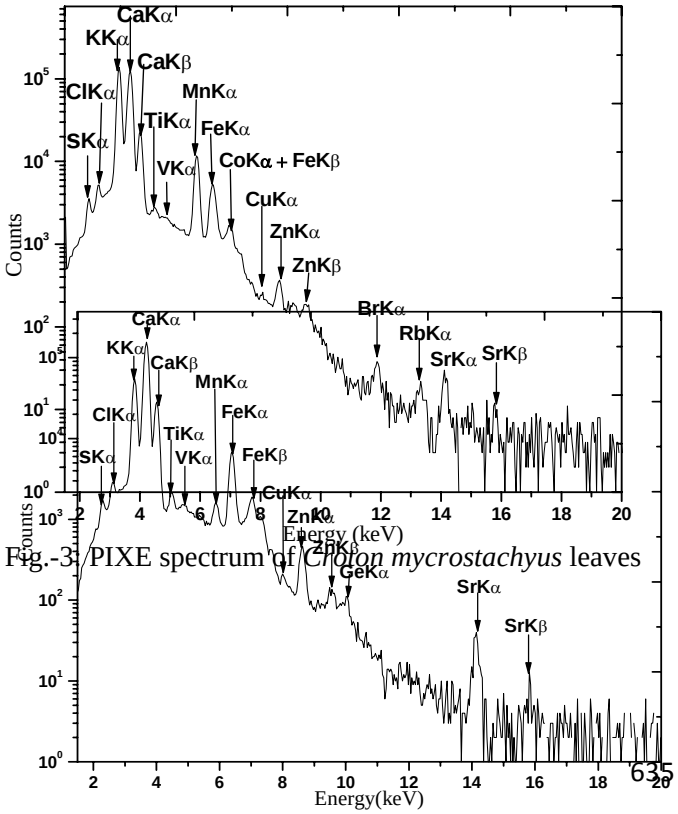

Fig.-3: PIXE spectrum of *Croton mycrostachyus* leaves

Fig.-4: PIXE spectrum of *Salivia nilotica* leaves

carbohydrate metabolism of the body

Chlorine is a constituent of sodium chloride. Hence the metabolism of chlorine and sodium are intimately related. Chloride is involved in the regulation of acid-base equilibrium, fluid balance and osmotic pressure. These functions are carried out by the interaction of chloride with Na + and K^+. It is necessary for the formation of HCl in the gastric juice. It activates the enzyme salivary amylase. [28]

Calcium builds and maintains bones and teeth; regulates heart rhythm, helps regulate the passage of nutrients in and out of the cell walls; helps prevent the accumulation of too much acid or too much alkali in the blood, assists in normal blood clotting, helps maintain proper nerve and muscle function; plays a part in secretion of hormones; activates some enzymes [29].

Copper is an essential constituent of several enzymes, it is involved in many metabolic reactions. It is required for maintaining a healthy heart and blood vessels, for the synthesis of hemoglobin, melanin and phospholipids, and for the development of bones and nervous system. It strengthens connective tissue and is involved in energy production, in the protection of cells from free radical damage and in brain neurotransmitters. It influences protein metabolism and general healing, improves vitamin C oxidation and is integral in the formation of RNA. One of the proteins, ceruloplasmin transports copper as well as help convert iron to a form that can be transported to other tissues. Copper is involved in the oxidation of Fe^{2+} to Fe^{3+} during hemoglobin formation. It is an important catalyst for iron absorption [30].

Cobalt helps with the repair of the myelin sheath; increases the effectiveness of glucose transport from the blood into body cells; increases the assimilation of iron and the building of red blood cells; stimulates many enzymes of the body by replacing manganese and zinc in those enzymes and normalizes the performance of other body cells [31].

Vanadium is reported to increase DNA synthesis and in vitro colonel growth. In vivo it may be needed for normal iodine metabolism and thyroid function [32].

These elements on their own or as important constituents of metalloenzymes or amino acids like in the case of sulphur may provide the curative properties of these medicinal plants.

V. SUMMARY AND CONCLUSION

Samples of three medicinal plants widely used in Ethiopia and other African countries were analyzed using the PIXE technique exciting the samples with a 3MeV proton beam. Data were analyzed using Gupix software. The elements Cl, K, Ca, Ti, V, Mn, Fe, Co, Cu, Zn, Ge, Br, Rb and Sr were detected and their concentrations determined to ppm level. Instead of administering these beneficial metals in the form of drugs prepared from chemicals, administering them in the form of plant based preparations is thought to be more effective. It is assumed that human physiology has a tendency for better assimilation of natural products.No toxic elements such as As,Pb,and Hg are detected in the plant specimens analyzed and or thus safe for human consumption.

Thus the present study helps to provide some scientific basis for the usage of these plants in the folk medicines and also adds to the data base on medicinal plants which will be useful for researchers working in the field of ethno-pharmacology.

ACKNOWLEDGMENT

The authors thank the Director and staff, Institute of Physics, Bhubaneswar, India, for providing facilities at their Ion beam laboratory for carrying out the PIXE work.

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