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Elemental analysis of some antioxidant-rich medicinal Zingiberales of Manipur using proton induced X-ray emission (PIXE) technique

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Abstract : Rhizomes of seven medicinal Zingiberales commonly used in Manipur have been analysed by the proton induced X-ray emission (PIXE) technique. The elements K, Ca, Mn, Fe, Cu, Zn, Rb and Sr are found to be present in varying concentrations. K and Ca have been estimated at percentage level while other elements are quantified at ppm level. Among the trace elements, Mn, an antioxidant rich element, is found to be present in high amount in most of the studied samples. Strong positive correlations are observed between K and Mn, K and Cu, Ca and Fe and Mn and Cu.

Keywords : PIXE, medicinal Zingiberales, antioxidant, trace element.

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1. Introduction

Medicinal herbs have been used from antiquity by humanity. Over three quarters of the world's population relies mainly on plants and plant extracts for primary heath care and there is a global resurgence of interest in the use of plant products to treat almost all diseases including cancer. Herbal medicines have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. They are rich source of synthetic and herbal drugs and contain a wide range of chemical compounds, commonly referred to as photochemicals. In addition, medicinal plants contain essential and trace elements, which can be available to the human body from any kind of consumptions of herbs and their extracts. Hence, it is desirable to establish the levels of elements in medicinal plants. Further, as certain elements at elevated levels are toxic, such an assessment would also be helpful in regulating their use.

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Medicinal Zingiberales are hot herbs today and a number of studies have shown that the rhizomes of these species possess a variety of potential antioxidant molecules [1-4]. These aromatic plants are not only used as food, spices and condiments, but they are also used in the traditional system of medicines for curing various human diseases [5,6]. The antioxidant and anticarcinogenic properties of curcumin, the chrome orange yellow coloring compound present in turmeric rhizome have been reported [7,8]. Rhizome extracts of *Alpinia galanga, Zingiber cassumunar, Kaempferia galanga, Curcuma caesia* and *Zingiber officinale* have been shown to possess antioxidant [9] properties. Supplementation of natural antioxidants through a balanced diet could be more effective and also more economical than the supplementation of an individual antioxidant, such as vitamin C or vitamin E, in protecting the body against oxidative damage under various conditions. Another advantage of using antioxidant agents of plant origin is that it can control the oxidative damage without any side effects. It is also reported that various elements present in plants have either direct or indirect role in the control and maintenance of the antioxidant defence system of the body. The trace elements copper, manganese, selenium and zinc act as co-factors of antioxidant enzymes to protect the body from oxygen free radicals that are produced during oxidative stress. It is necessary to maintain a balance between the harmful pro-oxidant components produced and the antioxidant compounds that counter these effects [10-12]. A delicate balance also exists for the redox trace elements such as copper, which can initiate free radical reactions but is also a co-factor of copper/zinc-superoxide dismutase, a free radical scavenging enzyme. Manganese is an antioxidant nutrient and is important in the breakdown of amino acids and the production of energy. It is an essential requirement for the metabolism of vitamin B1, C and E and for activation of various enzymes, which are important for proper digestion, and utilization of foods.

In the present study, the major and trace element content of seven medicinal Zingiberales commonly used in Manipur, a north-east state of India, have been determined by proton induced X-ray emission (PIXE) technique. PIXE is a powerful ion-beam analysis technique for the sensitive determination of elements and it has been used extensively in various fields [13-18].

2. Experimental

2.1 Sample preparation:

Fresh rhizomes of seven medicinal Zingiberales, namely, *Curcuma domestica, Zingiber officinale, Zingiber cassumunar* (Roxb), *Alpinia galanga, Heduichium marginatum* C.B Clarke, *Kaempferia galanga* Linn and *Curcuma caesia* were collected from different parts of Manipur. The outer skin of the rhizomes were removed, thoroughly washed with distilled water, cut into thin slices with a sharp steel knife, dried in an incubator at 40^0C and subsequently, ground into fine powder. The powdered samples were thoroughly mixed with high purity graphite powder in the ratio 4:1 by weight. This step is necessary for charge integration with better accuracy and eliminating the problems associated with charging during PIXE measurement. The samples thus obtained were further mixed with 200μl of

2-wt % polyvinyl alcohol, a binder, dried under an IR-lamp and subsequently pressed into pellets of 18mm diameter and 2mm thickness. The thick targets of Certified Reference Materials (CMRs) of cabbage (GBW 08504, China), wheat flour (NIST-8436) and bovine liver (NIST-1577b) used as calibration standards in the present study, were also prepared in a similar way.

2.2 Analysis :

PIXE measurement was performed at the Surface and Profile Measurement Laboratory at CCCM, BARC, Hyderabad using a 3MV Tandetron accelerator. A well collimated 2.4MeV proton beams of diameter 5mm and current 5-7nA was incident normally on targets placed inside an evacuated scattering chamber and the X-rays are detected by a planer high purity germanium (HPGe) (Eurisys Measures type EGX100-01, Be window thickness of 40 μm, FWHM of 150 keV at 5.9 keV) placed at 45^0 to the beam axis. A 25 μm mylar foil served as the X-ray exit window. The vacuum in the scattering chamber, pumped by a turbomolecular pump, was about 2×10^{-6} torr. An electron suppressor with a voltage of –900V was placed in front of the samples. The data were recorded on a PC based MCA and the spectral data were analysed by the GUPIX [19] software package which provides nonlinear fitting of the spectra.

In thick target PIXE (TT-PIXE), calibration standards having matrix composition identical to that of the sample under investigation are required for quantification with better accuracy. Therefore, three biological standard reference materials, as mentioned earlier, were used as calibration standards in the present study. In order to ascertain the accuracy of the analysis, the calibration standards were analysed against each other. The overall uncertainty in the estimation of all elements except Fe, on repetitive measurements, was observed to be 3-8%. It may be ascribed to errors in the measurement of peak areas and relative accumulated charges. In the case of Fe, the uncertainty was about 15%, which may be largely due to its inhomogeneous distribution in the targets. It is to be noted that sufficient care was exercised to avoid contamination during the preparation of the samples.

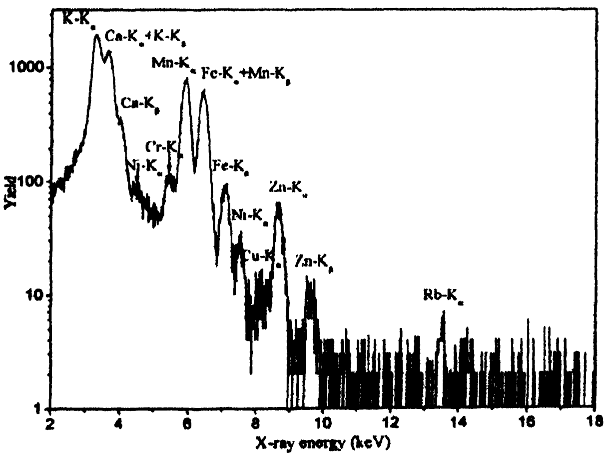

Figure 1. PIXE spectrum of *Zingiber officinale*

3. Results and discussion

The PIXE spectrum obtained from *Zingiber officinale* having peaks for various elements such as K, Ca, Mn, Fe, Cu, Zn, Rb is presented in Figure 1. The results of the elemental composition analysis of the samples are given in Table 1.

Table 1. Elemental concentrations of the medicinal rhizomes under study.

Sample	K (%)	Ca(%)	Mn	Fe	Cu	Zn	Rb	Sr
Curcuma domestica	4.84	0.329	24	142	8	46	5.3	...
Zingiber officinale	0.77	0.165	313.4	216.6	4.5	72.5	12.9	...
Kaempferia galanga Linn	3.92	0.22	632	177	8.4	66.5	16.4	...
Zingiber cassumunar (Roxb.)	2.33	1.94	203	83	2.7	45	...	....
Alpinia galanga	7.69	0.43	1803.3	231	28	60	....	....
Hedychium marginatum C.B Clarke	2.59	0.56	451	295	1.9	155.7	11.6	59.2
Curcuma caesia	1.62	0.069	215	27	3.4	153.5	8.2	...

Concentrations of elements are in ppm except for those major elements given in %.

From the data, it can be inferred that all the samples under study contain the trace elements Mn, Fe, Cu, Zn along with K and Ca as the major elements. The spectra of most of the samples also show the presence of Ti, Cr and Ni at very low concentration. Table 1 also shows the presence of high amounts of Mn, an antioxidant rich element, ranging between 203 ppm and 1803.3 ppm except in *Curcuma domestica* where the content is 24 ppm. But this value is also well within the common values reported elsewhere [20-25] for medicinal plants which range between 18 ppm and 700 ppm. *Alpinia galanga* contains a higher amount of Mn than the common values reported with 1803.3 ppm. An earlier analysis of tea leaves [26], reported values between 350 ppm and 900 ppm, concluding that tea drinks can provide a rich dietary intake of this metal. Another study [27] also reports an anomalous value of Mn reaching upto 2134 ppm in *Eucalyptus globules.* Among all the studied rhizomes, the K content is also highest in *A. galanga*. *H. marginatum* contains high concentration of Fe and Zn. As high levels of essential elements such as Fe, Mn, Zn and Ca have been demonstrated to influence the retention of toxic elements in animals or human beings [28, 29], we carefully searched for certain toxic elements such as Pb, As, Hg, Cd, *etc.* which are of prime interest in toxicological studies [28] but none was found in the rhizomes under study. Hence, these rhizomes could be considered safe to consume.

Table 2. Karl Pearson correlation matrix

Elements	K	Ca	Mn	Fe	Cu	Zn
K	1.000	.489	.757*	.273	.894*	-.409
Ca	.489	1.000	.452	.820*	.327	.157
Mn	.757*	.451	1.000	.443	.915*	-.146
Fe	.273	.820*	.443	1.000	.284	.054
Cu	.894*	.327	.915*	.284	1.000	-.378
Zn	-.409	.157	-.146	.054	-.378	1.000

Marked correlations are significant at $p< .05$

To investigate any kind of correlation between elemental concentrations and dependence of these concentrations amongst the studied Zingiberales, the Karl Pearson correlation matrix was calculated. Based on the correlation matrix (Table 2), it can be said that Mn is positively correlated with other elements except Zn. Ca and Fe have positive correlation with all the other elements. Correlations at statistically significant level are observed between K and Mn, K and Cu, Ca and Fe and Mn and Cu.

From the above discussions, it may be understood that the elements K, Ca, Mn, Fe, Cu and Zn might have played a significant role in the antioxidant property possessed by the medicinal Zingiberales. The high content of Mn, Fe, and Zn in these rhizomes may again support their effective use in the traditional system of medicine by the local people of Manipur. The low incidence of various tumors among the populations in north-east India, particularly in Manipur [30], may be linked with the high level consumption of these plant-based foods rich in high antioxidant elements in routine dietary intake. Further extensive investigation on the relationship of organic content and trace elements is in progress, and will be reported with better statistics in near future.

4. Conclusions

A PIXE investigation was carried out on some members of medicinal Zingiberales. The elements K, Ca, Mn, Fe, Cu, Zn Rb and Sr were found to be present in varying concentrations. The Karl Pearson correlation matrix of the common elements K, Ca, Mn, Fe, Cu and Zn was determined, from which the correlation co-efficient of two particular elements was observed. Most of the studied samples are found to contain high amount of Mn, Fe and Zn.

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