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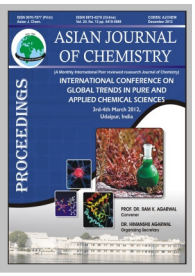


# Radioisotopes in Chemical Research: Neutron Activation Analysis of Leaves and Bark of Neem†

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Radioisotopes are chemical elements with specific mass number that emit characteristic radiation and have definite half-lives. George de von Hevesy was the first to use $^{210}$Pb for the determination of solubility of lead salts in water. Neutron activation analysis involves irradiation of the sample with thermal neutrons in a nuclear reactor followed by counting of its γ activity by high resolution γ-ray spectrometry. Upto 35 elements can be determined by adopting differential counting methodology developed in our laboratory. A non-destructive method has been developed for the determination of phosphorus in biological samples. It involves 5 min irradiation of -100 mg sample in a nuclear reactor, delay by 3 weeks followed by counting of β activity due to $^{32}$P using 27 mg/cm$^2$ Al absorber. A large number of medicinal herbs and herbal preparations have been analyzed for minor and trace element contents, which may be attributed to their therapeutic effects. Neem (*Azadirachta indica*) leaves and bark are widely used for the treatment of a variety of chronic ailments. Analysis of leaves and bark samples collected from different locations exhibit distinctive elemental profile and the contents are indicative of their therapeutic effects.

**Key Words: Radioactivity, Neutron activation analysis, Medicinal herbs, Neem leaves and bark, Elemental profile.**

## INTRODUCTION

**Radioactivity:** Henri Becquerel first observed in February 1896 that a double salt of uranium, $K_2UO_2(SO_4)_2 \cdot 2H_2O$ emitted some strange rays, which blackened a photographic plate. Initially these were called 'uranic rays' because of its origin from uranium atoms but Pierre and Mme Marie Curie coined the term 'radioactivity' to the new phenomenon of spontaneous emission of radiation by the uranium salt[1]. The trio was awarded physics Nobel prize in 1903 for this historic discovery. Subsequently this phenomenon was also observed in thorium containing compounds and three naturally occurring radioactive decay series corresponding to $^{235}$U ($t_{½}$ = 7.0 × 10$^8$ y), $^{238}$U ($t_{½}$ = 4.5 × 10$^9$ y) and $^{232}$Th ($t_{½}$ = 1.4 × 10$^{10}$ y) emitting α, β and γ radiations and decaying to finally yield $^{207}$Pb, $^{206}$Pb and $^{208}$Pb all stable lead isotopes were discovered. Mme Curie also discovered the two new radioactive elements, Po and Ra formed during successive decay of $^{238}$U and for this she was awarded chemistry Nobel prize in 1911. The year 1932, remains the most memorable in the history of scientific discoveries when neutron, positron and deuterium were discovered by J. Chadwick, C.D. Anderson and H.C. Urey respectively. Immediately thereafter artificial radioactivity was discovered by F. Joliot and I. Curie (1934) by the bombardment of aluminum foil (containing $^{27}$Al as target nucleus) with α-particle beam leading to the production of a new radioisotope $^{30}$P ($t_{½}$ = 2 min). Also a new artificial radioactive decay series starting from $^{237}$Np ($t_{½}$ = 2.14 × 10$^6$ y) decaying to stable $^{209}$Bi was discovered[2]. All these developments played a great role in understanding the structure of atom and the nucleus[3].

Radioisotopes of natural origin and artificially produced are chemical elements with specific mass number that emit characteristic radiations of definite energy and have definite half-lives[4]. Mme Curie was honored by naming the unit of radioactivity as Curie (1Ci = 3.7 disintegrations per sec and defined as the number of disintegrations emanating from 1 g $^{226}$Ra with $t_{½}$ = 1600 y) though SI unit has now been named after Becquerel as Bq (defined as equal to 1 disintegration per s). Earlier all the studies were carried out by using radioisotopes emitting α or β-particles and obtainable from the naturally

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occurring decay series only. However, forties saw the development of nuclear reactor and accelerators where new radioisotopes could be produced by particle (neutron, proton, α-particle, *etc*.) bombardment of a target nucleus thus fulfilling the need of suitable radioisotopes emitting different radiations and having different half-lives. By now more than 2500 radioisotopes of all the elements in the periodic table are known. Ever since, radioisotopes and radiotracers are being widely used for studying a variety of problems in various disciplines of science, medicine, agriculture and technology. Radiotracers have been extensively used in ascertaining reaction mechanism, mechanisms of catalysis and corrosion, diffusion and self-diffusion, liquid flow in a pipeline, diagnosis of a disease, distribution and metabolism of compounds in plants and animals, autoradiography, *etc*.[5].

**Applications of radioisotopes and radiotracers:** George de von Hevesy (1913) first used a radioisotope $^{210}$Pb for the determination of solubility of lead salts in water. He was also the first person to use radiotracers to follow biological processes, tracing the movement of radionuclides from soil into plants and the movement of food through animal systems. Radiotracers are chemical compounds having one or more radionuclide as cation or anion and used as marker to follow the course of a chemical reaction or physical process or to show the location of a substance[4,5]. Radiotracers are used in sub micro amounts (μg, ng or pg) and these can be employed for qualitative and quantitative analysis. Methods using radionuclides are more sensitive because of extremely low detection limits. Several radioisotopes are being used as nuclear medicines for the diagnosis of a disease or for therapeutic purposes. Choice of a suitable radioisotope depends on many factors as illustrated in Fig. 1. For example, $^{24}$Na ($t_{½}$ = 15 h) should be used for studying the flow of liquid in human body whereas $^{22}$Na ($t_{½}$ = 2.3 y) should be used for chemical investigations in a laboratory. Today radioisotopes are used in a myriad of ways in all disciplines of science and technology such as agriculture and plant biology, prospecting mineral resources, industry, dating of geological and archaeological specimen, environmental pollution and chemical research. Perhaps the most beneficial is their use in nuclear medicine for the benefit of people throughout the world. Several analytical procedures such as isotope dilution analysis, radiometric titration, radiorelease methods, radiochromatography and radioimmunoassay (RIA) have been developed for quantitative analysis. Looking at the importance of radiotracer research, International Conferences on Application of Radiotracers in Chemical, Environmental and Biological Sciences (ARCEBS)[6] have been organized at Saha Institute of Nuclear Physics, Kolkata. In India radiotracers are available through the Board of Radiation and Isotope Technology (BRIT), Mumbai though these are made in the Isotope Division of the Bhabha Atomic Research Centre (BARC), Mumbai. However, various radiopharmaceuticals used as nuclear medicine are made in the Radiopharmaceutical Division and marketed by BRIT, Mumbai.

**Neutron activation analysis:** After the discovery of artificial radioactivity, George Hevesy and Hilde Levy (1936) discovered the technique of neutron activation analysis (NAA) for the determination of Dy in a rare earth mineral[7]. The technique

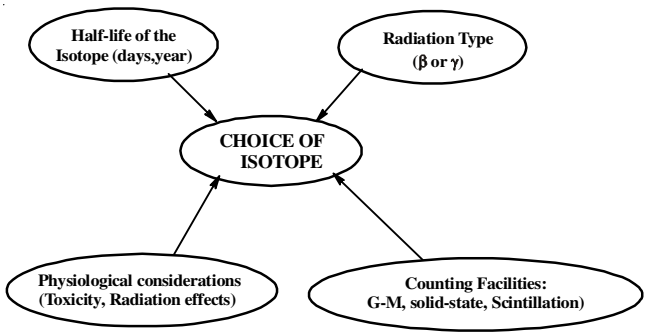


Fig. 1. Factors affecting the choice of a radioisotope as tracer

involves irradiation of the sample with thermal neutrons followed by counting of gamma activity as illustrated in Fig. 2. If gammas emitted by the the compound nucleus so formed are counted then the technique is called prompt gamma activation analysis (PGAA). However, this is not a commonly followed practice. Even after the sample is taken out of reactor, it is still radioactive and may decay by emitting β or γ which are counted. The activity so produced may be represented by the equation.

$$\lambda N_1 = A = N\sigma\phi(1-e^{-\lambda t_i})$$

where, N represents the number of target atoms, σ the neutron absorption cross section, φ the neutron flux and $t_i$ the time of irradiation. Following comparator method[7,8], concentration of element in the sample may be determined by using a simplified equation.

$$\text{Concn. of element in sample } (S_a) = \text{Concn. of element in standard } (S_t) \times \frac{\text{Activity in sample}}{\text{Activity in standard}}$$

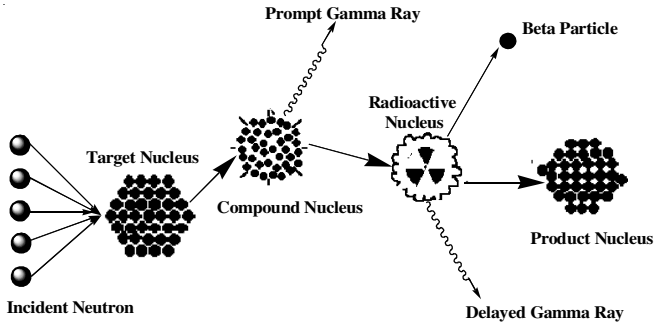


Fig. 2. Schematic illustration of neutron activation analysis showing irradiation of a target nucleus with neutron beam resulting in the formation of radionuclide emitting characteristic β and/or γ radiation

Reactor neutrons have wide range of energy, which plays an important role as different types of reactions *viz*. (n,γ), (n, p), (n, α), (n, n'), (n, 2n) are possible. For example $^{27}$Al on bombardment with different energy neutrons undergoes (n, γ), (n, p), (n, α) or (n, 2n) reactions yielding $^{28}$Al ($t_{½}$ = 2.24 min), $^{27}$Mg ($t_{½}$ = 9.45 min), $^{24}$Na ($t_{½}$ = 15 h) or $^{26}$Al ($t_{½}$ = 7.3 × $10^5$ y) respectively, each one having different nuclear reaction cross section[8].

Neutron activation analysis is a highly accurate, precise and sensitive method with nondestructive and multielemental character. It has been widely used for the analysis of a large number of elements in all kinds of matrices as illustrated in Fig. 3. We ourselves have been able to determine 30 to 35 elements by following short and long irradiations followed by

counting at different intervals up to 3 months. It has contributed significantly in the analysis of lunar samples, led to the recognition of several biologically essential trace elements and proved to be a valuable tool for the certification of reference materials by international agencies. During last four decades we have extensively used neutron activation analysis for the analysis of a large variety of geological, biological and environmental samples such as:

1) Lunar samples, meteorites, Lonar lake sediments, minerals and geological rocks.

2) Dietary constituents *viz.*, plant leaves, vegetables, cereals, spices, natural milk and branded milk powders including formulated and prepared diet.

3) Cancerous breast tissue of different age groups and histopathological stages, blood and correlation with diet.

4) Development of a nondestructive method for the determination of P in biological samples for biomedical and nutritional studies

5) Development of nondestructive methods for the determination of Al, Si and Fe in bauxite, Mn in pyrolusite and Cu in chalcopyrite ores using $^{241}$Am-Be neutron source.

6) Dust particulates from industrial, commercial and residential areas of metropolitan cities (Mumbai, Kolkata, Chennai, Hyderabad, Chochin and Delhi), cement factory, thermal power station, paper mill, *etc*.

7) Municipal waste, kitchen waste and sewage sludge of different metropolitan cities.

8) Fish from coastal areas of Visakhapatnam, Mumbai, Cochin and Nagpur.

9) Hair samples of locomotive workers and study of occupational exposure.

10) Medicinal herbs, herbal formulations and bhasmas from different locations and brands.

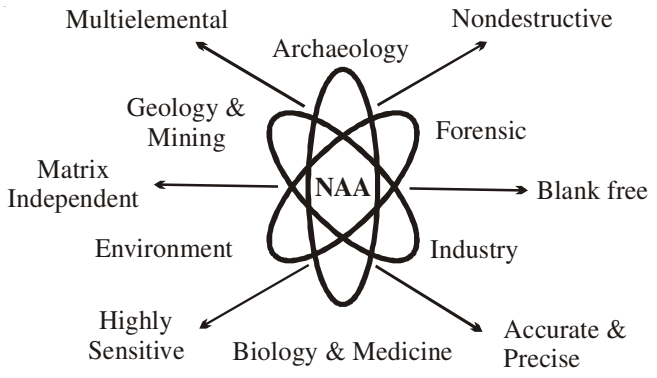


Fig. 3. Illustration of capabilities and applications of neutron activation analysis

Potentiality and applicability of neutron activation analysis will be illustrated by the analysis of leaves and bark of neem (*Azadirachta Indica*) widely used in traditional Indian medicine system.

**Analysis of leaves and bark of neem:** *Azadirachta indica*, commonly referred to as the neem tree, is a member of meliaceae family. The Latinized name is derived from the Persian word; *Azad-tree, Dirakht-tree, Indica*-Indian origin, meaning free tree of India[9]. It is considered as a 'wonder plant', 'village dispensary' or 'living pharmacy' as it is used by every Indian, from the poorest peasant using its twigs as toothbrush to wealthy individuals purchasing neem-based toothpaste. Neem is one of the world's most researched trees. Modern clinical studies have identified a number of compounds that effectively regulate immune system functions. Neem extracts and oil are clinically effective in curing psoriasis, eczema, acne, skin ulcers and other skin diseases. Neem leaves are used as vermifage, insecticide, astringent, tonic and antiseptic. It possesses antidiabetic, antibacterial and antiviral properties and used successfully in stomach worms and ulcers, arthritis, rheumatism, blood disorder, digestive and nervous disorders, cancer and especially chicken pox[10,11]. It is also used as a biopesticide that can help solve global environmental and health concerns. From environmental point of view, it has a reputation as a natural air purifier, exhaling out oxygen and keeping the oxygen level in the atmosphere balanced. Its ability to withstand extreme heat and water pollution is well known. It also helps to improve fertility of the soil and to rehabilitate degraded wastelands. The tree plays a vital role in controlling soil erosion, salination and preventing floods. Among its many benefits, most unusual and immediately practical is the control of farm and household pests. Clinical research suggests that imbalance of essential elements play a definite role in human metabolism.

Since all parts of neem tree have applications on domestic and medicinal front, several workers have reported trace element contents in its leaves. Sahito *et. al*[12] determined 15 essential and toxic elements in neem leaves from Pakistan by AAS. Ray *et al.*[13] determined 10 elements in neem leaves from Odisha by using energy dispersive X-Ray fluorescence (EDXRF). We have determined 7 minor and 15 trace elements in neem leaves and bark samples collected from different locations of Roorkee city. Also Cu, Ni, Cd and Pb were determined by atomic absorption spectrophorometry. An attempt has been made to correlate the elemental contents with its curative properties.

## EXPERIMENTAL

Eleven neem leaves samples were collected from various locations within IIT Roorkee premises. Further, three bark samples were collected from the same area. Its surface contamination was wiped with tissue paper and left for air-drying and then in an oven at -80 ºC. The samples were powdered in agate mortar and passed through 100-mesh sieve. RMs Mixed Polished Herbs (MPH-2) from the INCT, Poland and Peach Leaves (SRM-1547) from the NIST, USA were dried as per recommended procedure before use. About 30/80 mg aliquots each of powdered samples and RMs were accurately weighed and packed in Alkathene for short (1 min pneumatic) and in Al foil for long (7 h) irradiation at $5 \times 10^{13}/1.7 \times 10^{12}$ n cm$^{-2}$ s$^{-1}$ in Dhruva /APSARA reactors respectively. Short-lived activities were measured using an 80 cm$^3$ coaxial HPGe detector (EG and G ORTEC) with 8 k MCA in BARC, Mumbai. Long irradiated samples were air lifted to Roorkee and γ-activity was measured using a HPGe detector (FWHM = 1.8 keV at 1.33 MeV of $^{60}$Co) with 20 % relative efficiency and 8 k MCA with GENIE-2000 software (Canberra, USA). Counting was

followed for 1 h, 2 h, 6 h and 12 h at different intervals up to 2 months[14]. Typical gamma ray spectrum of neem leaves after 7 h irradiation in APSARA reactor is shown in Fig. 4. It shows various photopeaks of several radionuclides. Care was taken to obtain maximum number of elements from more than one counting. Elemental contents were calculated by using RMs as comparators. Phosphorus content was determined by measuring β- activity due to $^{32}P$ on an end window G.M. counter using 27 mg $cm^{-2}$ aluminum filter[15]. Contents of Cu, Ni, Cd and Pb were determined by AAS method using GBC Avanta Atomic Absorption Spectrophotometer using calibration plots.

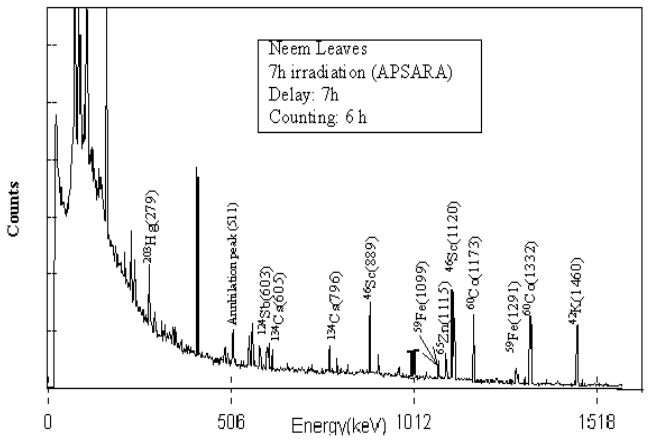


Fig. 4. Typical gamma ray spectrum of neem leaves after irradiation in APSARA reactor at BARC, Mumbai for 7 h, delayed by 7 h and counting for 6 h

## RESULTS AND DISCUSSION

Elemental concentrations in the RMs mixed polished herbs (MPH-2) and Peach Leaves (SRM-1547) by INAA were calculated using each other as comparator standard and then elemental contents in 11 leaves and 3 bark samples were calculated using both RMs as comparators[16]. Finally mean elemental contents in neem leaves (n = 11) and bark (n = 3) samples were calculated. Ranges and mean ± SD values for 22 elemental contents in leaves and bark along with those in SRM 1547 are listed in Table-1 where ± SDs were calculated on the basis of number of samples analyzed. Most elemental contents for SRM 1547 are in close agreement with the certified values within < ± 5 %. Hence our data should be accurate and precise within 95 % confidence limits. Since neem tree is widely found all around India and its leaves are extensively used as medicine, these have been analyzed by several workers[17-20] by employing neutron activation analysis. Earlier we have determined elemental contents in two branded neem leaves (from Himalaya Drugs and Pharmaceuticals, Bangalore and Vyas Pharmacy, Indore)[17] and another from Roorkee[18] as a part of wider study of medicinal herbs. So also Balaji *et al.*[19] and Mohanta *et al.*[20] have determined elemental contents in neem leaves collected from Mumbai and Tirupati and Guwahati respectively. A comparison of all the data of this work along with those reported in literature are given in Table-2. A cursory look of all the data shows that elemental contents vary in a wide range but not very significantly. This is quite obvious because the leaves analyzed were collected from

TABLE-1
MEAN ELEMENTAL CONCENTRATIONS IN NEEM LEAVES AND BARK AND SRM PEACH LEAVES

| Element | Leaves (n = 11) | | Bark (n = 3) | | Peach leaves (SRM-1547) | |
|---|---|---|---|---|---|---|
| | Range | Mean ± SD | Range | Mean ± SD | This work | Certified/Information* |
| **INAA** | | | | | | |
| Al (mg/g) | 0.14 - 0.36 | 0.25 ± 0.06 | 0.21 - 0.68 | 0.40 ± 0.20 | 242 ± 15 | 249 ± 7.47 |
| As (ng/g) | 131 - 285 | 190 ± 46 | 104 - 152 | 120 ± 22 | 63.8 ± 1.4 | 60 ± 18.0 |
| Ba (μg/g) | 22.5 - 42.6 | 33.8 ± 10 | 74.9 - 456 | 203 ± 160 | 112 ± 0.02 | 124 ± 4 |
| Br (μg/g) | 4.5 - 63.1 | 25.4 ± 19.8 | 3.34 - 15.9 | 10.9 ± 5.4 | 11.6 ± 1.0 | [11] |
| Ca (mg/g) | 3.4 - 6.7 | 4.4 ± 1.01 | 6.88 - 20.9 | 14.4 ± 5.9 | 16.1 ± 1.3 | 15.6 ± 0.2 |
| Cl (mg/g) | 0.84 - 4.33 | 2.43 ± 1.11 | 0.53 - 1.01 | 0.72 ± 0.25 | 0.39 ± 0.07 | 0.36 ± 0.02 |
| Co (ng/g) | 36.6 - 118 | 87.5 ± 26.9 | 68.4 - 115 | 85.0 ± 18.7 | 62.4 ± 4.2 | [70] |
| Cr (μg/g) | 0.47 - 3.31 | 1.34 ± 0.95 | 0.96 - 1.18 | 1.06 ± 0.08 | 1.03 ± 0.03 | [1.0] |
| Cs (ng/g) | 112 - 204 | 154 ± 31 | 61.9 - 85.2 | 79.3 ± 13.3 | 85 ± 2 | 79.7 ± 13.6 |
| Fe (μg/g) | 86 - 160 | 122 ± 21 | 143 - 247 | 186 ± 38 | 195 ± 2 | 218 ± 14 |
| Hg (ng/g) | 13 - 34 | 24 ± 5 | 28.0 - 202 | 84.4 ± 71.3 | 29.0 ± 0.03 | 31 ± 7 |
| K (mg/g) | 11.6 - 35.8 | 20.1 ± 7.7 | 1.56 - 2.59 | 2.10 ± 0.42 | 23.1 ± 0.01 | 24.3 ± 1.0 |
| Mg (mg/g) | 1.25 - 5.21 | 3.56 ± 1.19 | ND | ND | 3.98 ± 0.17 | 4.32 ± 0.08 |
| Mn (μg/g) | 12.0 - 25.8 | 19.2 ± 4.8 | 31.5 - 87.6 | 59.2 ± 0.6 | 107 ± 2.0 | 98 ± 3 |
| Na (mg/g) | 0.23 - 0.50 | 0.33 ± 0.08 | 0.36 - 0.44 | 0.40 ± 0.03 | 25 ± 0.02 | [24] |
| P (mg/g) | 1.16 - 3.34 | 2.16 ± 0.74 | 0.53 - 0.64 | 0.58 ± 0.04 | 1.44 ± 0.10 | 1.37 ± 0.07 |
| Rb (μg/g) | 17.4 - 29.8 | 24.2 ± 4.4 | 8.30 - 17.4 | 12.4 ± 3.5 | 20.8 ± 1.2 | 19.7 ± 1.2 |
| Sb (μg/g) | 18.9 - 53.4 | 35.1 ± 12.4 | 27.9 - 46.9 | 37.4 ± 6.81 | 22.6 ± 3.1 | [20] |
| Sm (μg/g) | 0.09 - 0.23 | 013 ± 0.04 | - | - | 0.89 ± 0.02 | [1] |
| Th (ng/g) | 147 - 279 | 191 ± 52 | 85.1 - 428 | 203 ± 139 | 47 ± 0.11 | [50] |
| V (μg/g) | 0.47 - 4.29 | 1.15 ± 1.06 | ND | ND | 0.33 ± 0.03 | 0.37 ± 0.03 |
| Zn (μg/g) | 22.1 - 52.8 | 37.7 ± 9.2 | 23.8 - 38.5 | 28.8 ± 4.5 | 18.8 ± 3.8 | 17.9 ± 0.4 |
| **AAS** | | | | | | |
| Cu (μg/g) | 2.58-8.90 | 6.14 ± 1.77 | 2.68 - 5.19 | 3.86 ± 1.08 | | |
| Ni (μg/g) | 0.99-4.79 | 3.17 ± 1.14 | - | - | | |
| Cd (μg/g) | 0.82-2.22 | 1.40 ± 0.44 | - | - | | |
| Pb (μg/g) | 4.5—36.4 | 18.2 ± 9.1 | - | - | | |

*Certificate of Analysis, Standard Reference Material 1547, National Institute of Science and Technology, USA, p. 5 (1993).
Values reported in [ ] are information values and ND = Not Detected

TABLE-2
COMPARISON OF ELEMENTAL CONCENTRATIONS WITH LITERATURE VALUES

| Element | This work | Neem (H) [Ref. 17] | Neem (V) [Ref. 17] | Roorkee [Ref. 18] | Tirupati [Ref. 19] | Mumbai [Ref. 19] | Guwahati [Ref. 20] |
|---|---|---|---|---|---|---|---|
| Al (mg/g) | 0.25 ± 0.06 | ND | ND | 0.96 ± 0.02 | 0.11 ± 0.01 | 0.31 ± 0.00 | ND |
| As (ng/g) | 190 ± 46 | 358 ± 29 | 211 ± 15 | ND | ND | ND | ND |
| Ba (µg/g) | 33.8 ± 10 | 204 ± 12 | 201 ± 14 | 21.7 ± 2.5 | ND | ND | ND |
| Br (µg/g) | 25.4 ± 19.8 | 398 ± 48 | 36.8 ± 1.6 | 28.8 ± 1.4 | 7.07 ± 0.52 | 3.86 ± 0.24 | 4.6 ± 1.0 |
| Ca (mg/g) | 4.4 ± 1.01 | 28.3 ± 1.6 | 24.8 ± 1.9 | ND | 30.6 ± 3.4 | 40.2 ± 0.5 | ND |
| Cl (mg/g) | 2.43 ± 1.11 | 9.21 ± 0.33 | 11.9 ± 0.9 | 2.01 ± 0.24 | ND | ND | ND |
| Co (ng/g) | 87.5 ± 26.9 | 59 ± 5 | 46 ± 6 | 120 ± 10 | ND | ND | 6100 ± 2500 |
| Cr (µg/g) | 1.34 ± 0.95 | 1.84 ± 0.07 | 1.58 ± 0.19 | 1.47 ± 0.26 | ND | ND | ND |
| Cs (ng/g) | 154 ± 31 | 88 ± 7 | 165 ± 8 | ND | ND | ND | 28 ± 1 |
| Cu (µg/g) | 6.14 ± 1.77 | 7.32 ± 0.33 | 4.24 ± 0.34 | ND | ND | ND | ND |
| Fe (µg/g) | 122 ± 21 | 173 ± 3 | 131 ± 1.2 | 256 ± 52 | ND | ND | 1230 ± 4 |
| Hg (ng/g) | 24 ± 5 | 67 ± 4 | 35 ± 2 | ND | ND | ND | ND |
| K (mg/g) | 20.1 ± 7.7 | 20.2 ± 0.6 | 15.7 ± 0.2 | 10.7 ± 0.6 | 28.4 ± 0.9 | 11.1 ± 0.6 | ND |
| Mg (mg/g) | 3.56 ± 1.19 | 0.58 ± 0.04 | 0.49 ± 0.03 | 0.9 ± 0.07 | 4.9 ± 0.3 | 7.6 ± 0.3 | 31 ± 1 |
| Mn (µg/g) | 19.2 ± 4.8 | 29.2 ± 1.2 | 32.9 ± 2.1 | 46.4 ± 3.3 | 16.9 ± 1.2 | 36.5 ± 2.1 | ND |
| Na (mg/g) | 0.33 ± 0.08 | 0.68 ± 0.02 | 0.21 ± 0.01 | 0.26 ± 0.08 | 0.11 ± 0.01 | 0.54 ± 01 | ND |
| Ni (µg/g) | 3.17 ± 1.14 | ND | ND | 1.33 ± 0.01 | ND | ND | 9.6 ± 1.3 |
| P (mg/g) | 2.16 ± 0.74 | 2.89 ± 0.03 | 1.86 ± 0.56 | ND | ND | ND | ND |
| Pb (µg/g) | 18.2 ± 9.1 | ND | ND | ND | ND | ND | 4.6 ± 1.0 |
| Rb (µg/g) | 24.2 ± 4.4 | 14.6 ± 0.9 | 27.5 ± 2.3 | 17.6 ± 1.5 | ND | ND | 30 ± 2 |
| Sb (µg/g) | 35.1 ± 12.4 | 20.2 ± 0.9 | 15.6 ± 1.0 | ND | ND | ND | ND |
| Sm (µg/g) | 013 ± 0.04 | ND | ND | 99 ± 7 | ND | ND | ND |
| Th (ng/g) | 191 ± 52 | 173 ± 11 | 314 ± 21 | 366 ± 23 | ND | ND | ND |
| V (µg/g) | 1.15 ± 1.06 | 1.77 ± 0.12 | 1.82 ± 0.07 | 1.19 ± 0.08 | 1.66 ± 0.06 | 4.26 ± 0.05 | 132 ± 2 |
| Zn (µg/g) | 37.7 ± 9.2 | 35.1 ± 1.4 | 28.6 ± 2.2 | 34 ± 1.6 | ND | ND | ND |

H = Himalaya Drugs and Pharmaceuticals, Bangalore; V = Vyas Pharmacy, Indore

different geographical locations and environmental conditions. Such variations may be attributed to geo-environmental factors and local soil characteristics[21] as also observed in our earlier studies on elemental contents in *Murraya koenigii* from 19 different Indian states[22].

Further we have developed the concept of elemental profiles which are unique for typical plant species or its parts even though elemental contents may vary in a wider range. These are shown in Figs. 5 and 6 respectively where vertical bars exhibit variability for that element in different samples. It is evident from these plots that despite wide variations in elemental contents of the samples collected from different locations, general characteristic features of the elemental profile remain unique.

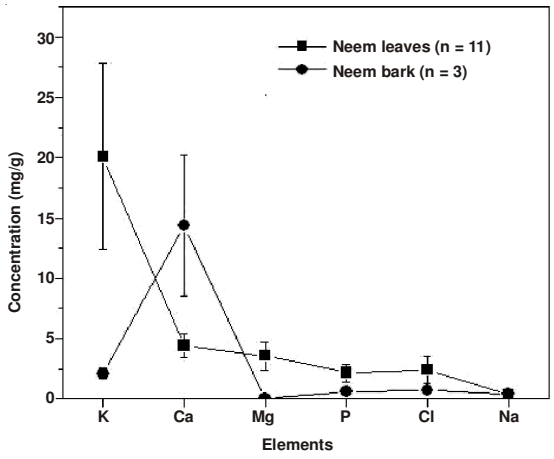


Fig. 5. Comparison of minor element concentrations in Neem leaves and bark

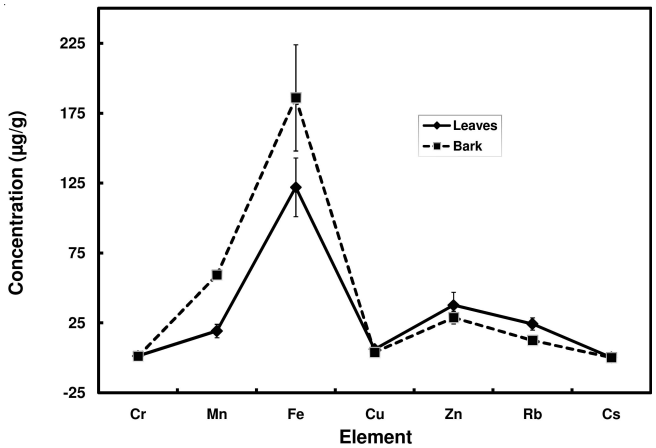


Fig. 6. Comparison of trace element concentrations in neem leaves and bark

**Elemental contents in Neem leaves:** A perusal of data in Table-1 shows that K is most enriched as it is found in >10 mg/g amounts whereas Ca, Mg, P and Cl were in the range of 1 to 6 mg/g. Mean Na and Al contents are much less, mean contents being 0.33 ± 0.08 mg/g and 0.25 ± 0.06 mg/g respectively. It is observed that Na, K, Ca and P contents do not vary significantly in 11 samples but Cl contents vary by almost 5-fold. Not much variation is observed amongst trace elements such as Ba, Cu, Fe, Mn, Rb, Sb, Th and Zn but Br, Cr, Ni, V and Pb exhibit large variations. Mean content of Fe was found to be 122 ± 21 µg/g compared to those of Mn and Zn, which were found to be 19.2 ± 4.6 and 37.7 ± 9.2 µg/g respectively. Such large variations may be attributed to geo-environmental factors and difference in age of the trees as suggested by Zaidi *et al.*[21].

**Elemental contents in Neem bark:** Mg and V in neem bark were found below detection limits of 0.50 mg/g and 0.10 µg/g respectively. Unlike K in leaves, Ca content in bark is highest with a mean value of 14.4 ± 5.9 mg/g. Na, Cl and P contents are also higher compared to leaves. In order to compare the elemental contents in leaves and bark, elemental profiles have been drawn in Figs. 5 and 6 respectively. It may be noted that the mean content of K in leaves is higher (20.1 ± 7.7 mg/g) by an order of magnitude compared to that in bark (2.10 ± 0.42 mg/g). However, Ca content in bark is higher (14.4 ± 5.9 mg/g) by a factor of more than three compared to that in leaves (4.4 ± 1.01 mg/g). On the contrary, other minor constituents such as P and Cl are much lower in bark Unlike in leaves, trace element profiles of leaves and bark are similar though most elemental contents in bark are higher except Cu, Zn, Rb and Cs. Particularly contents of Al, ba, Hg and Mn are higher by a factor of 3. However, Fe content in bark is somewhat higher (186 ± 38 µg/g) compared to that in leaves (122 ± 21 µg/g). Thus bark and leaves have distinctive elemental contents that may be responsible for its medicinal properties[10].

**Elemental correlations:** Several literature reports suggest interrelationship of various elements in plant species. We have observed a strong linear correlation between K and Cl (r = 0.96) and Rb and Cs with r = 0.97 in neem leaves. Rb and Cs salts enhance the absorption of insulin in lower respiratory tract as suggested in a recent US patent[23]. In this regard, V, Cr, Mn, Fe, Cu and Zn are of special importance in diabetes and all the elements have been found in µg/g amounts. V (1.15 ± 1.06 µg/g) and Cr (1.34 ± 0.95 µg/g), the two elements whose deficiency is intrinsically linked to diabetes[24] are found in comparable amounts but vary by almost an order of magnitude.

## Conclusion

Discovery of radioactivity and related techniques have played a major role in the scientific development of modern world. Besides providing the unique source of nuclear power, many technologies using radioisotopes and radiotracers have proved vital for diagnostic and therapeutic uses in modern medicine system and material characterization. Neutron activation analysis (NAA) is one of the most accurate, precise and sensitive method extensively used for the determination of major, minor and trace element contents in complex geological, biological and environmental samples. It has the advantage of being nondestructive and multielemental in character. Neutron activation analysis methodologies employing reactor irradiation followed by differential counting have been developed for the determination of 30 to 35 elements in medicinal herbs, herbal preparations and bhasmas. However, requirement of a nuclear reactor is a limitation in routine applicability of neutron activation analysis.

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