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Elemental analysis of medicinal plants from different sites by instrumental neutron activation analysis

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Abstract: A study was undertaken to evaluate the inorganic elements for humans in two Indian medicinal plants leaves, namely *Sphaeranthus indicus*, and *Cassia fistula* by Instrumental Neutron Activation Analysis (INAA). INAA experiment was performed by using 20 kW KAMINI Reactor at Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam. The emitted gamma rays were measured using gamma ray spectrometer. The concentrations of Al, Br, Ca, Fe, K, La, Mg, Mn, Na, Sc, V and Zn were determined in the selected medicinal plants. The medicinal leaves are using in treatment of various important ailments. The elemental content in selected medicinal leaves is various proportions depending on the soil composition, location of plant specimen and the climate in which the plant grows.

Key words: medicinal plants; reactor; HP (Ge) detector; neutron source and gamma assay.

Introduction

Medicinal plants are the oldest known health care products used for the treatment of various ailments. They are rich source of antioxidants and essential inorganic elements [1]. They may also contain toxic elements as well. The medicinal plants are gaining popularity as alternative and complementary therapies around the world because human body is more accustomed to natural products. Therefore, scientific studies of medicinal plants are required to test their potentials and characterize the medicinal properties. Moreover, medicinal plants contain several bio-chemical substances that produce definite physiological actions in the metabolism of the human body. Inorganic elemental levels present in medicinal plants are of great importance due to their pharmacological actions [2]. Quantification of essential and toxic inorganic elements is an important task in determining the effectiveness of the medicinal plants in treating certain diseases.

In the present investigation, sensitive analytical technique INAA has been applied to study the elemental content in leaves of *Sphaeranthus indicus,* and *Cassia fistula* from different sites. The leaves of *Sphaeranthus indicus,* and *Cassia fistula* were widely used in treatment of several diseases associated with inflammation, microbial infections, good wound healing affect, jaundice (viral infection), gastric disorders, piles, anemia, asthma, swelling, rheumatism, insect bites and facial paralysis.

Instrumental Neutron Activation Analysis (INAA) is one of the important and powerful nuclear activation techniques for simultaneous multi elemental analysis in diverse matrices [3]. INAA method has been applied, in the past to study several biological materials including plant samples [4, 5]. The principle of INAA is based on irradiation of a sample with neutrons. Different forms of neutron sources are available such as spontaneous-fission source, alpha-Beryllium source and nuclear reactor. Neutrons from the source are used to irradiate the samples to produce radioactivities. In subsequent measurement of the decay of the induced radioactivity will be used for quantify the concentrations of individual elements in the sample. INAA method has the advantages such as high sensitivity for many elements, less matrix effects, not influenced by the chemical state of the elements under investigation, accuracy and precision. INAA has one important limitation as needs a neutron source like nuclear reactor that is available at limited research centres. Several studies are available in the literature on elemental analysis in medicinal plants using INAA. The measured essential element levels and discussed their role in human body metabolism in traditional herbal medicines using INAA [5] and also used INAA method for study of essential trace elements in Indian medicinal herbs [1].

Sphaeranthus indicus Linn. (Synonym: *S. hirtusWilld.*) belongs to the family *Astraceae*, is usually known as Bodasaramu (Telugu) Munditika, Sravani (Sanskrit) and Indian Globe thistle (English). In general this plant is found to grow in paddy fields, dry waste places, damped areas and shady places in plains throughout India, Sri Lanka, Australia, Malaya and Africa [6].

The entire plant is appreciated as a general tonic, alterative, digestible, bitter, aphrodisiac, fattening,

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alexipharmic, emollient and anthelmintic [6, 7]. All the parts of the plant have medicinal value. Flowers are useful as blood purifiers in skin diseases [8], which are formed in October - January and fruits are produced in December-April. Root bark is reported as a precious remedy for piles [8]. The juice of the plant is styptic and supposed to be useful in liver and gastric disorders [9]. The sesquiterpene glycoside inaccessible from *Sphaeranthus indicus* is having immunostimulant effect [10]. The plant is reported to have potential of anti-inflammatory, antimicrobial activity and good wound healing [11, 12]. More over it was used in tuberculosis, bronchitis, anemia, asthma etc. In view of the ayurvedic uses and reported properties of the drug, it is proposed to explore its antiasthmatic potential using several experimental methods.

Cassia fistula Linn. (family *Fabaceae*, sub-family *Caesalpinioideae*), is well-known as golden shower tree, in English popularly called Indian Laburnum and as "rela" in telugu. It is very common plant is known for its medicinal properties and extensively used in Ayurvedic system of medicine for different ailments. Its medicinal properties are a semi-wild in nature. It is spread in several regions including Asia, South Africa, China, West Indies and Brazil [13]. It is deciduous and mixed-monsoon forests throughout superior parts of India, rising to 1300 m in outer Himalaya, is commonly used in traditional medicinal system of India [14].

Cassia fistula is a deciduous tree with greenish pale grey bark, trunk straight, complex leaves, leaves are 5-12 cm long arranged in pairs. The fruit is cylindrical pod and seeds numerous in black. The pods are pendulous, they are 40-70 cm long and 20-27 mm in diameter, straight or slightly curved shining, smooth, brown-black and indehiscent, with numerous (40-100) horizontal seeds. Seeds are 8 mm long and 5 mm thick as broadly ovate. The root, flower and pod is prescribed as a tonic, astringent, febrifuge and strong purgative [14] and moreover roots are useful in fever, heart diseases, joint pain, migraine and blood dysentery. The fruits are very important to be used for asthma [15], in the dealing of diabetes, chest complaints, liver complaints and eye diseases [16]. Seed powder also used in amoebiasis [17]. The leaves have laxative property and used externally as emollient, a poultice is used for chilblains, swelling, rheumatism, in insect bites and facial paralysis [14, 16 and 18]. The leaves are used in jaundice, piles treatment, rheumatism ulcers and also externally skin eruptions, ring worms, eczema.

Materials and Methods

Sample collection and preparation: In the present investigation samples were collected from different sites of agency region in East Godavari district, Andhra Pradesh, India. *Sphaeranthus indicus* was collected from three different sites whereas *Cassia fistula* was collected from two different sites. The sampling sites were located around Rampachodavaram and Maredumilli agency in East Godavari district as shown in Fig. 1. The samples were cut into small pieces. Surface contaminants of the samples were removed by washing with tap water twice and then with deionized double distilled water. The samples were dried at 70°C for overnight in an oven to the remove moisture content. The samples were powdered using agate mortar, pestle and passed through 100-mesh sieve. The biological reference material NIST CRM No: 7 (Tea leaves) was used as a comparator.

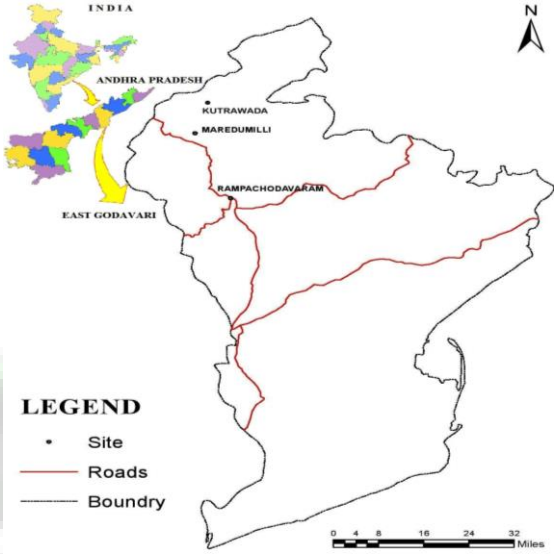

Figure 1: Map showing the sample collection locations in East Godavari district.

Irradiation and Analysis: INAA is based on the co-irradiation of sample and standard with neutrons from KAMINI nuclear reactor [19], IGCAR, Kalpakkam, India. The samples were weighed in the range 75-150 mg and heat sealed in clean polyethylene sheet. Samples and reference materials were packed in identical geometry and placed in the rabbit (20 mm diameter and 30 mm length). Samples were irradiated for 5 minutes for short lived radionuclides followed by 6 hours for long lived radionuclides at thermal neutron flux of $6.0*10^{11}$ $cm^{-2}s^{-1}$ at Pneumatic fast transfer system of KAMINI reactor, IGCAR [19]. The irradiation time and counting time were chosen based on the half-lives as well as properties of the produced radioactive isotopes of interest. Measurement of gamma-ray intensities from the decay of radionuclides, produced during the irradiation, was used to quantify the concentrations of various elements. The decay measurements of the radionuclides are used for the quantification of elements in the sample. Radionuclide *Reference Nuclear Data* used for the analysis are given in Table 1.

Table 1: Radionuclides used for the analysis and their gamma energies (Note: h-hours, d-days, m-minutes)

Nuclide (n, γ)	γ-ray energy (keV)	Half-life $T_{1/2}$
^{28}Al	1778	2.24m
^{82}Br	776	35.30h
^{49}Ca	3084	8.71m
^{59}Fe	1099	44.49d
^{42}K	1524	12.36h
^{140}La	1596	40.27h
^{27}Mg	1014	9.45m
^{56}Mn	846	2.57h
^{24}Na	1368	14.957h
^{46}Sc	889	83.79d
^{52}V	1434	3.75m
^{65}Zn	1115	243.9d

After appropriate cooling time, emitted gamma rays were measured using Hyper-Pure Germanium (HPGe) detector coupled to 8k-channel multi channel analyzer. HPGe detectors having good energy resolution which makes a INAA multi elemental technique. The gamma ray spectra were collected and analysed using APTEC and PHAST software's. Relative method was used for quantification of elemental concentrations in sample. In this method, elemental standard and sample are irradiated simultaneously and the induced radio activities from both sample and standard are measured in identical geometry with respect to HPGe detector. The mass of the element in sample is calculated by the equation (1), using the mass of the element in standard and count rates both of standard and sample. The mass of the element in sample is converted to concentration by dividing with sample mass.

$$m_s = m_{std} \, X \, \frac{N_s}{N_{std}} \, X \, \frac{D_{std}}{D_s} \text{ ---------- (1)}$$

m_{std} and m_s = masses of the element in standard and sample respectively
N_{std} and N_s = count rate of standard and sample respectively
D_{std} and D_s = Decay factor activation product of standard and sample respectively

Elements Br, Fe, K, La, Na, Sc, Zn were determined from long irradiation while Al, Ca, Mg, Mn, V were determined from short irradiation. Concentrations of elements were estimated in medicinal plants by measuring the intensities of the characteristic γ-ray energy as well as the half-life data. The typical gamma-ray spectra of long and short irradiations are shown in the Fig. 2.

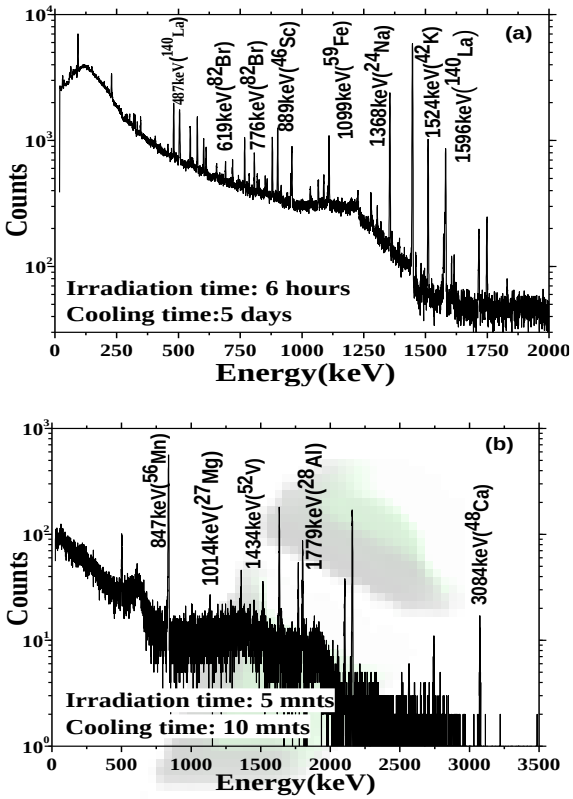

Figure 2: Typical gamma-ray spectra of (a) long and (b) short irradiation respectively

Results and Discussions

A total of twelve elements were measured using relative INAA method. Average concentrations (mg/kg) of elements in Sphaeranthus indicus and Cassia fistula at different sites are shown in Fig. 2. An examination of the Fig. 3 shows that Sphaeranthus indicus and Cassia fistula contain the elements Al, Br, Ca, Fe, K, La, Mg, Mn, Na, Sc, V and Zn in various proportions in different sites such as Maredumilli (site1), Rampachodavaram (site2) and Kutravada (site3). The major elements detected were aluminum (Al), calcium (Ca) magnesium (Mg) and potassium (K). The minor elements were iron (Fe), manganese (Mn) and sodium (Na). The trace elements were bromine (Br), lanthanum (La), scandium (Sc), vanadium (V) and zinc (Zn).

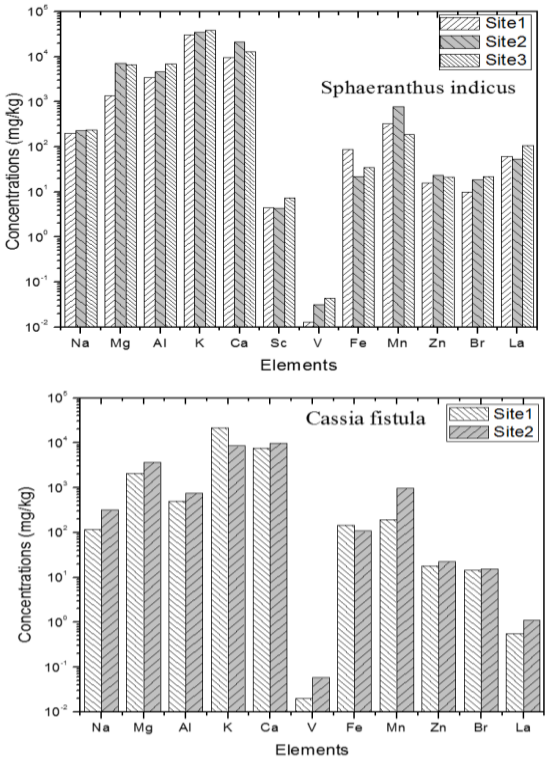

Figure 3: Comparison of average concentrations of elements in (a) Sphaeranthus indicus and (b) Cassia fistula from different sites.

In *Sphaeranthus indicus*, the concentration of aluminum varied between 3,483 and 6,816 mg/kg in three sites, highest was found at site3 whereas lowest was at site1. Bromine values varied similar range from 9 to 21 mg/kg in three sites. The maximum and minimum amount of Calcium found as 21,275 and 9,600 mg/kg at site1 and site2 respectively. The concentration of iron was ranging from 15.83 mg/kg at site2 to 23.20 mg/kg at site1. The concentration of potassium varied similarly in the range from 30,540 to 38,228 mg/kg in three sites. Lanthanum concentration was lowest 53 mg/kg at site2 and highest 105 mg/kg at site3. Magnesium levels varied between 1,375 and 7,171 mg/kg at site1 and site2 respectively. The concentration of Manganese was found highest 763 mg/kg at site2 and Mn was found lowest 191 mg/kg at site3. Sodium concentrations varied similarly between 198 and 234 mg/kg at site1 and site3 respectively. Scandium levels varied from 4.3 to 7.3 mg/kg at site2 and site3. The concentration of the vanadium was narrow range from 0.0133 to 0.0485 mg/kg at site1 to site3. Zinc content in these three sites varied similarly from 15.83 to 23.20 mg/kg.

In *Cassia fistula*, the concentration of aluminum is maximum 760 mg/kg at site2 and minimum 492 mg/kg at site1. Bromine levels are also varied in very similar way between 14.23 to 15.50 mg/kg from site1 to site2. Calcium has maximum concentration of 9,797 mg/kg at site2 and minimum concentration of 7,520 mg/kg at site1.The concentration of iron is ranging from 107 mg/kg at site2 to 147 mg/kg at site1. Potassium concentrations vary between 8,501 and 21,954 mg/kg, maximum concentration is found at site1 and minimum at site2. Lanthanum levels varied very similarly between 0.54 to 1.09 mg/kg from site1 to site2 respectively. Magnesium concentration is present at major levels, with a maximum of 3,700 mg/kg at site2 and minimum of 2,095 mg/kg at site1. The high concentration of manganese was found to be 974 mg/kg at site2 and low concentration was found to 190 mg/kg at site1. The concentrations of sodium are 117 mg/Kg and 319 mg/kg at site1 and site2 respectively. Vanadium is present at trace levels in both the sites, it was varied between 0.020 to 0.058 mg/kg. Concentration of zinc in the two sites varies from 17.9 to 22.3 mg/kg. It was found to maximum in site2 and minimum in site1.

The observed elemental concentrations in both leaves of *Sphaeranthus indicus* and *Cassia fistula* at different sites were different levels which are mainly attributed to the differences in the mineral composition of the soil in which the plants are grown. Other factors responsible for a difference in elemental concentrations are better absorbability of the plant, irrigation water and climatic conditions.

Conclusion

INAA is very sensitive and multi-elemental technique to analyze the biological samples like medicinal plants. A total of eleven elements have been determined by INAA. The precision and accuracy of the technique were secured by analysing the standard reference material. INAA is an important tool for ascertaining the elemental composition of medicinal plants. An assessment of these elements could be helpful in curative process of ill health.

The role of the inorganic elements are resolute in the body metabolism has been well-known and therapeutic action of medicinal plants through the alteration of metabolic processes has been recognized. Even though the direct relation between elemental concentration in medicinal plants and their reported restorative potentials is yet to be established, the levels of inorganic elements in medicinal plants were essential in understanding the pharmacological action of medicinal plants. The data generated on elemental concentration of the medicinal plants studied will be useful in the preparing different medicines in ayurveda and other herbal medicine systems of Indian traditional medicine.

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References

1. V. Singh and A. N. Garg. "Availability of essential trace elements in ayurvedic Indian medicinal herbs using Instrumental Neutron Activation Analysis." Applied Radiation and Isotopes, 48, (1997): 97-101.

2. R. Lokhande, *P. Singare, M. Andhele and R. Acharya.* "Study of some Indian medicinal plants by application of INAA and AAS techniques." Natural Science, 2, (2010): 26-32.

3. D. De. Soete, R. Gijbels and J. Hoste. "Neutron Activation Analysis." Wiley-Inter science, (1972) Jhon Wiley & Sons, London.

4. Funtua, L. A. Dim, S. Mu.azu, A. O. Oyewale, I. M. Umar, F. Grass and R. Gwozdz. "Instrumental neutron activation analysis (INAA) of Guiera senegalensis (Combrataceae) - a tropical medicinal plant." Nig. J. Phys, 9.15 (2003): 105.

5. Y. Serfor-amah, E. H. K. Akaho, B. T.B. Nyarko, A. W. K. Kyere and K. Oopm-Boachie. "Application of instrumental neutron activation analysis to plant medicines in Ghana: A review." J. Radioanal. Nucl. Chem., 257 (2003): 125.

6. P. K. Warrier. "Indian Medicinal Plants, a compendium of 500 species." Orient Longman; Kottakkal 5, (1994): 180-185.

7. G. S. Pande and K. C. Chunekar. "Bhavprakash Nighantu." Chaunkhambha Bharati Academy Gokul Bhavan: Varanasi, VIII Edn, (1999): 413.

8. K. M. Nadkarni. "Indian Material Medica." Popular Prakashan Pvt. Ltd. Bombay, III Edn, Vol I, (1976): 1162.

9. K. R. Kirtikar and B. D. Basu. "Indian Medicinal Plants." Lalitmohan Basu. Allahabad, India. Vol-II, (1981): 1346-1348.

10. M. S. Shekhani, P. M. Shah, A. Yasmin, R. Siddiqui, S. Perveen and K. M. Khan. "An immunostimulant sesquiterpene glycoside from Sphaeranthus indicus." Phytochemistry, 29.8 (1990): 2573-2576.

11. K. S. Dubey, A. H. Ansari and M. Harduha. "Antimicrobial activity of the extract of Sphaeranthus indicus." Asian journal of chemistry, 12.2 (2000): 577-578

12. F. Sadaf, R. Saleem, M. Ahmed, S. I. Ahmad and Navaid-UL-Zafar. "Heeling potential of cream containing extract of Sphaeranthus indicus on dermal wounds in Guinea pigs." Journal of Ethnopharmacology, 107.2 (2006): 161-163.

13. V. Prashanth Kumar, N. S. Chauhan, H. Padh and M. Rajani. **"**Search for antibacterial antifungal agents from selected Indian medicinal plants." J. Ethnopharmacol, 107 (2006): 182-188

14. R. K. Gupta. "Medicinal and Aromatic plants." CBS publishers & distributors, 1st edition (2010): 116-117.

15. Anonymous. "The Wealth of India." First Supplement Series (Raw Materials), National Institute of Science Communication and Information Resources, CSIR; Vol. 3 (2007): 340-342.

16. "The Ayurvedic Pharmacopoeia of India." New Delhi, Government of India Publication, Part 1, Vol. 5 (2001): 8-9.

17. C. P. Khare. "Indian medicinal plants." Springer publication, New York, (2007): 128.

18. K. M. Nadkarni. "Indian Materia Medica." Bombay Popular Prakashan, 1 (2009): 285-286.

19. J. S. Brhamajirao, E. Senthilvadivu, N. P. Seshadreesan, R. Acharya, C. R. Venkatasubramani, and A. V. R. Reddy. "Characterization of pneumatic fast transfer system irradiation position of KAMINI reactor for k_0-based NAA." J. Radioanal. Nucl. Chem. 294 (2012): 137–141.

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