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Ion Beam Analysis of Proton-Induced X-ray Emission (PIXE) Techniques for Elemental Investigation of Young Stage Neem Leaf of Southern India, Tamil Nadu

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Abstract

Young stage neem leaf (*Azadirachta indica*) was collected at Thiruvallur district in Tamil Nadu, South India. Multi-elemental analysis of neem leaf was carried out using non-destructive techniques (NDT) of proton-induced X-ray emission (PIXE, 2.5 MeV) which is one of the well-known surface chemical sophisticated analytical methods of ion beam analyses (IBA). From the emitted X-ray output of the target specimen specimen fingerprint multi-elements such as, aluminium (Al), silicon (Si), phosphorus (P), sulphur (S), chloride (Cl), potassium (K), calcium (Ca), titanium (Ti), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) and strontium (Sr) are found to be present in the leaf in different ppm levels. Among the elements, silicon (Si, 42034 $\pm$ 1198.0 ppm) and potassium (K, 28985 $\pm$ 747.8 ppm) showed the highest concentration. Minor elements (Mn, Sr, Fe, Zn, Cu and Ti) are observed in the neem plant. The variation in elemental concentration in the leaf may be due to soil, water, etc. However, there are no toxic elements observed like arsenic and lead in the leaf. Further, though the presence of different medicinal values in the target specimen chemical multi-elements observed in ppm level. However, there are more chemical analysis to be required for the functionalization of active biomedical applications for these kinds of medicinal species.

Keywords Elemental analysis · Biological specimen · Neem leaf · Ion beam technique · PIXE

Introduction

Medicinal plants contain both organic and inorganic compounds. Trace elements play a major role for the formation of bioactive compounds and the toxicity. Based on the trace element composition, the therapeutic efficacy and the dosage of the medicinal plants act as herbal drugs [1, 2]. *Azadirachta indica* (neem) is a traditional plant which

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contains various unique natural products, which proceed against various diseases. Neem has been extensively employed in siddha, ayurveda, unani and homeopathic medicines, which is a bigwig for modern medicine [3]. Neem oil and leaf extracts have been therapeutically employed to control leprosy, intestinal helminthiasis, respiratory disorders and constipation and used as a health promoter. More than 140 compounds have been isolated from the different parts of neem plant [4]. Nimbidin is a major crude compound extracted from the oil of seeds. Neem demonstrates several biological activities such as antifungal, antimalarial, anti-inflammatory, antibacterial and antiarthritic. From this crude, some tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid, have been isolated. Nimbidin and sodium nimbidate possess significant dose-dependent anti-inflammatory activity [5–7].

Various analytical techniques, such as XRF (X-ray fluorescence), TXRF (total reflection X-ray fluorescence), NAA (neutron activation analysis), and AAS (atomic absorption spectroscopy), have been employed to determine the composition of the medicinal plants. PIXE (proton-induced X-ray emission) was employed for rapid analysis of wide range of trace elements in ppm level [8–10]. Ion beam analysis (IBA) is a multi-elemental analytical method and used in different fields to provide information about elements present on the samples at ppm level. IBA of leaves of *Azadirachta indica* was showed higher concentration of Cr, Mn, Fe, Ni, Cu, Zn and Pb in the industrial areas in Vishakapatinam reported by Ravikumar et al. [11]. IBA was carried out on stem and bark of *Azadirachta indica* exhibited excess concentration of Cr, Pb, Ni, Co, Cd and As which exceed the permissible limit of World Health Organization (WHO) due to the cause of environmental pollution. However, to the best of our knowledge, no previous IBA on young stage of neem leaf has carried out. The main aim of the current study is to employ IBA for the identification and quantification of the average elemental concentrations (ppm) of young stage neem leaves in South India.

Materials and Methods

Sampling

Young leaves of *Azadirachta indica* (neem) were identified and collected from Thiruvallur district, near the industrial area Manali, Chennai, Tamil Nadu.

Sample Preparation

The first stage of young leaves of *Azadirachta indica* were washed and rinsed twice with distilled water to avoid

Fig. 1 Neem young stage leaf

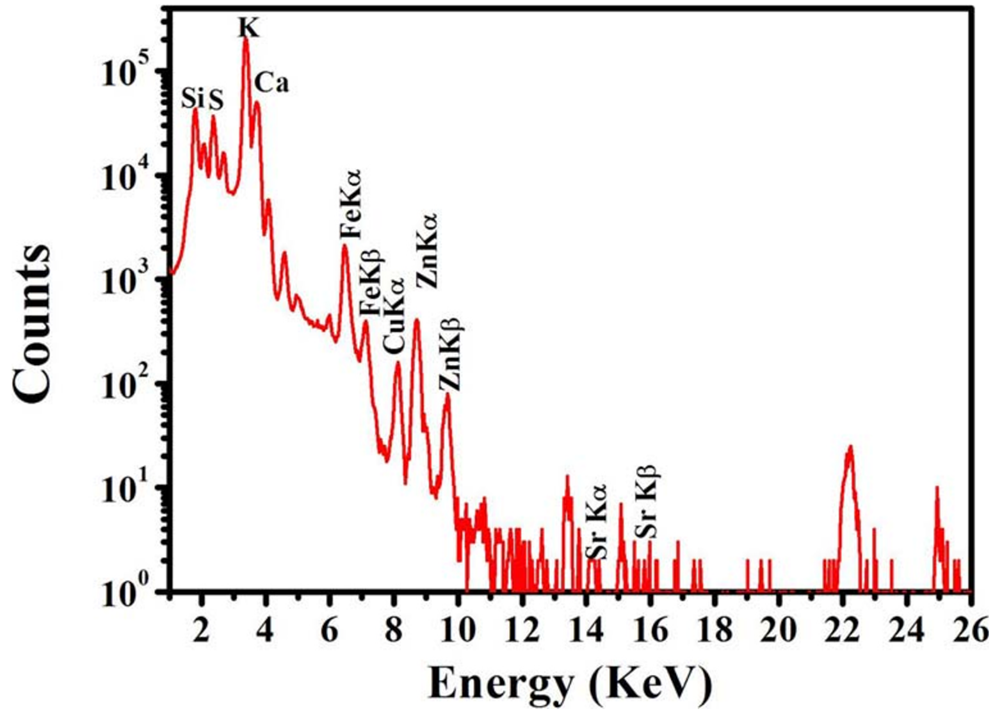

Fig. 2 Typical PIXE spectrum of young stage of neem leaf

contaminations and shade dried for 3–5 days and the powdered using mortar and pestle.

Plant description:

Order	Rutales
Sub-order	Rutinae
Family	Meliaceae (mahogany)
Sub-family	Melioideae
Genus	*Azadirachta*
Species	*indica*

Table 1 Multi-elemental composition in young stage of neem leaf

Elements	Concentration in ppm
Al	9671 ± 624.7
Si	42,034 ± 1198.0
P	5955 ± 284.1
S	6233 ± 185.1
Cl	1882 ± 64.2
K	28,985 ± 747.8
Ca	5213 ± 162.6
Ti	258.9 ± 10.3
Mn	54.18 ± 7.1
Fe	922.8 ± 32.1
Ni	0
Cu	157.8 ± 11.3
Zn	603.1 ± 27.6
Br	0
Sr	29.75 ± 14.6

PIXE Analysis

IBA (PIXE) was carried out using the 2.5-MeV Accelerator at GNS Science, New Zealand. The total accumulated charge for each sample around 9.92 μC. Characteristic X-rays generated from the sample were detected by a silicon (lithium) equipped with a 25-μm beryllium absorber. The PIXE spectra were analyzed by GUPIX8 software for quantifying the elemental concentration in ppm level (Figs. 1 and 2). From the Fig. 2 shows the PIXE spectrum observed the multi-elements such as Al, Si, P, S, Cl, K, Ca, Ti, Mn, Fe, Cu, Zn, Br and Sr were identified.

Results and Discussion

Trace elemental analysis was carried out in young stage of neem leaves collected from the industrial area near Manali, Thiruvallur district, Tamil Nadu, South India, using IBA. Elements such as Al, Si, P, S, Cl, K, Ca, Ti, Mn, Fe, Cu, Zn, Br and Sr were identified. The elemental concentrations of the collected young stage of neem leaves were mentioned in Table 1. The elements like Si and K are highly found in young stage neem leaves, whereas less concentration of strontium is found. Silicon (Si) plays a major role in the prevention of insomnia, skin disorders, tuberculosis and atherosclerosis. Silicon is the

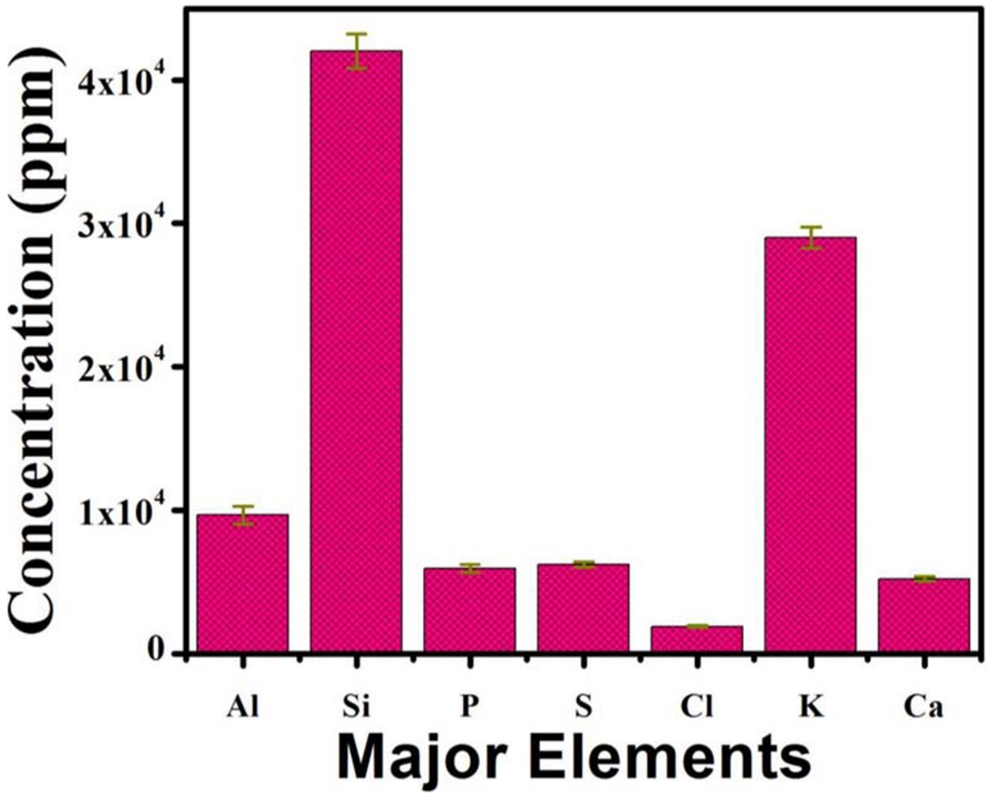

Fig. 3 Major elements in young stage of neem leaf

most important trace mineral required for the strong and flexi joints, glowing skin and stronger bones and also involved in assisting calcium for growth and maintenance of bones and joints. Silicon restores the natural glow of the skin and aids to increase healing process by enhancing the healing rate during fractures and by protecting against

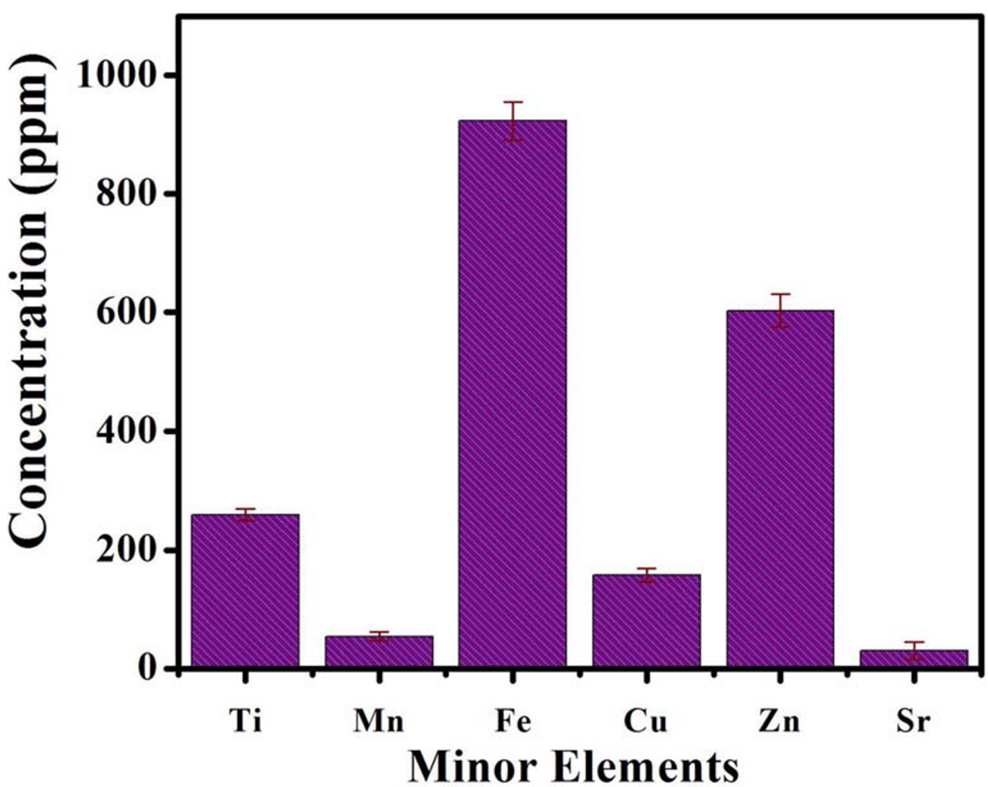

Fig. 4 Minor elements in young stage of neem leaf

Table 2 Comparison of Al concentration in young stage neem leaf with other herbal materials

Element	Present work (ppm)	*A. indica* (mg/kg) [21, 22]	*A. barbadensis* (mg/kg) [21]	*T. terrestris* (mg/kg) [21]	Hibiscus (mg/kg) [22]	Lemongrass (mg/kg) [22]	Ginkgo (female) (mg/kg) [22]	Rosehip (mg/kg) [22]
Al	9671 ± 624.7	134 ± 35	919 ± 58	1680 ± 165	272 ± 19	1080 ± 50	74.17 ± 1.5	157 ± 12

Table 3 Agricultural materials (powder form); additional information

Sr. no.	Elemental composition as mass fraction in mg/kg (ppm) unless noted by an * asterisk for %	
	Parameter	Value
1	Aluminium	286
2	Antimony	(0.013)
3	Arsenic	0.038
4	Barium	49
5	Boron	27
6	Bromine	(1.8)
7	Cadmium	(0.013)
8	Calcium	1.526*
9	Cerium	(3)
10	Chlorine	579
11	Chromium	(0.3)
12	Cobalt	(0.09)
13	Copper	5.64
14	Europium	(0.2)
15	Gadolinium	3
16	Gold	(0.001)
17	Iodine	(0.3)
18	Iron	83
19	Lanthanum	(20)
20	Lead	0.470
21	Magnesium	0.271*
22	Manganese	54
23	Mercury	0.044
24	Molybdenum	0.094
25	Neodymium	(17)
26	Nickel	0.91
27	Nitrogen	2.25*
28	Phosphorus	0.159*
29	Potassium	1.61*
30	Rubidium	10.2
31	Samarium	3
32	Scandium	(0.03)
33	Selenium	0.050
34	Sodium	24.4
35	Strontium	25
36	Sulphur	(0.18*)
37	Terbium	(0.4)
38	Thorium	(0.03)
39	Tin	(< 0.2)
40	Tungsten	(0.007)
41	Uranium	(0.006)
42	Vanadium	0.26
43	Ytterbium	(0.3)
44	Zinc	12.5

- Certified values are normal font

- Reference values are italicized

- Values in parentheses are for information only

The certificate is the only official source for values and uncertainties

many mucous membrane–related diseases, symptoms and also preventing aluminium toxicity, which aids to improve the quality of nails [12].

Potassium is the third most abundant mineral in humans, apart from acting as an electrolyte to relief from stroke, blood pressure, heart and kidney disorders, anxiety and stress, enhanced muscle strength, metabolism, water balance and nervous system. It acts as an activator of some enzymes and coenzymes which are involved in normal growth and muscle function [13]. Calcium has been used in cell walls, bones and other structural components. The deficiency of zinc is characterized by recurrent infections, lack of immunity and poor growth and to treat ulcers and wound healing applications [13–15].

The elements such as Ti, Fe and Sr are responsible for the treatment of gonorrhea, malaria, onchocerciasis, pile, hemorrhoids and dizziness. Bowen et al. reported that the concentrations of Cr, Co and other trace elements are well below the permissible limit [16]. Copper is the metallic trace element naturally present as a free metal, which is involved in the oxidation of Fe^{2+} to Fe^{3+} during the formation of the Haemoglobin and an important for iron absorption. Deficiency of copper leads to cardiac disorders, osteoporosis, anaemia and neutropenia. In young stage neem leaves, the concentration of copper is in permissible limit [17]. The essential role of copper in enzymes is related with oxidative metabolism and to contribute potential toxicity by free radical's generation [18]. Moreover, iron is an essential element to produce haemoglobin and oxygenation for red blood cells and also provided energy production and its deficiency results in anaemia. The function of titanium is not yet known; it is harmless to our body. Excess of iron storage in the body is associated with chronic diseases such as cancers, diabetes and heart disease [19, 20].

Figure 3 indicates the major elements in young stage neem leaves among which Si and K are present in higher concentrations in comparison with other elements such as Al, P, S, Ca and Cl. Figure 4 elucidates the minor elements in young stage neem leaves among which Sr and Mn are present in a least concentration when compared with Fe, Zn, Cu and Ti. In young stage neem leaves, no toxic elements like As, Cd, Hg and Pb were detected. The variation in elemental concentration in young stage neem leaves could be due to environmental conditions, water and soil. The Al concentration in *A. indica* is compared with other herbs (Tables 2 and 3) and showed high concentration as well. IBA data was also compared with the standard reference materials like NIST-1646A

(National Institute for Standard and Technology) samples, and the values are within the limit of detectable (PIXE spectra & tables are given in the Supplementary Materials) [23–28]. The variation in elemental concentrations of neem leaves is not yet clearly understood precisely.

Conclusions

Elements such as Al, Si, P, S, Cl, K, Ca, Ti, Mn, Fe, Ni, Cu, Zn, Br and Sr were found in young stage neem leaves (*Azadirachta indica*) and quantified in ppm level by IBA. Higher concentration of Si (42,034 $\pm$ 1198.0 ppm) and K (28,985 $\pm$ 747.8 ppm) is observed in the neem leaves compared with other elements. The minor elements such as Mn and Sr are found in the neem leaves. However, toxic elements like Pb, As, Ni and Cr are not noticed in young stage neem leaves. The variation in elemental concentration of the neem leaves might be due to environmental condition, soil, water, etc. Based on the overall results, the elements found in the young stage neem leaves are in permissible limit and further extensive deep chemical analysis to be performed for the functional herbal active drug applications.

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