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Research Article

PIXE TECHNIQUE: A HANDY TOOL FOR INVESTIGATION OF TRACE ELEMENTS IN SOME ANTI DIABETIC MEDICINAL PLANTS

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ABSTRACT

Proton Induced X-ray Emission (PIXE) technique was employed to determine the elemental concentration in leaves of 5 medicinal plants used in the treatment of diabetes mellitus. The samples were exited using a 2 MeV proton beam obtained from 3 MeV pelletron accelerator at IOP, Bhubaneswar, India. The plant species were found to contain reasonable amounts of potassium and calcium along with other trace elements such as Ti, Cr, Mn, Fe, Cu, Zn, Br, Rb, and Sr. The element vanadium was found in only one sample. All the samples under study contained very small amounts of chromium and lead. It is found that the concentration of the same element varies considerably with respect to conditions in which they grow.

Keywords: PIXE Technique, Pelletron accelerator, Diabetes mellitus, Traces elements, Medicinal plants.

INTRODUCTION

Medicinal plants are plants in which one or more of their parts contain substances that can be used for therapeutic purposes[1]. Hence such plants are often used either for prevention, relief of symptoms or treating various diseases worldwide. The plant as a whole or a part, or an extract of leaf, root, bark, fruit or flower may be used as a therapeutic drug. Research shows that overdose or prolonged ingestion of medicinal plants may lead to chronic accumulation of different elements which cause various health problems[2,3]. These elements may be essential or non essential. Just as essential metals induced in high concentration produce toxic effects, non essential metals induced even in very low concentration are also toxic for human health[4-6]. Medicinal plants posses some important elements in small doses which have both therapeutic and prophylactic properties. The elements are referred to as trace elements[7].Trace elements are necessary for physiological functions of cell, gene and play a vital role in secretion of enzymes. They regulate the production of various chemical constituents such as alkaloids, amines, glycosides etc. Trace element deficiency or abundance can result in many physiological disturbances including endocrine diseases in animals and humans[8,9]. Since plants absorb heavy metals, minerals and essential nutrients from soil, water or air, the geographical conditions in which they grow certainly influences the degree of distribution of these elements. The concentration of trace elements in medicinal herbs beyond permissible limit is a matter of great concern to public safety all over the world[10].

According to the World Health Organization (WHO), up to 90% of the population in developing countries uses plants and its products as traditional medicine for primary health care[11]. The WHO has listed 21,000 plants, which are used for medicinal purposes around the world. Among these, 2500 species are in India[12]. There are about 800 plants which have been reported to show anti diabetic potential[13].

Diabetes is a huge and growing burden: 415 million adults were living with diabetes in 2015 and this number is expected to increase to around 642 million or one in ten adults by 2040[14]. Diabetes is recognized as an important cause of premature death and disability. It is one of four priority non-communicable diseases (NCDs) targeted by world leaders in the 2011 Political Declaration on the Prevention and Control of NCDs[15]. The global prevalence of diabetes has grown from 4.7% in 1980 to 8.5% in 2014, during which time prevalence has increased or at best remained unchanged in every country[16].

Diabetes mellitus is categorized basically as two types:
Type I (insulin -dependent diabetes mellitus or IDDM)
Type II (non -insulin -dependent diabetes mellitus or NIDDM).

IDDM occurs due to insulin insufficiency because the pancreas fails to produce enough insulin and patients have to depend solely on insulin supplements from outside. NIDDM results from the body's ineffective use of insulin. Another type of diabetes which occurs when pregnant women without a previous history of diabetes develop high blood-sugar levels is called Gestational diabetes. All the types of diabetes are serious and become chronic if not addressed at an early stage and lead to increased risk of complications of renal failure, foot ulcers, amputation, heart attack and nerve damage, vascular and cerebral damage.

Hence a study has been undertaken to estimate the trace elements using PIXE technique in different plant species which are the basic essential ingredients used in preventing and treating diabetes.

MATERIALS AND METHODS

Plant collection and preparation of sample

Leaves of five Medicinal plants which show anti-diabetic properties and grow abundantly in and around Warangal district of Telangana State- India were identified and collected. The details of botanical name, family and local name of the medicinal plants used in the present investigation are given in the Table 1.

The leaves were washed thoroughly with doubly ionized water and then dried in an oven at a temperature of 60°C for about 24 hours and subsequently ground into fine powder by an agate mortar. The sample powder was mixed homogeneously with the graphite powder in 1:1 ratio. The mixture was pelletized into a thin pellet of uniform thickness having 10mm in diameter under a pressure of 100kg/cm^2. A graphite pellet was also prepared by taking 100 mg of pure graphite powder to make necessary correction.

Plants used in the present investigation

***Vinca Rosea* (*C.roseus*) Linn,** is a herbaceous subshrub also known as Madagascar periwinkle, *Vinca rosea*, or *Lchnerarosea* worldwide. Fresh leaf juice of *C. roseus* has been reported to reduce blood glucose in normal and alloxan diabetic rabbits[17]. Leaves and twigs of *Catharanthus roseus* have been reported to have hypoglycaemic activity in streptozotocin induced diabetic rats[18].

***Adhatoda vasica* Linn Nees,** is a small, evergreen shrub found in many regions of India and throughout the world *Adathoda vasika* is a shrub with lancet-shaped leaves 10 to 15 cm in length by 4 cm wide. The methanolic extract from the leaves of *Adhatoda vasica Nees* (Acanthaceae) showed a sucrose inhibitory activity with sucrose as a substrate. Results suggest the use of the extract of *A. vasica* as an antidiabetic agent and also show the possibility that the compounds, vasicine and vascinol could be a useful treatment for metabolic disorders [19].

Senna auriculata is a legume tree. *Senna auriculata(L.)Roxb* is widely used in Indian traditional medicines and flowers are used for diabetes[20]; leaves and flowers are also used to treat skin diseases[21]; leaf juice is used to reduce body heat[22]. The flower and leaf extracts have anti diabetic activity in experimentally induced diabetes rats[23,24].

Solena amplexicaulis is a perennial dioecious climber with tuberous root found throughout Asia mainly growing in hilly dry deciduous forests, scrub jungles. The tubers, leaves and seeds are extensively used in traditional system for various ailments. The whole plant is determined to be a potential source of natural antioxidant activity[25,26] and also used for the treatment of diabetes[27].

Andrographis paniculata (Burm.f.) Wall.ex Nees is a medicinally important annual herb essentially distributed in tropical Asia and West Indies. Since ancient times, *A. paniculata*, referred to as ‘‘Indian echinacea’’, has been used in India as a wonder drug in traditional siddha and ayurvedic systems of medicine as well as in tribal medicine[28]. The herb is found to have astringent and anodyne properties and is often used as an antidote for snake-bite. It is also used as a potential drug for dysentery, jaundice, cholera, diabeties, bronchitis, iches and piles.

Experimental system and data collection

A 2 MeV collimated proton beam of diameter 2mm obtained from the 3 MeV Tandem Pelletron Accelerator at Institute of Physics, Bhubaneswar, India, was used to irradiate the targets under vacuum condition (10^{-6} torr) inside the PIXE chamber. The targets were mounted on a multiple target holder ladder, oriented 45° to the beam axis and positioned vertically. The target holder was surrounded by a cylindrical electron suppressor held at negative potential with respect to the target. The ladder was moved vertically in the PIXE chamber for selecting the different targets maintaining the same projectile – target – detector geometry. Measurement was carried out with a low beam current of 20-30nA range in order to avoid pile up. For the detection of trace elements 50µm aluminum was used as an absorber to suppress prominent low energy K X-rays like Ca and a beam current of 40nA was used for it.

A Si (Li) detector [EG & GORTEC, active area 30 mm^2, beryllium window thickness of 12 µm, cooled at liquid nitrogen temperature i.e., 77K] with full width half maximum (FWHM) of 180 eV at 5.9 keV placed at 90° to the beam axis, was used to detect the characteristic X-rays emitted from the targets. X-rays exit the PIXE chamber through a 25µm Mylar window before entering the detector. Spectrum was recorded using a PC based MCA in 2K channel mode after being calibrating it with the Mn X-rays from the Fe^{55} radioactive source. The typical PIXE spectra obtained corresponding to different medicinal plants under study are shown in Fig. 1, Fig. 2, Fig. 3, Fig. 4 and Fig. 5 respectively.

Data analysis

These spectra were analyzed using GUPIX software package [29]. The versatile software very efficiently extracts X-ray intensities and converts them in to elemental concentrations. Non-linear least square fitting of the spectrum is done based on standardization technique which involves fundamental parameters, preset instruments constants and input parameters such as solid angle, charge collected, etc. Using this software package, different elements present in each of the anti-diabetic medicinal plants were identified and their concentrations were estimated.

RESULTS AND DISCUSSION

The concentration of various elements present in the medicinal plants under study are given Table 2. The elements potassium, calcium, titanium, vanadium, chromium, manganese, iron, copper, zinc, bromine, rubidium, strontium, and lead were found in varying compositions. It is also noted that the concentrations vary from sample to sample. Potassium, calcium, manganese, iron, copper and zinc were present in major concentration levels.

All the samples in the study contained appreciable amounts of potassium and calcium. The potassium content in the studied samples ranges from 11700 to 56700 ppm and calcium content varies in the range 17500 to 73400 ppm. *Adatoda vasika* contained highest amount of potassium and calcium followed by *Solena amplexicaulis* where as *Vinca Rosea* contained the least concentrations of these elements when compared to other plants under study. The study suggests that all the samples may also be administered to enhance the qualities of bone and teeth.

Table 1: List of anti-diabetic medicinal plants analyzed

Botanical name	Family	Local Name
Vinca Rosea	Apocynaceae	BillaGaneeru
AdatodaVasica	Acanthaceae	Vasaka
Senna Auriculata	Fabaceae	Tangedu
Solena Amplexicaulis	Cucurbitaceae	Creeping cucumber
Andrograhis Paniculata	Acanthaceae	Kalamegh

Table 2: Concentration of trace elements in ppm in the five medicinal in the present investigation

Elements	*Vinca rosea*	*Adatoda vasica*	*Senna auriculata*	*Solena amplexicaulis*	*Adregraphis paniculata*
K	11700	56700	44000	49800	47800
Ca	17500	73400	40400	73100	37800
Ti	3.18	9.88	8.99	10.23	23.35
V	-	-	-	-	12.75
Cr	0.33	1.32	0.96	1.57	1.11
Mn	53.43	108.44	100.10	138.4	177.36
Fe	57.79	106.69	72.45	137.30	355.77
Cu	6.30	44.10	6.30	126.00	25.20
Zn	23.10	121.82	23.84	181.43	87.55
Br	28.08	35.58	27.58	42.45	18.56
Rb	38.50	29.33	25.67	38.50	14.67
Sr	41.16	40.72	30.41	45.64	22.72
Pb	1.05	1.1	1.23	1.20	1.23

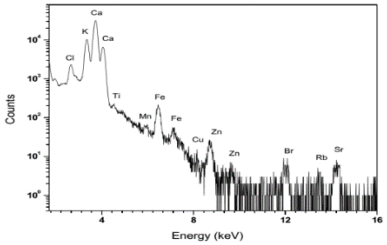

Figure 1: PIXE spectrum of *Vinca rosea*

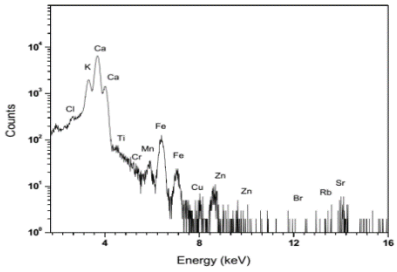

Figure 2: PIXE spectrum of *Adatoda vasica*

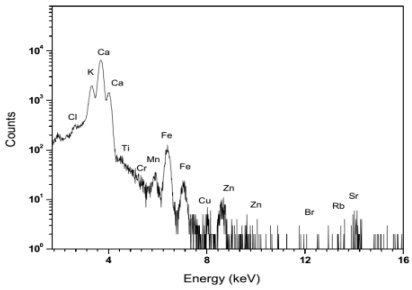

Figure 3: PIXE spectrum of *Senna auriculata*

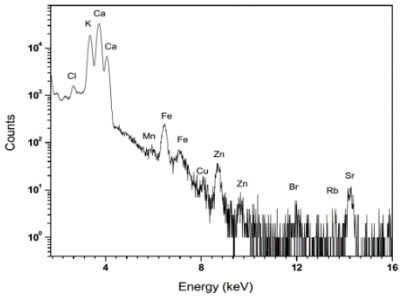

Figure 4: PIXE spectrum of *Solena aamplexicaulis*

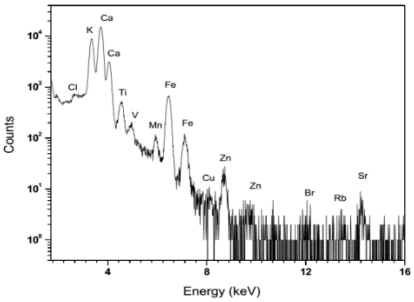

Figure 5: PIXE spectrum of *Andrograhis paniculata*

Study of the Table 2 clearly indicates that *Solena Amplexicaulis* contains highest amount of chromium (1.57 ppm), copper (126.00 ppm), zinc (181.43 ppm), bromium (42.45 ppm), rubidium (38.50 ppm) and strontium (45.64 ppm) when compared to that present in other plants. The concentration of trace elements, titanium, manganese and iron was also found to be fair in *Solena amplexicaulis.* The manganese content in various plants under study varies from 53.43 to 177.36ppm. Similarly, the concentration of Iron ranges from 57.79 to 355.77ppm.

A special attention has to be paid while studying the concentration of various elements present in *Andrographis paniculata*. As an Ayurveda herb it is known as *Kalmegh* or *Kalamegha*, meaning "dark cloud"**.** It is found that *Andrographis paniculata* had exceptionally high concentration of iron (355.77 ppm). The concentration of manganese was also found to be high in *paniculata* (177.36 ppm). Out of the five plants under study this was the only plant in which the transition metal vanadium was prominently found. The present study also reveals that *Vinca rosea* contained the least concentration of titanium, chromium, iron, copper, and zinc. Traces of lead were found to be present in all samples.

Various other studies show that potassium, calcium and trace elements especially chromium and zinc etc. are responsible for the secretion of insulin from β-cells of islets of langerhans[30] and also important for the glucose metabolic regulation[31,32]. Inorganic elements such as Cu, Zn, Cr, and Sr have been implicated in some metabolic disorders that include diabetes[33,34]. Zinc complex is the store house of insulin secreted from pancreas which plays an important role in glucose metabolism[35,36].

CONCLUSION

Since all vanadium compounds are considered toxic, the study suggests that vanadium should be isolated while administering the extracts of *Adrographis paniculata*. Keeping in mind the recommendations of Mayo Clinic Book of Alternative Medicine that pregnant women shouldn't use *Andrographis* because it could terminate pregnancy[37], care should be taken not to suggest *A. Paniculata* during gestational diabetes, though it contains high amounts of Mg and Fe which are much needed minerals for a pregnant woman for proper nourishment of the fetus. Of the five samples under study, *Solena amplexicaulis* contained the analyzed trace elements in recommended quantities and *Vinca rosea* contained the least.

However a noteworthy observation from the studies of other researchers on *Vinca rosea*, *Adatoda vasaka*, is that the concentration of the same element varies considerably from the reports of the present study though the same PIXE analysis was employed.[38,39]. Hence, further studies especially on these plants with respect to various conditions like the place where they grow, the climatic conditions, age, soil, water, etc. would certainly throw light on the elemental constitution and its variation to these conditions. Elemental analysis of different parts of these plants is also suggested for future scope in understanding the curative ability of the plants. This in turn would help the physicians to prescribe the type of extract, exact dosage, and combination of such medicinal plants. In light of the above discussion, an interdisciplinary research consisting of Biologists, Physicists and Pharmacologists certainly proves to be the need of the hour to make alternative herbal drugs available at a cut down cost for a common man and make the world a place for better living in-spite of looming, all pervasive lifestyle-induced diseases such as diabetes.

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