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Correlation of trace elemental content in selected anticancer medicinal plants with their curative ability using particle induced x-ray emission (PIXE)

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Trace elemental analysis employing particle induced x-ray emission (PIXE) technique was carried out in some selected medicinal plants used in the preparation of anti-cancer drugs. The 3MV Pelletron Accelerator at Institute of Physics, Bhubaneswar, India was used for the present PIXE measurements. A beam of 3 MeV protons, collimated to a diameter of 2 mm, bombarded the samples placed at an angle of 45° to the beam direction. The characteristic X-rays emitted by the elements present in the sample were recorded by a high resolution Si(Li) detector. The elements Cl, K, Ca, Ti, V, Mn, Fe, Cu, Zn, Br, and Sr were identified and their concentrations were estimated by using Guelph PIXE (GUPIX) software. These elements were found to be in widely varying concentrations in the specific parts of the analyzed anti-cancer medicinal plants. These medicinal plants can be considered as potential sources for providing a reasonable amount of the required elements other than diet to the cancer patients. The quantity of trace elements administered through these medicinal plants was found to be less than the recommended dietary allowance. The present data on elemental concentration in these medicinal plants will be useful to set new standards for prescribing the dosage and duration of administration of these herbal medicines to the cancer patients.

Key words: Trace elements, particle induced x-ray emission (PIXE), anticancer, medicinal plants.

INTRODUCTION

Herbal and natural products of folk medicine have been used for centuries in every culture throughout the world. Recently attention on plant research has increased all over the world and large body of evidence has been collected to show the immense potential of medicinal plants used in various traditional systems. Various parts of medicinal plants such as leaves, bark, root, fruits, and seeds are used in the treatment of several diseases. However, the ultimate objective of their use is that they should interact directly with our body chemistry and their active constituents must be absorbed into the body for deriving the required benefits.

Studies on the organic constituents of medicinal plants have been going on since a long time but recently the focus has been towards the inorganic constituents and their role in the preparation of drugs using the medicinal plants (Al-Omari, 2011; Rihawy et al., 2010; Negi et al., 2010; Devi et al., 2008; Naga Raju et al., 2006a). The role of inorganic elements in animal and plant metabolism has long been established. Most of the trace elements are essential to life, but they can have deleterious effects when present in excess. Death and disease related to acute or chronic exposure have been documented for some of the essential trace elements (Mertz, 1987; WHO,

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1996). It is essential to know the trace elemental concentration in medicinal plants from the point of view of nutritional requirement and intoxication risk associated with their consumption. The effects and influence of trace elements on administration of medicinal plants is also essential to understand the pharmacological action of herbs and to decide the dosage of the herbal drugs prepared from these plant materials.

Particle induced x-ray emission (PIXE) technique was first introduced at the Lund Institute of Technology by Johansson et al. (1970). It is a powerful and relatively simple analytical technique that can be used to identify and quantify trace elements. It has rapidly gained acceptance as a valuable analytical tool, because of its capability to detect simultaneously several elements present at very low concentrations. Owing to these advantages, PIXE technique was chosen for elemental analysis of specific parts of some selected medicinal plants known for their anti-cancer properties. Table 1 illustrates in detail the anti-cancer medicinal plants analyzed in this study, their common name, part studied, active principles underlying their curative properties, and their postulated mechanisms of action.

EXPERIMENTAL DETAILS AND DATA ANALYSIS

Ten to eighteen samples of the specific parts of six different anti-cancer medicinal plants, collected from Regional Forest Research Centre, Rajahmundry, were washed in tap water and rinsed thoroughly with double distilled water in order to remove surface contamination. Each plant sample was oven dried at 60℃. These samples were then homogenized in an agate mortar. Each powdered sample weighing 150 mg was mixed with high purity graphite powder, in order to monitor the beam current. A known quantity of yttrium with known concentration was then added an internal standard. The final powder was compressed using a 10 ton hydraulic press into pellets of 13 mm diameter and about 2 mm thickness. While pelletizing, both faces of the compression die were covered with thin disks of poly tetra fluoro ethylene to avoid direct contact between the powdered sample material and faces of the die. These pellets were then used as targets for the PIXE experiment.

A proton beam of 3 MeV energy generated by a 3MV Pelletron Accelerator was used to excite the samples in the present work. The samples were placed at an angle of 45° to the beam direction. The characteristic X-rays emitted from the sample were recorded with a high resolution Si(Li) detector (FWHM 160 eV at 5.9 keV energy), which was placed at an angle of 45° to the target position. The spectra were collected for a sufficiently long time so that good statistical accuracies were achieved. For each sample, the total charge collected and the average beam current were noted. The x-ray spectra corresponding to different medicinal plants are shown in Figure 1. The Guelph PIXE (GUPIX) software package (Campbell et al., 2010) was used to analyze the spectra of the anti-cancer medicinal plants. This package has provision to identify and quantify the different elements present in the sample. The accuracy of this technique and the use of GUPIX software were checked by analyzing NIST certified reference material (Apple leaf - Sample No. 1515) in the same experimental conditions. The obtained results, which are furnished in our earlier work (Naga Raju et al., 2006a), show good agreement between measured and certified values within experimental uncertainties.

RESULTS AND DISCUSSION

The specific parts of the anti-cancer medicinal plants listed in Table 1, were analyzed for their trace elemental content using PIXE technique. The elements Cl, K, Ca, Ti, V, Mn, Fe, Cu, Zn, Br, and Sr were identified and their relative concentrations were estimated in these anti-cancer medicinal plants. The average concentrations of the elements in different samples of each medicinal plant were estimated and are given in Table 2. The corresponding standard deviations for each element are also given in Table 2. Analysis of the present data reveals that the elements Cl, K, and Ca are found in major concentrations in these plants and Fe is observed in highest concentrations in all the medicinal plants when compared to the other trace elements.

From Table 2, it is observed that considerable levels of calcium (ranging from 10 mg/g in the leaves of *Andrographis paniculata* to 45.1 mg/g in the seeds of *Abrus precatorius*) are present in all the medicinal plants that are analyzed in the present work. The recommended dietary allowance (RDA) of calcium is 800 to 1200 mg per day. The daily dose of *A. precatorius* as prescribed by local physicians is 400 mg. Since 1 g of *A. precatorius* contains 45.1 mg of calcium, the amount of calcium supplemented to a patient through 400 mg of this medicinal plant is 18 mg. This suggests that the specific parts of the analyzed anti-cancer medicinal plants can be consumed without causing complications of metal toxicity.

Calcium is an essential structural and functional element in living cells. Among the numerous cell functions modulated by calcium, its participation in cell division and the regulation of cell proliferation and differentiation are particularly important (Whitfield et al., 1979). Numerous studies (Baron et al., 1999; Holt et al., 1999a, 1999b) have shown that calcium may reduce the risk of developing colon and breast cancer. It is proposed that calcium binds bile acids in the bowel lumen, inhibiting their proliferative and carcinogenic effects. For people with cancer, calcium intake may be very important for building and maintaining bone mass and strength, because some chemotherapy medications and radiation therapy cause osteopenia and osteoporosis. The traditional use of these plants in the preparation of anti-cancer drugs can well be correlated with significant concentrations of calcium present in them. When combined with vitamin D, calcium may have the potential to help prevent cancers of the breast and colon (Lipkin et al., 1999).

Vanadium was identified in almost all the anti-cancer medicinal plants that are analyzed in the present work. Leaves of *Catharanthus roseus* contain the least concentration (2.1 µg/g) of vanadium while the highest concentration (106 µg/g) was present in the roots of *Panax ginseng.* The dosage of *P. ginseng* prescribed daily by the local herbal practitioners for cancer treatment

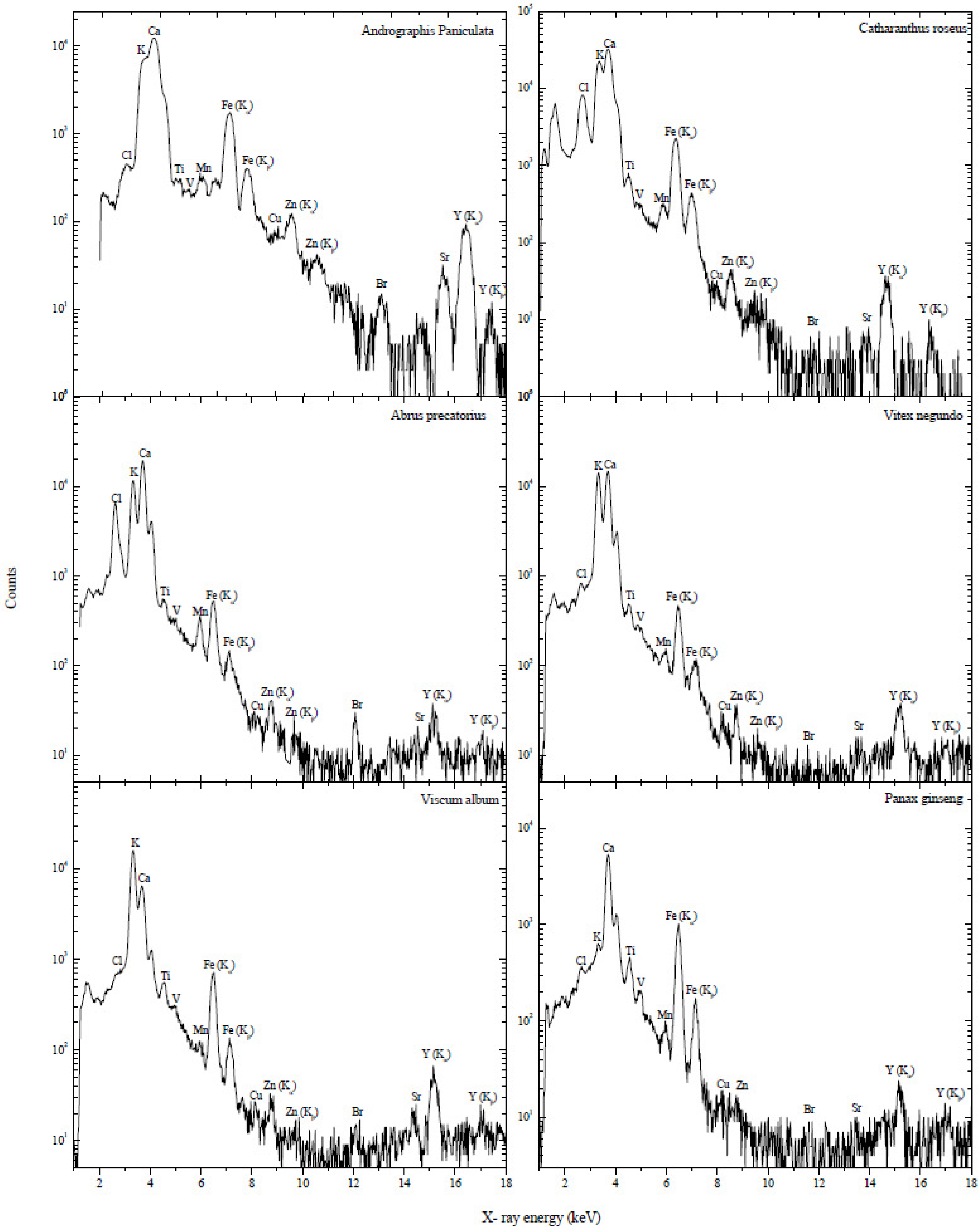

Figure 1. PIXE spectra of anti-cancer medicinal plants.

Table 1. List of anti-cancer medicinal plants analyzed in this study.

Botanical name	Common name	Part studied	Active Principles	Postulated mechanism of action
Andrographis paniculata	Creat, Kariyat, Indian Echinacea	Leaves	Andrographolide, Deoxyandrographolide, Neo-andrographolide	Stimulates immune system, induces cell differentiation activity and cytotoxic effects on cancer cells.
Catharanthus roseus	Cape periwinkle, Madagascar periwinkle	Leaves	Vinblastin, Vincristine, Vinorelbine, Vindesine	Halts mitosis of affected cells and causes cell death, probably prevents the growth of new blood vessels that support tumor growth.
Abrus precatorius	Jequerity, Rosary Pea	Seeds	Abrine, Glycyrrhizin, Lipolytic enzyme. Abraline, Abrasine, Abricin, Abrin, Abrusgenic-acid	Abrins have been used in experimental cancer research as they were found to be more toxic to cancer cells than normal cells in human tissue.
Vitex negundo	Nirgundi	Leaves	Betulinic acid, Ursolic acid, Luteolin, D-fructose, Casticin, β-sitosterol, p-hydroxybenzoic acid, Chrysophenol D, Isoorientin	Exhibits cytotoxic effects on cancer cells.
Viscum album	Mistletoe	Fruits	Viscotoxins, Glycoproteins, Lignans, Choline, Vitamin C, Triterpene Saponins, Caffeic acid, Histamine, Lectins Flavonoids,	Enhances immune system and exhibits cytotoxic effects on cancer cells.
Panax ginseng	Ginseng	Roots	Ginsenosides or Panaxosides Flavanoids, Daucosterin, Mucilaginous substances, , Bitter substances, Vitamins, Choline, Pectin, Fatty oil, Ethereal oil, Amino acids	Enhances the immune system , inhibits angiogenesis, and exhibits anti-mutagenic effects.

is 300 mg, which contains ~0.03 mg of vanadium. This amount of vanadium administered daily to cancer patients via these medicinal plants is safe to be taken considering the suggested daily RDA of vanadium to be 0.1 mg. This observation probably accounts for the anti-cancer property of these plants since various studies (Kanna et al., 2003; Ghosh et al., 2000; Narla et al., 2000; D'Cruz and Uckun, 2000) have revealed that vanadium acts as a promising anti-tumor agent. Vanadium based drugs were found to be effective against different cancer cell lines as well as cancer cells taken directly from patients. It is observed to be capable of exhibiting some unique beneficial effects particularly, its anti-carcinogenic potential under a very low dose without any adverse toxicity (Bishayee and Chatterjee, 1994; Chakraborty et al., 1995a, 1995b). Vanadium is not observed to be present in some medicinal plants which are used in the treatment of ailments other than cancer. The presence of vanadium in these medicinal plants might reduce the absorption of chromium by its antagonistic relationship with chromium, which is a well known carcinogenic agent. Interestingly, there were no traces of chromium observed in these plants. Administration of small amounts of vanadium through these medicinal plants can be attributed to the curative ability of these plants.

The concentration of zinc observed in the present study ranges from 13.4 to 79.5 µg/g in the different plants analyzed (Table 2). The highest levels were observed in the seeds of *A. precatorius* while the lowest concentrations were observed in the roots of *P. ginseng.*

The presence of zinc in these medicinal plants

Table 2. Concentrations of elements present in some anti-cancer medicinal plants (μg/g).

Elements	*Andogrophis paniculata*	*Catharanthus roseus*	*Abrus precatorius*	*Vitex negundo*	*Viscum album*	*Panax ginseng*
Cl	11900±150	38000±380	86100±640	2450±340	7690±140	2310±400
K	16500±210	23700±240	1840±140	1210±120	8420±280	3880±130
Ca	10000±800	15700±310	45100±350	17400±180	33000±140	15400±140
Ti	52.0±7.2	146.7±5.8	600±88	317±48	240±23	935±110
V	13.0±4.3	2.1±1.4	ND	19.4±12.9	18±14	106±43
Mn	30.0±1.8	52.0±3.2	493±55	72.8±15.6	28.0±6.8	105 ±24
Fe	386.0±14.3	682.0±13.6	900±90	458 ±57	472±21	2330±240
Cu	2.8±1.2	9.6±3.8	21.3±13.7	9.4±6.3	7.2±3.6	14.7±11.3
Zn	35.2±3.4	31.1±2.7	79.5±17.6	38.8±8.5	17.3±4.0	13.4±11.5
Br	14.4±2.8	1.83±2.7	160±34	6.1±1.6	14.9±7.4	ND
Sr	85.2±9.2	21.5±10	ND	ND	59±17	10.7±8.7

ND: Non detectable.

correlates with their use in the treatment of cancer since it was observed that there is a direct correlation between cancer and zinc deficiency (Naga Raju et al., 2006b, 2007; Rosas et al., 1995). Owing to the antagonistic property, zinc present in all the medicinal plants under study probably reduces the absorption of copper, which is a cancer-promoting agent (Goodman et al., 2004; Theophanides and Anastassopoulou, 2002).

It is to be noted that, on administering zinc through these anti-cancer medicinal plants some amount of copper also gets supplemented. But, the concentration of copper being supplemented is relatively less. From Table 2 it is observed that, except in *P. ginseng*, in all other plants the concentration of copper is nearly three to four times less than that of zinc, its antagonistic element. Therefore, whatever amount of copper enters the body through these plants is possibly prevented from being absorbed by the relatively high levels of zinc present in the plants. In view of the protective role of zinc against carcinogenesis, considerable amounts of zinc present in these plants support their usage for the treatment of cancer.

An interesting observation made in this work is the absence of chromium in the analyzed medicinal plants. This observation correlates well with the use of these medicinal plants in the treatment of cancer since chromium is a well known carcinogenic agent (IARC, 1998). However, a survey of literature (Al-Omari, 2011; Rihawy et al., 2010; Negi et al., 2010; Devi et al., 2008; Naga Raju et al., 2006a) related to elemental analysis of different medicinal plants used in the treatment of various ailments other than cancer shows that chromium is present in appreciable quantities in those medicinal plants. The absence of chromium in the work of Abdel-Wahab et al. (2009), where EDXRF technique was used to analyze elemental content of traditional anticancer medicinal plants, is in consonance with our observation.

Conclusions

PIXE technique has been used to identify and quantify 11 elements Cl, K, Ca, Ti, V, Mn, Fe, Cu, Zn, Br and Sr in six anticancer medicinal plants. The results obtained in the present study on trace elemental content of the analyzed anticancer medicinal plants provide a better understanding of the pharmacological action of these plants and they can also be used to set new standards for prescribing the dosage of the herbal drugs prepared from these plant materials. The supplementation of essential trace elements to cancer patients through these medicinal plants can be considered safe since the dosage prescribed by the local herbal practitioners does not present the risk of metal toxicity.

However, data on quantitative estimation of trace elements in different anticancer medicinal plants analyzed in this work can be used to administer these herbal drugs in an effective manner.

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