ANALYSIS OF ESSENTIAL TRACE ELEMENTS IN MEDICINAL HERBS BY RADIOCHEMICAL METHODS

A THESIS

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in

CHEMISTRY

By

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CANDIDATE'S DECLARATION

I hereby certify that the work being presented in the thesis entitled **ANALYSIS OF ESSENTIAL TRACE ELEMENTS IN MEDICINAL HERBS BY RADIOCHEMICAL METHODS,** in fulfillment of the requirement for the award of the degree of **DOCTOR OF PHILOSOPHY** and submitted in the **DEPARTMENT OF CHEMISTRY** of the Indian Institute of Technology Roorkee, Roorkee is an authentic record of my own work carried out by me during a period from **July, 2001 to May 2005** under the supervision of **Prof. A.N. GARG.**

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other Institute.

(ASHOK KUMAR)

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

Dated: 30th May 2005

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ABSTRACT

Herbs are nature's pharmacy and knowledge of its medicinal importance is recognized in most civilizations of world. However, it is more prevalent in Asia, Africa, Egypt, China, Latin America, Malaysia, Turkey etc. Indian society with strong cultural heritage and rich biodiversity attained this knowledge in a well organized form, systematically recorded and employed as a traditional health care system called *Ayurveda* (*Ayu* = life, *Veda* = knowledge) meaning *Science of Life*. It encompasses medicinal, psychological, cultural, religious and philosophical concepts with holistic approach towards long, healthy and disease free happy life. Present work was undertaken as a part of the Board of Research in Nuclear Sciences (BRNS) sponsored project on the *Development of Radiochemical Methods for the Analysis of Trace Elements in Medicinal Herbs.*

In recent years, antioxidant properties of dietary constituents and herbal products have created much interest. Many medicinal plants are reported to be rich source of antioxidants, which neutralize free radicals generated by certain biochemical reactions. Therefore, these have attracted attention of health professionals as well as of basic scientists interested in biomedical research. Thesis is divided into six chapters dealing with following aspects:

Ch I on ***Radiochemical Methods*** introduces the general aspects of radioanalytical methods including principle, classification and applications of NAA in various fields. Role of elements in life processes and its analytical importance is emphasized. A brief introduction of Indian medicinal system *Ayurveda* and its present status in global context is dealt. Importance of the availability of trace elements in biocompatible form and easy assimilability has been emphasized.

Ch II on **Experimental Methodology** deals with the sampling methods, its preparation and instrumentation used in NAA and AAS. Also details of data processing and bioassay studies are included. An important aspect of this work was the establishment of high resolution γ-ray spectrometry facility with lead shielding, associated hardware and software.

Ch III deals with our results on the **analysis of 25 elements in 15 medicinal herbs** commonly used in Indian household. In most cases K content is higher than Na by almost an order of magnitude. Similarly Ca and Mg are

found in much higher concentrations in some herbs. Fe contents in all the samples are > 200 μg/g though bakuchi (*P. corylifolia)* seeds and jatamansi (*N. jatamansi*) show much higher amounts of 923±123 and 1210±200 μg/g respectively. Jatamansi is particularly enriched in Cr (8.19±0.04 μg/g), Mn (474±5 μg/g), Fe (1210±200 μg/g), Cu (36.8 μg/g) and Zn (60.0±6.3 μg/g). According to *Ayurveda* it is recommended as antibacterial, antipyretic and heart tonic. K/P ratio was found to vary in a wide range of 2.07-24.2. Fe & Mn and Zn & Cr were linearly correlated in some herbs.

Ch IV deals with the **analysis of two herbal formulations; *Trifala and Pragya-peya***. Former is the most popular herbal formulation widely used as effective laxative, powerful antioxidant and antibacterial with anticancer properties. We have analysed 9 brands of *Trifala* including its 3 constituents, amalaki (*Embilica officinalis*), bibhitaki (*Terminalia bellirica*) and haritaki (*T. chebula*) for 29 elements. A perusal of elemental contents suggests that *trifala* as whole is enriched in K (12.6±1.1 mg/g), Mg (0.91±0.34 mg/g), Ca (4.88±7.62 mg/g), Fe (1.01±0.23 mg/g) and Zn (41.1±14.9 μg/g), though Se is also present in significant amounts (104±34 ng/g). Also gallic acid was separated by column chromatography and further confirmed by ir, NMR and GC-MS studies.

Pragya-peya, another herbal formulation from Shantikunj, Haridwar is a mixture of 12 herbs; *aagya-ghas*, *arjuna*, *bay leaves*, *brahmi*, *dalchini*, *fennel*, *nagarmotha*, *red sandal, shankhpushpi, sharpunkha, tulsi* and *yastimadhu*, widely recommended for cold and cough, as a nervine tonic and stimulant. It is especially enriched in several nutrient elements such as Ca (23.9±1.3 mg/g), K (9.37±0.92 mg/g), Mg (2.23±0.41 mg/g), P (1.20±0.04 mg/g), Mn (87.8±10.9 μg/g), Fe (676±176 μg/g), Cu (14.7±0.8 μg/g), Cr (1.56±0.24 μg/g), Co (0.62±0.09 μg/g) and Zn (34.5±4.0 μg/g). *Aagya ghas* is particularly enriched in Cr, Fe, Cu and Zn whereas fennel seeds are enriched in K, Mg, P and Se. It is possible that some metals exist as complex with macromolecules thus enhancing their bioavailability. Some toxic heavy metals such as Hg, Sb, Cd and Pb are also present but these are within WHO permissible limits. Fe is correlated with Fe/Zn and Co in all the herbs. It suggests that the concentration levels of many elements in the herbs are strongly affected by characteristics of plant, soil as well as environmental conditions.

Ch V describes **analysis of *Bhasmas*;** the unique metallic-herbal preparations known in Indian subcontinent for several thousand years. Use of metals in medicine is often associated with the question of toxicity. These are believed to be biologically produced nano-particles, which enter in the blood stream enhancing their efficacy as medicine. Twenty *bhasmas* based on Ca, Fe, Zn, Hg, Ag, K, As, Cu, Sn and gemstones were analysed for up to 21 elements including C, H, N and S. Besides the major constituent elements, several other essential elements such as Na, K, Ca, Mg, V, Mn, Fe, Cu, Zn, etc, have also been found in significant amounts. These are derived from herbs and seem to remain chelated with organic macromolecules acting as ligands. The *bhasmas* are taken along with milk, butter, honey or ghee (milk preparation) and thus make these elements easily digestible, eliminating their harmful effects and enhancing their biocompatibility. *Siddhamakaradhwaja,* and *swet parpati* correspond to the stoichiometry of HgS and KNO_3 respectively. K/P was found to vary in a wide range (0.23 to 12) though for most *bhasmas* (n=12) it lies in a close range of 2.3 $\pm$ 1.2. Further, Fe/Mn is linearly correlated (r = 0.96) with iron in 9 non-iron containing *bhasmas*.

Ch VI deals with **the bioassay studies of *brahmi* (*Bacopa monnieri*) extracts** in aqueous-methanol (BAM) for DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging, DNA strand break, antilipid peroxidation, superoxide anion scavenging, H_2O_2 scavenging activity and total phenol content. It also showed protective effect of γ-ray induced DNA damage. Elemental contents in respective extracts were also determined. It is observed that elemental contents of Na, K, Cl, Co and Zn were higher in the BAM extract.

In general, it is observed that no single herb is enriched in all the essential nutrients. However, some herbs are particularly enriched in elements such as Fe, Mn, Co, Cu, Zn, Se and V essential for various enzymatic processes and play a vital role in its pharmacological/curative properties. Also some elements in various medicinal herbs represent synergistic or antagonistic effects suggesting usefulness of herbal formulations.

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(ASHOK KUMAR)

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CHAPTER I

RADIOCHEMICAL METHODS

I.1 HISTORICAL DEVELOPMENTS

After the discovery of radioactivity, it was soon realized that individual radionuclides could be easily distinguished due to its characteristic radiation, energy and half-life. This led to the development of tracer techniques by George de von Hevesy in 1913. Later Chadwick's scintillating discovery of neutrons in 1932, led to the development of neutron activation analysis (NAA) by George Hevesy and Hilde Levi. Later Seaborg and Livingood (1938) discovered charged particle activation analysis (CPAA) followed by photon activation analysis (PAA). Thus, a new branch called ***Radioanalytical Chemistry*** came into being [1-5]. These new developments got tremendous boost due to many important discoveries such as nuclear fission, nuclear reactors, particle accelerators and semiconductors, which helped in the identification and accurate assay of radionuclides in a complex mixture. These developments were achieved by continued interaction between analytical chemists, nuclear physicists and electronic engineers leading to the developments in new instrumentation and methodologies. During last century, several new nuclear techniques such as Rutherford back scattering (RBS) [6], X-ray fluorescence (XRF) [7], energy dispersive X-ray fluorescence (EDXRF) [8], particle induced X-ray emission (PIXE) [9] etc have been developed. Besides, utilization of processes involved in electron inner shell excitations such as Auger electron spectroscopy [10] and Mössbauer spectroscopy [11] are also regarded as nuclear analytical techniques essentially because of similarity in equipments used in these techniques. Nuclear analytical techniques have made a great impact on the analysis of complex natural matrices including geological and biological systems.

Tian [12] reviewed on quality control in nuclear analytical methods and suggested that a combination of several nuclear analytical techniques can be a better way of characterisation. In the last seven decades of its discovery especially during sixties and seventies, many innovative instrumental and methodological developments [13-18] have taken place as summarized in Table I.1.

Table I.1. Stages in the development of NAA

1932-1944 (Induction period)	Discovery of Neutrons, Activation Analysis using isotopic sources.
1944-1964 (Nuclear Reactor)	Availability of nuclear research reactors, Exploration of applications
1950-1964 (Scintillation detector)	Fundamental advances in electronics/instrumentation, scintillation detectors and radiochemical neutron activation analysis of complex matrices.
1965-1975 (Solid state detector)	Si(Li), Ge(Li) and HPGe detectors, MCA, High resolution gamma ray spectrometry for multielemental analysis.
1975-1995 (Methodogical Developments & Applications)	Extensive applications in the analysis of Lunar rocks, meteorites, geological, archeological, biological, environmental, industrial and forensic studies – new era of methodological developments [14-20]; ➢ Epithermal neutron activation analysis (ENAA) ➢ Fast neutron activation analysis (FNAA) ➢ Cyclic neutron activation analysis (CNAA) ➢ Derivative neutron activation analysis (DNAA) ➢ Prompt γ-ray neutron activation analysis (PGNAA) ➢ Monostandard (k_0) NAA method ➢ Fast irradiation and measurement system (FIMS) ➢ In vivo activation analysis (IVAA) ➢ Development of Robotic systems
1995 - Present	Specific problem oriented studies in Biology, Medicine, Health & Nutrition, Environment,Industry.

The popularity and interest in nuclear analytical methods can be gauzed by a number of International Conferences held around the world as listed in Table I.2. The proceedings of MTAA, MARC and APSORC are published as special volumes of the *J. Radioanal. Nucl. Chem.* whereas for NAMLS, earlier these were being published in *Biol. Trace Elem. Res.* but for NAMLS8 these are proposed to be published in *J. Radioanal. Nucl. Chemistry.*

In this regard, a mention may be made about Indian efforts whereby first Nuclear and Radiochemistry Symposium was organized by the Bhabha Atomic Research Centre, Mumbai a part of Department of Atomic Energy (DAE) during sixties and these continued till seventies. After a gap of a decade, these were revived in 1980, when first Radiochemistry and Radiation Chemistry Symposium (RRC) was organized at the Andhra University, Visakhapatnam. It continued till 1992 when it got bifurcated into separate symposia as Nuclear and Radiochemistry Symposium (NUCAR) and

Table I.2. International Conferences on Nuclear Analytical Techniques

Name of the Conference	Location	Starting (Frequency)
◈ *Modern Trends in Activation Analysis (MTAA)* Recent MTAA-11 held in Guildford, UK, June 20-25, 2004	Around the world (MTAA-1 & 2 were held at TAMU, USA)	1961 (4 years)
◈ *Nuclear and Radiochemistry (NRC)* Recent NRC-6 held in Aachen, Germany, Aug 29-Sept. 3, 2004	Europe (NRC-1 was held at Lindau, Germany)	1984 (4 years)
◈ *Methods and Applications of Radioanalytical Chemistry (MARC)* Recent MARC-VI held, April 7-11, 2003 & next is due in April 3-7, 2006	Kona, Hawaii, USA	1967 (3 years)
◈ *Nuclear Analytical Methods in the Life Sciences (NAMLS).* Eighth in the series NAMLS8 held at Rio de Janeiro, April 17-22, 2005	Around the world (NAMLS 1 was held at Amsterdam, The Netherlands)	1967 (Irregular intervals of 4/6 yrs)
◈ *Nuclear Analytical Chemistry (NAC)* Recent NAC-III held in June 2001	Halifax, Canada	1985 (Irregular intervals)
◈ *Asia Pacific Symposium on Radiochemistry (APSORC)* Next APSORC-3 is due in Beijing, China during Oct 17-21, 2005	Asia (Japan & China)	1997 (4 years)

Trombay Symposium in Radiation and Photochemistry (TSRP) both being held in alternate years. Since 1995, NUCARs have been organized at the Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam, Saha Institute of Nuclear Physics, Kolkata (1997), BARC (1999, 2003), University of Pune, Pune (2001) and recently at the Guru Nanak Dev University, Amritsar (2005)

These conferences provide a forum at national/international level to presentation and discuss the present status and emerging trends in methodological, instrumentation and their applications in various disciplines. During last one decade very little methodological developments have taken place, though NAA has found many extensive applications in biological, environmental, biomedical, nutrition and health related research including characterization of high purity materials, new developments in Ion beams, chemical separations and special techniques. By now it has attained maturity

and its future is rather bleak because of lack of funding to research reactors essentially because several competitive techniques have come up.

Ever since first book "Neutron Irradiation and Activation Analysis" was published in 1964 [21], several monographs [3,5] and text books [1, 14, 22, 23] have been published. Recently a major reference work "Handbook of Nuclear Chemistry" has been published in five volumes [24]. Until 1994, biyearly reviews by Ehmann et al. [25] were in much prominence. In recent years several reviews describing present status of NAA and nuclear techniques have appeared [15, 26-28]. Bode [29] has raised some questions about the future of nuclear techniques.

I.2 NEUTRON ACTIVATION ANALYSIS (NAA)

(i) Principle: Primarily the technique of NAA involves two steps; irradiation of a sample with neutrons and subsequently measurement of the induced radioactivity [1,21]. The radionuclides are characterized by their characteristic γ-ray energy and half-life [30] as illustrated in Fig. I.1. In this process, the rate of formation of the product

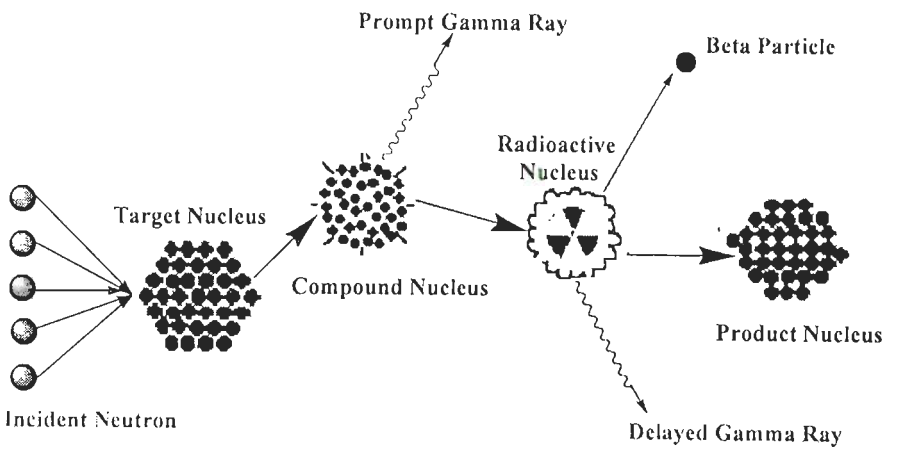


Fig. I.1. Principle of Neutron Activation Analysis

atoms (N_p) is represented as;

$$\frac{dNp}{dt} = N\sigma\phi - \lambda Np = N\sigma\phi - \lambda N_1 \qquad \ldots\ldots \qquad \ldots\ldots \qquad (I.1)$$

where N is the number of target atoms of an element in the sample $\left[\frac{i.w\,N_A}{M}\right]$,

i = isotopic abundance; w = concentration of element; M = mass of the target atom; N_A = Avogadro number; N = thermal neutron absorption cross section in barns (b = 10^{-24} cm^2); ϕ, neutron flux (n cm^{-2} s^{-1}) and λ, the decay constant (s^{-1}) = $\left(\frac{0.693}{t_{1/2}}\right)$ and $t_{1/2}$ = half life of the product nucleus. On integration, eqn (I.1) yields

$$\lambda N_1 = A = N \sigma \phi \left(1 - e^{-\lambda t_i}\right) \quad \ldots\ldots \quad \ldots\ldots \quad (I.2)$$

where t_i = the irradiation time. Since the sample is delayed for time t_d before counting using a counter with efficiency ε, and gamma-ray abundance γ, the complete equation [1,20,21] can be written as;

$$A = \left(\frac{wiN_A}{M}\right)\sigma \phi \left(1 - e^{-\lambda t_i}\right) e^{-\lambda t_d} \varepsilon \gamma = \left(\frac{wiN_A}{M}\right)\sigma \phi S D C \varepsilon \gamma \quad \ldots\ldots \quad \ldots\ldots \quad (I.3)$$

where $S = \left(1 - e^{-\lambda t_i}\right)$ is saturation factor, $D = \left(e^{-\lambda t_d}\right)$ is delay factor, $C = \left(1 - e^{-\lambda t_c}\right)/\lambda$ is the correction factor for decay during counting time t_c, and activity (A) is measured in terms of peak area after subtracting background. γ-activity of radioisotopes is measured using an HPGe detector though it could also be measured by using a scintillation detector [30]. Thus, concentration of an element may be determined by rearranging the left part of eqn. (I.3) as;

$$w = \frac{A.M}{i\, N_A\, \sigma\, \phi \left(i - e^{-\lambda t_i}\right) e^{-\lambda t_d} \varepsilon} \quad \ldots\ldots \quad \ldots\ldots \quad (I.4)$$

This way of calculating the concentration of an element is called the ***Absolute method*** where σ, ϕ, t_i, t_d and ε are the experimentally determined parameters [1,20]. After calculating w, the concentration of an element in the sample having weight (W) may be determined. However, the absolute method has uncertainties in the values of ϕ and σ [1-5]. It is well known that these may vary under experimental conditions depending on the location of the sample in the reactor or irradiation can. In order to eliminate these uncertainties, a ***Comparator method*** has been suggested where a standard (primary and/or secondary) with known concentration of element is simultaneously irradiated along with the sample under similar experimental conditions and counted in the same geometry [1]. Thus, an eqn. similar to (I.3) can be written for standard as;

$$A' = \left(\frac{w' i N_A}{M}\right) \sigma \phi \left(1 - e^{-\lambda_i}\right) e^{-\lambda_d'} \varepsilon \gamma = \left(\frac{w' i N_A}{M}\right) \sigma \phi S D' C \varepsilon \gamma \quad \ldots\ldots \quad \ldots\ldots \quad (I.5)$$

Dividing LHS of eqns. (I.3) and (I.5) we get

$$\frac{A}{A'} = \frac{w}{w'} \times \frac{e^{-\lambda t_d}}{e^{-\lambda_d'}} \quad \ldots\ldots \quad \ldots\ldots \quad (I.6)$$

This is applicable only if $t_{1/2}$ of the radionuclide is comparable with delay time (t_d) and counting time (t_c). However, if $t_{1/2}$ of the radionuclide is long enough compared to delay and counting times then, it can be further simplified to

$$\text{Concn. of element in sample } (S_a) = \text{Concn. of element in standard } (S_t) \times \frac{\text{Activity in sample}}{\text{Activity in standard}} \quad \ldots\ldots \quad \ldots\ldots \quad (I.7)$$

When many elements are to be determined in each sample, preparation of individual standards becomes tedious and impractical. Thus, either multielemental standards or the use of a single comparator for all the elements becomes necessary. In the eqn, (I.7), activity is replaced by specific activity to have the uniformity while considering weights of sample and the comparator standard. The standard/certified reference materials (RMs) are available from various agencies such as the National Institute of Standards and Technology (NIST, USA), International Atomic Energy Agency (IAEA, Vienna), National Institute of Environmental Studies (NIES, Japan), Institute of Nuclear Chemistry and Technology (INCT, Poland), wherefrom RMs of a variety of matrices are available but these are expensive. Therefore, many laboratories prepare their own standards by spiking with μg amounts of standard aq. solutions prepared from AR/GR/HP grade salts on to SiO_2/cellulose or Whatman filter paper strip. In order to achieve better accuracy and to eliminate errors due to secondary standard, a ***Monostandard (k_o) Method*** has been proposed by Girardi [20,31,32]. In this method a single element such as Au, Mn, Co with 100% isotopic abundance is used as a comparator for multielement NAA. Several schemes have been devised to accomplish this and the most important of these is k_o method [20,33-35]. The eqn. used to calculate concentration (w) using this method is

$$w(\mu g/g) = \frac{A_{sp}}{A^{*}_{sp}} \frac{1}{k_0} \frac{(f + Q^{*}_{0}(\alpha))\varepsilon^{*}}{(f + Q_0(\alpha))\varepsilon} \quad \ldots\ldots \quad \ldots\ldots \quad (I.8)$$

where A_{sp} = specific activity; f = the thermal to epithermal flux ratio

Q_0 = the ratio of the resonance integral to the thermal neutron cross section;

ε = the detector efficiency; α = the deviation of the epithermal flux from ideality

k_0 = the k_0 factor $\left(\frac{M^{*} i \gamma \sigma_{th}}{M i^{*} \gamma^{*} \sigma^{*}_{th}}\right)$; *corresponds to the comparator element

The k_o factor is an experimentally determined value that contains the values for the nuclear constants needed in the activation analysis. In eqn. (I.8) a new term K_{anal} is introduced so that it may now be written as

$$\rho(\mu g/g) = \frac{A'_p\ p}{A^{*}_{sp}} \cdot \frac{1}{K_{anal}} \quad \text{where } K_{anal} = k_o \frac{(f + Q_o)}{(f + Q_o)^{*}} \cdot \frac{\varepsilon}{\varepsilon^{*}} \quad \ldots\ldots \quad \ldots\ldots \quad (I.9)$$

where A'_p is the peak area of the i^{th} element corrected for saturation, cooling and decay during counting and normalised for 1 g sample. The k_o factors for many nuclides have been determined and published in a joint project involving the activation analysis laboratory of the Central Research Institute for Physics in Budapest and the Institute for Nuclear Sciences in Ghent using gold as a comparator element [32].

(ii) Neutron Sources: A wide range of devices is used to produce neutrons needed for activation analysis. Some are sophisticated and extremely expensive, while others are rather simple and modest in cost [1]. In Table I.3 are listed some commonly used isotopic neutron sources, machine type sources and nuclear reactors available for NAA work in India. The reactor neutrons have wide energy spectrum as illustrated in Fig. I.2

Energy of neutrons plays an important role as different types of reactions viz. (n, γ), (n, p), (n, α), (n, n'), (n, 2n) are possible depending on the energy of neutrons.

$$^{59}Co + {}^{1}n \longrightarrow [^{60}Co^{*}]$$

(n,γ) → γ + ^{60}Co ($t_{1/2}$ = 5.3 y)

(n,p) → ^{1}H + ^{59}Fe ($t_{1/2}$ = 44.5 d)

(n,d) → ^{2}H + ^{58}Fe (i = 0.282) *Stable*

(n,t) → ^{3}H + ^{57}Fe (i = 2.119) *Stable*

(n,α) → ^{4}He + ^{56}Mn ($t_{1/2}$ = 2.58 h)

(n,2n) → ^{2}n + ^{58}Co ($t_{1/2}$ = 70.8 d)

Table I.3 Common Sources of Neutrons for NAA [1, 36]

Reaction	Half life	Average Neutron Energy (MeV)	Neutron yield (ns^{-1} Ci^{-1}, unless otherwise stated)
I. Isotopic Sources			
(i) Photo neutron (γ, n)			
^{88}Y with ^{9}Be	106.6 d	0.16	$1x10^5$
^{124}Sb with ^{9}Be	60.2d	0.02	$1.9x10^5$
(ii) Alpha emitter (α,n)			
^{239}Pu with ^{9}Be	$2.4x10^4$ y	3.5	$\approx 10^7$
^{226}Ra with ^{9}Be	1600 y	3.6	$1.1x10^7$
^{241}Am with ^{9}Be	433 y	3.5	$2.2x10^6$
(iii) Spontaneous fission			
^{252}Cf	2.64 y	2.3	$2.3x10^{12}$ ns^{-1} g^{-1}
II. Machine Type Source			
(i) Cockroft-Walton n-generator $^{3}H(d, n)$ ^{4}He			
(ii) Cyclotron	-	14.7	$10^8 - 10^{11}$ n/s
10 μA of 30 MeV deuterons on Be	-	Broad distribution	$2x10^{11}$ ns^{-1}
[$^{239}Pu(\alpha,n)$ ^{242}Cm]			
III. Nuclear reactors	-	Broad distribution	
APSARA (1956) }			$\sim 10^{11}$ }
CIRUS (1960) } BARC, Mumbai			$\sim 10^{12}$ } n cm^{-2} s^{-1}
Dhruva (1985) }			$\sim 10^{13}$ }
Kamini (1999) – IGCAR, Kalpakkam			$\sim 10^{10}$ }

A. Thermal NAA: Thermal neutrons with energy 0.025 eV can induce radiative capture (n,γ) reactions in the target nuclei and delayed γ-rays from the sample are detected. An advantage of using thermal neutrons is their higher cross sections compared to those for other types. At higher flux of thermal neutrons, TNAA offers potentially higher sensitivity for a large number of elements except lighter elements such as C, N, O etc. and potentially toxic elements such as Tl, Pb, Ni etc. Concentration of up to 60 elements at trace and ultra trace level can be determined in a wide variety of samples.

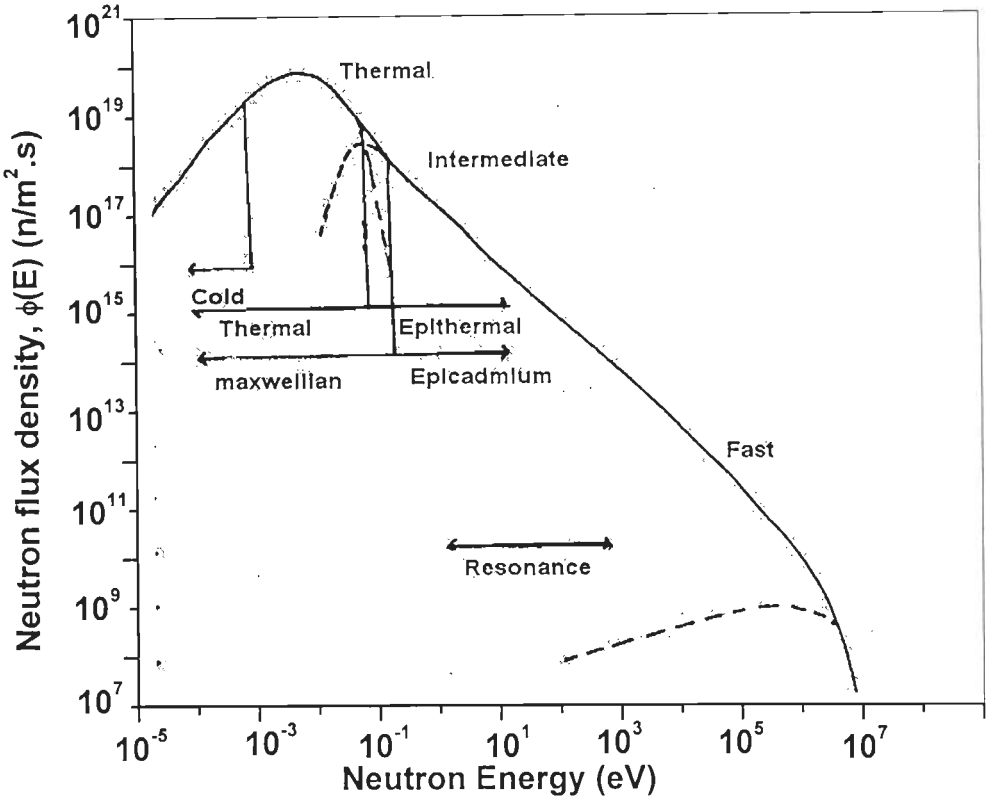


Fig. I.2 Energy Spectrum of Reactor Neutrons

B. Epithermal NAA: Epithermal neutrons are narrowly defined as those having energy in the range of 0.1 to 1.0 eV. In practice the lower end of the neutron energy range used in ENAA is closer to 0.5 eV, which is the Cd cutoff energy (~ 0.55 eV) and the higher end of the range extends through the resonance neutrons up to several keV [16]. Epicadmium neutrons, like thermal neutrons, also induce (n, γ) reactions and delayed gammas are detected for many elements such as Ag, As, Au, Ba, Br, Cs, Eu, Ga, Gd, Hf, Ir, La, Ni, Rb, Sm, Sn, Sr, Ta, Tb, U, W and Yb [27].

C. Fast NAA: The fast neutrons encompass the widest range of energy, 0.5 – 20 MeV. Neutrons of this energy range do not induce (n,γ) reactions but instead are responsible for (n, p), (n, α), (n, n') and (n, 2n) reactions. Mostly FNAA is performed using 14 MeV neutrons produced by Cockroft-Walton neutron generator using the reaction d(t, n)^{4}He. These are specially useful for the determination of oxygen using the reaction ^{16}O(n, p)^{16}N.

(iii) Interferences: NAA is based on nuclear phenomenon and hence no chemical interferences are possible. Possible nuclear interferences are primary, secondary and second order interference reactions, gamma ray spectral interference, neutron self-shielding, γ-ray self-attenuation, true and random coincidences during gamma ray measurement [1,2]. If same radionuclide is formed from other than the analyte element, then it is a primary interference reaction. For example, in the case of ^{56}Mn produced by (n, γ) reaction, the same radionuclide is also produced by (n, p) and (n, α) reactions on ^{56}Fe and ^{59}Co respectively. Thus, determination of Mn in steel containing iron and cobalt may be erroneous if higher energy neutrons are present. If the sample is irradiated with thermal neutrons only or Cd/B shielding is used then these interfering reactions may be eliminated. Other examples of such interferences are in the determination of Al in presence of Si and P or Na in presence of Al and Mg. Gamma-ray spectral interferences occur when similar γ-ray energies are produced by other radionuclides. For example: 846.8 keV of ^{56}Mn is often interfered by 844 keV of ^{27}Mg and 279.2 keV of ^{203}Hg with 279.5 keV of ^{76}Se. In such cases other indicator gamma rays can be used or one should wait for the decay of other nuclide. For example, ^{56}Mn has $t_{1/2}$=155 min. and it should be counted after an hour so that ^{27}Mg ($t_{1/2}$= 9.45 min) may undergo decay.

(iv) Sensitivity and Detection Limits: The sensitivity of an element in NAA is defined as counts μg^{-1} for particular experimental conditions viz. t_i (irradiation), t_d (delay) and t_c (counting) durations. Depending on the nuclear properties such as isotopic abundance (i) and neutron absorption cross section (σ) of the isotope of interest, the above parameters (t_i, t_d and t_c) can be decided. From the activity eqn., it is clear that the main parameters for sensitivity are σ, ϕ and ε. Since σ is fixed for a nuclide, high flux neutrons are needed and so also a detector of high efficiency. Because a sample may contain isotopes that are not active, the fraction of the atoms that are radioactive must also be known. Therefore, isotopic abundance (i) of the nuclide of interest plays an important role in detection limits. Detection limit in NAA varies from pg to mg depending on the element of interest, gamma ray background, sensitivity and sample matrix. The

detection limit (L_D) is defined as three times the standard deviation of the background counts (C_b) under the peak and is calculated using the expression

$$L_{D(counts)} = 2.71 + 3.29 \times \sqrt{C_b} \quad \ldots\ldots \quad \ldots\ldots \quad (\text{I.10})$$

The counts are then converted to μg g^{-1} with the use of S (sensitivity) sample mass (g). The typical detection limits using a flux of 10^{13} n cm^{-2} s^{-1} are available from the literature [3,27]. For elements like Eu and Dy, the L_D is about 1 pg whereas for Mn, In and Lu, it is 10 pg under normal experimental conditions.

I.3 CLASSIFICATION

The NAA technique can broadly be classified as instrumental and radiochemical NAA depending on whether the sample is counted directly after irradiation or after chemical separation and the resultant activity is used for calculating the concentration of an element [14]. Many methodological developments have become possible primarily due to the advancement in instrumentation, methodology and data processing procedures as illustrated in Fig. I.3. Some of these methodologies are described in following lines:

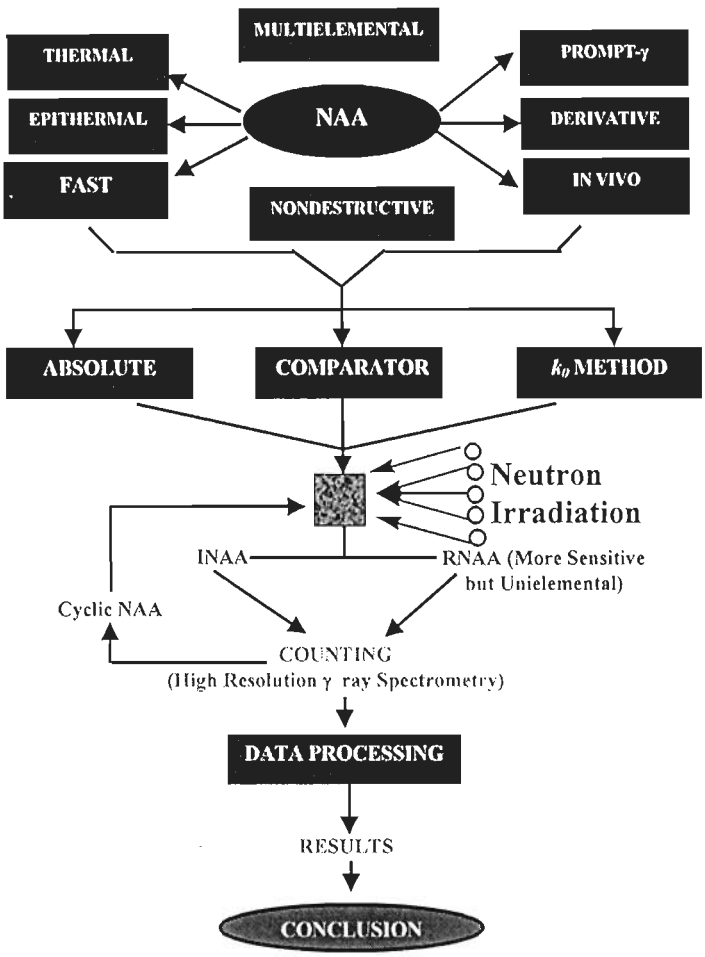


Fig.I.3 Schematic Illustration of NAA Methodologies

(i) Prompt Gamma (PG) NAA: In this methodology prompt gamma rays emitted during (n,γ) reaction as illustrated in Fig. 1.1 are used for analysis. This technique was first used in 1969 [37]. The method is especially suitable for determination of light

elements such as H, B, N, P, S, C and Si and elements of high neutron absorption cross section e.g, Hg, Cd, Sm and Gd. A majority of PGNAA work is carried out with isotopic sources though counting facility can be setup at the reactor site as well. Paul and Lindstrom [38] have described its fundamentals and applications. It is being increasingly used as a rapid instrumental, non-destructive and multielemental analysis technique. A further advancement of PGNAA is *in-vivo* PGNAA where the entire body or a segment of the patient/subject is irradiated with ^{241}Am-Be, ^{252}Cf or an accelerator based neutron source followed by counting of prompt/delayed gamma rays [39]. PGNAA has been widely used due to improved analytical sensitivity and detection limits specially by using low energy guided neutron beams [40]. Its most clinical use is the *in vivo* determination of ^{10}B concentration needed to measure the radiation dose received by patients undergoing neutron capture therapy for tumors [41].

$^{11}B(n, \gamma)^{12}B(t_{1/2} = 20.4$ ms,$E_\gamma = 4.44$ MeV); $^{48}Ca\ (n, \gamma)^{49}Ca(t_{1/2} = 8.72$ min,$E_\gamma = 3.08$ MeV)

$^{14}N\ (n, \gamma)\ ^{15}N$ STABLE, ($E_\gamma = 10.8$ MeV); $^{113}Cd\ (n, \gamma)\ ^{114}Cd$ STABLE, ($E_\gamma = 0.56$ MeV)

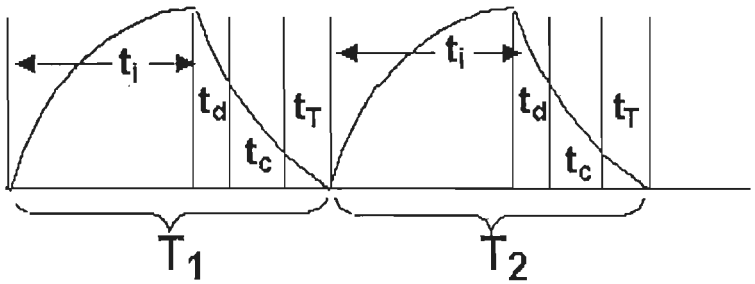
(ii) Short time activation analysis: This involves irradiation of the sample for a very short duration (1-600s) and counted after a delay of 20 ms. It is particularly useful for the determination of elements with very short half life of the order of few seconds. It has the advantage of low turn around time and high throughput. Grass [17] developed a fast irradiation and measurement system (FIMS) using TRIGA Mark II reactor at the Atom Institute, Vienna. Ochsenkuhn [42] reported that short time Instrumental NAA, with high throughput, sensitivity and accuracy without matrix interferences can be achieved in spite of the initial high count rate from both short and long lived nuclide activation. Freitas et al. [43] performed short time NAA ($t_i = 30$ s) for the analysis of trace elements in honey.

$^{11}B\ (n, \gamma)^{12}B\ (t_{1/2}=20.4$ ms,$E_\gamma=4.44$ MeV); $^{11}B(n, \alpha)^{8}Li\ (t_{1/2}= 844$ ms,E_{max} of $\beta = 12.5$ MeV)

$^{12}C(n, p)^{12}B\ (t_{1/2} = 20.4$ ms, $E_\gamma=4.44$ MeV); $^{13}C(n, p)^{13}B\ (t_{1/2}=17.3$ ms,$E_\gamma=3.68$ MeV)

(iii) Cyclic Neutron Activation Analysis (CNAA): In case of elements with short-lived activation products, sensitivity is often hampered by low activity and thus reliability of the measurement is at stake. This difficulty is overcome by repeating the

whole cycle of irradiation and counting several times and subsequently summing up the γ-ray spectra as typically illustrated below;



where t_i = irradiation time; t_d = delay time
t_c = counting time; t_T = transfer time

Givens et al. [44] gave its basic theory and derived an eqn. for the total activity as;

$$A = \frac{N\phi\sigma k}{\lambda}\left(1-e^{-\lambda t}\right)\left(1-e^{-\lambda t'}\right)\left(e^{-\lambda t''}\right)\left[\frac{n}{1-e^{-\lambda T}} - \frac{\left(e^{-\lambda T}\right)\left(1-e^{-n\lambda T}\right)}{\left(1-e^{-\lambda T}\right)^2}\right] \quad \text{(I.11)}$$

where A = total cumulative counts recorded in n cycles, *k*= factor containing the detector efficiency and branching ratio for the decay path, *t* = irradiation time for each cycle, *t'*= counting time for each irradiation, *t''*= delay time period to counting for each irradiation, T= the delay period from the end of a previous delay period to the end of the next delay period (cycle time, s) and n is the number of cycles. Thus, having statistically significant counts, sensitivity is enhanced.

Jayawickreme and Chatt [45] have used cyclic procedure to determine 23 elements in bovine kidney. In recent years, CNAA has developed into a useful analytical tool for the assay of short-lived isotopes. This technique has high potential for the analysis of composite samples having a wide range of elements that produces short lived and long lived isotope on neutron irradiation [46, 47].

(iv) Derivative Neutron Activation Analysis: This method also called as Molecular Activation Analysis, is used to enhance the sensitivity of NAA for more elusive elements, which are otherwise difficult to be determined by NAA and allow the determination of chemical speciation. In this technique, an element or chemical entity to be determined (U) is complexed with, or exchanged for another element, which has

higher sensitivity for NAA. Also the product radionuclide should have an interference free photopeak in low background region. Further, it is desirable that the compound formed should be stoichiometric and it should be easily separable quantitatively. Ehmann [48] et al. developed a DNAA procedure for the determination of P in a variety of samples. Recently, Scindia et al. [49] have reported determination of phosphorus using preconcentration chemical procedure accompanied by DNAA by extracting molybdovanadophosphoric acid in MIBK and measuring the activity of ^{52}V ($t_{1/2}$ = 3.4 min).

(v) In Vivo (IV) NAA: Anderson et al. [50] first suggested the possibility of applying NAA to determine whole body elemental levels in living organisms. Since then IVNAA has become an established technique in nuclear medicine and widely used for biomedical analysis. IVNAA found applications in basic physiological studies, clinical diagnosis of disease and monitoring of therapeutic interventions for diseases. Recently, Stamatelatos et al. [51] used PGNAA for *in vivo* body composition studies in small animals. In fact future developments in NAA depend on the diagnostic medical applications using *in vivo* activation.

$^{1}H\ (n, \gamma)\ ^{2}H$ (E_γ = 2.22 MeV)
$^{31}P\ (n, \gamma)\ ^{32}P$ (E_γ = 0.08 MeV)

(vi) Compton Suppression NAA: Gamma ray spectra of the activated samples in NAA usually suffer from a major drawback due to Compton scattering in the detector which results in increased background level and thereby decrease in analytical sensitivity. Improvement of analytical sensitivity for quantification of weak lines lying in lower part of a complex spectrum can be achieved through reduction of Compton continuum and the natural background. The use of Compton suppression spectrometers in activation analysis started when Cooper and Brownell [52] constructed Compton suppression system with a plastic scintillator shield (46 cm dia x 46 cm ht) surrounding a 35 cm^3 Ge(Li) detector. It was observed that Compton continuum was suppressed by a factor of 2 for ^{137}Cs.

(vii) Chemical treatment before Irradiation: In some cases chemical separations are required before irradiation as a special measure because the

concentration of analyte in the sample is too small or if speciation studies are to be carried out. This is often referred to as chemical neutron activation analysis (CNAA). These may be of following types;

A. Preconcentration NAA: In case of water sample especially preconcentration is essential because of low level of analyte elements present (at μg or ng/mL). For this purpose, water is passed through an ion exchange or some other adsorbent where the analyte elements are retained. In other cases, sample can be dry ashed whereby the sample size is reduced and analyte elements are concentrated. Yeh et al. [53] have reported the determination of rare earth and some other representative elements in polluted water by preconcentration over hydrated magnesium oxide.

B. Chemical Neutron Activation Analysis (CNAA): It was developed for determining trace amounts of analyte elements such as As and Hg in a variety of matrices including water, sedimentary rock, plant, animal organs etc. In this case, a chemical procedure is followed whereby trace elements are separated before irradiation. Nair et al. [54] determined Pd in U ores by CNAA using low energy photon spectrometry.

C. Speciation Neutron Activation Analysis (SNAA): The scope of NAA can be further extended for speciation studies by chemical separation of specie(s) prior to irradiation and then determining the elemental content. This is essential because the toxicity of an element depends significantly on its physiochemical form. There is an increasing interest in studying speciation, which involves two steps; first the separation of species from the sample followed by the detection of element by NAA. A variety of methods involving liquid chromatography, reversed phase chromatography, ion exchange, hydride generation, liquid-liquid extraction, solid phase extraction, co precipitation have been developed for the separation of various inorganic, organic and organometallic species. The main advantage of SNAA is that it can be applied for the simultaneous speciation of elements such as Cl, Br and I, which are rather difficult to be determined by other techniques. Shi et al. [55] developed an SNAA method in combination with HPLC for the determination of low levels of 5 arsenic species, As(III), As(V), monomethylarsonic acid (MMA), dimethylarsenic acid (DMA) and arsenobetaine (AsB) in natural water samples.

I.4 QUALITY ASSURANCE

Quality assurance programme is an essential part of a sound analytical protocol and should always be used to detect and correct problems in the measurement process or attain a state of statistical control. The objective of quality assurance programme for analytical measurements is to reduce measurement errors to agree upon limits and to assure that the results have a high probability of acceptable quality. Two concepts are involved in quality assurance: quality control, the mechanism established to control errors and quality assessment.

Based on the experience of scientific workers, NAA has been adopted as one of the reference techniques for the certification of Standard/ Certified Reference Materials (S/CRMs). Since NAA is a comparator method and also because samples analysed are of unknown composition, it is always advisable to simultaneously analyse SRMs for quality assurance and method validation [56, 57]. Therefore, it is essential to develop well-characterized RMs of natural matrices of geological/biological/environmental/ industrial origin.

Kucera and Soukal [58] used INAA for homogeneity tests and certification of coal fly ash standard from Czech Republic. In the IAEA Coordinated Research Programme. Byrene et al. [59] used INAA to improve certain disputed values in the certification of milk power A-11 and animal muscle H-4. Becker [60] has presented results of ASTM nuclear methods intercomparison of NIST SRMs Apple and Peach leaves. Heydorn has stressed the comprehensive nature of NAA for quality assurance [61]. In recent years, Suzuki et al. [62] determined 38 elements in a typical Japanese diet certified reference material by INAA. Wasim et al. [63] also used NAA for the analysis of 15 elements in an IAEA proposed certified reference material IAEA-407 (North Sea Fish Homogenate). Abugassa et al. [64] reported results of intercomparison test organized by the IAEA. Several methods have been employed for certifying the biological sample (IAEA-0140) and compared their relative performance.

During last seven decades NAA has attained sufficient maturity and is being adopted as a routine analytical method in many advanced countries where nuclear reactor is available. Despite limitations of requiring a nuclear reactor and associated

radiation hazards, NAA has travelled a long distance. It has a bright future in medicine, biology, environmental sciences and even industrial quality control.

I.5 APPLICATIONS

Importance of trace element analysis (TEA) in today's context of technological globalization is vital. A physicist working in the field of semiconductors or high purity materials, a geologist in mineral exploration, an analytical chemist dealing with the problems related to trace elements in biological systems or heavy metal pollutants in environment, a physician treating the patient suffering from a disease like Ca deficiency in bones, a curator in a museum interested in studying historical monuments and a criminologist dealing a the murder case, all require a suitable analytical technique to solve their problems at their respective work place. Though there are several alternative techniques such as XRF, ICP-MS, EDXRF, but the only common answer to all these problems is NAA provided a nuclear reactor is available. It is possible to determine up to 60 elements with high sensitivity, specificity, accuracy and precision. A scan of literature reveals publication of > 500 papers on NAA every year during last 5 years where different aspects of NAA and its extensive applications have been explored. For macro, micro, trace and ultra trace element analysis in the sample corresponding to diverse fields such as archaeology, biomedicine, animal and human tissues, environmental science, forensics, geology and cosmo chemistry, industrial products, nutrition, quality assurance of analysis and certification of RMs. Some important applications are primarily related with biology, nutrition and medicine as illustrated in Fig. I.4. NAA had been extensively used to determine trace and ultra trace constituents in rocks, minerals, ores, coal, sediments, tissues, hair, blood or even whole body. After the arrival of lunar samples in 1969, NAA played a

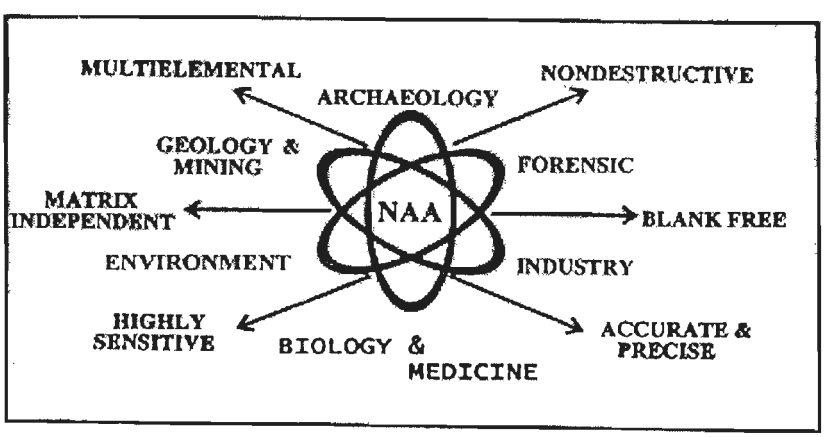


Fig. I.4 Illustration of Capabilities and Applications of NAA

major role in trace element analysis and thereby providing information on the evolutionary aspects of solar system [65]. Landsberger [27] has reviewed recent developments (1997-2002) and applications in the fields of archaeology, geochemistry, engineering materials, environment, plants and crops, medical and clinical studies. Pi diagram in Fig. I.5 illustrates the use of radiochemical methods on the basis of 13,858 papers published during 2001-04. It clearly shows tough competition with AAS and ICP-MS methods, which are becoming more popular because of radiation hazards associated with radiochemical methods.

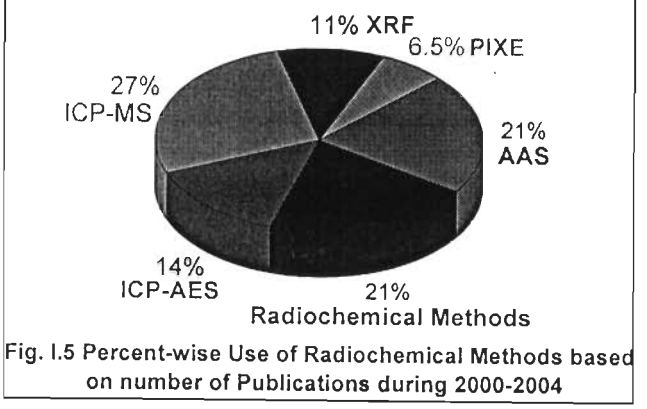


Fig. I.5 Percent-wise Use of Radiochemical Methods based on number of Publications during 2000-2004

(i) Forensic science: Non-destructive nature of NAA has proved to be a boon in forensic studies. Some marker elements such as Ba, Sb, Cu, Sb, Ag, Fe, Br, Zn and K are analysed depending on the sample type. Pioneering work was carried out by Guinn and coworkers [66] who solved a typical case of As poisoning. Krishnan and Jervis [67] used NAA to find the muzzle distance of bullet fire and to trace the culprit using Ba and Sb in gun shot residue swapped from hands. Looking to the potentials of NAA, Central Forensic Science Laboratory (CFSL), Kolkata has set up NAA unit at the Analytical Chemistry Division of BARC, wherefrom Chattopadhyaya et al. [68] have surveyed Indian scenario. In USA, Federal Bureau of Investigation (FBI) has set up NAA laboratory in New York where extensive analysis of forensic objects is carried out since more than two decades.

(ii) Environmental Sciences: NAA has made significant contributions in the determination of heavy metals and other inorganic pollutants in a variety of matrices. NAA has emerged as a basic analytical tool by environmentalists against its earlier recognition as a nuclear technique. A number of books [69], monographs [70] and reviews [71] have discussed the potentiality of NAA for environmental studies. INAA has

been used by analysing hair and fish to monitor occupational exposure and aquatic environmental pollution [72]. A large population exposed to subsoil water contaminated with As showed clinical features of arsenic toxicity called arsenicosis [73]. On the other hand airborne particulate matter (PM) from industrial and vehicular emissions has been associated with a vide variety of environmental problems including detrimental health effects [74]. Athari et al. [75] employed INAA for determining 21 elements in the total suspended particulates (TSP) in the ambient air of Tehran city. There could be different aspects of such studies where biosphere and urban/industrial monitoring could be carried out by analysing hair, air/water/soil or fish.

(iii) Biology, Nutrition and Medicine: It is now well recognized that in addition to the principal body building elements (Ca, Mg, P, Na, K etc.) a number of trace elements, including V, Cr, Mn, Fe, Co, Cu, Zn, Se, Mo, Rb and I are essential for growth and health in animals and humans [76]. Just as important however, are some toxic elements for the same group of species e.g. As, Br, Ag, Cd, Sb, Ba, Pb though to widely differing extent. Activation analysis has proved to be a very effective technique for the determination of most of these trace elements [23]. The collaboration between activation analysts and medical researchers has resulted in a much deeper understanding of the function of trace elements in the body [76,77]. Cesareo [78] has written an excellent monograph describing different methods and their advantages in trace element analysis in medicinal studies. Leung et al. [79] observed variation in trace element levels in hair, blood and tissue samples of nasal-pharynx cancer patients. Ehmann et al. [80] extensively studied brain tissue of age related neurological diseases. Sarmani [81] used NAA to study trace elements in kidney stones. Garg and coworkers [82] used NAA in the determination of trace element levels in human blood and excision biopsy samples of breast cancer to correlate with clinical stages and further correlated it with dietary intake. As a part of clinical trace element research almost all parts of the human body viz. brain, hair, eyes, teeth, muscle, lung, liver, blood, kidney, urine, nails, bones and toenails etc. have been analyzed. [83].

Another biologically important area where NAA is being extensively used for multielemental analysis is nutritional studies where Iyenger [84] has not only written a monograph on compositional aspects of trace elements but he has advocated the need

for mixed total diet reference material [85]. In a recent study, Zaidi et al. [86] have reported trace element evaluation of some medicinal herbs whereby variation in trace element contents of same plant species from different origins were attributed to ecological and geographical variations.

I.6 ROLE OF ELEMENTS IN LIFE PROCESSES

Bulk of living matter consists of H, C, N, O and S, and macro-minerals Na, Mg, P, Cl, K and Ca serve as structural components of tissues. However, many elements such as Fe, Co, Mn, Cu, Zn, Mo etc. at trace level play a vital role in biochemical and enzymatic processes. These elements occur at such low concentration that they cannot be quantified by ordinary analytical methods [87]. All the trace elements can be classified in two categories.

I. Those whose essentiality has been established by accepted scientific standards, and

II. Those elements whose proof of essentiality does not exist.

Trace elements play a major role in health, since they are essential in the assimilation and utilization of vitamins and other nutrients. They aid in digestion and provide the catalyst for many hormones, enzymes and essential body functions and biochemical reactions. They also aid in replacing electrolytes lost through heavy perspiration or extended diarrhoea and protect against toxic reactions and heavy metal poisoning. Table I.4 shows the classification of all the elements found in human body [76,77,87,88].

During last few decades researchers have further added Se, Cr, Ni, V, Si, I and As in the list of essential elements suggesting their essentiality at optimum concentration. An essential element is defined as one required for maintenance of life; its absence results in deleterious effects causing death of the organism [77, 88]. Severe deficiency or an excess of an element may result in death. A chronological order of the establishment of essentiality of trace elements and their normal metabolic functions including deficiency symptoms are listed in Table I.5. These elements, generally, have an optimum range of concentration below which deficiency symptoms are observed and their excess amount may also be harmful [89].

Table I.4 Classification of the elements found in biological systems

Structural Elements	C, H, O, P, N, S, Ca
Electrolyte Elements	Ca, Cl, K, Mg, Na, HCO_3^-, SO_4^{2-}, HPO_4^{2-}
Trace Elements	
Essential	
a. Biologically	Co, Cr, Cu, Fe, I, Mn, Mo, Se, Zn, As, F, Ni, Si, Sn, V
b. Clinically	Cr, Cu, Fe, I, Se, Zn
Toxic	
a. Potentially Toxic	As, Br, Cd, Hg, Pb, Se, Tl
b. Environmental contaminants	Cd, Hg, Pb
c. Industrial Hazards	As, Be, Cr, Hg, Ni, Mn, Pb, Sb, Si, Ba, Sr

In order to maintain essential metabolic functions of enzymes and hormones nutritional supply of trace elements must be sufficiently high. As illustrated in Fig. I.6, the supply status of a trace element may be divided into five concentration ranges [89].

1. Severe deficiency, characterized by clinical symptoms
2. Marginal deficiency, where

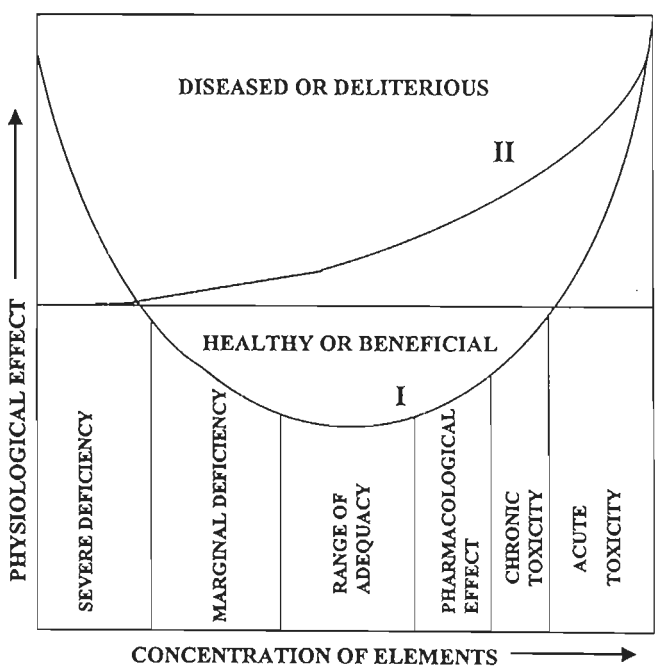


Fig. I.6 Correlation of Elemental Concentration with its Physiological Effects

Table I.5. List of essential elements, their metabolic functions and deficiency symptoms [76]

Element	Function	Deficiency Symptoms
Fe	Oxygen and electron transport, constitutes hemoglobin	Anaemia, Stomatitis, Dysphagia, brittle nails
Cu	Constituent of oxidative enzymes, interaction with iron, cross linking of elastin	Anaemia, Change of ossification, elevated serum cholesterol, diarrhoea, nervous system damage
Mn	Mucopolysaccharide metabolism, constituent of superoxide dismutase	Disturbance in bones and cartilage formation, deafness, dizziness
Zn	Constituent of enzymes involved in energy metabolism and transcription	Growth depression, poor healing of wounds, sexual immaturity, skin lesions, menstrual problems, change of taste, acuity
Co	Constituent of vitamin B_{12}	Loss of appetite, vitamin B_{12} deficiency
Mo	Constituent of xanthine, aldehyde and sulfide oxidases	Human dental caries, Male impotence
Se	Constituent of glutathione peroxidase, interaction with heavy metals	Endemic cardiomyopathy (Keshan disease) premature aging, eye and nerve disorders, infertility
Cr	Potentiation of insulin	Relative insulin resistance, impaired glucose tolerance, elevated serum lipids, fatigue and lack of energy, indigestion
Sn	Possibly an essential element for animals, but no specific role for tin in human health has been identified	Possible growth depression
As	DNA repair mechanism	Growth depression
V	Gene regulations and in various enzymatic systems	Several physiological malfunctioning including thyroid, glucose and lipid metabolism.
Ni	Interaction with iron absorption, activates enzymes	Anaemia, inconsistent growth
Ca	Essential for formation of healthy bones and teeth, regulates blood clotting, muscle function, nerve transmission	Muscle cramps, brittle bone disease, dental problems
Mg	Help in absorption of other minerals, stimulates bone growth and promotes the body use of vitamin B, C, and E	Lack of energy, muscle spasms, weakness asthma, cardiovascular disorders
K	Essential for growth, stimulates nerve impulses, promotes healthy skin, boosts kidney function, combines with Na to regulate heart beats	Irregular heart beats, dry skin, nervous disorders
P	Cell repairs, vital to growth of bones and teeth, helps digest proteins, fats and carbohydrates	Poor growth, arthritis, loss of appetite

no conspicuous clinical symptoms are measurable and supplementation may work

3. Optimal functioning of metabolism, promoting normal health and performance
4. Sub toxic where intake is in excess of the optimal supply and is characterized by biochemical changes with pharmacological aspects manifesting clinical symptoms and
5. Toxic, characterized by clinical symptoms and finally acute toxicity may cause death.

In order to study the effect of metal concentration in different parts of the body for proper functioning, several workers have analysed essential and toxic elements in various body parts. Wasowicz et al. [90] determined blood concentration of essential trace (Se, Zn, Cu) and toxic (Pb, Cd) elements as markers of antioxidant and prooxidant processes in workers exposed to Pb and Cd. In view of the limited data available from the Asian region on the daily intake of nutritionally essential trace elements, Iyengar et al. [91] have estimated intake of some minor and trace elements in Asian diet as a part of IAEA Coordinated Research Project (CRP). In a recent review, Baran [92] presented the general aspects related to the essentiality of inorganic elements in living organisms followed by a detailed discussion of the supplementation of Fe, Cu, Zn, Cr, Se, Mg and some other minor trace elements. According to Windisch [93], the trace elements are usually converted into metabolically recognizable forms inside the body, channeled to their biological functions, or subject to homeostatically controlled excretion. Nordberg et al. [94] presented nutritional and toxicological information in a monograph. Hostetler et al. [95] reviewed essentiality of trace minerals in many enzymatic and metabolic pathways critical for conceptus development during pregnancy in livestock species. Nandakumaran et al. [96] reported the transport characteristics of essential trace elements such as Zn, Cu, Se, and Fe in studying the maternal foetal direction in normal pregnancies, using in vitro perfusion of human placental lobules. Goldhaber [97] reviewed essentiality of trace element risk assessment vs toxicity. Walsh et al. [98] reported nutritional deficiencies or excess of trace elements including Fe, Zn, Cu and Mn affecting bone health. Chandra [99] reported the impact of nutritional status and nutrient supplements on immune responses and incidence of infection in older

individuals. Keen et al. [100] discussed the immune defects associated with deficiencies of specific essential trace elements and minerals.

I.7 INDIAN MEDICINE SYSTEM; *AYURVEDA*

Ancient Indian traditional medicine system, *Ayurveda*, an indigenous system of medicine, dates back to the Vedic age (1500-800 B.C.) and has been an integral part of Indian culture. The name is derived from the ancient Indian language Sanskrit; *Ayus* (life) and *Veda* (knowledge) meaning science of life. In principle, Ayurveda advocates a holistic approach to the human health care having its goal a long, healthy and happy life [101-103]. According to Rao [104], *Atharvaveda* deals with deep understanding by ancient Indians about the role of natural environment viz., vegetation, oxygen, hydrogen, nitrogen, carbon and products formed by their synthesis in sunlight, in the maintenance of human health and increasing the longevity.

The earliest systematic approach to the development of Ayurvedic medicine and surgery begins with the valuable contributions made by ***Charak*** [105] and ***Sushruta*** [106] who recorded a detailed account of the properties of herbs and their effect on the diseases and pioneering work done on surgery, respectively. These *Samhitas* written by the pioneers indicate the use of some primary metals like gold, silver, copper and iron and their alloys including bronze and brass. Joshi [107] and Dixit [108] have quoted the selective use of these metals even for serving food, vegetables, etc. and recommended the storage of cooked food in metal vessels to avoid their surface interaction. *Ayurveda* emphasizes on the strengthening of human immune system followed by treatment unlike allopathy, the western system of medicine where the drug is administered only for curing. Another important aspect of *Ayurveda* is the dietary restriction, which considerably affects efficacy of medicine.

The majority of medicines and tonics mentioned in *Ayurveda* are plant based where good health is a state in which man has balanced *doshas* (physical units of the body) namely *vata* (dealing with fluidity and motion), *pitta* (deals with energetics) and *kapha* (expresses static and passive constitution). Any imbalance in these three states causes illness. Many civilizations of the world viz. Africa, Asia, China, Russia and Latin America have been practicing the use of plants for treatment of diseases. [109-111].

Looking to the importance of herbs, *A Compendium of Indian Medicinal Plants* in 6 volumes has been published [112]. The popularity of medicinal plants in other cultures is ever increasing as complementary medicines become more widespread. Natural food and medicine are in greater demand in today's life. Human body is accustomed to natural products and there are many constituents in the plants that afford opportunity to the digestive system as well as regulate the action by absorption, thus helping to reduce the side effects as well as incidences of ailments [113].

Generally, Ayurvedic medicines are a rational combination of various plant drugs and are manufactured under different pharmaceutical processes in order to get their typical form i.e. powder, tablet, decoction, expressed juice etc. but also to modify and intensify its inherent properties [114]. Ayurvedic formulations contain more than one crude drug and have, in general, a good record of safety and efficacy. Some of these formulations contribute immensely to the treatment of human ailments and to promote health world over. India is a large tropical biodiversity country with rich heritage. Besides China and Africa, India is probably the only country where traditional medicine system is most developed in the entire subcontinent of India, Pakistan, Bangladesh, Nepal and Sri Lanka. Large populations, especially from rural area, wholly depend on herbal medicines. There is increasing awareness and interest in the revival, scientific development and consolidation of Indian medicines so that it may occupy the status of national system, which can be adopted by other developing countries as well.

Bhasmas are unique Ayurvedic preparations composed of metals and herbs. In a few instances they also contain marine and animal products. *Bhasmas* are converted to a powder form by calcination/incineration achieved by blending the metal with specified plant juices and heating in a specially prepared earthen crucible [111]. These *bhasmas* are biologically produced nano particles taken with honey/butter/ghee and once ingested seek moisture from the body and thus assimilated. *Bhasmas* carry the effect of the herbs further into the tissues and significantly magnify the properties for potentiating deeper and powerful action [115]. The Indian medicine system of *Ayurveda* has been used as a means for the prevention of the effects of aging and generation of diseases in organs [116]. Many western scholars such as Hankey [117] consider *Ayurveda* as

complementary and alternative medicine (CAM) along with naturopathy and homoeopathy.

In order to emphasize the importance of rich biodiversity with a large number of plants with medicinal properties forming a backbone of *Ayurveda* in India, the Department of Posts have brought out a series of four postal stamps of Rs. 5 each featuring four medicinal plants Ashwagandha (*Withania somnifera*), Amalaki (*Emblica officinalis*), Brahmi (*Bacopa monnieri*) and Guggulu (*Commiphora weightii*), out of which we have analysed first three as described in Ch. III, IV and VI respectively. Such plants have greatly helped India in developing its own healing system several hundred years ago.

I.8 AYURVEDIC FORMULATIONS: PRESENT STATUS

Commercial production of Ayurvedic traditional medicines in India must be just about 150 years old. Prior to this, an Ayurvedic physician often called as *Vaidya* used to collect raw material and process the required formulation according to family tradition in small quantities and distribute it to his patients. During freedom movement, enthusiasm on Ayurvedic medicines was revived with a view to inculcate patriotism. During that period, to overcome shortcomings in the profession, commercial production of Ayurvedic medicines was initiated by some manufacturing firms such as Baidyanath, Dabur, Hamdard, and Zandu etc. in different parts of the country [118, 119]. This initiative had its own positive and negative influence on the *Ayurveda* in terms of modern context especially with regard to following points of special significance.

(i) Globalization of product delivery: Mass scale production of these products has entailed in an increase in the availability of Ayurvedic medicines. Thus, flora and

fauna limited to certain geoclimatic zones could be delivered through medical stores to all corners of the country or world.

(ii) Professionalization of pharmaceutics: As a result of such mass production, Bhaishajya Kalpana (pharmaceutics) could be segregated from the scope of *Vaidyas*. They need not waste their time and energy on the production of medicines. Eventually, the Vaidya could be just consultant physicians and lay emphasis on the diagnosis and treatment.

(iii) Creative marketing strategies: Because of dynamic marketing approach, it has been possible to make certain Ayurvedic medicines a household name. Such brand popularization has resulted in greater enthusiasm on the system of *Ayurveda* as a whole. At the same time efficacy of same products marketed by different manufacturers could be tested, which may result in competitive character and availability of better product in the market.

(iv) Scope for research and developments: Due to the competitive spirit, the industry opted for research and development to enhance their venues. These developmental ventures may help to realize the potential area of Ayurvedic medicines. However, there are some demerits of Ayurvedic medicines especially with regard to following points

A. Lack of standardization; Many processes described in the ancient literature are not designed for industrial scale. Initial phase of this transition, has been a critically tough job due to lack of standardization. All the manufacturers may not follow same standardized procedure in terms of preservatives, additives or putting expiry date.

B. Pressure on material resources; Due to spurt in the demand, there has been a sudden load on the resources of raw materials. Thus, trading of raw material has become a point of suspicion and its collection from prohibited zones is important from environmental contamination point of view. There is a need to develop 'herbal gardens' using organic manures and be away from city in a pollution free zone.

C. Lack of Pharmacopoeia; Due to the advantages of non-existent pharmacopoeia and quality norms, short-term players could have their day. This entailed in weakening the growing enthusiasm on the system itself. Thus, a regulatory commission administered by the state/central government is needed.

In fact, lots of research efforts in terms of money and manpower have to be put in and a multidisciplinary approach for quality control using modern instrumentation should be adopted. In this regard, efforts made by China in the popularization of their traditional Chinese medicines (TCM) have to be appreciated. Similarly in Latin America and African countries also lots of research efforts are being directed to make their traditional medicines system much stronger with R & D base.

I.9 LITERATURE SURVEY

Steiner [109] has edited an excellent monograph published by the American Chemical Society where researchers from India, Fiji, Africa and China have contributed about the medicinal plants and their therapeutic uses including cancer. Most studies on medicinal plants reported in the literature pertain to the organic constituents viz. essential oils, glycosides, vitamins, alkaloids, polyphenols and other active components and their pharmacological effects. It is supposed that since many of these compounds are naturally synthesized, these are more effective as drugs. However, little is reported about their minor and trace element composition besides major constituents of C, H, O, N and/ or S. though in some cases minor constituents such as Ca, Mg, Fe, P are known in older literature [103, 113]. Several Chinese and Japanese workers [120,121] have used high performance liquid chromatography (HPLC) for simultaneous analysis of organic constituents in medicinal plants from their respective countries.

Herbal medicines are widely used by a majority of rural population from Asia including entire Indian subcontinent and China, Africa, Egypt, Latin America etc. where these are based on a trust between the physician and the patient. In recent years these medicines have found their market in Western world where the Asian population has been growing and hence their use has been steadily increasing because these are more economic. Whereas their efficacy is unquestionable locally, many westerners feel scary about their harmful effects, primarily due to heavy metal toxic contaminants such as Cd, Hg, Pb, etc. [122,123]. Besides heavy metals, herbal products may be contaminated with excessive or banned pesticides, microbial contaminants, chemical toxins or adulterated drugs [124]. Therefore, regulatory standards have to be imposed on these products manufactured using good particles.

In view of this, an awareness has to be created for quality control of these medicines. For this purpose, a variety of analytical techniques such as neutron activation analysis [125], X-ray fluorescence (XRF) [7], proton induced x-ray emission (PIXE) [9], atomic absorption spectrophotometry (AAS) [125], inductively coupled plasma (ICP-AES & ICP-MS) [125], energy dispersive X-ray fluorescence (EDXF) [8] spectrophotometry [124] including electroanalytical methods have been extensively used.

Perhaps Hess et al. [126] in 1968 first explored the potentiality of NAA for qualitative determination of Na, K and Mn in several plant seed species. Since then a large number of researchers [127-150] from various countries have analyzed medicinal plants for elemental composition. A careful search of literature has revealed extensive use of NAA for multielemental analysis of medicinal herbs e.g. Kist et al. [142] from USSR (now Uzbekistan) determined 32 elements in five species of medicinal plants; Kanias et al. [134] from Greece determined trace amounts of Sb, Cs, Cr, Fe, Eu, Rb, Sc, Sr, Th and Zn in the *Eucalyptus camaldulensis* leaves; Vega-corrillo et al. [138] from Mexico reported elemental distribution in medicinal plants used in folklore medicines from Mexico; Sarmani et al. [137] from Malaysia determined 29 elements in herbal preparations; Serfor-Armah et al. [133] analysed five Ghanian medicinal plants for 17 elements. *Ginseng* is an extensively used Chinese herb for vigour and strength and widely available in US stores. Razic et al. [145] from Yugoslavia analysed trace elements in *Echinacea purpurea*. In an unique study Wesolowski and Konieczynski [140] from Poland used thermal decomposition and determined metal and non-metal contents. Chizzola et al. [127] analyzed medicinal, aromatic and spice plants from Austria for Cd, Cu, Fe, Mn, Pb and Zn contents. Ajasa et al. [139] determined the concentration levels of Fe, Mn, Cu, Pb and Zn and macronutrients (Na, K, Mg, and Ca) along with P in some Nigerian herbal plants. Saiki et al. [129] from Brazil employed INAA for the determination of 15 elements in plant extracts used as medicines. Spectrophotometry has been used for the determination of Cd, Co, Ni, Pb and Hg in 42 Chinese herbal medicinal plants [130]. Khan et al. [146] analysed 21 products in *Panax ginseng* as a part of quality assessment program.

In a recent study Zaidi et al. [86] have reported trace elements evaluation of some medicinal herbs from Pakistan. In India primarily three groups of workers from the universities at Nagpur, Pune and Tirupati have employed INAA for multielemental analysis of a variety of herbal medicines. Garg and co-workers [147] employed INAA for analysing peacock's feathers, orange peel offs and medicinal herbs for up to 20 essential and trace elements. Rajurkar and Damame [148] determined 14 elements in some medicinal plants used in the treatment of cardiovascular disease and urinary tract disorders. Balaji et al. [149] determined essential elements in Ayurvedic medicinal leaves by INAA. Rai et al. [150] studied the accumulation of heavy metals in Indian herbal drugs.

I.10 AIM AND SCOPE OF PRESENT WORK

Besides micronutrients such as Na, K, Mg, Ca, P, Cl and Fe, many trace elements play a vital role in life processes. Their excess or deficiency causes illness or toxic effects. In order to keep healthy, it is essential to have these elements in bioavailable and assimilable form at optimum concentration in various body organs. In many civilizations of Africa, Asia, China, Latin America including Indian sub continent medicinal herbs have long been in use for the treatment of many diseases. *Ayurveda*, the ancient Indian system of medicine prescribes the use of herbs and herbal preparations including *bhasmas* where essential elements are supplied in bioavailable and assimilable form. Present work was undertaken as a part of the Board of Research in Nuclear Sciences (BRNS), Department of Atomic Energy (DAE), Government of India research project entitled ***Development of NAA and other Radioanalytical Methods for Trace Elements in Medicinal Herbs and other Herbal Preparations.*** Our first aim was to establish a high-resolution gamma ray spectrometry facility to make it useful for neutron activation analysis (NAA) of essential, trace and toxic elements in medicinal herbs and herbal preparations. It was proposed to perform multielemental analysis for 25-30 elements using short and long irradiations in a nuclear reactor followed by γ activity measurements using HPGe detector, MCA system and associated software. Though NAA methodology is now well developed during last seven decades, we have optimized various experimental parameters for the analysis of complex biological

matrices. Also, we participated in the analysis of candidate reference materials (RMs) from the NIST (USA), IAEA (Vienna) and INCT (Poland). Besides NAA, atomic absorption spectrometry (AAS) was also used for the determination of Ni, Cu, Cd and Pb because of their environmental importance. Following samples were analysed;

(i) Nine different *Trifala* brands (Baidyanath, Dabur, Hamdard, Himalaya, Surya, Zandu and two local) powder including a tablet (Zandu), herbal formulation of *amalaki* (*Emblica officinalis*), *bibhitaki* (*Terminalia belerica*) and *haritaki* (*Terminalia chebula*) have been analysed for 29 elements by NAA and AAS. Also gallic acid has been separated/identified by thin layer chromatography, elemental analysis, ir and NMR spectral methods including GC-MS.

(ii) A nervine tonic and vitalizer, *Pragya-peya*, from Shanti Kunj, Haridwar and its 12 constituent herbs have been analyzed for up to 26 elements. Three different batches of the sample, collected over a period of one year, were analysed for sample homogeneity. Similarly three samples of *brahmi* (*Bacopa monnieri*) and four samples of tulsi (*Ocimum sanctum*) were analysed.

(iii) Bioassay of *brahmi* extracts in methanol, (1:1) aqueous methanol and aqueous medium were carried out for DPPH radical scavenging activity, DNA strand break, anti-lipid peroxidation, H_2O_2 scavenging, super oxide scavenging activity and total phenol content.

(iv) Fifteen medicinal herbs [*C. rhombifolia* (*amaltas*), *W. somnifera* (*ashwagandha*), *P. corylifolia* (*bakuchi*), *T. cordifolia* (*guduchi*), *M. fragrans* (*jaiphal*), *N. jatamansi* (*jatamansi*), A. *paniculata* (*kalmegh*), *H. anticlysentrica* (kutaj), *T. chebula* (*laghu Haritaki*), *S. racemosa* (*lodhra*), *A. indica* (*neem*), *V. negundo* (*nirgundi*), *H. indicus* (*sariva*), *A. calamus* (*vach*) and *E. ribes* (*vidang*)] of common usage were analysed for up to 28 elements.

(v) Twenty Ayurvedic metallic-herbal preparations *bhasmas* based on calcium (4), iron (4), zinc (3), mercury (2), potassium (1), silver (1), copper (1), tin (1), arsenic (1) and two gem-stone were analyzed for the main constituent and 17 more elements by NAA besides C, H, N and S contents for organic constituents.

(vi) Attempts have been made to inter-relate elements such as Na *vs.* K, K *vs.* P, Ca *vs.* P, Fe *vs.* Mn, and Cr *vs.* Zn in several groups of medicinal herbs. Also variations in elemental ratios (K/Na, and K/P) and elemental contents in different constituents were studied.

(vii) We participated in the Inter Comparison study of Tea leaves (TL-1) and Mixed Polish Herbs (MPH-2) from INCT, Poland; Pine Needles (SRM-1575a) a depleted SRM from NIST, USA and Marine Sediment (IAEA-433) from IAEA, Vienna. It was observed that our data for several elements were well accepted for certification in the Recommended mean and ranges.

It has been observed that many herbs are enriched in one or more essential trace elements, which remain bound with organic molecules so as to make them biocompatible. Some herbal preparations, which represent combination of two or more herbs, make them especially useful for enhancing vitality and resistance to the immune system. An attempt has been made to correlate the elemental contents with their therapeutic effects.

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CHAPTER II

METHODOLOGY AND INSTRUMENTATION

Nutrient element analysis of complex biological samples in general and medicinal herbs in particular has been a challenging task before analytical chemists. A general procedure involves several distinct stages, viz. sampling, sample preparation and analytical procedure. Since we have adapted two methodologies NAA and AAS, we shall limit our discussion to these two only. However, bioassay methodologies are described separately in Ch VI. In case of NAA, it involves packing, irradiation, activity measurements, and calculations of results and data interpretation. On the other hand AAS involves sample dissolution, instrumental measurements followed by data analysis. As already described in Ch.I, sample contamination, spectral interferences and inappropriate data analysis may lead to serious errors in NAA procedure [1,2]. The different stages in the NAA and AAS procedures with emphasis of instrumentation, data analysis and sources of error including application to samples of plant origin analysed.

II.1 SAMPLING

It is one of the most critical steps in biological sample analysis and more so in case of medicinal herbs where specific parts of a plant such as leaves, stem, bark, fruits, seeds, roots which are used as medicine. In view of this, sampling means the process of collecting individual parts of a plant, which is truly representative of that part. For example in case of *neem* (*Azadirachta indica*) its leaves, flowers, bark and seeds including thin stem have different medicinal value both externally and internally. The chronic, non-healing wounds and ulcers are dressed with the aqueous paste of its bark. The oil of its seeds or leaves is effective in healing the diabetic wounds. Dental infections are well controlled with the gargle of decoction of its leaves. Even thin stem part is used as chewing stick for cleaning teeth in place of brushing with toothpaste. External application of the paste of its leaves, alleviates itching and burning sensation of the skin. Dried leaves are added when storing grains to keep them free from pests and insects. Internally, *neem* is a great medicament for various diseases. It is recommended in the treatment of rheumatism, skin diseases like scabies, eczema and ringworm, diabetes, obesity, piles and jaundice. Even tender and mature leaves of *neem* have different medicinal value. Its bitterness (*tikta rasa*) has an important role in blood purification [3,4].

If the sampling is not done properly, then all the labour and time spent in a careful analysis may be completely wasted. Therefore, it is essential to follow standard sampling protocol depending on the nature of analysis with utmost care [5]. Curren and King [6] have reviewed modern methods and recently developed techniques for sampling. Rossbach et al. [7] have described representative sampling from environmental monitoring point of view and its possible impact on human health. As the consumption of herbal medicines has been increasing world wide, the number of patients experiencing negative health consequences has also been increasing. One of the major causes of reported adverse effects is directly linked to the poor quality of herbal medicines, including raw material, which may have been sprayed with pesticides or chemically treated during storage. Quality control directly affects the safety and efficacy of herbal medicinal products. Medicinal plant material derived from the same species can show significant differences in quality when cultivated in different environment owing to the influence of soil, climate and other factors such as irrigation, plant maintenance and protection. In this regard, environmental pollution caused by nearby industrial emissions and effluents is also very important. Under the overall context of quality assurance and control of herbal medicines, World Health Organization (WHO) developed the guidelines on good agricultural and collection practices (GACP) of medicinal plants for the sustainable production of herbal products. The guidelines are also related to the protection of medicinal plants aiming promotion of sustainable use and cultivation of medicinal plant [8,9].

Medicinal plants should preferably be grown in a pollution free atmosphere, away from highways and in separate gardens away from city. Similarly, artificial fertilizers and use of insecticides, fungicides should be avoided. In the present study, most samples were from Haridwar and Rishikesh region near *Ganga River* where not many heavy industries are located.

Versieck [10] emphasized the transfer of extraneous elements from inventory such as rubber stoppers, steel/ aluminium containers, glass, quartz, teflon, storage equipments etc. Woittiez and Sloot [11] presented a review on the theoretical and practical aspects of trace element sampling and sample preparation, mainly for the biomedical and environmental studies. This include causes (or precautions) of change

in trace elemental composition due to sample preparation (contamination, losses, in homogeneity), sample pretreatment (washing, drying etc) and sample decomposition (wet and dry ashing). Park and Pohland [12] described the factors affecting the ability the sampling plan to accomplish this goal, which include nature of analyte of interest, distribution of the analyte throughout the lot, physical characteristics of the product and sample size. Several IAEA technical reports deal with the precautions in sampling for NAA [2,13].

II.2 SAMPLE PREPARATION

Samples in NAA or AAS cannot be analysed on site. Hence their storage is also essential though it may be for a short period. In general, sample must not be contaminated by any chemical treatment or destabilized during sample handling and storage. For this purpose, clean room conditions, clean vials and other equipment must be used. All the samples analysed in this study were solid powders free from any contamination. Following factors should be considered:

(i) Removal of Surface Contamination: It is a primary requirement for the biological samples from where all the unwanted dust material should be removed before processing. It is important to follow strict measures to avoid contamination sources during sample collection, which include good agricultural practice (GAP). Some of these measures include the use of talc free gloves, laminar hood or clean hood and dust free laboratory including good laboratory practices (GLP). In case of leaves, fruits, stem, roots etc, surface contamination was removed by washing with double distilled deionised water, 0.01 M HCl or high purity acetone. It is generally recommended to avoid any violent treatment like scrubbing the sample in a medium to avoid intermixing of elements from the sample and medium. Any dirt/ moisture content was swiped using tissue paper. These were first air dried in sunlight or under IR lamp well below 80 ^{0}C to avoid any charring or loss of volatile elements [2,14].

(ii) Sample Homogenization: The solid samples were crushed, ground/pulverized in an agate mortar. The dry ashing of sample at appropriate temperature is also practiced for human, animal and plant tissues. In this process,

however, significant amounts of volatile elements such as I, Br, Sb, Cd, Hg, Sn etc. may be lost if the temperature is high. Recently Lafargue et al. [15] reported that sample used in proficiency testing scheme (PTS) need to be homogeneous to confirm the results of our laboratory with that of others. Its error can be attributed to the analytical method and not the sample. Esbensen et al. [16] described typical problems encountered with homogenization of biological and environmental samples. Koh et al. [17] reported loss in elemental contents in some plant materials over the temperature range, 150 to 400 °C. However, no significant loss in Ca, Sm, Mn, Fe, Co, Cu, Zn, Sb contents has been observed.

The most straightforward way to decrease loss of volatile elements is oven drying at ~80 °C to avoid matrix decomposition and loss of volatile elements. During our experimental work all the samples were dried in an oven or under IR lamp in a specially fabricated dust free chamber as shown in Fig. II.1. Here the temperature could be adjusted depending on the height of placement of the sample. After crushing (in an agate mortar) and drying the samples were sieved to uniform particle size by passing through 100-mesh sieve.

Fig. II.1 Dust Free Chamber for Sample Preparations

(iii) Preservation and Storage: It includes retaining the morphological features of a specimen, whereas storage, specially from trace element point of view may be understood to imply an overall safe, contamination free and non-degraded contaminant [18]. The matrix properties, humidity, pH, temperature, duration of storage and the container material are linked to the contamination and loss of originality of the sample because of adsorption, leaching and biochemical interaction. The method of preservation by freezing is emerging as a better choice [19]. Subramanian [20] has emphasized the need for preservation and storage of biological fluids collected for trace

element determination. To avoid the microorganism affected degradation of the samples of biological origin it should be irradiated with ^{60}Co γ-rays at 3-5 Mrad [2,13]. The dose required depends on the sample matrix. Some biological samples (medicinal plants, and herbal preparations) analysed in our study were irradiated in a ^{60}Co Gamma Chamber-900 at ~ 2.5 Mrad at a dose rate of ~ 0.3 Mrad/h at the Nagpur University.

II.3. PREPARATION OF STANDARDS

In this study NAA has been used in the comparator mode except in some cases where k_0 monostandard method was used. This eliminates many uncertainties in the analytical determination of elemental contents [21-23] though it has its own problems of inherent errors. The choice of proper comparator standard is of great significance and depends upon the matrix, elements to be determined and their concentrations. A standard may be primary (synthetic), secondary/ SRM and gold (monostandard) in case of k_0 method.

(i) Primary (Multielemental Synthetic) Standard: These are prepared by weighing known amount of the respective element or compound in purest form (with an assay of 99.9% or better) and with known stoichiometry, deposited on specific and inert substrate such as Whatman filter paper, alumina, SiO_2, magnesia, cellulose, resin etc. [23]. An ideal standard should be of high purity with quantitative characterization of impurities, soluble in common solvents (preferably aqueous) with comparable elemental concentration.

In Table II.1, the constituents of a typical synthetic standard used in this study are listed. These were prepared in a clean glove box by depositing aq. acidic solutions of 1 to 5 μg of AnalaR/GR/HP grade salts on Whatman filter paper No. 42 strip. All solutions were prepared in doubly distilled water and standardized by standard procedure.

For k_0 method, however, a monostandard of gold was prepared as already described [24]. A high purity (5N, 99.999%) gold foil was dissolved in aqua regia. The solution was slowly evaporated to dryness under IR lamp and dissolved in 0.1M HNO_3. Finally the concentration was made to 100 μg/mL. 50 μL (5μg) solution was dried on

alkathene taking care that the spot portion did not exceed 5 mm in diameter and packed in a similar manner as the samples.

Table II.1 Constituents of a typical synthetic multielemental standard

Element	Compound/ Reagent	Firm and Purity	Amt. of Element deposited
As	As_2O_3	BDH, England, 99.8%	2 μg
Cd	$Cd(NO_3)_2.4H_2O$	Merck, Germany, 99%	10 – 15 μg
Co	$Co(OOC.CH_3)_2.4H_2O$	E. Merck, India, 99%	~5 μg
Cr	$Cr(NO_3)_3.9H_2O$	Fluka, Germany, 98%	4 –6 μg
Fe	$(NH_4)_2Fe(SO_4)_2.6H_2O$	E. Merck, India, 99%	~10 μg
Hg	$(CH_3COO)_2Hg$	E. Merck, India, 99%	~2 μg
Mn	$KMnO_4$	E. Merck, India, 99.5%	~20 μg
Sb	$KSbO\ C_4H_4O_6$	BDH, England, 99.9%	~5 μg
Se	SeO_2 in HNO_3, 1 μg /mL	Merck, Germany, 998±5 mg/L	3 – 5 μg
Zn	$Zn(OOC.CH_3)_2.2H_2O$	Merck, Germany, 99.5%	10 – 15 μg

(ii) Standard Reference Materials (SRMs): Perhaps the simplest quality assurance procedure in NAA is to include two or more Standard/Certified Reference Material in each batch of unknown samples for short or long irradiation. If the results obtained for the RM agree for each other with the known composition within the expected uncertainty, the corresponding result for the unknown sample can be considered with confidence. SRMs such as those available through the international agencies viz. NIST (USA), IAEA (Vienna), INCT (Poland) and NIES (Japan) have been widely used as primary standards in many INAA studies [22,24]. These reference materials are usually the homogenized natural matrices. Das [25] has recommended the simultaneous analysis of two or more SRMs preferably of similar matrix but from different agencies and known analysis by different techniques. Recently, in order to address the measurement and standard needs of the food and nutrition community, Wise et al. [26] of NIST developed a suite of food-matrix SRMs characterized for

nutrient concentrations. Dybczynski et al. [27] prepared and characterized two new reference materials of biological origin, Tea leaves (INCT-TL-1) and Mixed Polish Herbs (INCT-MPH-2). Ratner and Vernetson [28] evaluated different INAA techniques (parametric, comparative and k_0 standardization) using SRMs. In all our studies, we have used at least three different SRMs all having similar matrix of plant origin but from different agencies. All the SRMs used in our studies are listed in Table.II.2.

Table II.2. List of Standard Reference Materials

Standard	Identification Number	Source
Biological		
Apple Leaves	SRM-1515	NIST, USA
Cabbage	IAEA-359	IAEA, Vienna
Citrus Leaves	SRM-1572	NIST, USA
Oriental Tobacco Leaves	CTA-OTL-1	INCT, Poland
Peach leaves	SRM-1547	NIST, USA
Pine Needles	SRM-1575	NIST, USA
Tomato Leaves	SRM-1573	NIST, USA
Virginia Tobacco Leaves	CTA-VTL-2	INCT, Poland
Geo-Environmental		
Estuarine sediment	IAEA-405	IAEA, Vienna
Pond Sediment	No.2	NIES, Japan
Soil	Soil-5	IAEA, Vienna
Basalts	W-1 and BCR-1	USGS, USA

Analysis of SRMs and INAA has become complimentary to each other because of its inherent characteristics. On one hand, it is recommended to use SRMs to give good quality data in samples analysed by NAA and on the other hand, NAA is referred to as a reference method available to produce good quality data in SRMs for multielemental analysis [29]. Dybczynski et al. [30] reported that in the inter-comparison studies of Polish Standard CTA-OTL-1, NAA led with 42% as distributed over the various analytical techniques. Orvini et al. [31] have characterized three new sediments of environmental matrix developed by Bureau Communautaire de Reference, UK.

II.4 NAA METHODOLOGY

(i) Packing: All the samples and standards each of 30-50/ 100 mg were accurately weighed and packed in polyethylene or polypropylene vial/ high purity aluminium foil for irradiation. A variety of packing materials have been used depending on the duration of irradiation, neutron flux and nature of matrix. Care should be taken to ensure that no packing material constituent be activated by neutrons. We have used alkathene (pure form of polypropylene) for short-term irradiation of 1 min, 5 min and 1-2 hrs. For longer irradiation of 7-14 hrs in APSARA reactor and 1-3 d in Dhruva reactor high purity aluminium foil (superwrap) was used. For 1-5 min. irradiation, each set set contained 2 SRMs and 3 samples, doubly sealed in alkathene and then encapsulated in polyethylene bottles. All the packing and sealing of samples were carried out in a dust free and clean chamber (Fig. II.1).

(ii) Irradiation: All irradiations were carried out in APSARA/Dhruva reactors at the Bhabha Atomic Research Centre (BARC), Mumbai. Some samples were also irradiated for 1 min in Dhruva reactor by using pneumatic carrier facility (PCF). This has been the most beneficial aspect of our work as it generated sufficient activity to determine up to 11 elements without much radiation hazards. Other short irradiations of 5 min, and 1-2 h were carried out in APSARA reactor followed by counting at the reactor site itself and later at the Radiochemistry Division of BARC. Long irradiated samples (in DHRUVA and APSARA) were air lifted to Delhi and then brought to our laboratories at Roorkee. All the irradiations were carried out with thermal neutrons (10^{12} – 10^{13} n cm^2 s^{-1}) in E-8 position. In Table II.3 are listed irradiation and counting schedule including the radionuclides identified/determined.

(iii) Post Irradiation Treatment: It involves decontamination of irradiated sample followed by the transfer to fresh butter paper bag. As soon as the irradiated sample is received, the container is cut open and the samples are unpacked. These are decontaminated so as to remove any extraneous activity. In case of short irradiation work, surface, decontamination was done by swiping the alkathene with cotton, or tissue paper soaked with acetone/ methanol. These are then mounted on Perspex or aluminium sheets with suitable and reproducible geometry. For longer irradiations of a

few hours or days, Al-foil wrapper is first carefully cleaned, recognized by weighing and then counted. After initial counting these were unwrapped and the samples were transferred to pre-weighed, clean butter paper packets (1 cm x 1 cm). This is to avoid many impurities in Al-foil, which may get activated and interfere with the sample activity though an Al blank was also used on some occasions.

Fig. II.2 Glove Box for Handling of Irradiated Samples

Table II.3: Irradiation, delay and counting schedule

Time	Reactor	Delay	Counting	Nuclides Identified
1 min. (*n=10)	Dhruva	~3 min. ~8 min. ~15 min. 20 d	50 s 50 s 300 s 2000 s	^{28}Al, ^{139}Ba, ^{49}Ca, ^{27}Mg, ^{51}Ti, ^{52}V. ^{28}Al, ^{139}Ba, ^{49}Ca, ^{27}Mg, ^{51}Ti, ^{52}V ^{38}Cl, ^{56}Mn, ^{24}Na, ^{42}K, ^{76}As. ^{32}P(β^-)
1 d (*n =12)	Dhruva	10 d 12d 20 d 25 d 40 d	1h 2h 6h 1 h 12 h	^{140}La, ^{233}Th(^{233}Pa), ^{86}Rb, ^{82}Br. ^{75}Se, ^{124}Sb, ^{51}Cr, ^{131}Ba, ^{141}Ce, ^{203}Hg, ^{181}Hf, ^{59}Fe, ^{60}Co, ^{152}Eu. ^{32}P(β^-). ^{65}Zn, ^{134}Cs, ^{60}Co, ^{152}Eu.
5 min. (*n=30)	APSARA	~3 min	50 s 100 s 300 s	^{28}Al, ^{49}Ca, ^{27}Mg, ^{52}V. ^{28}Al, ^{49}Ca, ^{27}Mg, ^{52}V ^{38}Cl, ^{56}Mn, ^{24}Na, ^{42}K
1 h (*n=15)	APSARA	2 h 4 h 30 h	5 min. 30 min. 1 h	^{24}Na, ^{42}K, ^{56}Mn, ^{64}Cu. ^{24}Na, ^{42}K, ^{56}Mn, ^{64}Cu. ^{24}Na, ^{42}K, ^{140}La, ^{82}Br, ^{76}As.
7 h (*n=1)	APSARA	10 d 20 d 25 d	1 h 2 h 4 h	^{24}Na, ^{42}K, ^{82}Br. ^{51}Cr, ^{60}Co, ^{59}Fe, ^{65}Zn. ^{60}Co, ^{59}Fe, ^{65}Zn
2x7 h (*n=1)	APSARA	6 d 10 d 20 d 25 d	1 h 2 h 6 h 1 h	^{140}La, ^{233}Th(^{233}Pa), ^{86}Rb, ^{82}Br, ^{51}Cr. ^{140}La, ^{233}Th(^{233}Pa), ^{86}Rb, ^{82}Br, ^{51}Cr. ^{65}Zn, ^{59}Fe, ^{46}Sc, ^{60}Co, ^{233}Th (^{233}Pa), ^{131}Ba. ^{32}P(β^-)

*n represents number of irradiations performed in this study

Extreme care was taken in transferring the irradiated samples for repacking. All the operations with activated sample were carried out inside a glove box as shown in Fig. II.2. During our experiments, we were able to recover 80-95% material though in some cases large losses (up to 40 %) were also noticed.

(iv) Activity Measurement: For the counting of γ activity two pronged approach was adopted whereby short lived nuclides were counted at the reactor site itself or at the Radiochemistry Division of BARC and further counted for longer duration after bringing them to our laboratory at Roorkee. Long irradiated samples however, were air lifted to Delhi and then brought to Roorkee.

A. ***At BARC:*** Activity due to short lived radionuclides were counted using 80 cm^3 coaxial HPGe Detector (EG & G ORTEC) and 4k MCA. At least three countings of 50 s, 50 s and 100/300 s were performed to derive maximum information. In some cases, 1 h counting was also followed depending on the availability of equipment at that time.

B. ***At Roorkee:*** γ-activity was measured using an HPGe detector with 8k MCA and GENIE-2000 gamma spectroscopy software from Canberra, USA. Counting was followed for 1k, 2k, 5k, 10k and 20k s at different intervals up to 3 months. Care was taken to obtain maximum elemental information from more than one countings and the reproducibility of data was checked. For example, data for ^{65}Zn ($t_{1/2}$ = 244 d) and ^{60}Co ($t_{1/2}$ = 5.27 y) were obtained from 20 k counting and after an interval of 8-10 weeks so that interferences from short lived nuclides may be eliminated.

In both cases radionuclides were identified by prior calibration of the spectrometer. In some cases however, half life was also followed. Characteristic E_γ , $t_{½}$, and other characteristics are listed in Table II.4.

(v) Instrumentation: The γ-ray spectrometry forms the basis of NAA though β-activity measurements were also carried out especially for the determination of P via ^{32}P. In the present study, a coaxial HPGe detector was used because of its better performance in terms of energy resolution, efficiency, peak to Compton ratio, linear response of pulse height and energy of gamma photons [32]. It consists of HPGe detector with all its accessories as schematically shown in Fig. II.3 Following are the

specifications and performance data of HPGe detector (Canberra, USA) used in this study.

Detector Model: GC2018,

Cryostat Model: 7600 SL,

Serial Number: 05017312

Preamplifier Model: 2002 CSL

Resolution: 1.8 keV (FWHM) at 1332 keV & 0.9 keV (FWHM) at 122 keV

Relative Efficiency: 20%,

Peak/Compton Ratio: 50:1

Physical Characteristics;

Diameter = 60.5 mm,

Length = 29.5 mm

Distance from window = 5 mm

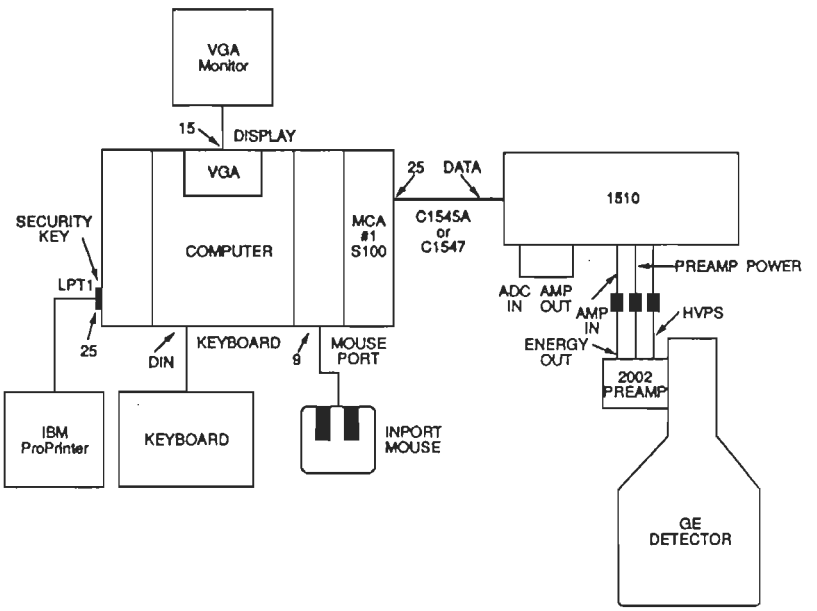
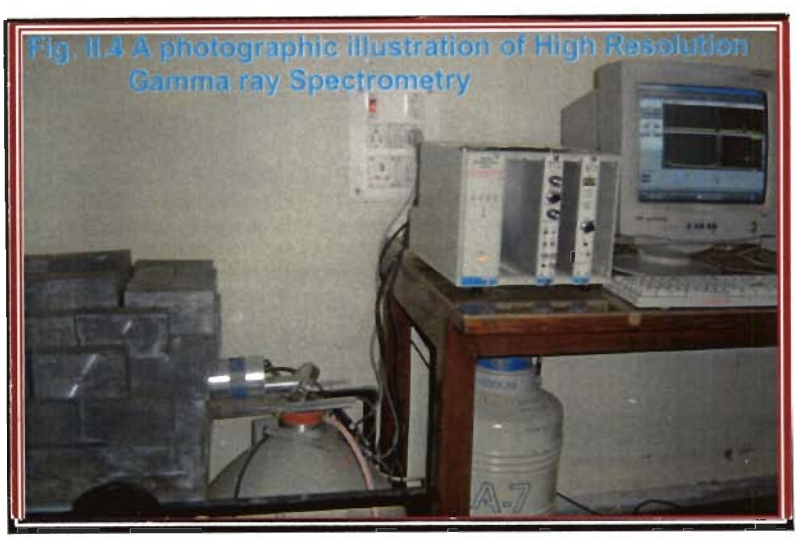


Fig. II.3 A Schematic Diagram of the Counting Setup



Recommended bias voltage = (+) 4000 V dc was obtained through 5 kVA UPS system (on-line) from Stellar, New Delhi. It had fifteen 12 V batteries (FURUKAWA, Black Gold) with 4 hr backup. A photographic illustration of the counting set up with lead shielding and computer are shown in Fig. II.4.

(vi) Calculation and Data Processing: With the development of high resolution semiconductor detectors and multichannel analyzers, γ-ray spectrometry has become an important tool for the detection of natural and artificial radioactivity and activation analysis of complex samples [32-34]. The spectra of irradiated samples of natural matrices are usually complex with closely lying multiple peaks. The peaks show Gaussian distribution as the first contribution to the photo peak in a semiconductor

Table II.4: Nuclear Characteristics of the Radionuclides Identified/Determined by (n,γ) Reaction

Target Element (Nuclide)	Isotopic Abundance (%)	Product Nuclide	Half Life ($t_{1/2}$)	Cross Section σ (b)	Energy E_γ (keV)
Al (^{27}Al)	100	^{28}Al	2.241m	0.23	1779
As (^{75}As)	100	^{76}As	26.3h	4.3	559, 657
Au (^{197}Au)	100	^{198}Au	2.7d	98.8	412
Ba (^{138}Ba) and ^{130}Ba	(71.7), 0.1	(^{139}Ba), ^{131}Ba	(83.1m), 12d	(0.36), 8.8	(166), 373, 496
Br (^{81}Br)	49.5	^{82}Br	35.4h	3.31	554, 776
Ca (^{48}Ca)	0.187	^{49}Ca	8.718m	1.1	3084
Ce (^{140}Ce)	88.5	^{141}Ce	33d	0.6	145
Cl (^{37}Cl)	24.4	^{38}Cl	37.2m	0.43	1643, 2167
Co (^{59}Co)	100	^{60}Co	5.27y	37.2	1173, 1332
Cr (^{50}Cr)	4.35	^{51}Cr	27.8d	15.9	320
Cs (^{133}Cs)	100	^{134}Cs	2.06y	30	605,796
Eu (^{151}Eu)	47.8	^{152}Eu	13.5y	5900	244, 1408
Fe (^{58}Fe)	0.33	^{59}Fe	44.6d	1.2	1099, 1291
Hf (^{180}Hf)	35.1	^{181}Hf	42.4d	10	482
Hg (^{202}Hg)	29.7	^{203}Hg	46.6d	3.8	279
K (^{41}K)	6.88	^{42}K	12.5h	1.30	1524
La (^{139}La)	99.9	^{140}La	40h	8.93	487, 1596
Mg (^{26}Mg)	11.17	^{27}Mg	9.46m	0.038	1014
Na (^{23}Na)	100	^{24}Na	15h	0.5	1368
Mn (^{55}Mn)	100	^{56}Mn	2.58h	13.3	846
P (^{31}P)	100	^{32}P	14.3d	0.19	1708(β^-)
Rb (^{85}Rb)	72.1	^{86}Rb	18.7d	0.91	1077
Sb (^{123}Sb)	42.8	^{124}Sb	60d	3.3	603, 1691
Sc (^{45}Sc)	100	^{46}Sc	83.8d	27.2	889, 1120
Se (^{74}Se)	0.89	^{75}Se	120d	52	264
Sr (^{84}Sr)	0.56	^{84}Sr	65.2d	1.4	514
Th (^{232}Th)	100	^{233}Th(^{233}Pa)	27d	7.4	312
Ti (^{50}Ti)	5.18	^{51}Ti	5.76m	0.179	320
V (^{51}V)	100	^{52}V	3.74m	4.9	1434
Zn (^{64}Zn)	48.6	^{65}Zn	244d	0.76	1115

detector is due to statistical fluctuations in sharing the absorbed energy between ionization and heating the crystal network It is not practical to analyse these spectra

manually thus, the use of computers with suitable software is essential. Najafi [35] reviewed the analysis of γ-ray spectra using mathematical methods and computers. The continuum under the peak is due to Compton effect from the γ-rays of higher energies and the background. Following steps are involved in the computerized analysis of a peak.

a) Smoothening of experimental data
b) Peak searching
c) Selection of fitting intervals
d) Peak energy calculation
e) Peak area calculation

For NAA, the most important of all these factors is peak area calculation. Several methods [36-43] have been proposed to determine the total peak area (TPA) represented as: $A = \sum_{-n}^{+n} \alpha_i - \frac{n+1}{2}(b_{+n} + b_{-n})$ where, n = number of channels on right (+) and left (-) of the peak channel, α_i = total counts in i channels, $b_{\pm n}$ = background counts in 2n channels as determined from a straight line drawn between the channels to left and right to peak channel. An illustration of basic approach followed in activity measurement in terms of peak area is shown in Fig.II.5.

However, the superimposition of photopeaks on each other or the high Compton background makes it difficult to precisely determine the net counts in the region of interest (ROI). The base area is mostly taken as trapezoidal summation of the average counts in each channel giving the background over which the photo peak is situated. Czauderna [36] suggested a digital method for the net peak area

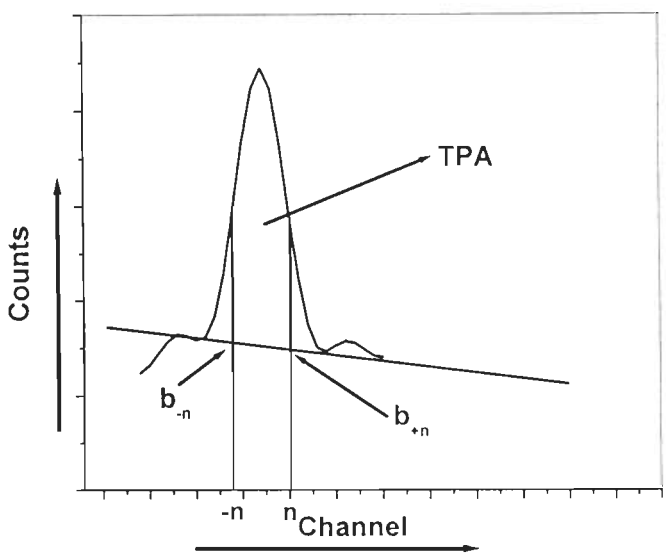


Fig. II.5 An Illustration of the Measurement of Total Peak Area (TPA)

determination and compared his results with TPA method. Heydorn and Damsgard [37] reported the validation and loss free counting system for NAA with short lived indicators, where the use of peaks in the corrected spectrum has been emphasized for the concentration calculation, while the data from an uncorrected spectrum determines the counting statistics. Several softwares have been developed whereby closely lying multiplets can be resolved into individual photo peaks. These include NADA [38], SAMPO 90 [39], DECHAOS [40] etc. The developments in this field include,

a) System of programmes for activation analysis calculations (SPAAC) [41]
b) Development of an interactive version of the well known fully automatic gamma ray spectrum analysis code HYPERMET [42].

Keeping in view of the ever increasing demand for nuclear data libraries and softwares, IAEA started operating the most comprehensive collection of nuclear data library world wide and many of them have been relevant to radiochemistry and activation analysis applications. Jing-ye et al. [43] designed an automatic data processing system having functions of radionuclide identification, flux ratio; k_o value measurement, quantitative calculation of elemental concentrations, nuclide data management and experimental design etc. for NAA in DOS environment.

II.5 DETERMINATION OF PHOSPHORUS

Phosphorus is an important element in biological, geological and environmental samples. It is essential to all living matter as it is present in bones, plants and animal tissues [44,45]. A variety of methods for its determination at mg/g to μg/g level are described in literature [46-48]. Weginwar et al. [46] first developed a NAA procedure whereby β-activity due to ^{32}P formed by (n,γ) reaction was determined by using a gas flow proportional counter and 27 mg cm^{-2} Al filter after a delay period of ~ 3 weeks. Pierre and Kennedy [47] proposed the use of plastic scintillator and determined P in polymers up to detection a limit of 2 μg/g. Scindia et al. [48] reported its determination using preconcentration chemical procedure accompanied by derivative NAA by extracting molybdovanadophosphoric acid in methyl isobutyl ketone and determines the activity of ^{52}V ($t_{1/2}$ = 3.4 min). We have slightly modified our earlier method by counting β activity using an end window G.M. counter (Model GC602A Nucleonix, Hyderabad) and Al absorber of 27 mg cm^{-2}. Our group has been using this method for the last 15 years

and reported phosphorus content in a variety of RMs and biological samples with a detection limit of 0.1 mg/g [49]. It has been observed during our participation in the Intercomparison studies that our data have been found to be the most acceptable within <±5% of the certified values in most cases.

II.6 ATOMIC ABSORPTION SPECTROPHOTOMETRY

AAS method has been widely used for the determination of trace elements in the environmental, geological and biological samples. Herber and Stoeppler [14] have written an excellent monograph on the trace element analysis in biological samples. Though it has the disadvantage of being destructive and mostly unielemental, it is the most sought for analytical technique for samples that are most easily collected as solutions [50]. In a recent handbook, Rosenberg and Panne [51] reviewed AAS and AES for quantitative analysis and discussed its limitations. We have used it for the determination of elements like Ni, Cu, Cd and Pb, which are difficult to be determined by thermal NAA. It may be pointed out that while Ni and Cu are considered as essential elements, Cd and Pb are highly toxic and environmental contaminants.

(i) Sample Decomposition: Most analytical techniques routinely used for the determination of essential elements in plants require destruction of the sample organic matrix before measurement. Many decomposition methods are available for mineralisation of plant material, before the determination of these elements. Dry ashing and wet decomposition in both classic and modern versions are still the more frequent methods. Under the controlled conditions, dry and wet decomposition techniques are comparable for their analytical performance [52]. Polkowska-Motrenko et al. [53] showed that the classical open wet mineralisation procedure in case of certain biological materials may lead to negative systematic errors of up to 14%. Even much higher negative systematic errors were observed when the amount of HF in the mineralisation procedure was too small Smrkolj and Stibilj [54] decomposed plant samples in a digestion mixture of $H_2SO_4/HNO_3/H_2O_2/HF/V_2O_5$ and sensitive detection of Se was achieved by hydride generation atomic fluorescence spectrometry (HG-AFS). Carrilho et al. [55] proposed microwave-assisted acid decomposition of samples derived from animals and plants. In this study, 2 g each of sample and RMs were accurately weighed

and digested in a mixture of nitric acid and perchloric acid (5:1) as reported in literature [56]. After digestion, dilution and filtration using Whatman filter paper, 2-3 drops of HCl was added and the solution was made up to 25 mL. In some *bhasma* samples Ca and K were also determined by flame photometer (ELICO model CL-361, Hyderabad).

(ii) Instrument: An atomic absorption spectrophotometer (GBC Avanta, Australia) at the Institute Instrumentation Centre (IIC) was used. It uses acetylene-air/acetylene-nitrous oxide mixture to ignite the flame. For each element the instrument was first calibrated using standard solutions of specific concentration range prepared from high purity grade salts of respective elements. The wave-length, concentration range and sensitivity for the elements determined in this study are given in Table II.5. For most medicinal herbs, only Ni, Cu, Cd and Pb were determined by AAS. However, for *bhasmas,* some other elements such as As, Fe, Hg, Sn and Zn were also determined. After calibration, stock sample solutions were suitably diluted to get absorbance in the calibration range.

Table II.5 Experimental parameters for elements analyzed by AAS

Element	Wavelength (nm)	Concentration Range (µg/mL)	Sensitivity (µg/mL)
As	193.7	30-190	0.64
Cu	324.7	1.0-5.0	0.025
Cd	228.8	0.2-1.8	0.009
Fe	248.3	2.0-9.0	0.05
Hg	253.7	73-290	1.6
Ni	232.0	1.8-8.0	0.04
Pb	217	2.5-20	0.06
Sn	235.5	35-135	0.72
Zn	213.9	0.4-1.5	0.008

II.7 PARTICLE SIZE MEASUREMENTS

In view of some reports about nano particle size of metallic herbal preparations called *bhasmas* [57], its particle size was determined by transmission electron microscope (TEM) of Philips Model EM 400 at IIC. It had line-to-line resolution of 1.4 Å

and point-to-point resolution of 3.0 Å with magnification range from 50 to 8,00,000, which was set as per sample requirements. All measurements were recorded at room temperature. Other details are mentioned in Ch.V. 7 (vii).

II.8 SEPARATION OF ORGANIC CONSTITUENTS

Some workers have shown the presence of gallic acid and polyphenols [58] in *Trifala* with which metals may be bonded thus enhancing their bioavailability. In order to confirm gallic acid and quantify it, column chromatography was performed using a mixture of ethyl acetate and methanol (7:3). Further, details are given in Ch,IV.6(vi).

It was further confirmed by elemental analysis using Elementar Vario – EL III, Germany, Analyzer. ^{1}HNMR and ^{13}CNMR spectra were recorded on Brucker 400 MHz spectrometer at Jubliant Organosys, Noida. GC-MS was recorded using a Perkin-Elmer Clarus-500 gas chromatograph coupled with a mass spectrometer having 30 m x 0.32 mm fused silica capillary column (5% phenyl methyl siloxane), (film thickness=0.25 μm) in a temperature program from 50°C for 0.5 min to 250°C (no hold) at a rate of 10°C/min. The injector temperature was 250°C, and the flow rate of He as carrier gas was 1mL/min. IR spectrum in KBr was recorded on a Thermo Nicolet (Nexus, USA) FT-IR spectrometer.

II.9 BIOASSAY

Indian medicinal plants are rich source of antioxidants and require a thorough investigation. Various methods have been used to monitor and compare the antioxidant activity in foods [59]. We have studied bioassay of *brahmi* (*Bacopa monnieri*), highly recommended in Ayurvedic therapy as a brain and nervine tonic that benefits both the mind and spirit and improves the intellect and consciousness [3,4]. In the present study we evaluated different extracts of *brahmi* towards DPPH radical scavenging activity, anti-lipid peroxidation, H_2O_2 scavenging, super oxide anion scavenging and DNA strand break assay. Antioxidant activity is correlated significantly and positively with total phenolics. The antioxidant activity of phenolics is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors and metal

chelators. In view of this, total phenolics were determined using the Follin's reagent. In order to understand the role of elements in respective extracts, these were also analysed for their elemental contents by NAA. All the experimental details are described in Ch. VI.

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CHAPTER III

MEDICINAL HERBS

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III.1 HERBS AS MEDICINES

Herbs have been called ***nature's pharmacy*** though their action can in some cases be similar to modern drugs [1,2]. Herbal remedies, often called as health food or neutraceuticals, are generally gentler and safer than any other drug form. According to WHO survey, about 70-80 % of the world populations rely on herbal medicines in their primary health care. Many of the drugs used in conventional medicine are derived from herbs, but herbalism uses the whole plant or its part such as the leaves, the flowers, the stem or the roots rather than isolating the "active agent" [3,4]. Frequently plants contain constituents that work together synergistically and are easily bioassimilable. Sometimes, use of the whole plant helps decreases the side effects that may occur when using isolated components. A large and increasing number of patients in Asia, Africa, China, Latin America, and Middle East use medicinal herbs because of economic reasons and easy availability. More than one third of Indians and Latin Americans use herbs as health tonics, yet patients (and physicians) often lack accurate information about their safety and efficacy. It is believed that ancient Indian sages had full knowledge about the efficacy of herbs and is well described in older literature in Sanskrit [4-7] but burgeoning interest in medicinal herbs has increased scientific scrutiny of their therapeutic potential, pharmacological action and safety. Thus, scientific study of herbs provides physicians with data that may help patients make wise decisions about their safe use. The public's belief that herbal and herbal products are safer than synthetic drugs can only be ascertained by imposing regulatory standards on these products that these should be manufactured using good practices of supply, manufacturing and storage [8].

III.2 LITERATURE SURVEY

As already mentioned in Ch.I.9, a large number of workers from different countries have analysed medicinal herbs for its organic and inorganic constituents. We shall briefly describe scientific studies reported in literature on 15 medicinal herbs analysed here and reported in this chapter.

Amaltas (*Cassia rhombifolia*) is indigenous to India and grows all over the country. It is a medium size deciduous tree, 8-10 m in height. The pods, which are used as medicine,

are cylindrical, 3-60 cm in length and thick. The pulp of pods contains sugar, gum, astringent matter, gluten, colouring matter and oil [3,7]. The fruits pulp is used as antibacterial and beneficial in common skin infection due to fungi and bacteria. Internally, it is a mild laxative being especially useful to children, weak individuals and in pregnancy. Kapoor and Farooqi [9] explored the possibility of its use as a potential source of commercial gum.

Ashwagandha (*Withania somnifera*) is used in Ayurvedic formulations for a variety of health promoting effects. It grows widely in dry and subtropical parts of India. It is 0.3-1.5 m in height. The plant extract contains anaferine and lactones with anolides with adaptogenic and antistress activity. Being a potent painkiller and anti-inflammatory, it is used as an effective remedy in rheumatic disorders. The mild sedative effect of *ashwagandha* has a claiming effect on mind so as to alleviate the mental stress and hence it promotes sound sleep [3,4]. Rani et al. [10] evaluated the anti-genotoxicity of its leaf extracts and reported that it does not impart any protection against oxidative damage caused by high glucose and H_2O_2 to human tumor cells suggesting its use as an anti-tumor. Kaur et al. [11] extracted a biological active constituent with anti-stress activity. Jayaprakasam and Nair [12] isolated four novel withanolide glycosides showing cyclooxygenase-2 enzyme inhibition.

Bakuchi (*Psoralea corylifolia*) seeds are useful in bilious infections. Its plant grows throughout India, especially in the plains of central and eastern India. The seeds have great medicinal values. The plant is used both internally as well as externally. The seed oil is extremely beneficial externally in skin diseases [3,7]. Its powder is specially recommended by Vaidyas in leprosy and leucoderma internally and is also applied in the form of paste or ointment externally. This herb has been given the name "KUSHTANASHINI" (leprosy destroyer) because of its efficacy in the treatment of leprosy. Chen et al. [13] reported the behavioral and biochemical studies of total furocoumarins isolated from the seeds of *bakuchi*. Turel et al. [14] determined Cu, As, Sb and Se by thermal NAA using substoichiometric technique.

Guduchi (*Tinospora cordifolia*) is a widely used shrub in folk and Ayurvedic system of medicine. It is one of the most versatile rejuvenative herbs. *Guduchi* grows throughout India in deciduous as well as dry forests. It accords longevity, enhances memory,

improves health, bestows youth, betters complexion, voice energy and luster of skin. Several alkaloids, diterpenoids lactones, glycosides, steroids, sesquiterpenoids, phenolics, aliphatic compounds and polysaccharides have been isolated from this shrub. It has anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, antioxidant, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomudulatory and anti-neoplastic activities [15]. Upadhyay et al. [16] studied its use for hepatic disorders on the hydraulic permeability of water in the presence of bile salt through a transport cell model.

Jaiphal (*Myristica fragrans*) or nutmeg fruit is a spice as well as medicine for increasing absorption, particularly in the small intestine. The plant is indigenous to Molucco islands and cultivated in Indonesia, West Indies and tropical countries. In India, it is cultivated in Kerala and Tamil Nadu. It is an evergreen tree of 10-12 m height. The fruits are broadly pyriform, yellow, broadly ovoid, blunt, covered by fleshy arillus. It has the property of good appetizer and digestant. The stable oil in *jaiphal* is known as 'butter of nutmeg' and contains mesin [3,7]. Dorman and Deans [17] reported its chemical composition including antimicrobial and in vitro antioxidant properties. Pino et al. [18] isolated its essential oil and reported 86.6% terpine by GC. Rasheed [19] isolated the pharmacologically active constituents of jaiphal oil and identified by GC-MS and ^{13}CNMR spectral studies. Results showed the difference in the components of jaiphal oil of different origins, especially in eugenol content. Borrowman et al. [20] found that diarrhoea and steatorrhoea in thyroid medullary carcinoma, responded to the treatment with *jaiphal* after thyroidectomy.

Jatamansi (*Nardostachys jatamansi*) is a wonderful nerve stimulant and promotes appetite. This root is bitter and aromatic [21]. The plant is about 10-60 cm in height and grows in Alpine Himalaya and is cultivated in Punjab to Sikkim and in Bhutan. It is used for the treatment of various disorders like convulsions and heart palpitation, as a sedative in high blood pressure and cardiac arrhythmias and to prepare hair tonics [2,3]. Mody et al. [22] developed a process for dry powder extract after grading, shredding, solvent extraction and spray drying of the rhizome extract.

Kalmegh (*Andrographis paniculata*) grows throughout India in moist and shaded place in the plains. It grows to a height 0.5-1 m, with quadrangular branches. The fruits are

linear capsules with numerous seeds. It is an appetizer, liver stimulant, vermicidal and cholegogue in properties. It is used as a bitter tonic in convalescence and in many liver preparations. It is a commonly incorporated ingredient of many liver preparations, available in the market. Ashok et al. [23] studied the effect of aging on its andrographolide content. Patel et al. [24] determined andrographolide and Fe content. Talukdar and Dutta [25] suggested a fast quantitative method for the estimation of andrographolide by TLC of its liquid extract.

Kutaz (*Holarrhena antidysenterica*) plant is indigenous to India and found all over the country in deciduous forests up to 900 m. Kutaz leaves contain O-containing alkaloids (kurchiphyllamine and kurchiphylline). It is antidysenteric, bitter, stomachic, febrifuge and anthelmintic. It has been used as a household remedy for the treatment of diarrhoea and dysentery associated with bleeding [3,7]. Niranjan et al. [26] developed a spectrophotometric method for the estimation of total alkaloids in the stem bark, seed and formulations. Raman et al. [25] isolated an alkaloid holarrifine-24ol from its stem bark and screened its antibacterial and antifungal activity against 10 pathogenic bacteria.

Laghu Haritaki (*Terminalia chebula*) is used in cough, asthma, abdominal distention, tumors, heart disease, skin disease and itching. Haritaki tree is found in the sub-Himalayan tracks from Ravi to West Bengal, Assam and in all deciduous forests of Madhya Pradesh, Bihar and Maharashtra. It is a moderate size tree, 15-25 m in height. The fruit contains about 30% astringent substances such as chebulinic acid, tannic acid, gallic acid and chebulagic acid etc. It is commonly used in gastrointestinal, ascites, piles, enlargement of liver-spleen, worms, colitis respond very well [3,7]. Barthakur and Arnold [28] analysed the edible fruit tissue for organic and mineral nutrients and found that 100 g raw fruit can supply 14 macro- and micronutrients at the minimum Recommended Dietary Allowance (RDA) level of Se, K, Mn, Fe and Cu (100, 63.5, 32, 30 and 28.5 % respectively).

Lodhra (*Symplocoa racemosa*) is useful in bowel complaints such as diarrhoea, dysentery, dropsy, eye diseases, fever, cough, ulcer and swelling. Tree grows all over India with 6-8 m in height and grayish bark containing alkaloids, leupeoridin, glycosides, loturin, loturidine, colloturine and sodium carbonate [3,7]. De Silva et al. [29] reported

that its petroleum ether and ether extracts afforded a high yield of butilinic acid with smaller amounts of acetyloleanolic and oleanolic acids.

Neem (*Azadirachta indica*) is one of the most powerful blood purifier and detoxifier among traditional herbs [3,4]. The plant grows throughout India in the plains and grows wildly in the sub-Himalayan tract at an altitude of 800-1000 m. Each part of this tree including leaves, bark, fruit and seed has medicinal value [4]. The leaves are useful for diabetes, leprosy, intestinal worms, liver enlargement and headache. *Neem* oil and soap are used as bug repellent and a natural insecticide. The constituents of *neem*, studied extensively, include margosic acid, nimbin, nimbidin, nimbinin, azadirone, kaempferol, quercetin, β-sitosterol, vanillic acid, meliacins etc. The tender branchlets of *neem* are used as toothbrush in rural India. These are similar to the analysis of chewing sticks reported by Osubiojo [30] from Nigeria. Sahito et al. [31] determined 15 essential and toxic elements (Zn, Cr, K, Mg, Ca, Na, Cu, Fe, Pb, Al, Ba, Mn, Co, Ni and Cd) in water extract of its different parts by AAS. Coventry and Allan [32] characterized a laboratory prepared seed extract along with a commercially available formulated product using HPLC, and showed it to be effective against a range of bacteria in an agar diffusion assay.

Nirgundi (*Vitex nigundo*) grows all over India, in wastelands, up to 1500 m elevation. It is 2-4 m in height with quadrangular branches and thin gray bark. It is helpful in strengthening eyes, hair, reduce swelling and nausea, and many types of ear diseases. The plant contains alkaloids, glycosides, flavonoids, reducing sugars, sterols, resins and tannins. Ono et al. [33] isolated a new phenyldihydonaphthalene type lignan, vitedoin A (I), a new phenylnaphthalene type lignan alkaloid, vitedoamine A (II), and a new trinorlabdane-type diterpene vitedoin B (III), were isolated from the seeds of *Vitex nigundo* along with five known lignan derivatives.

Sariva (*Hemidesmus indicus*) grows all over India and Sri Lanka. A twining shrub grows 1.5-3 m tall with very slender, woody stems. The tuberous roots are dark brown in colour and have camphor like fragrance when fresh. The components isolated from its roots are essential oil containing 80% hydroxy-4-methoxy benzaldehyde, fatty acids, ketone, saponin, tannins sterol, β-sterol, stigmasterol and sarsapic acid. The fragrant roots of *sariva* are used in Indian native medicine and herbal tea preparations. The

paste of the root is applied on the skin in case of swelling associated with burning sensation due to pitta (acidity). Dutta et al. [34] analysed the *sariva* roots collected from Bengal and it contained ~0.225% essential oil of which 80% is a crystalline substance identified as 2-hydroxy-2-methoxybenzaldehyde.

Vacha (*Acorus calamus*) grows throughout India, in marshy land up to 1800 m height. It is mainly cultivated in Kashmir, Manipur and Nagaland. The perennial herb with branched rhizomes immersed in the mud, grows 1-2 m in height. The rhizome contains calamediol essential oil, tanning substances and vitamin C. It is a bitter stimulant and has been used for loss of appetite and flatulence. Govindarajan et al. [35] evaluated pharmacological action of the dried rhizomes collected from Dehradun and Lucknow.

Vidang (*Embelia ribes*) is used to expel the intestinal worm, specially tape-worm. It grows throughout India, especially in hilly regions up to 1200-1500 m elevation. It is a small shrub with long, slender, flexible branches and whitish gray stem. An alkaloid christembine was isolated from the fruit, which is really a boon to the digestive system [3,4].

Jain et al. [36] analysed some medicinal plants for Ca, Cu, Fe, Mg, Zn, Ba, Na, K, Al, Mn, Ce, Sr, V, Hg, Cr, Ni, Co, Cd and Ag by using flame photometer, AAS and ICP-AES. Garg and co-workers [37] analysed several medicinal herbs including *haritaki*, *amaltas*, *jaiphal*, *neem ashwagandha* and *bakuchi* seeds for 20 elements by INAA. Rajurkar and coworkers [38] reported the analysis of *ashwagandha* and *neem.* by NAA and AAS. Naidu and co-workers [39] determined 20 macro, micro and trace nutrient elements in Indian medicinal and vegetable leaves including *neem*. Singh and Mittal [40] used INAA to study trace elements in *neem* and *tulsi*. It was reported that the concentrations of potentially toxic elements such as Cd, Cs, Cr, Hg, Sc and Se were twice in samples from Patiala compared to those from unpolluted areas. Ekinci et al [41] employed EDXRF for trace elements in *vacha*.

III.3 PARTICIPATION IN RENEWAL OF SRM-1575a

National Bureau of Standards (NBS now NIST), USA first developed Pine Needles (SRM-1575) during seventies [42]. After that research workers all around the world used it extensively for the standardization of materials and data validation of

similar matrices. As a result, its supply got depleted and hence there arose a need to prepare and analyze a new SRM. Dr. Donald A. Becker of NIST initiated its renovation as SRM-1575a. The Pine Needles were collected from Loblolly pine tree (*Pinus taeda*) in North Carolina from freshly felled trees of approximately the same age and origin. The needles were dried at 70 ^{0}C for 48 h, coarse ground to pass through a 2 mm sieve and shipped to NIST where the material was jet milled to pass a 100 μm sieve, blended, radiation sterilized and bottled. One unit of SRM 1575a consists of approximately 50 g of dried, jet milled, radiation sterilized and blended Pine Needles [43].

III.4 PRESENT STUDY

We have determined 28 elements (Al, Au, Ba, Br, Ca, Ce, Cl, Co, Cr, Eu, Fe, K, La, Mg, Mn, Na, P, Rb, Sb, Sc, Sm, V, Th, Zn by INAA and Cu, Ni, Cd and Pb by AAS) in 15 medicinal herbs of common use for the treatment of various ailments in Indian household. These are *amaltas* (*C. rhombifolia*), *ashwagandha* (*W. somnifera*), *bakuchi* (*P. corylifolia*), *guduchi* (*T. cordifolia*), *jaiphal* (*M. fragrans*), *jatamansi* (*N. jatamansi*), *kalmegh* (A. *paniculata*), kutaj (*H. anticlysentrica*), *laghu haritaki* (*T. chebula*), *lodhra* (*S. racemosa*), *neem* (*A. indica*), *nirgundi* (*V. negundo*), *sariva* (*H. indicus*), *vacha* (*A. calamus*) and *vidang* (*E. ribes*). INAA using short and long irradiation in a nuclear reactor was followed by high-resolution gamma ray spectrometry. Fig. III.1 shows the pictures of all the relevant parts of the medicinal plants analyzed in this study. Nomenclature and uses of these medicinal herbs are listed in Table III.1. Additionally Ni, Cu, Cd and Pb were analysed by AAS as described in Ch II.6. We also participated in the inter-laboratory comparison study for the renewal SRM-1575a Pine Needles of NIST (USA).

Fig. III.1 Photographs of medicinal herbs

Table III.1. Common uses of medicinal herbs as described in Ayurvedic literature

General Name	English Name	Family	Botanical Name	Medicinal Uses (Ref. 1, 3, 6)
Amaltas (Fruit)	Indian Laburnum	Caesalpinceae	Cassia rhombifolia	Laxative, Anti-inflammatory, Antipyretic, Expectorant, Antileprotic, Analgesic, Cures Ringworms, Rheumatism, Constipation.
Ashwagandha (Leaves & root)	Winter cherry	Solanaceae	Withania somnifera	Aphrodisiac, Rejuvenator, Diuretic, Narcotic, Antipurgative, Nervine tonic, Anti-inflammatory, Analgesic, Antifungal, Antibacterial, Antiarthritic.
Bakuchi (seeds)	Malaya tea	Leguminosae (Papilionaceae)	Psoralea corylifolia	Nervine tonic, Appetizer, Expectorant, Antibacterial, Stimulant, Wound healer, Anthelmintic, Laxative, Diuretic, Leprosy, Asthma, Fever.
Guduchi (Leaves & fruit)	Tinospora	Menispermacea e	Tinospora cordifolia	Astringent, Antipyretic, Rejuvenator, Blood purifier, Nervine tonic, Diuretic, Anti-dote, Anti elegric, Anti-inflammatory, Gout, Pyrexia.
Jaiphal (Fruit)	Nutmeg	Myristicaceae	Myristica fragrans	Stomachic, Astringent, Heart tonic, Carminative, Stimulant, Aphrodisiac, Appetizer, Blood purifier, Anti thirst, Intestinal trouble.
Jatamansi (Root)	Spikenard	Valerianaceae	Nardostachys jatamansi	Aromatic, Stimulant, Antispasmodic, Nervine & heart tonic, Diuretic, laxative, Antipyretic, Arthritis, sedative, Hypotensive, Antibacterial, used in Colic, Ulcer, Hysteria.
Kalmegh (Fruit)	The Creat	Acanthaceae	Andrographis paniculata	Hepatic stimulant, Cholagogue, Antileprotic, Stomachic, Nutritive, Anorexia, Malaria, Oedema, Skin disease, Blood purifier.
Kutaz (Leaves)	Tellicherry	Apocynaceae	Holarrhena antidysenterica	Astringent, Antibillous Digestive, Diarrhoea, Haemorrhoid, Rheumatic arthritis, Skin disease, Blood purifier, Piles, Asthma.
Laghu Haritaki (Fruit)	Chabulic Myrobalans	Combretaceae	Terminalia chebula	Digestive, Laxative, Stomachic, Rejuvenator, Anti-inflammatory, Tonic, Appetizer, Anti-diabetic, Malaria and eye infections.
Lodhra (Bark)	Symplocos bark	Symplocaceae	Symplocos racemosa	Astringent, Antipyretic, Blood purifier, Tonic, Piles, Anti-inflammatory, Antimucilagenous, Wound healing, Dysentery, Leucorrhoea, Liver & eye trouble, Fever, Leprosy, Ulcer.
Neem (Leaves)	Margosa tree	Meliaceae	Azadirchta indica	Astringent, Purgative, Demulcent, Anti-inflammatory, Liver stimulant, Eczema, Antiseptic, Blood purifier, Anthelmintic, and used in Fungal infections & Rheumatism,
Nirgundi (Leaves)	Fiveleaved chaste	Verbenaceae	Vitex negundo	Analgesic, Expectorant, Vermifuge, Anti-inflammatory, Stomachic, Carminative, Rejuvenator, Diuretic, Anthelmintic.
Sariva (Root)	Sarsaparilla	Asclepiadaceae	Hemidesmus indicus	Antibacterial, Antifungal, Antiviral, Diuretic, Blood purifier, Anti-inflammatory, Leucoderma, Diarrhoea, Asthma.
Vacha (Leaves & root)	Sweet flag	Araceae	Acorus calamus	Stomachic, Anticonvulcent, Antipyretic, Antibiotic, Diphoretic, Anti-inflammatory, Expectorant, Spasmolytic, Nerve tonic, Musclerelaxent.
Vidang (Fruit)	Barbreng	Myrsinaceae	Embelia ribes	Appetizer, Stomachic, Rejuvenator, Nervinetonic, Blood purifier, Tapeworms infestation, Toothache, Dentalcarries.

III.5 EXPERIMENTAL

(i) Sample Preparation: Some of the medicinal herbs were procured from Shantikunj, Haridwar / Yogi Pharmacy in powder form. Fruit of *amaltas* and *neem* leaves were collected within the campus. All the rest were procured from local medicine shops and after cleaning, the samples were powdered in an agate mortar, passed through a 100 mesh sieve and oven dried at 80^0C for 2 h. Various RMs used as comparators, were procured from NIST (USA), IAEA (Vienna) and INCT (Poland), and used as such. A synthetic multielemental standard was prepared by spiking 2-5 μg amounts of Cr, Fe, Co, Zn etc in aqueous solution of their respective AR/high purity grade salts as described in Ch.II.3.

Pine Needles, SRM-1575a (~ 10 g, # 1292-1) and Peach Leaves, SRM-1547 [43] (~3g) were sent by Dr. Donald A. Becker NIST (USA). Its moisture content was determined as per recommended procedure by desiccator drying in glass vial using fresh $Mg(ClO_4)_2.xH_2O$ (E. Merck, Germany) for 120 h and found to be 4.7 and 4.2% respectively. This is somewhat higher than reported (2.9%) in the Final report [43]

(ii) Irradiation and Counting: About 50 mg each of powdered samples and RMs were weighed accurately and packed in polythene/aluminum foil (Superwrap) for short (5 min)/long (7x2 h & 3 d) irradiations. Irradiation details are same as described in Ch. II.4(ii). Details of counting set up are same as mentioned in Ch. II.4(iv). Irradiation and counting schedule followed and elements determined are given in Table II.3. Procedure for the determination [45] of phosphorus is same as described in Ch.II.5.

III.6 RESULTS

Typical gamma ray spectra of *jatamansi* for short and long irradiated samples are shown in Figs.II.2 and 3 respectively. Elemental concentrations

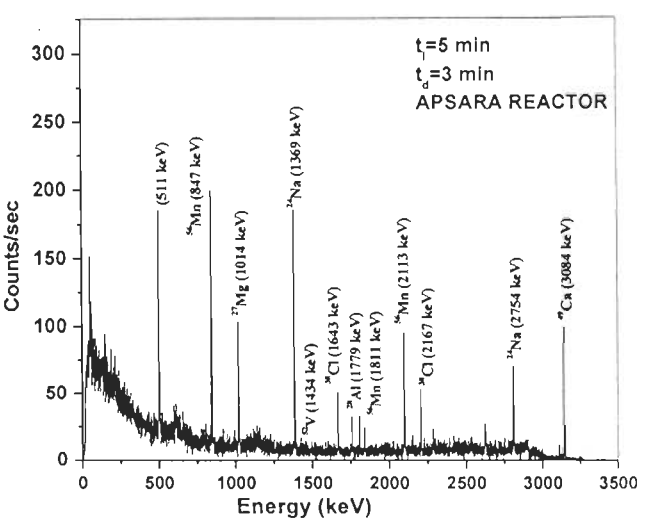

Fig.III.2 Typical Gamma ray Spectrum of *Jatamansi*

in 15 medicinal herbs were measured by relative method using different RMs and multielemental standards as comparators. Only if the values for elemental concentrations in various RMs matched well within ±5-10% of the certified values, the values for samples were considered. These are listed in Table III.2. Concentrations of Ni, Cu, Cd and Pb as determined by AAS are given in Table III.3. Also included in the same Tables are our data for participation for the analyses of depleted RM Pine needles (1575a) and Peach leaves (SRM-1547) used as control. In each case triplicate analyses were made using 100 mg sample. It is observed that standard deviations for most of the elements were small (≤10%) suggesting a good precision in our measurements. On the basis of good agreement, it is presumed that our values as listed in Table III.2 should be accurate and precise within ± 10%. Mean elemental concentrations, ranges and their implications are discussed in following lines.

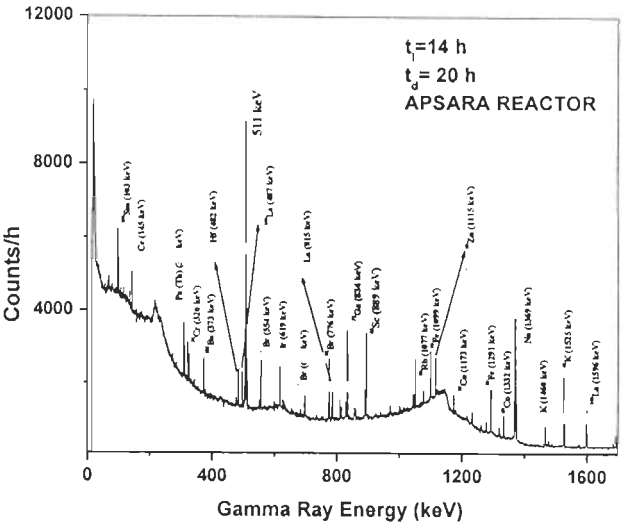

Fig.III.3 Typical Gamma ray Spectrum of *Jatamansi*

III.7 DISCUSSION

Out of 28 elements determined in 15 medicinal herbs, some are essential macro and micronutrients whereas others are toxic elements. Their contents and variations are discussed here.

(i) Nutrient Contents in Medicinal Herbs: A perusal of data in Tables III.2 & 3 shows that no single herb is enriched in all the elements. The electrolytic elements Na and K responsible for maintaining normal fluid balance across the cell membrane are generally found as minor (≥ 100 µg/g) and major (≥ 10 mg/g) constituents respectively [46-48]. All the samples have shown much higher concentration of K (4.21-18.6 mg/g)

Table III.2A. Elemental concentrations in some medicinal herbs

Medicinal Herbs	Al (mg/g)	Au (ng/g)	Br (µg/g)	Ca (mg/g)	Cl (mg/g)	K (mg/g)	Mg (mg/g)	Na (µg/g)	P (mg/g)	V (µg/g)	Zn (µg/g)
Amaltas	0.20±0.01	0.48	4.45±0.44	<0.5	1.41±0.05	12.2±0.3	0.83±0.05	64.2±1	3.29±0.15	ND	38.9±4.1
Ashwagandha	1.00±0.02	ND	23.1±2.6	14.9±1.3	1.30±0.02	14.1±0.07	1.22±0.06	290±10	0.72±0.01	ND	43.6±4.2
Bakuchi	1.34±0.02	ND	5.24±0.41	0.73±0.06	1.02±0.12	13.5±0.3	0.47±0.03	438±4	2.04±0.04	1.74±0.14	56.5±5.9
Guduchi	0.42±0.01	0.47	1.58±0.01	16.3±0.06	0.45±0.02	18.1±0.4	1.97±0.06	23.8±0.6	1.87±0.27	ND	13.5±1.9
Jaiphal	0.027±0.003	1.36	0.51±0.01	1.40±0.05	0.23±0.01	4.21±0.17	1.94±0.06	195±5	2.03±0.05	ND	40.0±0.5
Jatamansi	1.70±0.03	ND	7.28±0.57	1.63±0.12	1.19±0.08	8.85±0.2	0.47±0.04	1490±13	1.35±0.06	ND	60.0±6.3
Kalmegh	2.47±0.24	ND	8.45±0.39	2.29±0.14	2.46±0.22	18.6±0.9	1.88±0.06	443±135	0.90±0.05	1.44±0.06	30.9±1.4
Kutaz	1.04±0.06	ND	23.9±1.1	5.30±0.07	2.34±0.24	6.75±0.3	2.12±0.31	255±78	0.70±0.06	ND	27.5±1.3
Laghu Haritaki	0.73±0.01	ND	3.39±0.19	3.88±0.14	3.52±0.16	11.2±0.3	2.63±0.03	81.3±1	0.64±0.04	1.70±0.15	35.1±3.7
Lodhra	1.08±0.02	ND	13.6±0.6	0.89±0.06	1.91±0.36	6.24±0.3	2.45±0.20	240±74	0.91±0.02	3.04±0.06	26.7±1.7
Neem	0.96±0.02	3.84	28.8±1.4	3.59±0.10	2.01±0.24	10.7±0.6	0.90±0.07	262±80	1.33±0.01	1.19±0.08	34.8±1.6
Nirgundi	1.91±0.07	1.15	0.88±0.03	6.48±0.56	0.37±0.01	13.1±0.5	1.74±0.05	35.5±0.9	1.87±0.27	ND	13.8±1.7
Sariva	0.25±0.03	0.38	1.73±0.16	11.0±0.8	2.40±0.01	9.45±0.44	1.32±0.03	195±2	0.39±0.02	ND	41.1±0.5
Vacha	1.36±0.16	0.18	9.78±0.8	4.25±0.53	1.93±0.08	14.1±0.7	1.55±0.04	466±10	2.78±0.06	0.66±0.02	37.9±0.4
Vidang	1.24±0.15	0.26	15.0±1.2	2.46±0.11	5.94±0.09	17.6±0.8	1.65±0.05	457±8	1.43±0.03	3.55±0.08	37.7±1.0
SRMs Pine Needles (SRM-1575a)	0.600.02 (0.58±0.03)	1.51±0.3 (-)	3.08±0.15 (-)	2.82±0.21 (2.50±0.10)	0.462±0.051 (0.421±0.007)	4.31±0.44 (4.17±0.07)	1.01±0.02 {1.06±0.17}	91±34 {63±1}	1.12±0.08 (1.07±0.08)	ND (-)	36.2±3.2 (38±2)
Peach Leaves (SRM-1547)	0.28±0.03 (0.25±0.008)	1.50±0.01 (-)	12.3±0.3 {11}	17.5±1.5 (15.6±0.20)	0.340±0.020 (0.360±0.019)	26.5±2.0 (24.3±1.0)	4.15±0.35 {4.32±0.08}	22.6±1.6 (24±2)	1.32±0.13 (1.37±0.07)	0.52±0.05 (0.37±0.03)	20.5±1.5 (17.9±0.4)

ND = Not Detected, { } Information values, ( ) Certified values

Table III.2B. Elemental concentrations in some medicinal herbs

Medicinal Herbs	Ba (µg/g)	Ce (µg/g)	Co (µg/g)	Cr (µg/g)	Fe (µg/g)	Eu (ng/g)	La (µg/g)	Mn (µg/g)	Rb (µg/g)	Sb (ng/g)	Sc (ng/g)	Sm (ng/g)	Th (ng/g)
Amaltas	ND	1.29±0.28	0.55±0.09	1.47±0.01	697±113	7.91±1.4	1.69±0.05	6.44±0.06	18.1±0.7	196	9.30±1.1	133±10	493
Ashwagandha	12.5±2.3	1.11±0.5	0.07±0.01	1.86±0.37	221±11	33.2±1.2	0.93±0.01	17.4±1.0	15.5±1.5	155±12	44.9±2.2	150±11	158±12
Bakuchi	28.7±2.5	3.10±0.7	0.86±0.15	1.97±0.01	923±150	40.5±6.9	2.65±0.08	161±2	12.1±0.5	252	54.4±6.6	336±30	157
Guduchi	ND	1.09	0.07±0.01	0.961	277±8	41.2±5.6	2.38±0.08	47.3±1.9	14.7±0.3	14.7±1.8	39.0±4.8	218±16	28.5
Jaiphal	ND	0.92±0.20	0.19±0.02	1.91±0.07	63.2±1.5	145±19	0.48±0.08	43.6±1.7	6.64±0.39	82.6±2.7	93.5±6.5	87.6±2.2	135±20
Jatamansi	17.0±1.8	1.40±0.3	1.26±0.22	8.19±0.04	1210±200	273±47	6.19	474±5	52.7±2.1	310	1420±20	1051±60	2604±64
Kalmegh	16.9±1.3	0.38±0.1	0.16±0.01	1.11±0.24	250±52	10.3±1.8	1.00	51.3±3.7	21.1±2.0	ND	190±10	125±20	428±26
Kutaz	12.5±0.78	ND	0.14±0.01	1.30±0.23	255±52	7.89±1.4	0.80	34.1±2.4	ND	ND	180±10	112±18	422±26
Laghu Haritaki	14.4±1.72	1.00±0.2	0.54±0.09	1.28±0.01	656±107	12.6±2.2	0.81±0.08	12.2±0.3	14.8±0.6	133	290±20	103±8	202±5
Lodhra	24.9±1.35	1.55±0.4	0.15±0.01	1.20±0.24	233±49	8.10±1.4	0.82	191±13	13.9±1.2	ND	220±20	155±25	2154±90
Neem	21.7±2.5	0.50±0.1	0.12±0.01	1.47±0.26	256±52	11.4±2.0	0.68±0.01	46.4±3.3	17.6±1.5	ND	185±10	99.0±7.0	366±23
Nirgundi	ND	2.52	0.07±0.01	1.07	263±5	59.8±7.6	3.08±0.09	44.2±1.7	21.1±0.5	22.1±2.8	63.3±7.7	341±25	57.1
Sariva	72.0±3.6	0.94±0.12	0.19±0.02	2.00±0.07	64.5±0.8	771±100	0.62±0.12	19.1±0.9	7.56±0.44	84.1±2.9	104±10	95.0±2.0	162±25
Vacha	43.2±2.2	1.55±0.18	0.19±0.02	2.01±0.07	80.2±1.7	21.6±2.9	0.63±0.12	121±6	6.69±0.39	56.3±1.9	154±13	121±6	180±25
Vidang	ND	1.58±0.18	0.23±0.02	3.02±0.10	78.1±1.6	57.3±7.5	0.65±0.12	50.2±2.9	7.93±0.46	49.9±1.7	297±30	126±2	160±24
SRMs													
Pine Needles (SRM-1575a)	7.60±0.54 (6.0±0.2)	0.75±0.08 (-)	0.50±0.03 {0.06±0.01}	1.33±0.1 (-)	192±17 (46±2)	ND (-)	0.34±0.01 (-)	506±40 {488±12}	16.4±0.4 (16.5±0.9)	33.0±6.0 (-)	96.7±4.5 {101±3}	41.6±2.6 (-)	205±26 (-)
Peach Leaves (SRM-1547)	116±4 (124±3)	10.2±0.2 {10}	0.05±0.01 {0.07}	1.27±0.19 {1}	182±16 (218±6)	190 {170}	9.37±0.60 {9}	106±6 (98±3)	19.2±0.1 {19.7}	71.0±2.0 {20}	114±4 {40}	890±20 {1000}	60.2±5.0 {50}

ND = Not Detected, { } Information values, ( ) Certified values

Table III.3: Concentration of Cu, Ni, Cd and Pb analysed by AAS

Sample	Cu (μg/g)	Ni (ng/g)	Pb (μg/g)	Cd (ng/g)
Amaltas	2.69	907	1.42	1240
Ashwagandha	9.95	169	0.79	757
Bakuchi	12.0	99.0	0.89	318
Guduchi	3.88	157	1.01	62.4
Jaiphal	4.24	76.0	1.68	150
Jatamansi	36.8	75.0	1.59	12.5
Kalmegh	12.8	78.0	1.71	172
Kutaz	4.05	260	0.98	723
Laghu Haritaki	5.20	186	0.68	474
Lodhra	4.18	133	1.08	104
Neem	6.50	297	1.40	933
Nirgundi	3.85	299	1.36	932
Sariva	1.59	78.0	1.51	1557
Vacha	6.55	111	1.05	368
Vidang	14.0	89.0	0.88	12.0

by almost an order of magnitude compared to that of Na (23.8-1490 μg/g). Similarly Ca (0.5 - 16.3 mg/g) and Mg (0.50 - 2.63 mg/g) are also present in higher concentrations. So also Cl, possibly associated with Na and K was found at 0.3 – 3.5 mg/g level. These may be responsible for the absence of side effects as regards stomach lesions. Also Ca and Mg compounds are recommended in the prevention of osteoporosis [49]. Incidentally, mean concentration of K is highest (11.9±4.4 mg/g) followed by Ca (5.36±5.10) amongst all the minor constituents. Thus, all the herbs are likely to provide strength and act as vitalizer.

Transition elements Cr (0.96-8.19 μg/g), Mn (6.44-474 μg/g), Fe (63.2-1210 μg/g), Co (7-1260 ng/g), Cu (1.59-36.8 μg/g), and Zn (13.5-60.0 μg/g) were found at varying concentrations. Iron contents of all the samples are > 200 μg/g though *bakuchi* seeds and *jatamansi* have much higher amounts of Fe, 923±123 and

1210±200 µg/g, respectively. Zinc is an important element responsible for many enzymatic processes and is involved in the working of genetic material, proteins, immune reactions, wound healing, development of the foetus, and sperm production [47,48]. It has been suggested that normal levels of zinc can prevent diarrhoea [49]. In all samples, Zn content is found to be > 30 µg/g, being highest in *jatamansi* (60.0±6.3 µg/g). Manganese is another essential element required for biochemical processes. In most samples, it is found to be < 100 µg/g barring *vacha*, *lodhra*, *bakuchi* and *jatamansi*, where Mn content is > 100 µg/g. However, Mn is highest for *jatamansi* (474±5 µg/g). Thus, *jatamansi* is particularly enriched in Na (1490±13 µg/g), Cr (8.19±0.04 µg/g), Mn (474±5 µg/g), Fe (1210±200 µg/g), Cu (36.8 µg/g) and Zn (60.0±6.3 µg/g). In Ayurvedic literature, *jatamansi* is recommended as antibacterial, antipyretic and as heart tonic [3,4,7]. *Bakuchi* seeds are commonly used for many herbal preparations, which are widely recommended as appetizer, tonic with antibacterial and stimulant properties [3,4]. Many gold and vanadium compounds have been described to possess therapeutic properties. These elements could be detected in a few herbs only suggesting their special importance. The variation in elemental contents as observed in Tables III.2 & 3 could be due to preferential uptake of the elements by the plant species from the soil [50]. Therefore, soil characteristics together with environmental conditions also play an important role in the elemental contents.

(ii) Correlations of elemental contents: Histograms of mean elemental contents of minor constituents (Na, K, Mg, Ca, P and Cl) are shown in Fig.III.4. It is observed that in general, all medicinal herbs are enriched in K and Ca, though P, Mg and Cl are also present in significant amounts. As mentioned earlier, Na content is lowest whereas K content is highest in all the herbs [37]. Further, K/Na ratio is lowest in *jatamansi* (5.94) and highest for *guduchi* (761) though for 9 samples it is below 50. Histograms of Mn, Fe

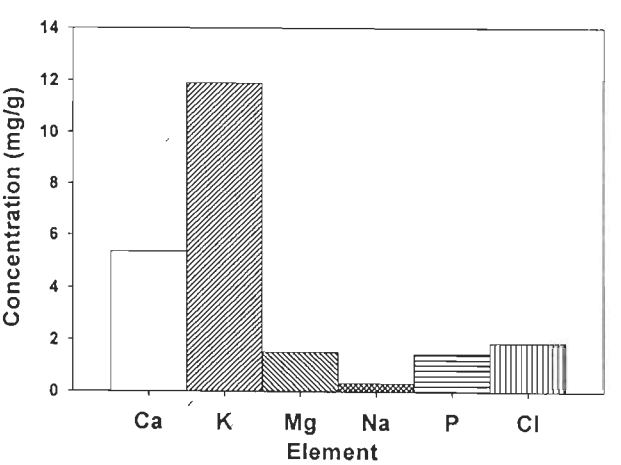

Fig.III.4 Mean elemental concentration of minor elements

and Zn in all the medicinal herbs are shown in Fig.III.5. Though Fe content in *jatamansi* and *bakuchi* seeds is ~1 mg/g but *amaltas* and *laghu haritaki* also exhibit significant amounts (>0.6 mg/g) whereas all others have < 0.3 mg/g. Apparently Zn content varies in a small range of 13.5-60.0 μg/g. Similar histograms of Cr, Co and Cu in medicinal plants are shown in Fig.III.6. Apparently *jatamansi* is most enriched in Cu (36.8μg/g) whereas other herbs contain Cu in a much smaller range of 2.69-14.0 μg/g. Same pattern was observed for Cr with *jatamansi* exhibiting highest (8.19 μg/g) amount, whereas all other herbs contain < 3 μg/g. In most herbs, Co content is the lowest (≤ 1 μg/g) but varies in a wider range (0.07-1.26 μg/g) with highest content in *jatamansi*.

There are several literature reports suggesting interrelationship of K and P. We have plotted K/P ratio as shown in Fig.III.7. *Ashwagandha*, *kalmegh* and *sariva* exhibit K/P ≥20 whereas in 8 herbs it is in the range of 5-10 and only two samples show K/P <5. In general, it can be said that K

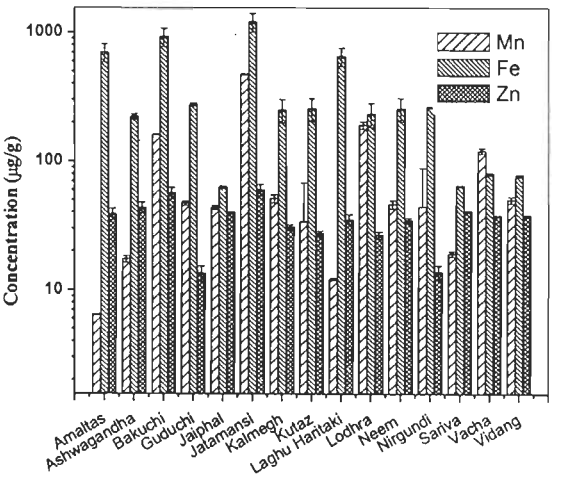

Fig.III.5 Variation in concentrations of Mn, Fe and Zn in medicinal herbs with ±1σ as error bars

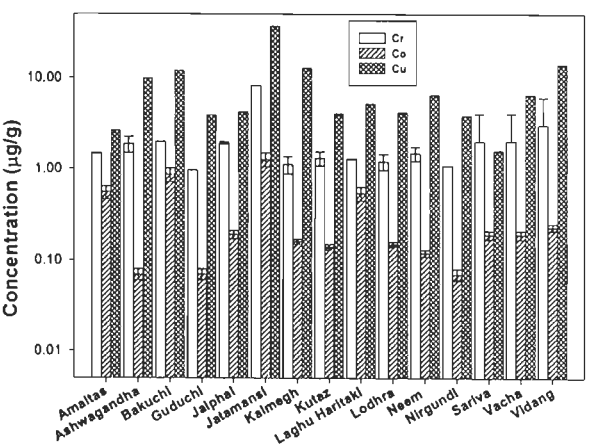

Fig.III.6 Concentration of Cr, Co and Cu in medicinal herbs

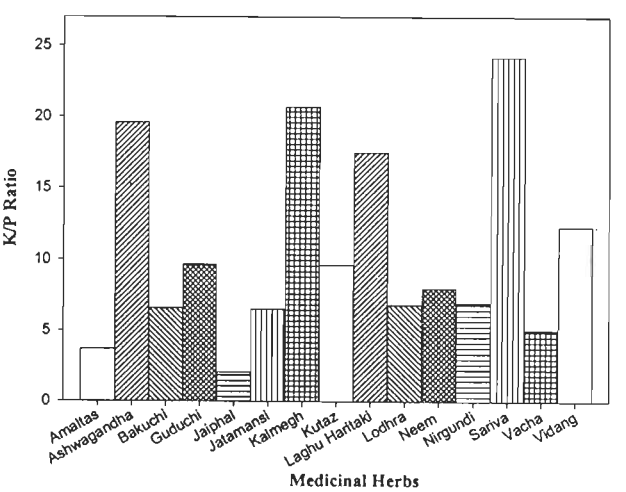

Fig.III.7 Variation of K/P ratio in medicinal herbs

content in plant samples is ≥ 10 times higher than P content. Fe and Mn, both essential elements for biochemical processes, are poorly correlated in medicinal plants. A plot of Fe with Mn shows poor relationship (Fig.III.8) with r = 0.645. This may perhaps be due to the fact that we are considering different parts of plants such as fruits, seeds, leaves, stem and roots of the herbs. Interestingly, when Zn and Cr contents in 6 leaves samples only were considered then a near linear relationship with r = 0.926 was observed as shown in Fig.III.9. In general, it may be mentioned that interrelationship of several of the medicinal herbs suggest synergistic or antagonistic effect among the elements present, thus providing various elements to the body in a balanced manner.

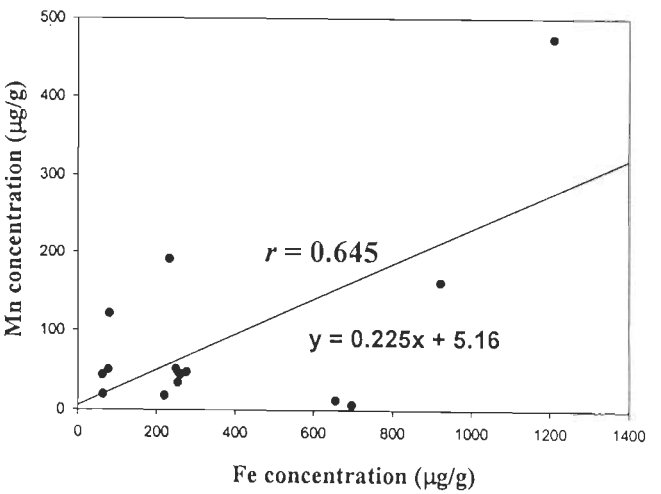

Fig.III.8 Variation of Fe with Mn in different medicinal herbs

These crude drugs used over centuries might have been acting like multi drug therapy in restoring the ionic balance and as the trace element fortification in its natural form. Although little is known about the precise molecular mechanism of the trace element contents in medicinal herbs, the sustained use of these medicinal herbs by the patients and the relief in curing suggests that these herbs provide trace elements in bioavailable/assimilable form that might be responsible for the pharmacological

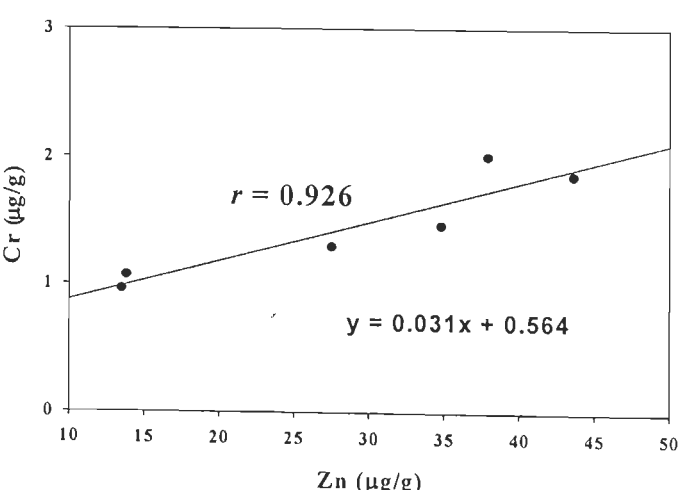

Fig.III.9 Plot of Zn vs Cr in six leaves samples of medicinal plants

action. The elucidation of elemental speciation in medicinal herbs will help to interpret the therapeutic action and in designing chemically pure medications. It has been well demonstrated that INAA, with the multi elemental capability over a wide range of concentrations, its blank free nature and minimum sample preparation is unique for such studies. Toxic trace elements Sb, Cd, Pb including radioactive element Th are present in all the samples but these are well within WHO permissible limits.

(iii) Inter laboratory Comparison study: Elemental concentrations in SRM-1575a (Pine Needles) and SRM-1547 (Peach Leaves) are listed in Table III.2. Standard deviations were calculated on the basis of triplicate analyses and different irradiation sets. Comparison of our analytical data as illustrated in Fig.III.10 with the certified values for candidate standard reference material of biological origin shows that;

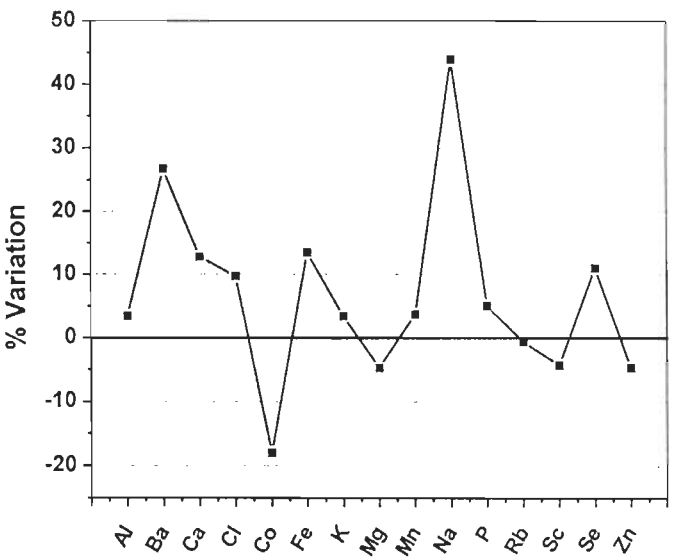

Fig. III.10 Percent variation in Element Concentrations with Certified Value

- Our data for at least eight elements (Al, K, Mg, Mn, P, Rb, Sc and Zn) are in excellent agreement (< ±5%) with the certified values.
- Our data for P obtained by neutron irradiation followed by β^- counting in both cases are in excellent agreement (±5%) with the certified values. Thus our method for phosphorus determination seems to provide good quality data for biological samples.
- Our data for Cl is in agreement within ±10 %.
- However, our data for Ca, Fe, and Se are on the higher side within ±10-15 %.
- Our data for Na is just off by ~40% but the final report mentions only information value. It may also be due to some contamination from sweat. However, data for Ba are higher by ~25%.

- We had also reported our data for Br, Ce, Cr, La, Sb and Th (Table III.2) for which no certified or information values are available.

III.8 CONCLUSION

From our analytical data on 15 commonly used medicinal herbs (6 leaves, 5 fruits, 2 roots and 1 each of seed and bark), following generalizations can be made;

- Elemental contents vary in a wide range depending on the nature of herb and the plant part.
- *Jatamansi* (root) is most enriched in Mn (474±5 μg/g), Fe (1210±200 μg/g), Cu (36.8 μg/g), Cr (8.19±0.04 μg/g), Co (1.26±0.22 μg/g) and Zn (60±6 μg/g) and these contents may be responsible for to its pharmacological action.
- K/P ratio for most herbs (n=8) lies in a narrow range of 5-10 whereas for 5 herbs it varies in a large range of 10-25.
- Fe and Mn are poorly correlated (r=0.645) but Zn and Cr in leaves (n=6) alone are better correlated (r =0.926).
- Toxic elements such as Cd, Ni, Sb and Pb are present in insignificant amounts and well below WHO permissible limits.
- Our data for SRM-1575a are, in general, good agreement with the recommended/information values. Out of 24 elements, our data for 3 elements (Na, Ba and Co) were, however, in poor agreement. For 9 elements where no recommended/information values were available, our data may be considered as reference by future workers.

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CHAPTER IV

HERBAL PREPARATIONS

1. A part of this work on *Trifala* has been published in ***J. Radioanal. Nucl. Chem.*, 263,** 751-758 (2005).
2. A part of this work on *Pagya-peya* has been published in ***J. Pharmaceut. Biomed. Anal.*, 37,** 631-638 (2005).

Herbal medical care, considered as complementary or alternative medicine (CAM), is a discrete clinical discipline with least adverse effects and well distinguished by British Medical Association [1]. Ancient Ayurvedic and Chinese healers knew that herbs were the repositories of the most concentrated form of nature's intelligence [2]. Researchers today are confirming that herbs contain the richest mixture of photo chemicals-natural chemicals such as bioflavonoids that offer medicinal and nutritional value. Herbal formulations made with whole herbs, as *Ayurveda* supplements, work more like foods than like drugs in the body. They offer potent nourishment to the cells and tissues and correct imbalances in physiology over time and safety, without causing side effects. Ayurvedic formulation as a whole is an expression of a blessing from *Rigveda*, "*Jeevema sharadah shatam*". It also represents the quest of mankind for a "panacea" which could address a wide array of health issues from aging to cough and common cold [3].

IV.1 SCIENCE OF HERBAL COMBINATIONS

Herbal formulations are blends rather than single herbs or oils. They epitomize the science of combining herbs at its sophisticated best. The herbs in formulations are carefully selected and combined to strengthen the formula in different ways [4]:

- ✓ Primary herbs offer targeted benefits for a specific area of health such as energy or a part of body.
- ✓ Supporting herbs reinforce the healing action of the primary herbs.
- ✓ Bioavailability of nutrients in herbs helps the body assimilate them easily.
- ✓ Herbs 'co-factors' remove impurities and the toxic byproducts of incomplete digestion from the body.
- ✓ Balancing herbs cancel out any potential discomforts or side effects that can come with the benefits of a particular herb.
- ✓ Combination of herbs in a definite proportion works in a synergistic way to enhance their efficacy for the cure of a disease.

Together these different types of herbs create a whole that is greater than the sum of its parts. The ancient Ayurvedic texts maintain, how a formulation is prepared and that is crucial to its efficacy. Processing of Ayurvedic herbs is, therefore, a meticulous and highly developed science that ranges from instructions on when to harvest a specific herb to the sequence in which ingredients are to be added to a formulation. The formulations are prepared meticulously as laid down in the Ayurvedic texts and taken in a specific way with dietary restrictions.

Some combinations like *Trifala* (*amalaki, Emblica officinalis; bibhitaki, Terminalia belerica* and *haritaki, Terminalia chebula*), *Trikatu* (black *pepper, Piper nigrum; pipali, Piper longum* and dried *ginger, Zingiber officinale*), *Trimad* (*Nagarmotha, Cyperus rotundus; Chitrak, Plumbago zeylanica* and *vidang, Embelia ribes*), *Chaturbeej* (fenugreek seeds, *Trigonella foenumgraecum*, *chandrasur*, *kala jaji* and *yawami*) etc. are the herbal preparations well known for curing of varióus chronic diseases [4,5]. We have analysed two formulations, *Trifala*-a mixture of three fruits and *Pragya-peya*, a herbal tonic of 12 constituents.

IV.2 *TRIFALA*

Trifala is among the most common formulations used in Traditional Ayurvedic Medicine (TAM). It is composed of (*Tri* means three, *Fala* means fruit) three fruits of Indian gooseberry (amalaki, *Emblica officinalis*), belleric (bibhitaki, *Terminalia belerica*) and chebulic myrobalan (haritaki, *Terminalia chebula*) in equal amounts. Each of the constituents (Fig.IV.1) has a variety of chemical constituents and uses [3] as listed in Table IV.1. It also acts as a powerful antioxidant and antibacterial with anticancer properties, which support the body strength [6-8]. Because of its high nutritional value, *Trifala* uniquely cleanses and detoxifies at the deepest organic level without depleting the body's reserves. It possesses some anthroquinones, which help to stimulate bile flow and peristalsis. The nutritional aspect is more in the form of its high vitamin C content, the presence of linoleic oil and other important nutrients, which it makes more

Fig. IV.1 Photograph of Fruits of Amalaki, Bibhitaki and Haritaki

of a tonic [9]. Some of the scientific researches and practical experiences of people using it down through the ages has demonstrated that *Trifala* is an effective blood purifier that significantly reduces serum cholesterol and lipid level. It is regarded as a kind of universal panacea and it is the most widely prescribed herbal formula throughout India. Its anti-microbial and anti-inflammatory activity is useful in the treatment of ophthalmic disorders [10]. Mahdisihassan [11] has suggested that *Trifala* has Arabic and Chinese synonyms. Patel [12] patented a *Ayurvedically* prepared herbal formulation of several medicinal herbs including *Trifala*, which is used for relieving headache, blood constipation, tape worm, haemorrhoid, constipation and stomach acidity symptoms.

Table IV.1. Uses of *Trifala* constituents as described in Ayurvedic literature

Sanskrit/Hindi Name (English) Botanical	**Chemical Constituents** [13]	**Uses** [5,13]
Amalaki/Amala (Emblic Myrobalan) *Emblica officinalis*	Vitamin C, Nicotinic acid, Tannins, Gallic acid, Ellagic acid, Linoleic acid, Linolenic acid, Oleic acid.	Diabetes, Hysteria, Jaundice, Eczema, Piles, Diarrhoea, Menarrhagia, Scurvy, Rebuilds and maintains new tissues and increases red blood counts,
Bibhitaki/Baheda (Belliric Myrobalan) *Terminalia belerica*	β-sitosterol, gallic acid, Ellagic acid, Chebulic acid, Manitol, Oxalic acid, Galloyl, Galactose, Fructose.	Cough, Asthma, Anorexia, Vomiting, Arthritis, Throat disorder, Fever, Epilepsy, Spleenomegaly, Piles, Diarrhoea, Leprosy, Brain tonic and laxative.
Haritaki/Harad (Chebulic Myrobalan) *Terminalia chebula*	Tannic acid, Gallic acid, Chebulinic acid, Anthroquinine, phosphoric acid, Succinic acid.	Constipation, Haemorrhoid, Skin disease, Asthma, Dysentery, Uterine debility, Anaemia, Diabetes, Leucoderma, Tumors and Heart disease.

It helps in healing ulcers in cases of pyroderma gangrenosum and in the treatment of Lipoma. It is effective in preventing superoxide induced haemolysis of the red blood cells. It also prevents lipid peroxidation induced by Fe^{3+}/ADP/Ascorbate system in liver mitochondria [4]. Water extract of *Trifala* is also widely used for eye diseases including the treatment of conjunctivitis, progressive myopia, the early stages of glaucoma and cataract. It is taken daily both internally as well as externally as eyewash. It can be used as a powder or a paste (by mixing with ghee) or as a decoction [9]. *Trifala* and its constituents prevent aging and impart longevity, immunity and body

resistance against many diseases, improve mental faculty, vitality and luster to the body.

According to a research review by Pandey et al. [14] *Trifala* has been found to be non-toxic. Animal studies have shown that *Trifala* increases growth in young animals, and stimulates the increased consumption of feed and water. *Trifala* has been shown to increase the mass and contractile strength of the heart, with no adverse effects noted through blood analysis. *Trifala* was found to reduce inflammation caused by toxins and arthritis, and reduce pain and writhing in animals poisoned with acetic acid. In large doses, *Trifala* was found to be hypoglycemic [12] and is shown to cure diabetes.

Because of its widespread uses, it is now available in raw, powder as well as tablet form and marketed by a large number of pharmaceutical firms and it available at grocery stores. Walimbe and Kulkarni [15] studied the toxicology and safe dose of *Trifala* and found to be useful even for long periods. Though its safe dose is 8.0 g/kg in human beings of 70 kg wt. but physicians prescribe varying doses depending on physical condition of the patient and other factors.

(i) Literature Survey: In recent years, several researchers have investigated various properties of *Trifala* using modern instrumental methods and clinical practices over animal models or humans [6-12,14-25]. Bahulikar et al. [16] employed thin layer chromatography (TLC) to demonstrate the presence of gallic acid used as a marker for standardizing the formulation. Lalla et al. [17] used high performance TLC for qualitative and quantitative evaluation of tannin containing herbs. Kaur et al. [7] evaluated antimutagenic potential of its water, chloroform and acetone extracts. These authors have further used UV, ir, ^{1}H and ^{13}C NMR for the identification of polyphenolic compounds in acetone extract. Vani et al. [6] studied in vitro antioxidant potential of *Trifala* by DPPH radical scavenging, superoxide and peroxyl radical scavenging and linoleic acid peroxidation to show its efficacy in preventing superoxide-induced haemolysis of red blood cells. The extracts also prevented lipid peroxidation induced by Fe^{3+}/ADP/ascorbate system in rat liver mitochondria. Gaind et al. [18] reported its anthelmintic activity and reported that *haritaki* was the most potent of three components. Jagetia et al. [19] studied its radioprotective effect in the mice exposed to gamma radiation. The highest protection against gastrointestinal (GI) death was observed for a

dose of 12.5 mg/kg *Trifala*, where highest number of survivors were reported up to 10 days post irradiation of 10 Gy. Sabu and Kuttan [20] used 75% methanolic extract of *Trifala* for inhibiting lipid peroxide formation and to scavenge hydroxyl and superoxide radicals. It has been shown that the oral administration of the extracts (100 mg/kg body wt) reduced blood sugar level in normal and alloxan diabetic rats. The three fruits, viz. *Emblica officinalis*, *Terminalia belerica* and *Terminalia chebula*, have proven to have antioxidant and anti-HIV capacity [19]. *Trifala* is an efficacious cardiotonic mixture, which is also prescribed for symptoms of inflammation, heat, infection, obesity, anaemia, fatigue, candida, poor digestion, tuberculosis, pneumonia and AIDS [7,22]. Kaur et al. [8] examined the cytotoxic, antimutagenic and apoptotic activities of chemically defined fractions of *Trifala*. Jayajothi et al. [23] evaluated it for antioxidant activity by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging method from total phenolic content by Folin-Ciocalteu method and gallic acid equivalent by HPTLC method. In a recent study Bahulikar et al. [24] used IR spectrophotometry for the identification and characterization of *Trifala* based on characteristic IR frequencies for the herbal drug. Jagetia et al. [25] examined nitric oxide (NO) scavenging activity of the extracts of various polyherbal drugs including *Trifala* by using sodium nitroprusside as in vitro NO donor. These findings have helped in explaining the pharmacological activities like rejuvenating, adaptogenic, antiinfection, anti-inflammatory, cardioprotective and neuroprotective characteristics of this clinically non-toxic drug.

IV.3 *PRAGYA-PEYA*

Pragya peya is a product of Shantikunj, Haridwar (India). It preserves energy, enhances vitality and memory, and also acts as safeguard against body disorders. *Pragya-peya* is often recommended for cold and cough and widely used as nervine tonic and stimulant. It is a mixture of 12 herbs as shown in Fig. IV.2. three of

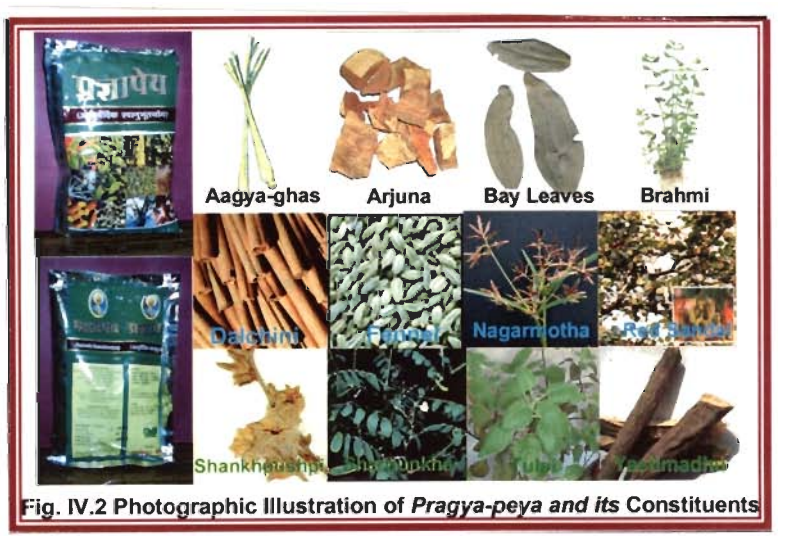


Fig. IV.2 Photographic Illustration of *Pragya-peya and its* Constituents

Table IV.2: Nomenclature and medicinal uses of constituents of *Pragya-peya*

General Name	English Name	Family	Botanical Name	Uses [2,3,11]
1. Aagya-has	Rosha Grass	Poaceae (Graminae)	Cymbopogon schoenanthus	Heart disease, Cough, Colic, Rheumatism, Cold, Fever, Indigestion,
2. Arjuna	Arjuna bark	Combretaceae	Terminalia arjuna	Heart and Liver disease, Cardiovascular system, Styptic, Tuberculosis, Cough, Dyscrasia, Fever, Ulcer
3. Bay Leaves	Bay Leaves	Lauraceae	Cinnamomum tamala	Asthma, Abdominal disorder, diarrhoea, Diabetes, Dyspepsia, Cough and anti-inflammatory
4. Brahmi	Bacopa	Scrophulariaceae	Bacopa monnieri	Skin disease, Nervous disorder, Memory enhancement, Leprosy, Tuberculosis, Anaemia, Cough, Increases blood protein and RBC, Hepatoprotactive
5. Dalchini	Cinnamon	Lauraceae	Cinnamomum zeylanicum	Flu, Indigestion, Mouth wash, Cough, Heart Disease
6. Fennel	Fennel	Umbellifereae	Foeniculum vulgare	Fever, Urine inflammation, Thirst, Dysentery, Diarrhoea, Cholera, Spleenomegaly, Analgesic, Brain tonic, Antacid, Kidney disease
7. Nagarmotha	Nutgrass	Cyperaceae	Cyperus rotundus	Dyspepsia, Skin disease, Diarrhoea, Thirst, Fever, Stimulant, Wound healer, Hair growth
8. Red Sandal	Red Sandal	Leguminosae	Pterocarpus santolinus	Cough, Blood purifier, Fever, Headache, Skin Disease
9. Shankh Pushpi	Shankh Pushpi	Convolvulaceae	Convolvulus pluricaulis	Brain and heart tonic, Reduces blood pressure and tension, Effective on thyroid high secretion, Epilepsy, Insomnia
10. Sharpunkha	Wild Indigo	Leguminosae	Tephrosia purpurea	Flatulence, Indigestion, Diarrhoea, Cough, Asthma, Liver-spleenomegaly, Oedema, Leprosy, Dyspepsia
11. Tulsi	Sacred Basil	Lamiaceae	Ocimum sanctum	Bronchitis, Malarial fever, Asthma, Urinogenital disorder, Vomiting, Indigestion, Ear ache, Dyscracia, Hepatoprotective, Spermatopoitic, Antibacterial, Antituberculosis
12. Yastimadhu	Liquoric Root	Leguminosae	Glycyrrhiza glabra	Cough, Vomiting, Wound healing, Haematemesis, Thirst Cholera, Skin disease, Spermatopoitic, Heart disease, Epilepsy

these are commonly used spices. Each one of these has its own medicinal importance in day-to-day life as mentioned in Table IV.2. Unlike *Trifala, Pragya-peya* is not so well known and therefore its properties have not been investigated scientifically or clinically. However, the constituents are well known and in some cases, their scientific properties have been investigated. A brief discussion of individual constituents follows.

(i) Aagya-ghas (*Cymbopogon schoenanthus*) grows wildely in Thar deserts of Jaisalmer. Shahi et al. [26] studied its chemical and pharmacological properties. It yields essential oils, rich in terpenoids such as sesquiterpene, hydrocarbons and limonene, which are abundantly used as odourants in perfumeries. Ketoh et al. [27] studied the insecticidal activity of essential oil extracted by steam distillation and found significant insecticidal activity at 6.7 μL/L.

(ii) Arjuna (*Terminalia arjuna*) is a long tree 20-25 m high and grows in downhill areas of Himalaya, Bihar, Madhya Pradesh and Bengal. It is rich in glucosides, tannin, ellagic and arjunic acids [5,13]. It is a well known heart tonic and is used extensively in cardiac debility [13]. Prasad et al. [28] studied antiallergic and anti-asthmatic activities of its alcoholic extracts and related it to membrane stabilizing potential and inhibition of antigen induced histamine and acetylcholine release. Naidu and Swamy [29] determined changes in Ca, Mg, S and Na concentrations at monthly intervals in leaves, stem and roots of *Arjuna*. Siddiqui et al. [30] prepared extract of its bark and studied its hypotensic activity. Also a new phyto constituent was isolated and its structure was elucidated. Kaur et al. [31] studied antimutagenic activity of acetone and methanol fractions.

(iii) Bay leaves (*Cinnamomum tamala*) tree grows in Himalaya region at ~1500 m height and is primarily a spice well known for its typical aroma. Mir et al. [32] analysed hydrodistilled oil for chemical composition by capillary GC and GC-MS. The leaf volatile oil contained 40 constituents, of which high proportions were monoterpenes (65.6%). Semwal et al. [33] reported pro-oxygenic activity of various organic solvent fractions.. Ahmed et al. [34] analysed its water distilled essential oil by GC-MS and identified 63 compounds including β-caryophyllene (25.3%), linalool (13.4%) and caryophyllene oxide (10.3%). Singh et al. [35] isolated several flavonoids such as kaempferol, quercetin, myricetin, kaemoferol-3-O-rhamnoside and quercitrin.

(iv) Brahmi (*Bacopa monnieri*) grows throughout India up to 1200 m elevation in moist or wet places such as on borders of water channels, well, irrigated fields. It has been traditionally used for nervous disorders, epilepsy, senility, premature aging, hair loss, chronic and obstinate skin conditions and venereal diseases. It is excellent for eczema, fever, immune system problems, rheumatism and ulcers. Brahmine and herpestine alkaloids have been isolated from its leaves [5,13]. Deepak et al. [36] determined the major saponin mixture bacoside A by HPLC and found concentration ranges in samples collected from different regions of India. In Ch. VI are described details of bioassay and other properties.

(v) Cinnamon (*Cinnamomum zeylanicum*) is an important spice tree cultivated in India and Sri Lanka. On steam distillation, cinnamon bark yield volatile oils of varying composition. Kaul et al. [37] investigated the essential oil profiles from pedicels of buds, flowers and fruits by GC, GC-MS. Kitazuru et al. [38] studied the antioxidant activity by using β-carotene/linoleic acid co-oxidation. Irradiation up to a dose of 25 kGy did not affect the antioxidant potential of cinnamon compounds.

(vi) Fennel (*Foeniculum vulgare*) is a scented shrub of 2-3 m height. Its seeds are rectangular/cylindrical of 6-7 mm length. Angelova et al. [39] studied the accumulation of heavy metals from contaminated soil. Raina et al. [40] analysed the fennel seeds by GC and GC-MS and identified anethole, mechavicol, l-fenchone and l-limonene as major constituents in oils.

(vii) Nagarmotha (*Cyperus rotundus*) grows all over India up to 2000 m altitude, especially in the river banks. A perennial herb grows 0.30-1 m tall. The essential oil of the plant contains at least 27 components comprising sesquiterpene hydrocarbons, epoxides, ketones, monoterpenes and aliphatic alcohols [5,13]. Its paste relieves itching and reduces foul odour due to excessive sweating. It is one of the best herbs in digestive disorders, toothache, vomiting, diarrhoea colitis etc. It is a keen stimulant for appetite. Haider et al. [41] analysed *nagarmotha* for the essential trace elements and heavy metals, which depend on geo-climatic conditions of the region.

(viii) Red sandal (*Pterocarpus santolinus*) is found mainly in southern parts of India in the hills of Cuddapah, S. Kurnool, N. Arcot and Chunglepet. It is a 10-15 m long tree, which grows up to 500 m in Mysore, Kurg and Tamilnadu [2]. There are several varieties of

sandalwood and considered to be very auspicious. It is also used as a natural colorant and its paste has soothing effect in hot summers [13].

(ix) Shankhpushpi (*Convolvulus pluaricaulis*) plant grows widely in open grassy places throughout India. It is small, diffuse, much branched, hairy with prostrate branches. The leaves are small elliptic to oblong, lanceolate, densely clothed with silky hairs and 1-2 cm long. It is very helpful in nervous debility conditions, urinary disorders and hypertension. It is one of the best herbs used as a general tonic and rejuvenative to pregnant ladies [13]. Srivastava and Deshpande [42] showed the presence of d-glucose and maltose and analysed it for fatty acids (C_{14}- C_{28}) and waxy constituents with straight chain hydrocarbons (C_{22}- C_{33}) and fatty alcohols (C_{24}- C_{32}). It is astringent, pungent and bitter in taste, sweet in the post digestive effect and has hot potency. The plant juice is traditionally used in treating mental disorders.

(x) Sharpunkha (*Tephrosia purpurea*) grows throughout India and western Himalayas, up to an elevation of 1500 m a much-branched perennial with 30-60 cm in height. Raghu [43] carried out multi-element analysis and calculated biological absorption coefficient, which were explained on the basis of bio-geochemical cycling, exclusion mechanism and bioavailability. Bhatnagar and Kapoor [44] isolated N-hentriacontanol from pod husk as well as seeds and characterized on the basis of spectral studies.

(xi) Tulsi (*Ocimum sanctum)* grows all over India in most Hindu households, temples and gardens. An erect annual grows 0.5-1.5 m in height. It has two varieties; green and purple, the latter one being more useful from medicinal point of view. Essential oil from the leaves contain eugenol, eugenal, carvacrol, methylchavicol, limatrol and caryophylline [5,13]. Reshma et al. [45] extracted ocimum flavonoids showing promising results as radioprotective in rodents. It is offered as benediction to Indian Gods, considered as very auspicious and has many medicinal properties.

(xii) Yastimadhu (*Glycrrhiza glabra*) is cultivated mainly in Punjab and sub-Himalayan tracts. A tall perennial herb grows about 1-2 m in height, with a thick rootstock. Out of 12 triterpenoids isolated from roots some are glabrolide, deoxoglabrolide, isoglabrolide, glycyrrhetol etc. [13]. Its stem part is good for cough and used as a medicine. Sato et al. [46] cultured licorice in a hydroponic system to examine the relation between the concentration of nutritional solution applied and glycrrihizin content to determine the

optimal nutrient solution for licorice production. Statti et al. [47] determined the variability of active constituents of antibacterial and antifungal activities of the extracts.

Besides organic constituents in various medicinal herbs, several Indian workers have analysed individual herbs such as *tulsi, brahmi, arjuna* etc for their elemental contents by using NAA, AAS and EDXRF. Rajurkar and coworkers [48] reported the analysis of *tulsi*, *brahmi* and *nagarmotha* for Mn, Na, K, Cl, Al, Cu, Co, Pb, Ni, Cr, Cd, Fe, Ca, Zn and Hg contents by NAA and AAS. Singh and Garg [49] analysed *amalaki, bibhitaki, haritaki, tulsi, brahmi, shankhpushpi, fennel, nagarmotha* and *arjuna* bark for 20 elements (As, Ba, Br, Ca, Cl, Co, Cr, Cu, Fe, K, La, Mn, Na, P, Rb, Sb, Sc, Se, Sr and Zn) by INAA. Naidu et al. [50] also analysed *tulsi* for 20 elements by INAA. Miyamoto et al. [51] employed INAA, RNAA and IPAA for the determination of up to 26 elements in cinnamon. Ekinci et al. [52] from Turkey analysed *yastimadhu* for P, Cl, K, Ca, S, Al, Ti, V, Rb, Sr, Zr, Nb, Mo, In, Sn, I and Ce by using EDXRF. Zaidi et al. [53] studied the trace element contents in fennel and *yastimadhu* where elemental variations have been attributed to difference in origin and other factors.

IV.4 PARTICIPATION IN INTERCOMPARISON STUDIES

During past one decade Prof. R. Dybczynski from INCT, Poland has been developing RMs of biological origin. In continuation of tobacco leaves standards CTA-OTL-1 and CTA-VTL-2, he proposed two candidate RMs Tea Leaves (INCT-TL-1) and Mixed Polish Herbs (INCT-MPH-2). First one was prepared from black tea processed in Argentina and procured through AGROS (Poland) [54] and the second (MPH-2) was prepared by mixing 9 herbs (Table IV.3) supplied by HERBAPOL S.A. (Poland) respectively. MPH-2 forms a medicament called "Cholegran" promoting excretion of bile and showing antiphlogistic and antispasmodic action and stimulating digestion [55]. We were invited to participate in the analysis of these RMs and results were submitted in May 2001. Along with these a control material was also sent for method and data validation.

Table IV.3 Constituents of Mixed Polish Herbs (INCT-MPH-2)

Polish name/ Part used	English name	Latin name	Proportion
Rzepik pospolity Whole plant	Common Agrimony	Agrimonia eupatoria	7.0
Szanta zwyczajna Whole plant	White Horehound	Marrubium vulgare	7.0
Serdecznik Whole plant	Motherwort	Leonurus cardiaca	7.0
Rumianek Flower	Wild Chamomile	Matricaria chamomilla	7.0
Kruszyna Bark	Alder Buckthorn	Frangula alnus	11.0
Kocanka Flower	Everlasting	Helichrysum arenarium	7.0
Mi' ta Leaf	Peppermint	Mentha piperita	11.0
Rzewie1 Root	Medicinal Rhubarb	Rheum officinale	7.0
Lubczyk Root	Garden Lovage	Levisticum officinale	7.0

IV.5 PRESENT STUDY

In order to investigate the claims of Ayurvedic physicians regarding possible role of essential and trace elements in the cure of many diseases, it is essential to analyze the herbal formulations for their authentication in a systematic manner. In view of this, present work was initiated to analyze two herbal formulations. Nine different brands of *Trifala* powder including a tablet were analysed for 29 elements by NAA and AAS. As some researchers have reported gallic acid and used for its standardization, it was separated chromatographically and identified by various spectroscopic techniques including ir, ^{1}HMNR, ^{13}CNMR and GC-MS including elemental analysis. Similarly, another formulation *Pragya-peya* - a herbal tonic with its twelve constituents were analyzed for 26 essential and toxic elements by INAA and AAS. In order to study variation in batch products, elemental contents were determined in three batches collected at different intervals. Also four samples each of *brahmi* and *tulsi* collected from different locations including a sample from a pharmaceutical firm were analysed. Some inter-elemental correlations were attempted.

IV.6 EXPERIMENTAL

(i) Sample Collection and Preparation: Out of 9 brands of *Trifala,* 7 were from different pharmaceutical firms as mentioned in Table IV.4.and two were local brands. Two batches each of three constituents in powdered form were procured from Shanti Kunj, Haridwar, India. *Pragya-peya*, a product of Shantikunj, Haridwar is a mixture of 12 herbs. Three different batches of samples were procured with a time interval of 6 months each in a year. All the individual constituents in dried and raw form were procured from Shantikunj and Yogi Pharmacy. Surface contaminants of raw products were wiped with tissue paper and then useful parts were separated. All these were dried in an oven at ~80 ^{0}C and were powdered in agate mortar.

Table IV.4 Details of *Trifala* brands

Brand	Name	Manufacturer	Batch No.
# 1	Baidyanath	Shree Baidyanath, Ayurved Bhawan Ltd., Gwalior Road, Jhansi	11, 7/2001
# 2	Dabur	Dabur India Ltd., P.O. Daburgram, Jharkhand- 814 132	1076, 11/01
# 3	Hamdard	Hamdard (Wakf) Laboratories, B/1-2, III, Industrial Area, Ghaziabad (U.P.)	41, 06/01
# 4	Himalaya	The Himalaya Drug Company, Makali, Bangalore-562 123	20110-ML, Jan. 2002
# 5	Surya	Surya Herbal Limited, C-33, Sec-59, Noida-201 301	0049, 09/2001
# 6	Zandu (powder)	Zandu Pharmaceutical Works Ltd.,	MTC-133, Mar. 01
# 9	"(Tablet)	70, Gokhale Road South, Mumbai-25	MTF-501, Apr. 2004
	Local	Roorkee	
# 7	Local	Powdered constituents were mixed.	
# 8			

All the samples of *Trifala, Pragya-peya* and their constituents were further powdered in agate mortar and passed through 100 mesh sieve to keep the particle size

uniform. All the samples were oven dried at 80 °C for 2 hrs. Two candidate RMs TL-1 and MPH-2 sent by Prof. R. Dybczynski were used as such. Other RMs of biological origin were Apple Leaves (SRM-1515), Oriental (OTL-1) and Virginia Tobacco Leaves (VTL-2) [56-58].

(ii) Irradiation and Counting: 30-50 mg each of powdered samples and RMs were weighed accurately and packed in alkathene/aluminum foil (super pure) for short (1 min) and long irradiation (3 d) respectively. All irradiation details are same as described in Ch. II. 4(ii) except that 1 min Dhruva irradiation was carried out using pneumatic carrier facility (PCF). Short lived nuclide activities were measured using a 80 cm^3 coaxial HPGe detector (EG & G ORTEC) and 4 k MCA at the reactor site and later at the Radiochemistry Division of BARC, Mumbai. Long irradiated samples were airlifted to Delhi and brought to Roorkee where γ-activity was measured using a detector system described in Ch. II. 4(iv). Irradiation and counting schedule followed and elements determined are the same as mentioned in Table II.3. Comparator method was adopted for calculating elemental contents. Details of phosphorus determination are the same as described in Ch. II.5

AAS was used for the determination of Cu, Ni, Cd and Pb as mentioned in Ch II.6

(iv) Separation of Gallic Acid: 50 g of *Trifala* (from Dabur) powder was Soxhlet extracted in ethanol. Insoluble off white crystals and dense oily parts were obtained. Of these, white crystalline material was soluble in water. The off white crystalline solid was mixed with silica gel in a petridish and dried in an oven. After drying the off white crystals along with silica gel were loaded on a silica gel column (60x2.5 cm^2) and separation was carried out using a set of solvents with increasing polarity in the order; petroleum ether< chloroform< ethyl acetate< ethanol< methanol< water. A total of 15 fractions were collected of which 6,7 & 8 fractions obtained with ethyl acetate gave a single spot on TLC plate (R_f = 0.86) in ethyl acetate: methanol (7:3) solvent system. The fractions were combined and distilled whence a colourless crystalline solid was obtained. It gave sooty flame suggesting aromatic character. Phenolic and carboxylate groups were tested and elemental analysis matched with that of gallic acid.

IV.7 RESULTS

Mean elemental concentrations of minor and trace elements as determined in 9 different brands of *Trifala* and its 3 constituents (in duplicate) are listed in Tables IV.5A, B and 6 respectively. Mean elemental concentrations of 7 minor and 15 trace elements in *Pragya-peya* and its 12 constituents are listed in Tables IV.7A & B. The concentrations of Ni, Cu, Cd and Pb in *Trifala, Pragya-peya* and their constituents as determined by AAS are listed in Tables IV.8. Typical data for two candidate reference materials Tea Leaves (INCT-TL-1) and Mixed Polish Herb (INCT-MPH-2) including three RMs, Apple Leaves (SRM-1515), and Tobacco Leaves (OTL-1 & VTL-2) are listed in Table IV.9. A comparison of our data with the certified/information values of RMs shows good agreement (within ±10 %) for most elements. Also the observed standard deviations are <±10 % suggesting good precision. Therefore, it is presumed that our data for herbal preparations *Trifala*, *Pragya-peya* and its all the constituents in Tables IV. 5, 6 & 7 must also be accurate and precise within ±10 %. C, H analysis data for the standard gallic acid (SRL Mumbai) and the separated fraction were found as follows;

For the standard (SRL, Mumbai) gallic acid (separated fraction); C = 44. 6 (45.2) % and H = 4.48 (4.57)% against calculated values of 44.68% and 4.26% respectively. On the basis of analytical data, molecular formula $C_7H_6O_2$ may be suggested.

IV.8 DISCUSSION

Implications of elemental data in two herbal formulations including variations in elemental contents and inter-elemental correlations are discussed here.

(i) *Trifala*: As already mentioned 9 different brands including a tablet were analysed. A perusal of elemental contents in Table IV.5 shows wide variations for all the elements, which may be attributed to their different origins of its constituents and processing methodology adopted by manufacturers.

Table IV.5A Elemental concentrations in different brands of *Trifala.*

Trifala Brand	Al (mg/g)	Br (µg/g)	Ca (mg/g)	Cl (mg/g)	K (mg/g)	Mg (mg/g)	Mn (µg/g)	Na (mg/g)	P (mg/g)	V (µg/g)	Sc (ng/g)	Se (ng/g)
#1	0.30±0.01	7.30±0.50	1.31±0.32	1.68±0.15	11.5±0.5	0.48±0.02	29.1±1.0	1.51±0.02	1.19±0.01	0.84±0.04	236±3	107±14
#2	0.31±0.01	5.85±0.91	0.79±0.09	0.55±0.05	13.3±0.9	0.31±0.03	32.3±2.0	0.13±0.01	0.81±0.01	0.89±0.06	226±3	113±14
#3	0.34±0.06	4.94±0.36	2.41±0.18	2.46±0.18	13.6±1.3	0.86±0.07	21.0±7.6	0.16±0.03	0.88±0.01	1.24±0.25	201±6	124±18
#4	0.13±0.01	242±27	25.1±2.0	3.25±0.11	10.9±0.2	1.19±0.06	26.0±1.0	1.29±0.09	17.1±0.1	2.20±0.10	195±3	<50
#5	0.58±0.02	29.1±1.5	2.63±0.14	2.42±0.11	12.5±1.0	0.99±0.06	18.7±1.8	0.23±0.01	0.70±0.02	1.94±0.08	150±2	130±18
#6	0.98±0.01	5.45±0.40	3.14±0.24	2.43±0.09	14.2±0.3	1.20±0.11	43.4±1.6	0.20±0.02	0.75±0.02	2.28±0.04	236±2	140±15
#7	0.76±0.06	5.30±0.61	3.20±0.20	2.00±0.11	13.2±0.8	1.20±0.20	24.6±1.5	0.25±0.01	0.76±0.01	1.58±0.15	208±10	111±4
#8	0.80±0.01	39.3±1.0	2.50±0.45	2.11±0.08	12.0±1.1	1.03±0.04	21.1±1.2	0.18±0.01	0.76±0.02	1.85±0.10	104±14	50.6±8.4
#9	0.17±0.01	5.78±0.01	2.85±0.31	3.06±0.52	12.2±0.2	<0.20	146±14	0.17±0.01	0.84±0.05	<0.50	86.5±5.5	52.9±3.1
Mean±SD	**0.49±0.30**	**38.3±77.4**	**4.88±7.62**	**2.22±0.79**	**12.6±1.1**	**0.91±0.34**	**40.2±40.4**	**0.46±0.54**	**2.64±5.42**	**1.60±0.56**	**183±56**	**104±34**

Table IV.5B Elemental concentrations in different brands of *Trifala.*

Trifala Brand	Ba (µg/g)	Co (µg/g)	Cr (µg/g)	Cs (ng/g)	Eu (ng/g)	Fe (µg/g)	Hf (ng/g)	Hg (ng/g)	La (µg/g)	Rb (µg/g)	Sb (ng/g)	Th (ng/g)	Zn (µg/g)
#1	22.4±0.4	1.04±0.07	2.85±0.62	108±3	23.1±1.7	1320±7	149±6	43.3±0.5	0.84±0.01	20.6±0.6	43.3±4.1	375±11	56.7±8.3
#2	20.2±0.3	0.92±0.06	2.58±0.55	105±3	23.7±2.7	1190±8	149±10	40.7±0.9	0.57±0.02	22.1±0.4	51.2±1.8	326±7	50.8±7.4
#3	12.3±0.2	0.92±0.07	2.57±0.56	89.3±5.3	20.1±0.4	1200±16	162±8	29.5±0.4	0.45±0.19	20.2±0.5	32.2±1.5	288±10	53.3±7.9
#4	25.7±0.7	0.63±0.09	2.03±0.38	<30	9.60±0.40	825±25	357±10	104±2	2.00±0.01	22.8±0.8	86.0±4.5	407±13	37.8±4.0
#5	22.6±2.0	0.62±0.08	1.76±0.33	67.0±9.0	11.4±0.6	900±20	227±6	88.0±2.0	2.38±0.02	21.1±0.8	97.0±5.0	116±7	37.6±4.0
#6	23.7±0.4	0.92±0.05	3.99±0.85	87.9±4.1	23.2±2.3	1170±15	158±2	25.0±1.0	0.70±0.02	20.3±0.5	68.6±6.5	400±10	49.1±7.2
#7	17.2±0.3	0.76±0.04	1.99±0.44	91.1±7.3	19.6±2.1	970±43	117±6	38.3±0.9	0.71±0.02	17.4±1.2	39.9±7.5	274±10	41.7±6.5
#8	23.3±1.5	0.32±0.02	2.61±0.31	124±6	12.2±1.1	975±75	36.0±8.0	52.7±0.5	0.43±0.04	21.3±1.4	62.3±1.8	172±12	36.0±3.8
#9	18.2±0.8	0.25±0.01	1.10±0.04	63.1±2.7	<2.0	563±26	<30	<5.0	0.85±0.05	17.3±0.1	32.5±0.6	234±9	6.69±0.35
Mean±SD	**20.6±4.1**	**0.71±0.28**	**2.39±0.81**	**91.9±20.5**	**17.9±5.9**	**1013±234**	**169±93**	**52.7±28.3**	**0.96±0.70**	**20.3±1.9**	**57.0±23.3**	**288±101**	**41.1±14.9**

Table IV.6 Elemental concentrations in *Trifala* constituents (in duplicate)

Element	*Amalaki*		*Bibhitaki*		*Haritaki*	
	#1	#2	#1	#2	#1	#2
Al (mg/g)	0.05±0.001	0.27±0.01	1.16±0.01	0.65±0.01	0.38±0.03	0.28±0.01
Ba (μg/g)	8.64±0.13	6.99±0.92	22.9±0.4	22.1±3.5	20.1±0.3	18.7±3.1
Br (μg/g)	13.8±1.8	ND	11.3±1.3	ND	4.31±0.55	ND
Ca (mg/g)	1.18±0.11	1.04±0.07	5.23±0.37	3.96±0.37	1.57±0.29	0.79±0.06
Cl (mg/g)	0.65±0.06	1.43±0.02	3.09±0.11	3.84±0.17	1.65±0.08	1.95±0.07
Co (μg/g)	0.70±0.04	0.54±0.12	3.04±0.2	0.43±0.10	0.92±0.04	0.44±0.10
Cr (μg/g)	2.18±0.48	2.11±0.19	2.47±0.54	1.70±0.10	2.31±0.50	1.58±0.14
Cs (ng/g)	5.10±0.80	ND	451±7	138±13	63.2±4.4	ND
Eu (ng/g)	6.07±1.42	18.1±3.6	27.5±0.9	15.1±3.6	17.8±2.7	34.0±4.0
Fe (μg/g)	899±4	904±104	1120±6	847±27	1150±49	732±79
Hf (ng/g)	103±2	131±18	139±5	126±16	136±8	110±14
Hg (ng/g)	27.4±0.4	ND	36.9±0.7	ND	50.7±1.7	ND
La (μg/g)	ND	0.78±0.04	1.11±0.02	0.81±0.04	0.44±0.05	0.64±0.03
K (mg/g)	10.6±0.3	9.19±0.9	19.6±1.3	17.2±0.4	13.0±0.4	11.0±0.5
Mg (mg/g)	0.41±0.06	0.63±0.03	1.50±0.20	1.51±0.12	<0.20	0.55±0.01
Mn (μg/g)	8.6±0.4	19.4±0.9	22.9±1.4	16.9±1.3	9.2±0.05	7.01±0.4
Na (mg/g)	0.06±0.01	0.17±0.01	0.38±0.03	0.17±0.02	0.11±0.01	0.08±0.01
P (mg/g)	1.00±0.02	0.50±0.01	1.01±0.01	1.19±0.02	0.69±0.01	0.62±0.01
Rb (μg/g)	4.8±0.1	17.7±1.2	34.5±0.1	31.7±7.9	18.1±1.2	19.8±3.2
Sb (ng/g)	53.2±0.9	35.7±6.6	16.0±2.6	12.2±1.2	41.2±4.4	65.7±3.3
Sc (ng/g)	122±1	175±4	263±4	157±3	189±11	148±3
Se (ng/g)	76.4±10.2	ND	240±34	ND	110±12	ND
Th (ng/g)	200±12	200±19	372±9	200±18	413±20	162±15
V (μg/g)	0.64±0.04	0.65±0.04	2.00±0.15	1.36±0.03	0.96±0.14	0.57±0.05
Zn (μg/g)	44.3±6.6	43.2±4.2	51.5±7.7	32.6±3.3	51.7±8.0	33.2±3.7

Table IV.7A Elemental concentrations of minor and trace elements in *Pragya-Peya* and its constituents

Sample	Al (mg/g)	Br (μg/g)	Ca (mg/g)	Cl (mg/g)	Fe (μg/g)	K (mg/g)	Mg (mg/g)	Na (mg/g)	P (mg/g)	V (μg/g)	Se (ng/g)
Pragya-peya*	2.05±0.27	9.18±0.98	23.9±1.3	2.71±0.41	676±176	9.37±0.92	2.23±0.41	1.02±0.17	1.20±0.04	2.78±0.38	126±51
Aagya-ghas	1.08±0.05	3.70±0.30	2.50±0.06	2.53±0.23	940±103	9.40±0.74	< 1	0.25±0.02	1.40±0.04	1.62±0.03	ND
Arjuna (bark)	0.38±0.02	13.6±1.5	102±4	3.81±0.41	203±25	6.86±0.52	1.97±0.24	0.29±0.09	0.55±0.07	0.97±0.02	96.4±19.3
Bay Leaves	0.27±0.01	2.30±0.20	6.56±0.21	0.14±0.02	915±109	9.18±0.71	1.59±0.11	0.09±0.01	1.15±0.01	< 0.5	ND
Brahmi	1.88±0.86	35.5±5.8	13.6±0.8	4.76±0.07	767±453	14.6±0.7	4.99±0.58	0.28±0.01	1.86±0.03	2.78±0.11	512±72
Cinnamon	0.13±0.01	62.5±6.0	5.46±0.22	0.57±0.04	922±50	5.06±0.61	1.13±0.09	0.09±0.01	0.70±0.01	0.80±0.02	ND
Fennel	0.25±0.05	35.0±0.8	15.1±0.1	4.88±0.34	744±20	20.0±2.0	5.11±0.42	1.69±0.06	4.18±0.34	1.09±0.01	602±52
Nagarmotha	2.93±0.10	21.7±2.0	2.74±0.10	3.23±0.05	219±15	8.27±0.52	2.05±0.09	1.06±0.03	0.85±0.05	5.19±0.08	ND
Red sandal	3.93±0.20	13.9±1.7	6.91±0.75	6.12±0.61	324±16	6.08±0.41	2.08±0.20	2.61±0.08	0.32±0.03	4.52±0.30	ND
Shankhpushpi	2.55±0.30	14.0±1.7	11.5±0.7	2.55±0.04	283±18	18.5±0.6	4.79±0.40	0.39±0.02	0.97±0.02	2.42±0.08	ND
Sharpunkha	1.83±0.10	9.80±1.20	10.4±0.8	0.99±0.12	217±28	8.85±0.63	2.59±0.32	0.31±0.03	1.92±0.11	5.08±0.19	ND
Tulsi	0.24±0.01	29.0±1.0	20.9±0.7	5.57±0.25	160±5	15.5±0.6	3.72±0.02	13.3±1.8	2.92±0.15	0.61±0.01	176±10
Yastimadhu	0.40±0.02	3.70±0.30	12.6±1.0	1.36±0.07	610±25	8.06±0.52	4.77±0.31	0.45±0.04	0.70±0.02	1.07±0.08	ND

* Mean of three different batches with SD of the mean, ( ) Certified value, { } information value

Table IV.7B Concentration of trace elements in *Pragya-Peya* and its constituents

Sample	Ba (µg/g)	Co (µg/g)	Cr (µg/g)	Cs (ng/g)	Hg (ng/g)	La (µg/g)	Mn (µg/g)	Rb (µg/g)	Sc (ng/g)	Th (ng/g)	Zn (µg/g)
Pragya-peya*	37.7±16.8	0.62±0.09	1.56±0.24	227±16	44.8±29.8	1.69±0.94	87.8±10.9	10.5±3.3	273±91	115±6	34.5±4.0
Aagya-ghas	ND	0.77±0.10	6.70±0.02	214±14	38.5±2.5	0.89±0.02	201±13	12.2±1.2	238±9	322±17	56.9±6.0
Arjuna	55.2±11.0	0.13±0.01	1.10±0.21	85.4±8.0	49.2±6.4	2.65±0.22	7.45±0.50	11.8±0.5	39.8±2.1	253±44	26.5±0.1
Bay Leaves	ND	0.72±0.10	1.90±0.01	ND	39.4±2.6	0.68±0.03	440±19	17.7±1.8	180±17	355±38	47.0±5.0
Brahmi	79.4±46.7	0.84±0.12	3.97±2.04	526±41	168±17	2.48±0.22	134±24	28.5±5.0	411±91	422±55	50.8±0.4
Cinnamon	167±6	0.83±0.10	2.07±0.01	ND	33.0±7.0	0.74±0.09	631±30	14.4±1.2	155±21	165±14	48.0±6.0
Fennel	10.7±1.0	0.54±0.10	1.50±0.35	50.1±4.7	70.4±5.8	2.58±0.23	72.3±3.0	19.8±0.9	161±22	213±41	34.5±4.4
Nagarmotha	40.7±4.0	0.16±0.01	1.22±0.31	ND	53.0±3.0	3.72±0.44	140±7	22.6±2.3	372±12	305±10	29.9±0.1
Red sandal	53.6±5.2	0.17±0.01	1.47±0.33	761±68	40.0±4.0	3.36±0.05	48.4±2.1	14.9±1.5	747±105	104±12	35.7±0.2
Shankhpushpi	ND	0.23±0.01	0.95±0.22	ND	31.0±3.0	1.47±0.07	71.8±7.8	8.80±0.90	336±20	515±15	32.2±0.1
Sharpunkha	57.7±5.5	0.14±0.01	1.53±0.41	42.8±4.3	25.8±3.2	1.74±0.04	82.1±5.0	15.3±1.6	369±19	70.0±8.0	43.0±0.2
Tulsi	68.0±3.3	0.07±0.01	1.23±0.12	32.5±3.5	115±13	1.56±0.16	40.5±2.8	9.70±0.41	127±16	22.5±3.0	30.8±4.1
Yastimadhu	20.0±2.2	0.44±0.04	2.10±0.19	ND	18.8±3.1	0.75±0.19	19.8±2.0	4.52±0.11	153±19	229±37	31.1±1.0

* Mean of three different batches with SD of the mean, ND = Not Detected, ( ) Certified value, { } information value

Table IV.8 Elemental concentrations in *Trifala, Pragya-peya* and their constituents by AAS

Sample	Ni (µg/g)	Cu (µg/g)	Cd (ng/g)	Pb (µg/g)	Sample	Ni (µg/g)	Cu (µg/g)	Cd (ng/g)	Pb (µg/g)
Trifala brand									
#1	0.78	8.20	25.0	0.65	Pragya-peya*	5.62±3.43	14.7±0.8	334±34	3.79±0.83
#2	9.55	12.8	200	2.17	Aagya-ghas	2.78	15.0	184	1.52
#3	1.25	7.20	100	1.37	Arjuna (bark)	0.61	3.80	195	0.79
#4	0.38	1.06	500	1.32	Bay Leaves	1.37	9.63	122	1.08
#5	1.50	5.60	25.0	1.24	Brahmi	4.00	14.5	425	1.40
#6	2.20	7.60	100	1.80	Cinnamon	ND	4.46	240	1.72
#7(Local)	0.70	8.50	620	1.54	Fennel	1.46	10.9	86.1	0.95
#8(Local)	0.45	9.57	330	1.42	Nagarmotha	0.76	3.77	ND	1.58
#9(Tablets)	0.70	2.56	280	1.15	Red sandal	1.54	6.85	ND	1.71
Mean±SD	**1.95±2.91**	**7.01±3.56**	**242±211**	**1.41±0.42**	Shankhpushpi	1.91	5.92	292	0.57
Amalaki #1	0.57	5.00	320	1.55	Sharpunkha	1.44	9.68	193	1.36
#2	0.59	4.02	300	1.75	Tulsi	0.65	20.1	410	1.83
Bibhitaki #1	1.23	8.38	500	1.72	Yastimadhu	ND	8.30	60.2	1.70
#2	1.05	8.16	280	1.55					
Haritaki #1	0.15	8.40	500	1.45					
#2	0.20	4.02	540	1.20					

* Mean of three different batches with SD of the mean

Table IV.9 Elemental concentrations in candidate reference materials from INCT

Element	Tea Leaves *(INCT-TL-1)*	Mixed Polish Herb *(INCT-MPH-2)*	Apple Leaves *(SRM-1515)*	Tobacco Leaves *(CTA-OTL-1)*	Tobacco Leaves *(CTA-VTL-2)*
Al	2140±150	640±40	260±15	1650±70	2180±70
(μg/g)	(2290±280)	(670±111)	(286±9)	(1740±290)	[1682]
Ba	35.4±0.5	32.6±0.5	55.0±5.0	85.5±6.1	43.0±1.2
(μg/g)	(43.2±3.9)	(32.5±2.5)	(49.0±2.0)	(84.2±11.5)	(42.7±6.6)
Br	13.4±0.7	8.10±0.45	2.50±0.32	8.96±1.1	14.0±1.5
(μg/g)	(12.3±1.0)	(7.71±0.61)	[1.8]	(9.28±1.06)	(14.3±1.4)
Ca	6.01±0.65	11.2±0.8	15.5±1.0	31.7±1.5	39.1±1.2
(mg/g)	(5.82±0.52)	(10.8±0.7)	(15.3±0.2)	(31.7±1.2)	(36.0±1.5)
Ce	0.802±0.06	1.16±0.20	2.82±0.32	2.40±0.35	1.39±0.25
(μg/g)	(0.790±0.076)	(1.12±0.10)	[3.00]	(2.69±0.30)	(1.91±0.29)
Cl	0.550±0.051	2.96±0.32	0.580±0.060	0.277±0.024	7.68±1.3
(mg/g)	(0.573±0.048)	(2.84±0.20)	(0.579±0.023)	[0.298]	(7.43±2.80)
Co	357±24	246±125	85.5±7.5	630±10	400±20
(ng/g)	(387±42)	(210±25)	[90.0]	(879±39)	(429±26)
Cr	2.16±0.47	1.78±0.45	0.35±0.03	2.33±0.3	1.97±0.18
(μg/g)	(1.91±0.22)	(1.69±0.13)	[0.30]	(2.59±0.32)	(1.87±0.16)
Cs	3240±180	64.5±5.4	ND	186±13	525±15
(ng/g)	(3610±370)	(76.0±7.0)		(177±22)	(515±46)
Fe	524±33	515±98	90.0±10.0	1050±30	1020±70
(μg/g)	[432]	[460]	(83.0±5.0)	[989]	(1083±33)
K	17.5±0.5	18.7±0.6	16.1±0.10	15.6±0.1	9.97±0.3
(mg/g)	(17.0±1.2)	(19.1±1.2)	(16.1±0.20)	(15.6±0.5)	(10.3±0.4)
La	1.25±0.15	0.580±0.02	19.5±1.5	1.42±0.01	1.10±0.15
(μg/g)	(1.00±0.07)	(0.571±0.046)	[20.0]	(1.44±0.16)	(1.01±0.10)
Mg	2.34±0.14	2.88±0.18	2.75±0.30	4.36±0.25	5.30±0.80
(mg/g)	(2.24±0.17)	(2.92±0.18)	(2].71±0.08)	(4.47±0.21)	(5.10±0.23)
Mn	1585±36	185±5	55.0±5.0	414±26	77.2±3.0
(μg/g)	(1570±110)	(191±12)	(54.0±3.0)	(412±14)	(79.7±2.6)
Na	27.0±4.0	401±20	26.0±2.4	350±10	330±17
(μg/g)	(24.7±3.0)	[350]	(24.4±1.2)	[345]	[312]
P	1.93±0.04	2.39±0.03	1.60±0.10	2.89±0.18	2.21±0.13
(mg/g)	[1.80]	[2.5]	(1.59±0.11)	(2.89±0.13)	(2.20±0.08)
Rb	77.6±5.3	10.6±0.7	9.85±1.02	9.97±0.17	47.5±2.3
(μg/g)	(81.5±6.5)	(10.7±0.7)	(10.2±1.5)	(9.79±1.27)	(48.6±2.3)
Sb	ND	62.8±4.7	15.0±2.0	105±3	300±25
(ng/g)		(65.5±9.1)	[13.0]	[75]	(312±25)
Sc	266±13	136±14	35.0±4.0	314±10	212±23
(ng/g)	(266±24)	(123±9)	[30.0]	[380]	[268]
Se	84±5	182±16	55.0±4.5	135±15	162±20
(ng/g)	[76]	{-}	(50.0±9.0)	(153±18)	[188]
Th	ND	121±12	28.0±3.0	346±2	350±20
(ng/g)		(154±13)	[30.0]	(348±54)	(378±31)
Zn	37.3±5.0	32.7±8.3	13.0±0.5	53.3±5.6	38.8±2.0
(μg/g)	(34.7±2.7)	(33.5±2.1)	(12.5±0.3)	(49.9±2.4)	(43.3±2.1)

In parentheses ( ) are certified values, [ ] are information value, in {-} no data available, ND= not detected

A. Variation in Elemental Contents in different Brands: In order to visualize the variation, elemental data for different brands of *Trifala* are plotted in Fig. IV.3 to 6. A perusal of data in Table IV.5 and Figs. IV.3 & 4 shows that in spite of the fact that different brands are derived from different pharmaceutical firms from different parts of the country, most elemental contents are in a close range without any gross difference except in a few cases. This is particularly evident from small values of standard deviations of mean (RSD, 20-40%) in Table IV. 5 & 8. It is observed from Fig. IV.3 that Ca and P contents in # 4 (Himalaya) are highest On the contrary # 6 from Zandu has higher contents of Al, Cr and Mn. Similarly # 1 from Baidyanath has highest contents of Fe, Co, Sc and Zn. In some brands Na and Cl contents were found to be higher, which may be attributed to deliberate addition of common salt so as to suppress its bitter taste. Similarly calcium phosphate may have been added as a preservative. In order to further see the large variations, ranges and means were plotted in Figs. IV.5 & 6 where Na, P, Ca, Mn, Ni and Cu exhibit variations by an order of magnitude. Br is an environmental contaminant [59] due to processing methodology as observed for Indian tea brands [60]. The large differences may primarily be attributed to their differences in origin (with regard to soil) [59] and

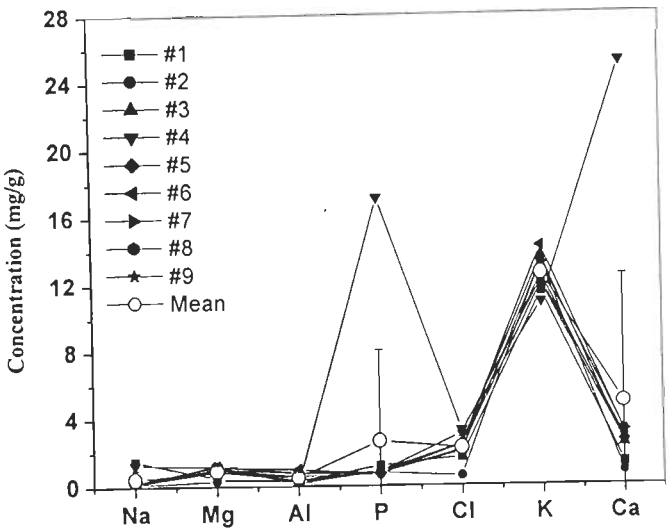


Fig. IV.3. Elemental Profiles in different *Trifala* Brands

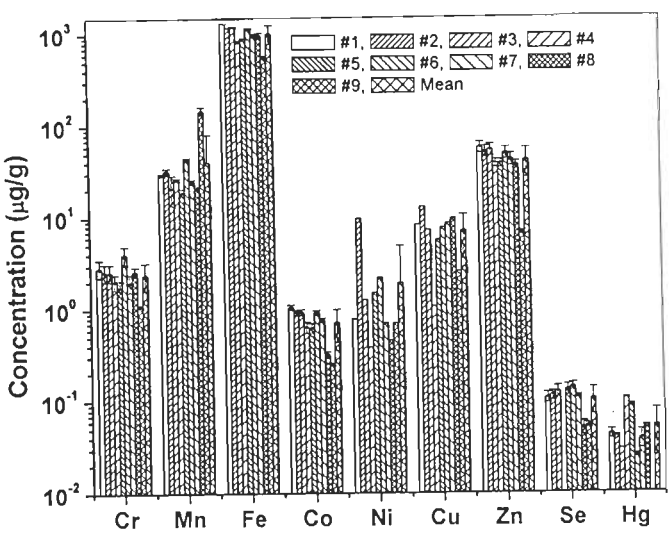


Fig. IV.4 Variation in Elemental Content in various Brands of *Trifala*

processing methodology being followed by different manufacturers. Large variation in Fe contents may possibly be due to soil content and differential contamination from iron containers where these are crushed/powdered. Some other minor differences in elemental contents especially of Se may be attributed to soil characteristics wherefrom individual constituent herbs have been derived. Therefore, it is apparent that in spite of differences in origin of different brands, elemental contents in various brands of *Trifala* are primarily similar and these are likely be due to its constituents.

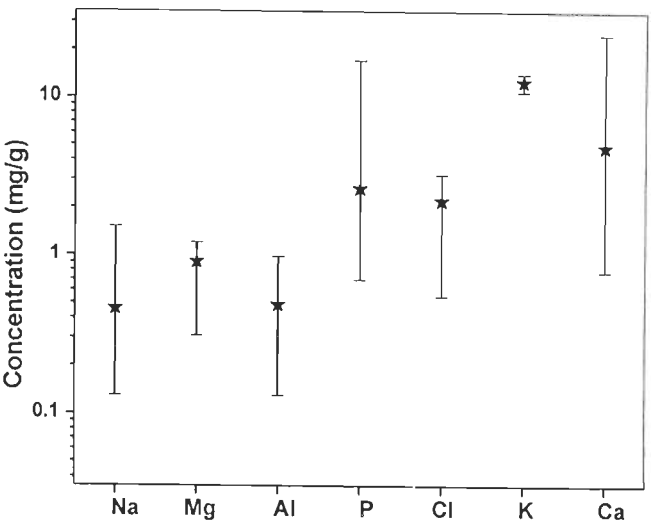


Fig. IV.5 Range and Mean Elemental Content of Nine Brands of *Trifala*

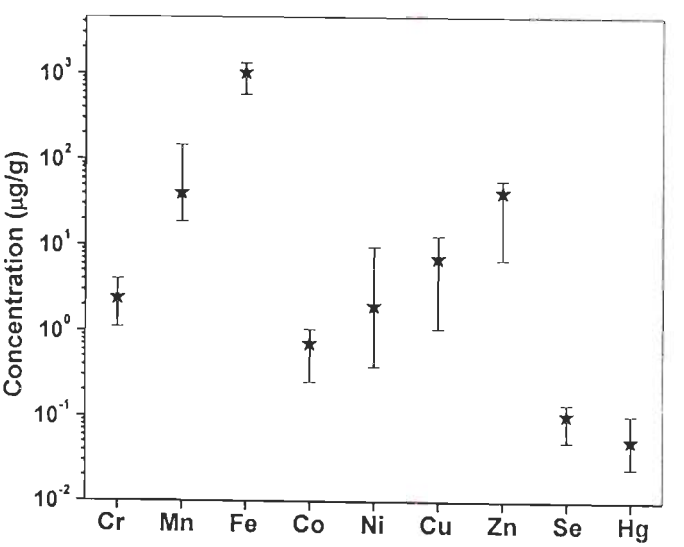


Fig IV.6 Range and Mean Elemental Contents in Nine Brands of *Trifala*

A perusal of elemental contents in Tables IV.5 & 8 suggests that *Trifala* as a whole is rich in Mg, K, Ca, Fe, and Zn, though Se is also present in significant amounts (104±34 ng/g). Particularly higher amounts of Ca (4.88±7.62 mg/g) and Fe (1013 ± 234 µg/g) could be especially useful in the treatment of constipation and for enhancing vigour and memory. Availability of Zn in the range 6.69-56.7 µg/g in various *Trifala* brands and its constituents makes it especially useful with regard to its mean daily requirement of 10-15 mg/day [61]. This is especially important because zinc deficiency has been correlated with acute and chronic mal absorption states [62] and diabetes [63]. Similarly, small amounts of Se in all the samples may be

responsible for its anticancer properties [64]. There appears to be an association between Se deficiency and protein malnutrition disease, multiple sclerosis, cancer and heart disease. It has been suggested that Se as glutathione peroxidase inhibits the replication of tumour viruses and prevents the malignant transformation of cells [64]. All *Trifala* brands and its three constituents have shown V content in the range 1-2 μg/g, which has been suggested to play vital role in the treatment of cancer and diabetes [65].

It is further observed that besides several nutrients, some toxic heavy metals such as Hg, Sb, Cd and Pb are also present. These are primarily environmental contaminants arising out due to industrial activity and automobile emissions in the area where these medicinal plants must have grown [53]. However, these are found at very low concentrations and within permissible limits [59, 61,66-68]. There are literature reports of heavy metals being detected in Chinese herbal plants [69].

B. Elemental Contents in Trifala Constituents: As already evident from Table IV.1, all three constituents of *Trifala* have widespread uses for treating a variety of ailments. In order to check homogeneity, two different batches each of the three constituents were analyzed. A perusal of data in Tables 6 & 8 shows sample homogeneity with respect to Ca, P, Se, Cr, Mn and Zn. However, a comparison of elemental contents in three constituents shows that *bibhitaki* is most enriched in Ca, K, Mg, Mn, P, Rb and Se contents. Its active constituents are gallic acid, ethyl gallate, ellagic acid, glucose, chebullic acid including 38% fixed oils and 40% proteins. It has purgative properties with higher risk for developing coronary heart diseases. Promising antimutagenic/ anticarcinogenic potential of this herb may possibly be due to potentially bioavailable form of these elements [4,5].

C. Elemental Contents in Powder vs. Tablet: Powder as well as tablet forms of *Trifala* are marketed by Zandu Pharmaceuticals, New Delhi and we analysed both so as to see the difference in elemental contents, if any. A comparison of elemental contents in *Trifala* tablet and powder is shown in Fig. IV.7 showing that most elements are in comparable range within ± 10%. However, Br, Cl, Fe, Mn, P and Zn, differ by more than 50%. It is observed that Mn and Cl are particularly high in tablet whereas Br, Fe, and Zn are much lower. This may probably be due to differences in the method of processing during preparation and also the three components coming from different geographical

locations. On correspondence, we were informed by Dr. J. M. Pathak, Manager, R & D, Zandu Pharmaceuticals informed [70] that starch paste and magnesium stearate along with talcum are added in tablet and these may be responsible for lower elemental contents in tablet. In view of these data, it may be said that by and large elemental composition in all *Trifala* samples remains same irrespective of the brand except minor differences due to geo-environmental factors.

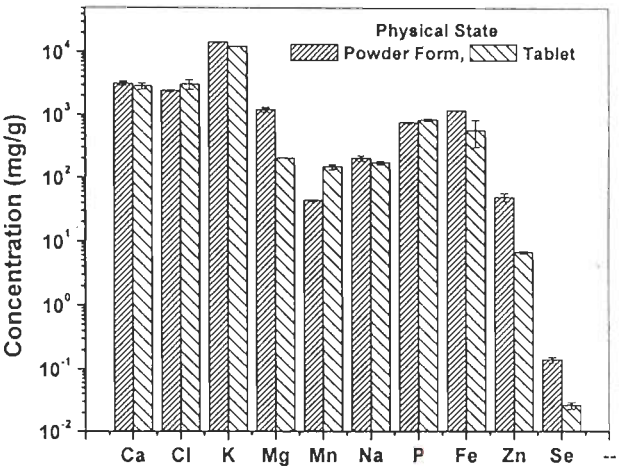


Fig. IV.7 Variation in the Elemental Contents in Powder and Tablet form of Trifala of same Brand

(ii) *Pragya-peya*: Unlike *Trifala*, *Pragya-peya* is manufactured and marketed by one firm i.e. Shanti Kunj, Haridwar only. Therefore, variation in elemental contents in different brands could not be studied. However, we did try to study the test for homogeneity in three different batches of samples collected at different intervals. Some prominent features of elemental contents and their variations are discussed here.

A. Test for Homogeneity: In order to test the homogeneity of the batch products of the herbal preparation, three different samples collected over one year period were analysed. A plot of minor constituents (Na, Mg, Al, P, Cl, K and Ca) at mg/g level in Fig.IV.8 shows very little variation with almost overlapping behaviour for all the elements. Further, the points corresponding to mean values vary in a small range of ±10%. Similarly bar plots of some trace elements (Cr, Mn, Fe, Co, Ni,

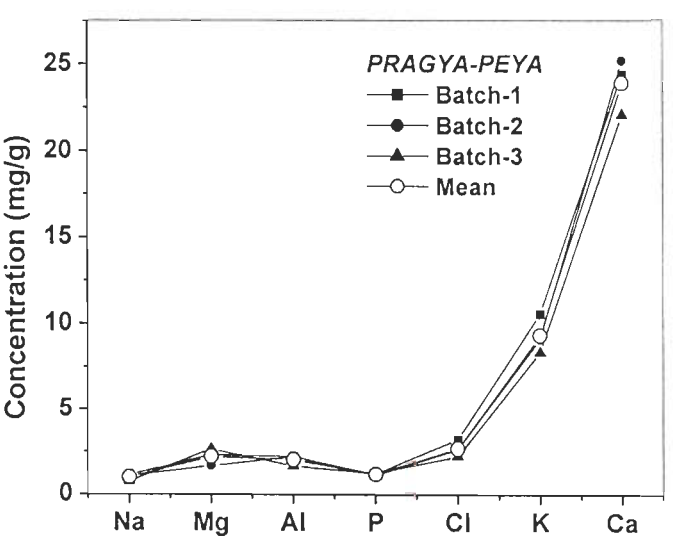


Fig.IV.8 Variation of Elemental Concentrations in *Pragya-peya* from different Batches

Cu, Zn, Se, Hg and Ba) in Fig.IV.9, also show little variation in elemental contents of three batches within a small range of ±10% except for Fe, Mn, Ni and Hg, which, however, vary in a wide range. Such variations may be attributed to soil and ecological variations under which the individual constituent herbs must have been grown [53]. Thus, our observations are in accordance with a recent study by Zaidi et al. [53] where variation is elemental contents have been attributed to ecological and geographical variations. In the present study, all three samples were procured from the same source so that large variation due to different geographical origins is not expected.

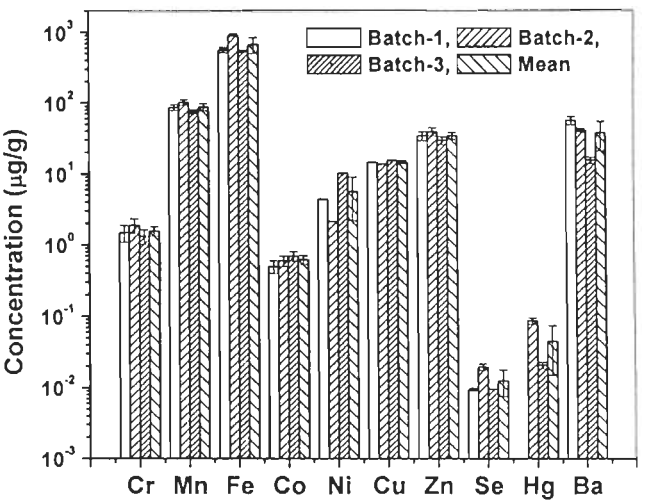


Fig. IV.9 Variation in Elemental Concentrations in *Pragya-peya* from different Batches

B. Elemental Contents in *Pragya-peya*: It contains several nutrient elements in significant amounts and in bioavailable form, easily digestible in our body system. It is observed that Ca and K contents are higher (≥10 mg/g) whereas those of Na, Mg, Al and P are much lower, in a small range of 1-2 mg/g. All these are primarily electrolytic elements responsible for maintaining balance in extra-cellular fluid of the body. Besides, structural elements Ca, Mg and P are sources of strength and vitality [71,72]. The human body needs Ca more than any other mineral as it stimulates enzymes in the digestive process and coordinates function of all other minerals (daily dietary intake, 0.4-0.6 g of Ca). As already mentioned, *Pragya-peya* has been found to be highly effective as a nervine tonic, for curing cold and cough, and enhance body resistance against many diseases whereas trace elements such as Fe, Mn, Zn, Rb, V, Se, Cu etc may be responsible for other biochemical functions. It is most enriched in Fe (676±176 μg/g), an important mineral that enters into the vital activity of blood and glands. Mn and Zn, found at ~90 and ~30 μg/g level respectively, are especially important for several

enzymatic processes. These help in eliminating fatigue and reduce nervous irritability [61,66,71]. Of special importance are V and Se whose compounds have been found to be antidiabetic [72] and cancer protective [64] respectively. Also some toxic elements such as Cd, Hg, Th, Pb are environmental contaminants from soil and water [73]. However, the small amounts present are well within WHO permissible limits [59].

C. Elemental Contents in Constituents of Pragya-peya: Even though *Pragya-peya* is prepared by mixing 12 constituent herbs, each one of which has its own medicinal importance in day-to-day life (Table IV.2). Therefore, we analysed all the twelve herbs. A perusal of elemental data in Tables IV.7 & 8 shows that no single constituent herb is enriched in all the elements. In general, fennel seeds most often used as a spice and mouth freshener [5,74] are enriched in K, Mg, P and Se whereas *brahmi* is most enriched in Co and Rb besides Cd and Hg, which are essentially environmental contaminants [72]. Fennel seeds are available in different varieties (small, medium and, thick) and recommended as antacid, for treatment of urine inflammation, diarrhoea and kidney disease. Its Se content (602±52 ng/g) is of special interest because of cancer preventive properties [64]. Also *aagya-ghas* is most enriched in Fe (940±103 μg/g), Cr (6.70±0.02 μg/g) and Zn (56.9±6.0 μg/g), which are essential for enhancing vitality, regulating glucose metabolism and enzymatic processes respectively [61,66]. Surprisingly K concentration of most herbs is much higher than that of Na except in *tulsi* where these are in comparable amounts (K/Na = ~1.2). Mg and Ca are major essential minerals in bones and teeth. Ca is also involved in normal muscle (including heart muscle) contraction and relaxation, blood clotting, proper nerve functioning, improvement in the body immune defense and in prevention of osteoporosis [71]. *arjuna* bark with highest Ca content (~ 10 %) is used widely in many herbal preparations [74] and is considered of high medicinal importance.

Many transition elements such as Cr, Mn, Fe, Co, Cu and Zn were found at varying concentrations (<1 to >900 μg/g). Most herbs contain Fe > 500 μg/g except *arjuna, nagarmotha*, *red sandal*, *shankhpushpi, sharpunkha* and *tulsi* which all contain Fe in a smaller range of 160-324 μg/g. Zn is another important element responsible for many enzymatic processes and is involved in the working of genetic material, proteins, immune reactions, wound healing, development of foetus and sperm production [75,

76]. Our studies have shown that Zn level in blood of diabetic patients is lowered [63]. In all the constituents, Zn content is found to be in the range of 26.5-56.9 μg/g with the lowest and highest contents in *arjuna* and *aagya-ghas* respectively. Mn is also an essential element required for various biochemical processes [61,66]. In most of the constituents, Mn is found to be < 200 μg/g but in *bay leaves* and *cinnamon* it is much higher, being highest in *cinnamon* (631±30 μg/g). In Ayurvedic literature, *cinnamon* is recommended for flu, indigestion, cough and heart diseases [5,13]. Se content was generally found at ng/g level in several constituents such as *arjuna, brahmi, fennel*, and *tulsi.* Mercury is highly toxic, and environmental contaminant. It is found in the range of 18.8-168 ng/g with highest content in *brahmi* [59,68]. This may be essentially due to contamination from the soil. Many vanadium compounds, described to possess therapeutic properties are specially being used for the treatment of diabetes [72,77]. Vanadium was also detected in all the constituents in the range of 0.5 to 5.2 μg/g with highest content in *nagarmotha*. The variation in elemental contents as observed in this study could be due to preferential uptake by a particular plant species from the soil. Therefore, soil characteristics together with environmental conditions play an important role in the nutrients contents [78].

(iv) Inter-elemental Correlations: In general, all the constituent herbs are enriched in K, P, Na and Ca considered as essential for maintaining fluid balance and body strength. Several literature reports suggest interrelationship of various elements [67,79]. Therefore, we attempted some inter-elemental correlations in the analysed samples. Bar plot of Fe/Zn ratio shown in Fig. IV.10 for seven brands lies in a close range of 21.8 to 23.9 except for local and tablet

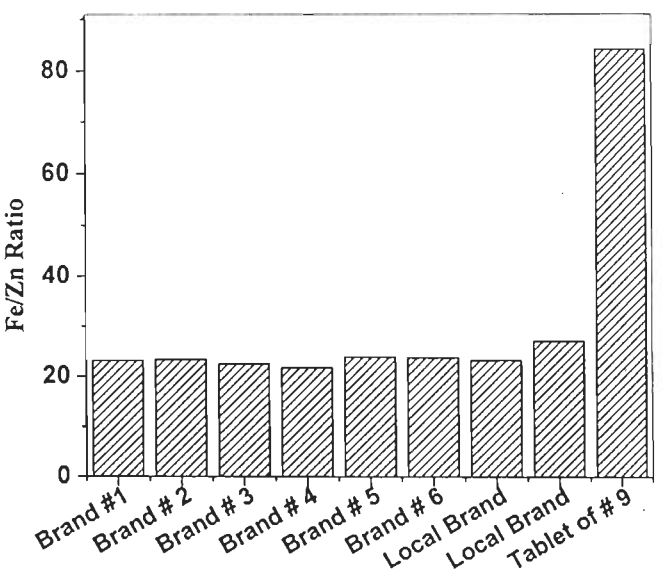


Fig. IV.10 Variation in Fe/Zn Ratio in different Brands of *Trifala*

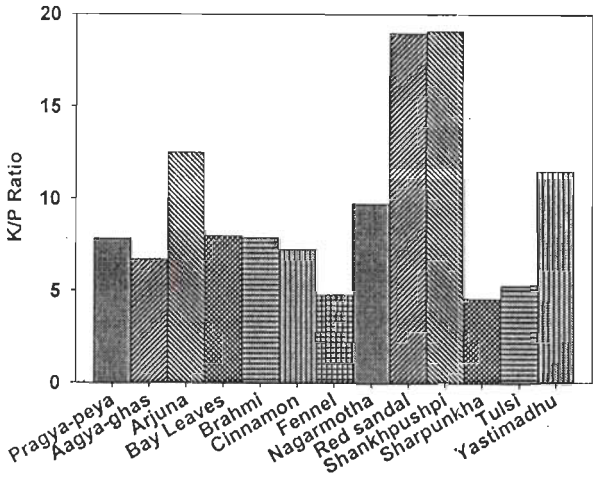


Fig.IV.11 Variation in K/P Ratio in *Pargya-peya* and its Constituents

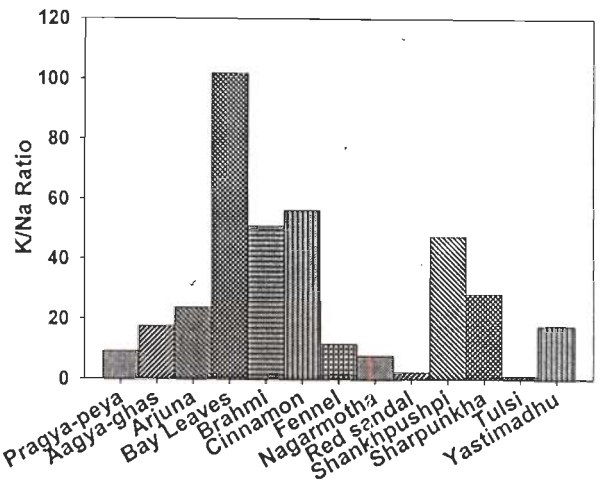


Fig.IV.12 Variation in K/Na Ratio in *Pragya-peya* and its 12 Constituents

brands of *Trifala* where it is 27.1 and 84.1 respectively. This is essentially due to variation in geographical and environmental location.

We have plotted K/P ratio as shown in Fig.IV.11 in *Pragya-peya* and its constituents. It is observed that *red sandal* and *shankhpushpi* exhibit K/P ~19.0 whereas in *arjuna* bark and *yastimadhu,* it is in the range of 10-15 but in all other cases it is < 10. Therefore, it can be said that K content in plant samples is ~10 times higher than that of P content. A plot of K/Na ratio in Fig. IV.12 was drawn with wide variation in the range of 1-100, the highest being in *bay leaves* (102). Only 4 constituent herbs exhibit K/Na in a comparable range of 35-55. A plot of Ca/P, both structural elements, is shown in Fig. IV.13. Most of the constituents exhibit Ca/P ratio in a narrow range (< 10), except *arjuna* which shows the highest ratio (185). *Red sandal, shankhpushpi and yastimadhu* exhibit a ratio of ~20. It may be noted that *arjuna* has highest content and that may be responsible for high Ca/P ratio.

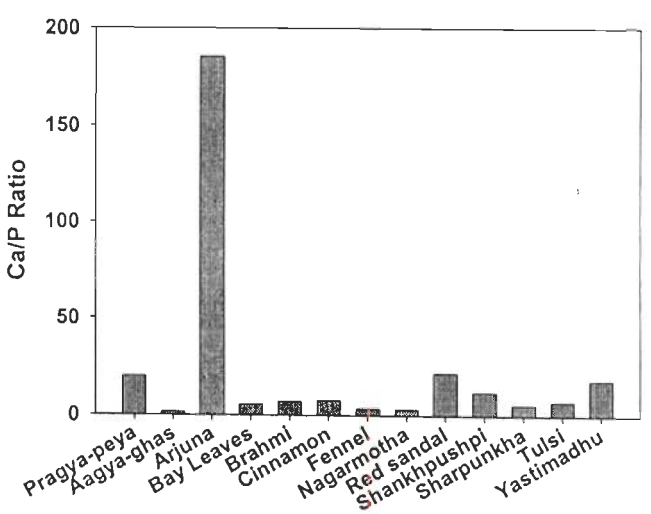


Fig.IV.13 Variation in Ca/P ratio in *Pragya-peya* and its 12 Constituents

Fe, Co and Zn, essential elements for biochemical processes [80,81], are well correlated in *Pragya-peya* and its

constituent herbs. A plot of Fe vs. Fe/Zn ratio shows linear relationship (Fig. IV.14) with r = 0.899, which is more like two clusters and represents not so good relationship. It may possibly be due to the fact that all parts of the herbs are different e.g. leaves, stem, bark, root etc as reported by Razic et al. [80]. However, Fe and Co (r = 0.969) show excellent relationship in *Pragya-peya* and its 12 constituents as shown in Fig. IV.15. In general, it may be mentioned that inter elemental relationships in medicinal herbs suggest synergistic or antagonistic effects, thus providing various elements to the body in bioavailable form in a balanced manner with almost no harmful effects [4,13] except some environmental contaminants. These, however, could be avoided by collecting herbs grown in a clean and well-controlled environment in herbal gardens away from city. For this, agricultural practices (GAP) are required followed by supply and manufacturing process [73].

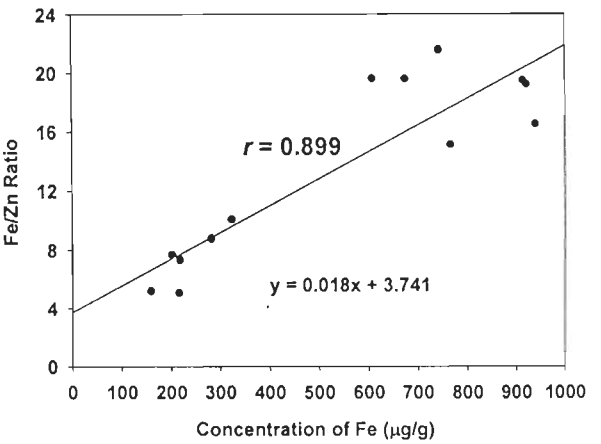


Fig.IV.14 Variation in Fe/Zn Ratio with Fe Concentration Pragya-peya and its Constituents

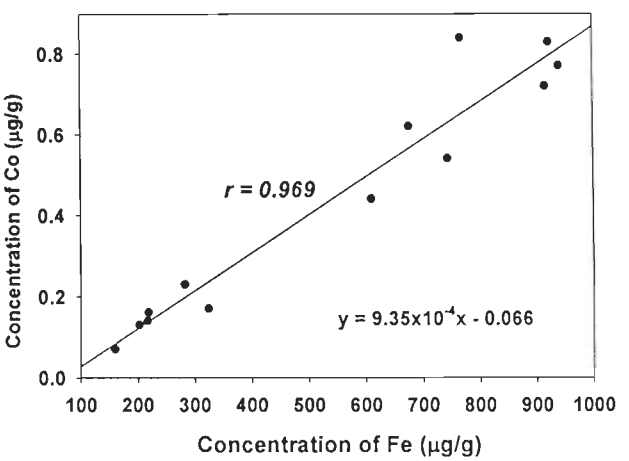


Fig.IV.15 Relation between Fe and Co Concentrations in Pragya-peya and its Constituents

(v) Effect of Origin on Elemental Contents: In order to study the effect of origin on the elemental contents in medicinal herbs, we analysed 4 samples each of *brahmi* and *tulsi* collected from different locations including a sample from a pharmaceutical firm (Himalaya). Though only mean elemental contents are mentioned in Table IV.7A & B but individual elemental profiles of the two samples are plotted in Figs. IV.16 & 17. A perusal of Fig. 16 A & B shows that in general, minor and trace elemental contents are similar in the 4 samples of *brahmi* but minor (Na, K, Ca, P & Cl) elemental contents in sample # 4 from Himalaya are somewhat higher. Similarly Cr, Mn,

Fe, Zn and Rb contents in 4 samples differ by a factor of 2 to3. In case of *Tulsi* leaves, all the minor elements except Mg differ though within a range of ±10-20%. Similarly, Fe,

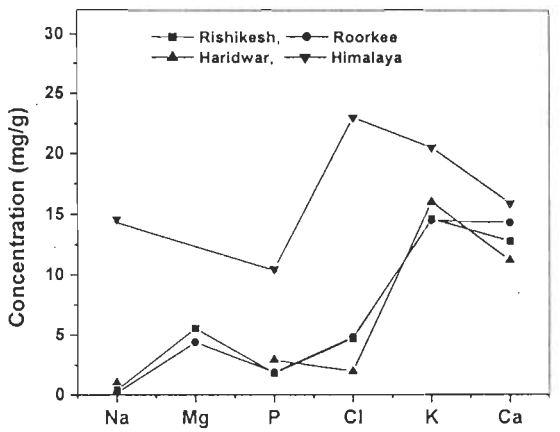


Fig. IV.16A Effect of Origin on Elemental Contents in *Brahmi* Collected from Different Places

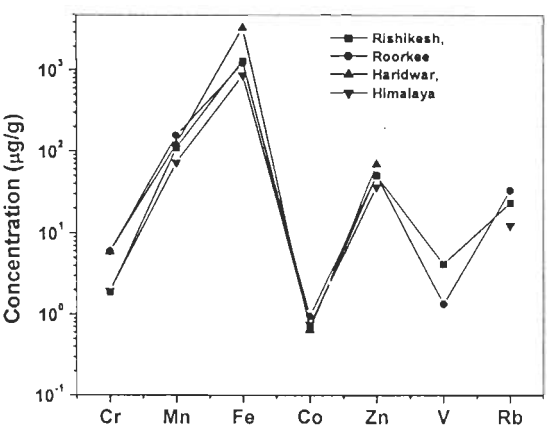


Fig. IV.16B Effect of Origin on Elemental Contents in *Brahmi* Collected from Differerit Places

Rb and to some extent Zn contents in a sample from Himalaya are also higher. It may be mentioned that Himalaya Drug Company is from Bangalore in southern India where

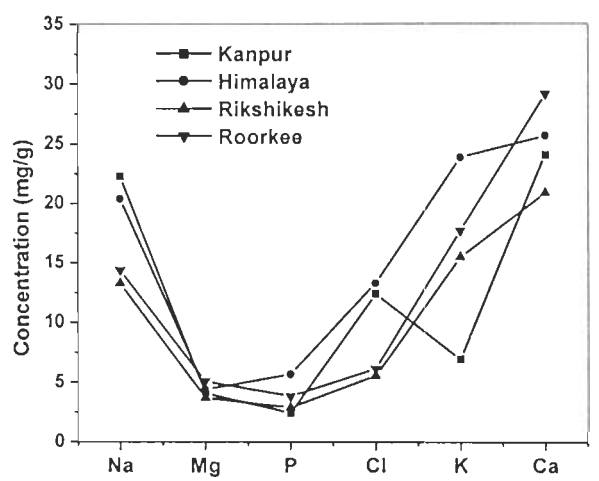


Fig. IV.17A Effect of Origin on Elemental Contents in *Tulsi* Collected from Different Places

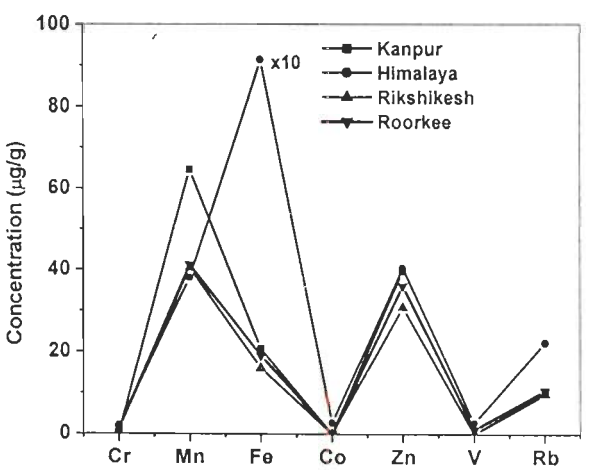


Fig.IV.17B Effect of Origin on Elemental Contents in Tulsi Collected from Different places

geo-climatic conditions are much different than in northern part where from other samples were derived. Apart from the fact that particular plant specie accumulates an element from the soil, elemental uptake also depends on the soil characteristics

including ecological and geographical variations [51]. In spite of these minor differences, elemental profiles, in general, are similar in 4 samples each of two medicinal herbs.

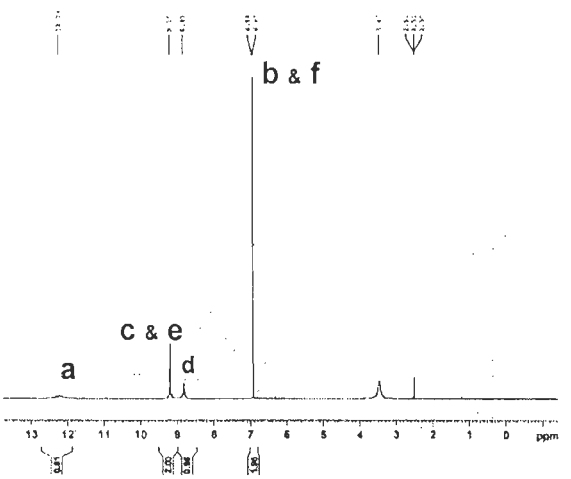
Tulsi leaves being so important medicinally that these have been also analysed by other workers around the country [48-50]. It is observed that our data for ca, Cl, Co, Cr, Fe, Mn, P, Rb and Zn are in general of the same order of magnitude. However, Fe content reported by Naidu et al. [49] and Zn content by Rajurkar and Pradeshi [48] are too high and may be attributed to local soil characteristics. In spite of the fact that that tulsi leaves analysed by other workers are from different parts of the country but they all are comparable.

(vi) Identification of Gallic Acid: Structure of gallic acid was elucidated on the basis of infrared, ^{1}H (Fig. IV.18) & ^{13}C NMR (Fig. IV.19) and GC-MS (Fig. IV.20) studies. IR spectrum in KBr shows a broad band in the region 3500-2500 cm^{-1} attributed to ν (OH) of carboxylic (–COOH) as well as phenolic (φ–OH) groups. A characteristic band at 1703 cm^{-1} is due to ν(C=O) and another at 1618 cm^{-1} may be attributed to ν(C-O) [82]. These assignments match well with gallic acid reported in Aldrich Library of FT-IR [83]. Proton magnetic resonance spectrum in Fig. IV.18 shows a most intense peak at δ 6.96 ppm due to aromatic protons of benzene. A low intensity broad peak at δ12.24 ppm is due to –COOH proton. Further, the peaks at δ 9.20 and 8.83 ppm with area ratio of 2:1 may be due to two –OH at m and on other –OH at p- positions. The –OH group can not be in o- positions of –COOH otherwise H-bonding between the –OH group and carboxylic acid would have shifted the δ value downfield appreciably. Overall, the spectrum suggests four different types of six protons, of which two aromatic protons (b and f) are somewhat equivalent, the small difference (δ = 0.03) being due to free rotation of -COOH group. No splitting is observed in the spectrum as only very long range coupling is taking place between adjacent –OH groups [84]. ^{13}C NMR spectrum in Fig. IV.19 shows an intense peak at δ145.3 and another at δ 137.93 attributable to two C atoms (n and p) attached to –OH groups. A peak at δ167.4 ppm justifies the presence of C=O moiety of –COOH. Another peak at 120.39 ppm indicates the C atom attached to -COOH group. A singlet at δ108.7 ppm may be due to two C atoms, which remain unsubstituted (m and q) in the aromatic ring. The data along with assignments are listed

in Table IV.10. These assignments compare well with gallic acid reported in Aldrich Library of FT-NMR [85]

Table IV.10 ^{1}HNMR and ^{13}CNMR data of Gallic acid fraction separated from *Trifala*

Chemical Shift (δ) in ppm	Assignment	Chemical Shift (δ) in ppm	Assignment
12.2 (1H, s)	-OH^{a} (Carboxylic)	167.4 (1C, s)	-COOH (k)
9.20 (2H, s)	-OH$^{c\&e}$ (Phenolic)	145.3 (2C, s)	C-O (n and p)
8.83 (1H, s)	-OH^{d} (Phenolic)	137.93 (1C, s)	C-O (o)
6.96 (2H, s)	C-H$^{b\&f}$ (Benzene)	120.4 (1C, s)	C-C (l)
		108.7 (2C, s)	C-C of benzene (q & m)



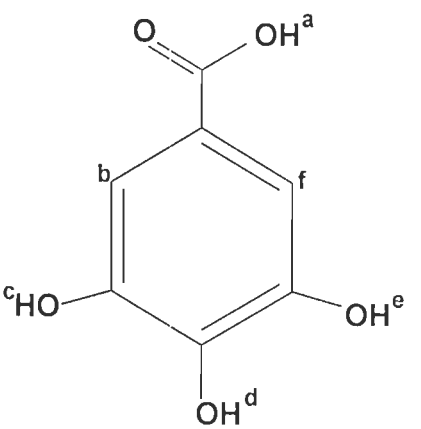
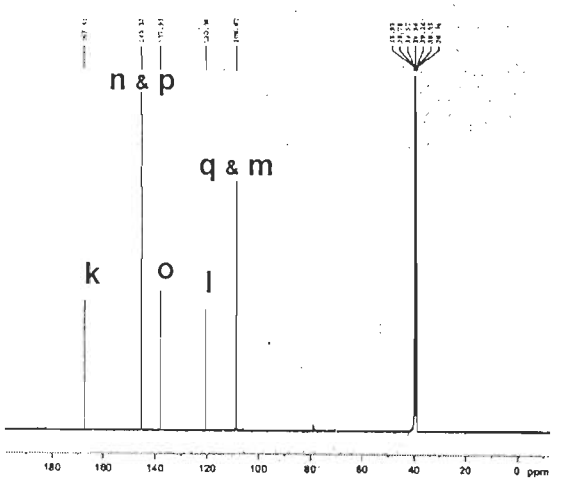


Fig. IV.18 ^{1}HNMR of Separated Fraction in d_6-DMSO



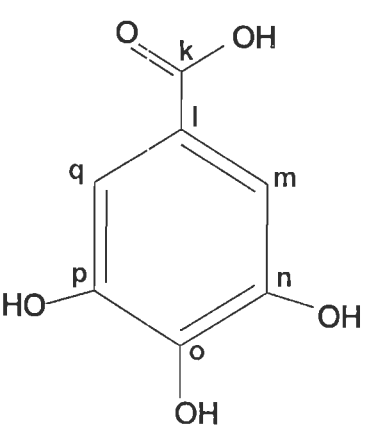


Fig. IV.19 ^{13}CNMR of Separated Fraction in d_6-DMSO

TLC for standard gallic acid and separated fraction were run in ethyl acetate: methanol (7:3) system and both gave R_f =0.86 (Fig. IV.20). After isolation GC-MS was recorded (Fig. IV.21) exhibiting 5 prominent peaks corresponding to m/z at 126, 108, 97, 80 and 52. The base peak at m/z =126 is likely to be formed by the elimination of CO_2 from -COOH group of gallic acid. Hence the molecular wt. of the compound should be 170 which compares well with C, H analysis and also with the mol. wt. of gallic acid. Thus, it can be concluded that the separated fraction from the ethyl acetate extract of *Trifala* is gallic acid (3,4,5-trihydroxy benzoic acid) as already suggested in literature [16,24]. All other peaks in GC-MS at 108, 97, 80 and 52 may be explained due to resonating structures as per fragmentation pattern shown in Fig. IV.22. The main peaks match well with NIST spectra library [86]. Further, quantitative estimation showed ~2% gallic acid of *Trifala.*

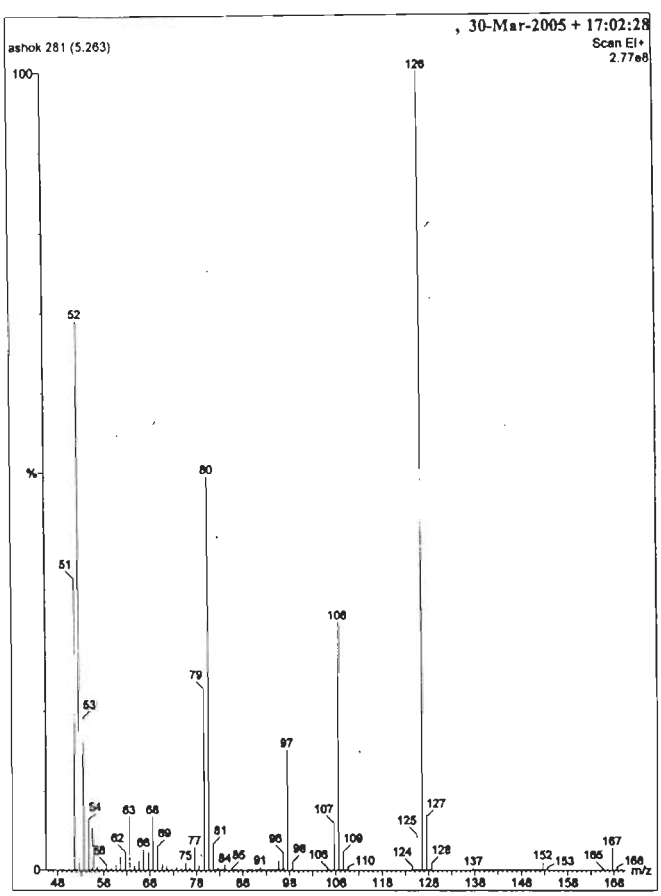


Fig. IV.21 GC-MS of Gallic acid Fraction

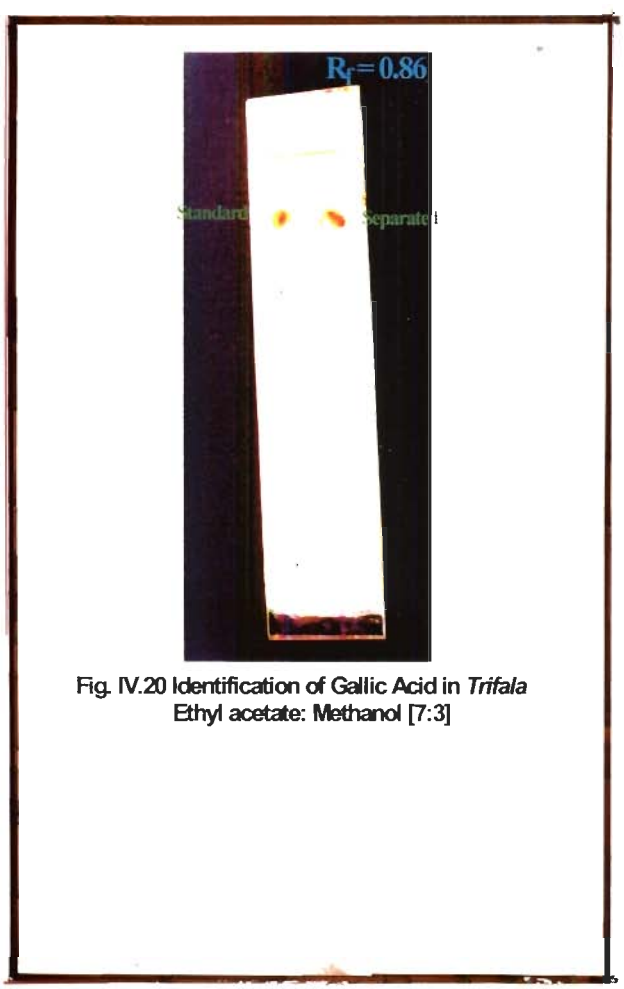


Fig. IV.20 Identification of Gallic Acid in *Trifala* Ethyl acetate: Methanol [7:3]

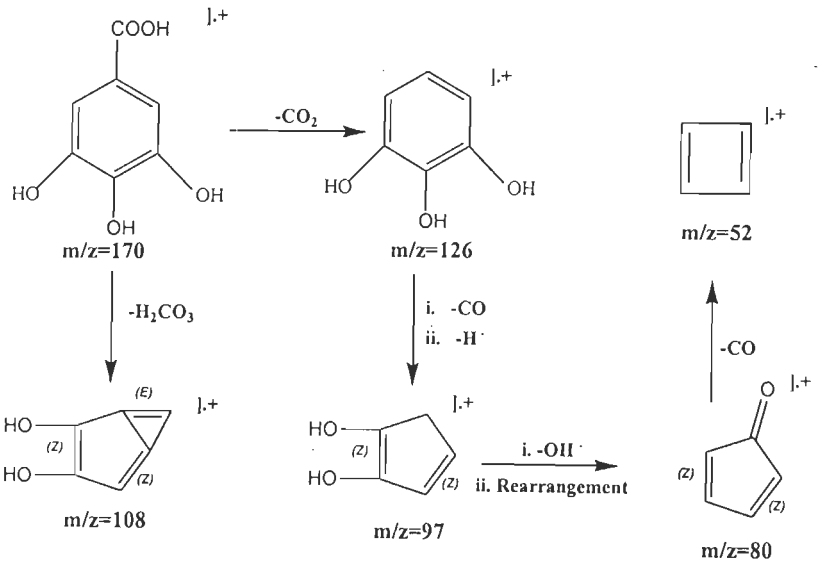


Fig. IV.22 Fragmentation Pattern for Gallic Acid

(vii) Inter-laboratory Comparison Study: We participated in the inter-comparison study of Tea Leaves (INCT-TL-1) and Mixed Polish Herbs (INCT-MPH-2) sent by Prof. R. Dybczynski, Poland. We submitted our data for 22 elements (Al, Ba, Br, Ca, Ce, Cl, Co, Cr, Cs, Fe, K, La, Mg, Mn, Na, P, Rb, Sb, Sc, Se, Th and Zn) by INAA. Also Apple Leaves (SRM-1515) and Tobacco Leaves (CTA-OTL-1 & CTA-VTL-2) were analysed as comparators and for data validation/quality assurance. The data are listed in Table IV.9 where recommended and information values as provided in the final reports [54,55]/certificates are also included. A perusal of our datafor various RMs, shows agreement within ±10% with the certified values for various RMs. Comparison of our analytical data for both the candidate RMs, TL-1 and MPH-2 as submitted agree with the certified values [55]. This is further illustrated in Fig IV.23. It is observed that;

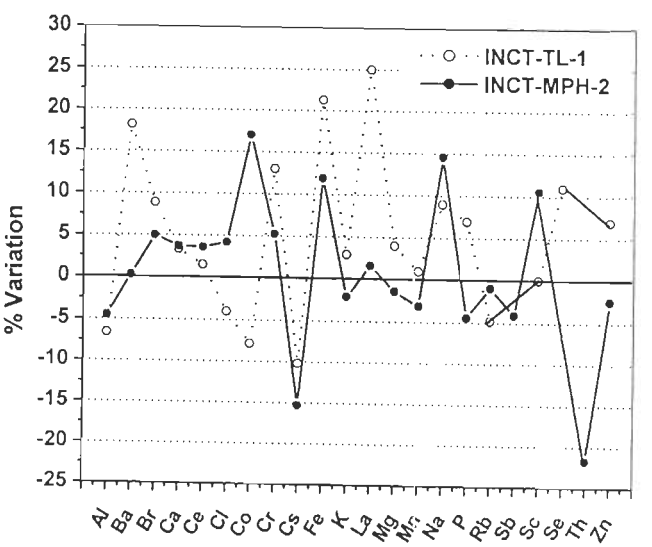


Fig. IV.23 Percent Variation in Elemental Content with their Certified Values

- Our data for Ca, Ce, Cl, K, Mg, Mn & Rb in both TL-1 and MPH-2 including Al, Sb & P are in excellent agreement within ±5% of the recommended values.
- Our data for P in both RMs as obtained by the method developed in our laboratory are in excellent agreement (±5%) with the recommended values.
- Our data for Br, Co, Sc and Se agree within ±15% of the certified value.
- However, our data for Ba, La and Th do not agree so well as these are within ±20 % of the certified values.

It may be further seen from Fig. IV.22 that majority of analytical data are on both sides of the recommended values except in a few cases where positive or negative deviations are observed. Despite these deviations, it may be concluded that elemental data provided by our laboratory are of reasonably high quality and it provides further confidence about the quality of our analytical data for herbal; formulations.

IV.9 CONCLUSION

On the basis of analytical data on minor and trace elements in two herbal preparations *Trifala* and *Pragya-peya* along with their constituent herbs by INAA and AAS, following generalizations may be made;

- The ranges of elemental concentrations vary in a wide range of mg/g to ng/g in both the herbal formulations.
- Essential elements such as K, Ca, Cl, Mg and P are present at mg/g levels whereas V and Se are found at ng/g level in both the herbal formulations and their constituents.
- Different *Trifala* brands exhibit similar elemental profiles except minor differences. It is enriched in Ca (~0.5%), K (~1.3%), Mg (~0.10%), P (~0.25%) and Fe (~0.10%) contents.
- Out of three constituents of *Trifala*, *bibhitaki* is most enriched in nutrient elements such as Ca, Fe, K, Mg, Mn, and P.
- *Pragya-peya,* having come from a single firm, is homogeneous in terms of most elemental contents and seems a standard formulation as a nervine tonic. This is due to the fact that it is from a single source and all the ingredient herbs are grown in polluted free environment.

- *Pragya-peya* is enriched in nutrient elements such as K (~1%), Fe (~0.7 mg/g), Ca (~2.4%), Mg (~0.22%), P (~0.12%), Cu (14.7 μg/g) and Zn (34.5 μg/g), which are responsible for various biochemical and enzymatic processes.
- V and Se detected in *Trifala* and *Pragya-peya* suggest that these formulations may be used as ant diabetic and cancer protective but clinical studies are essential.
- *Arjuna* bark contains ~10 % Ca along with Cr, Zn and Se in significant amounts.
- The herbal formulations, which are high in nutrient elements such as Na, K, Ca, Mg, Mn, Fe, Co, Se and Zn can influence changes in the functioning of some body organs.
- It is possible that many extraneous elements get into the herbal formulation during processing, so the knowledge of elemental composition of herbal formulations is essential as it helps in ascertaining its usefulness, safety and toxicity before use.
- In all *Trifala* brands, Fe/Zn ratio varies in a narrow range. Gallic acid (organic constituent) and Fe/Zn (inorganic constituents) ratio can be used for the standardization of *Trifala* formulation.
- K/P ratio changes in a wide range of 4.6-19.1 in *Pragya-peya* and its constituents.
- Several interesting correlations have been observed between Fe vs. Fe/Zn, Fe vs. Co, in all the herbs. It suggests that the concentration levels of many elements in the herbs are strongly affected by plant characteristics as well as soil and climatic conditions.
- Heavy toxic metals such as Cd, Hg and Pb were detected in both the formulations but these are well within WHO permissible limits.
- Besides the essential elements, the concentrations of several other elements were also determined so as to obtain information about their uptake from the soil and their possible role in biochemical processes of the body system.
- It is essential to investigate the pharmacological activity of *Pragya-peya* for its usefulness in the treatment of various ailments.

- Since *Trifala* is being manufactured and marketed by many pharmaceutical firms, and wide ranging elemental variations have been observed, it is essential to have quality control and follow standard protocol with regard to additives, stabilizers and methodology (including cleaning of raw material).

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CHAPTER V

BHASMAS: METALLIC- HERBAL PREPARATIONS

A part of this work has been accepted for publication in ***Biol. Trace Element Res.*** and also it was presented at ***NAMLS8***, Rio de Janeiro, April 17-22, 2005

V.1 WHAT ARE BHASMAS?

Metallic preparations have not been used directly for the cure of ailments in any other world civilization where herbal medicines have long been in use. *Ayurveda,* the Indian system of medicine was perhaps the first and still remains the only one where metallic-herbal preparations often called *bhasmas* were widely recommended for the treatment of many chronic ailments since 7^{th} century BC [1,2]. *Siddha Nagarjuna* is considered to be the father of Indian alchemy and *Rasa Sastra* is one of the disciplines where *bhasmas* were first described as intriguing formulations of metals such as gold, silver, copper, iron, zinc, mercury, lead, tin and some of their alloys apparently associated with organic macromolecules derived from the herbal extracts by alchemic processes making these biologically assimilable [3]. There exists a reference of the use of metals as artificial limbs in *Rigveda*. *Charak* and *Sushrata Samhitas* indicate the use of some primary metals for serving food, vegetables and fruits and recommend for the storage of cooked food. *Bhasmas* are often prepared by repeated incineration of metals or their salts (preferably oxides) with medicinal herbs or their extracts so as to eliminate their harmful effects and are taken along with honey, milk, butter or ghee (a preparation from milk) [4]. *Bhasma* literally means "ash" and is an Indian mineral preparation made from precious metals and their naturally occurring salts by calcination process called *putas*. However, before calcination, the metal must go through two main processes, physical purification (called *shodhan*) and chemical detoxification (called *maran),* both derived from ancient Indian language Sanskrit). The preparation methods involve crushing, boiling and repeated incinerations in earthen crucible at specified temperature to make the minerals ready for human consumption. This purification is different from chemical purification and is also called *putas* carried out in a special vessel under the earth and incinerated using cow dung's dry cake. In chemical purification only the foreign matter is eliminated whereas in Ayurvedic purification its objective is aimed at -

i) Elimination of harmful effects from the crude material.

ii) Modification of undesirable physical properties of the crude material.

iii) Conversion of the some of the characteristics of the crude material.

iv) The enhancement of its therapeutic action so that it may be biologically assimilable and maintaining its potency indefinitely.

When *bhasma* is finally prepared it should be tested in the following ways-

a) There should be no metallic luster (*Nishchandrika*).

b) When the *bhasma* is rubbed between the index finger and thumb, it should be so fine that it goes easily into the grooves (*Rekhapurit*).

c) When a small quantity of *bhasma* is sprinkled onto cold water, still it should float on the surface (*Varitaram*).

d) The *bhasma* should not revert to its original state (*Apurnabhava*).

In *Ayurveda* minerals are combined with herbs that assist the assimilation and delivery of the ingredients to the human body [5,6]. It is well established that several metals play a vital role in the biochemical processes as well as in the cure of many diseases [7,8]. Minerals are essential constituents of the bone, teeth, muscles, blood and nerves and play a vital role to our overall mental and physical well being [9,10]. The human physiology is unable to manufacture minerals like it manufactures vitamins, proteins and enzymes. Iron, calcium, magnesium, manganese, copper and zinc are all found in the soil wherefrom these are absorbed directly in different parts of plants [11], which supply these to our body through the food chain. Perhaps the metal in bhasma binds itself to carrier macromolecule, acts as a catalyst or alters the membrane fluidity. Apparently, the organic ligands derived from the herb render the metal easily assimilable. Another aspect of Ayurvedic preparations is synergism, which is apparently achieved by selectively blending of many plants, minerals and animal products, and thus maintaining the active ingredients at a minimum level and reducing or eliminating its side effects.

V.2 ANCIENT METHODOLOGY OF *BHASMAS*

Ayurvedically prepared metals and minerals do not react with the body tissues and hence *bhasma* is considered to be more powerful than almost any other healing preparation [1,6]. It is believed that widely used heavy metals such as Hg and Pb in traditional medicine system act as a catalyzer, which stimulate catalytic activity by their presence in the intestines without ever reaching the blood stream [12]. It is probably this property that renders many of the highly toxic metals into non-toxic form. It is believed

that greater the number of *putas*, lesser the toxicity and higher the efficacy. Further, the toxic effects of these medicines are neutralized by the medium of honey/ghee/milk/butter and provide a natural and effective alternative to synthetic allopathic drugs. Honey is the most frequently suggested vehicles in Ayurvedic texts. *Bhasmas* may be considered as biologically produced nano-particles which are more likely to be biocompatible than those produced chemically [5]. In fact many metals especially the gold *bhasma* is shown to exist as nano-particles by immersing geranium leaves in a solution containing chloroaurate ion [13]. Fricker [14] reviewed the medical uses of gold compounds focussing on their anticancer and antimicrobial properties. It has been suggested that gold containing drugs affect gene expression.

Ancient literature describes a variety of ways and apparatus called *Yantra* for the preparation of these *bhasmas*. A typical *Kupipaka yantra*, used for collecting volatile products is shown in Fig. V.1 where metals such as Hg, As and S are interacted by putting in the bottom of the refractory lined bottle heated in a sand bath or using a muffle furnace. This is done such that upper part remains outside the heating source so that after heating the volatile material present in the herb or SO_2 (in case of S) come out from the bottle. At this stage the bottle mouth is sealed with a refractory plug so as to allow the formation of As_2S_3 or HgS which get volatilized and condensed in the bottle's neck. After the processing is over, the bottle is carefully cut from the centre taking precaution not to disturbing the condensed particles or to avoid its contamination. Similarly, many other types of vessels are described in literature [15,16]. Of course, there are no uniform methods followed by physicians. A typical methodology followed by

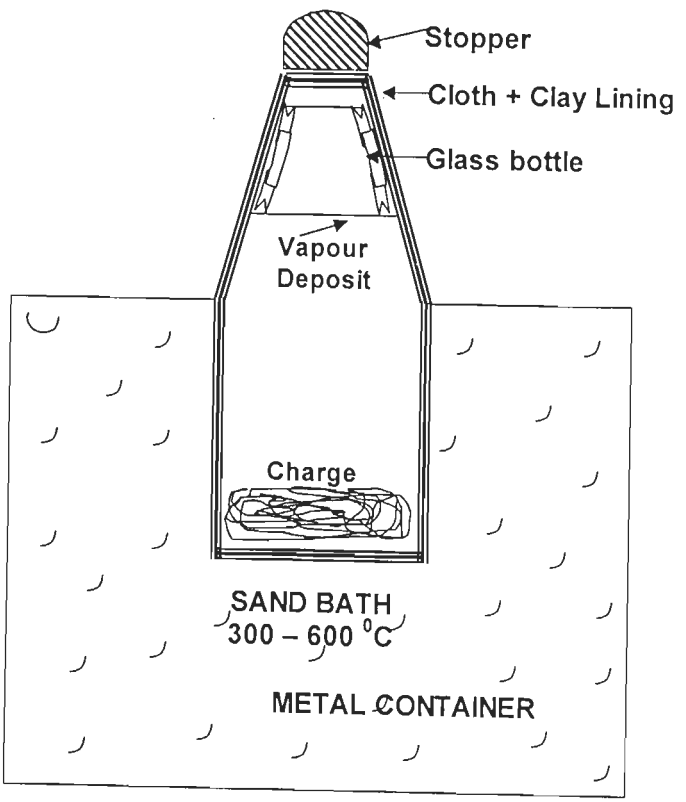

Fig. V.1 Schematic Drawing of *Kupipaka Yantra* in Sand bath

M/s Shree Dhootpapeshwar Ltd, Mumbai for the preparation of zinc *bhasma* is schematically illustrated in Fig. V.2.

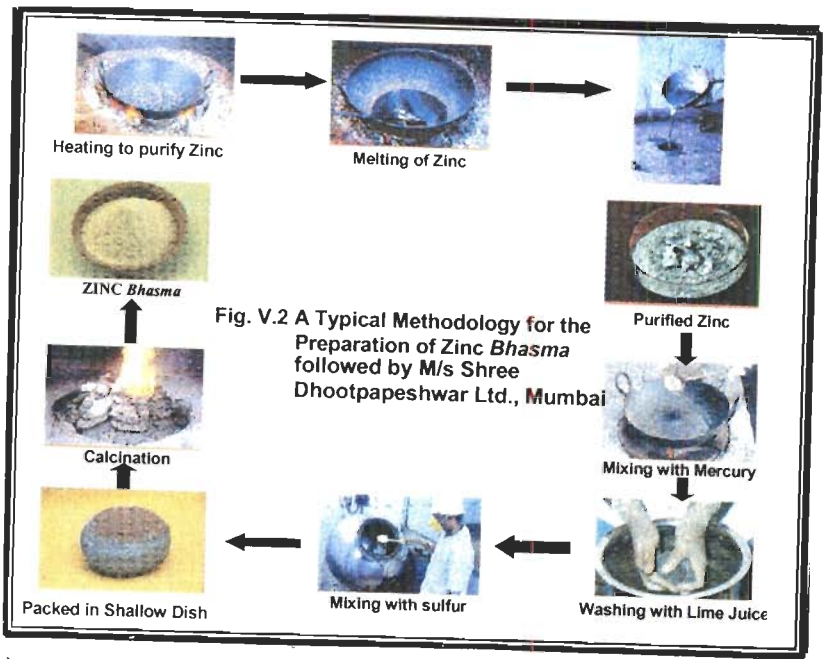

Fig. V.2 A Typical Methodology for the Preparation of Zinc *Bhasma* followed by M/s Shree Dhootpapeshwar Ltd., Mumbai

In the Ayurvedic literature *lauh* (iron) *bhasma* prepared after 1000 *putas* is considered to be more potent and is expected to consist of almost pure iron particles resulting due to the reduction of the oxide by carbon from *Trifala* extract. The large spherical particles seen are formed due to the interaction of iron oxide and the ashe [17]. Zinc metal was melted (450 ^{0}C) and then poured in the limewater for *shodhan*. This quenching treatment causes development of micro-cracks at the grain boundary of the metal pieces and some compound of pinhole porosity at certain structural planes are separated. This helps in the fragmentization of the metal pieces curing trituration [18].

Similarly, a mercury preparation *Siddh Makardhwaja* is reported to initiate stimulant action of Hg ions in highly diluted state [19]. A well-made *bhasma* enters the system faster and stays there for longer duration than any other drug. *Bhasmas* made from different gemstones are often mixed with cardamom, cinnamon, cloves and other spices and used extensively to promote health [20].

In earlier days, the quality of the herbs and herbal preparations were not subjected to much review, but was based on sacred trust between the physician and the patient [12,20]. However, in modern times a patient or a physician (more so because of the Government regulations) seeks assurance for the quality, safety and efficacy of an herbal *Ayurvedic* medicine especially for export in the western market where many Asians still want to practice these alternative medicines. Therefore, herbal preparations and *bhasmas* need very stringent quality control of the finished product as many of them contain toxic inorganic elements such as As, Hg, Pb, well known for their highly

toxicity [21,22]. Most herbal preparations and in particular *bhasmas* do not undergo scientific quality control and safety protocol. There are no well established/documented reports on the variation in the amount of major constituent elements. Therefore, a strict quality control of the finished product is needed as most of them contain inorganic elements in concentrated form [1,6].

V.3 LITERATURE SURVEY

Most literature studies on *bhasmas* are from Indian scholars only though some recent references of western scholars could also be found. According to ancient literature the necessity and effect of metals on human health was recognized as early as 7^{th} century BC. However, by the 7^{th} century AD, the manufacture of nontoxic drugs from the metals like gold, silver, copper, iron had begun and the processes were improved with the increasing understanding of the alchemical processes. Between 8^{th} and 16^{th} century AD, many new processes of *Satvapatana* and *bhasma* preparation were developed using their own tools, *Yantras* and *Puta* furnace for the conversion of metals into drugs [1,2]. The modern analysis of these processes confirms the high level of understanding of the physicochemical principles and the skill achieved at that time. As Rao [23] has quoted from *Atharvaveda,* ancient Indians had developed a deep understanding about the role of natural environment viz. vegetation, oxygen, hydrogen, nitrogen carbon and the products formed by their synthesis in sunlight, in the maintenance of human health and increasing the longevity.

During ancient times the exact control of the drug composition was achieved by controlling the proportion of various drugs and minerals added to the charges during processing and the types as well as number of *putas* given to the mixture. Finally each product was judged by visual examination, taste and special tests described for each case. However, with the development of modern techniques of metallic drug preparation extensive use of chemical analysis, XRF and XRD analysis, Auger study and optical as well as scanning electron microscopic studies [16] have made it possible to gain better understanding of the ancient processes. This has helped in identifying the nature of the metallic compound formed and used as drug and also improve its quality control procedures.

Several workers have analysed *bhasmas* for the chemical characterization of various elements associated with the main constituent as well as other trace elements [24-27]. During early thirties, Chopra et al. [24] were probably the first to have reported analysis of unique inorganic preparations of indigenous medicines-*bhasmas* of Fe, Sn, Ca, Au and Ag for their main constituents by employing classical chemical methods. Vasanth et al. [25] analysed *talaka* and *naga bhasmas* containing As_2S_3 and PbS respectively for several other minor constituents such as Ca, Mg, As, Sb, Al, and Fe including some trace elements. Dixit and Shivahare [26,27] analysed *pearl* and *cowrie bhasmas* for their minor constituents viz. Mg, Ca, Fe, Zn etc by employing modern instrumental techniques. In recent years several methods such as spectrophotometry, flame photometry, atomic absorption spectrophotometry (AAS), inductively coupled plasma-atomic emission spectrometry (ICP-AES), including particle induced x-ray emission (PIXE) and x-ray diffraction have been used for trace characterization of *bhasmas* [28-33]. Recently Lalla et al. [28] have described preparation, characterization and analysis of *shankh bhasma* and studied its antacid activity. Garg et al. [29] used PIXE for determining trace element contents in several *bhasmas* so as to understand their biocompatibility. Sondhi and co-workers [30,31] have analysed 20 *bhasmas* for 15-20 elements (Al, Na, K, Ni, Mn, Pb, Cr, Cu, Fe, Hg, Si, Mg, Cd, Ce, Co, Ag, Sr and Ca, Zn) by flame photometry, AAS and ICP-AES. Pandit et al. [32] have also used AAS for determining 12 elements (Mg, Ca, Na, Ba, K, Pb, Cu, Mn, Zn, Ni, Co and Cd) besides the main constituent (Fe) in *Lauh bhasma* and evaluated its pharmacological action. Krishnamurthy and Sane [33] also analysed *lauh bhasmas* from different pharmaceutical manufacturers to understand composition and structural characterization. Mitra et al. [13] have determined 12 elements (As, Pb, Ca, Mg, Ba, Sr, Fe, Al, Cu, Zn, Ni, Co) besides the main constituent (Au) in *Swarnabhasma* by AAS. Such studies have played vital role in providing scientific evidences to Ayurvedic physicians and Research and Development departments of pharmaceutical units so as to have better standardization of these indigenous medicines. Singh [34] studied *Naga bhasma* based on lead and investigated its *Rasayana effects.* It has been suggested that better chemical reaction with quality control could be achieved in the muffle furnace heating method than the traditional *putas* method where earthen pots are

recommended. Recently, a conference on "Metals in Medicine: *Ayurvedic* and Modern View" was held at Parbhani, Maharashtra, during Sept. 4-5, 2004 where several workers presented their work on the use of metallic/mineral medicinal preparations of Cu, Ag, Au, Hg etc in the management of diseases. Toxicity and standardization of these metallic preparations have been a subject matter of controversy and discussion and need thorough investigation.

V.4 PRESENT STUDY

In the present study we have employed instrumental neutron activation analysis (INAA) for determining up to 21 elements including C, H, N and S in twenty *bhasmas,* four each based on Ca and Fe, three of Zn, two each of Hg and gemstones, one each of K, Ag, Cu, Sn and As with respect to their chemical composition. The main constituent element was further analysed by complexometry, gravimetry, flame photometry and AAS. In Table V.1 are listed the names and uses of various *bhasmas* described in literature [1-6]. The *bhasmas* analysed in this study were found to contain the main constituent element at percentage level along with some other nutrient and toxic elements at minor and trace level. These additional elements derived from herbs do not find any mention in *Ayurvedic* literature but seem to be quite useful for maintaining fluid balance and biochemical enzymatic processes in the body system [35]. The purpose of this study was the detailed analysis, determine stoichiometry if possible and to point out the importance of quality assurance by way of trace element analysis in Ayurvedic practice vis-à-vis its product utilization in the national/international market. Also we participated in the Intercomparison study of Reference Material (RM) Marine Sediment, IAEA-433 [36].

V.5 EXPERIMENTAL

(i) Sampling: Twenty *bhasmas* in fine powder form were procured from reputed Pharmaceutical firms and purchased locally. These are likely to have been prepared by standard procedure as described in Ayurvedic literature and used as such for analysis.

TABLE V.1: *Bhasmas*, ingredients and uses

Bhasma (Metal)	Ingredient/ Description	Uses (Ref. 1-6, 54)
1. Mukta Moti (Ca)	Pearls and Ghee (milk preparation)	Cough, impotency, eye disorders, tuberculosis, sprue, nervine sedative, used in hyperacidity, asthma, cough and nervous excitement in growing children and pregnant women.
2. Mukta Shukti (Ca)	Pearls and Rose water	Respiration, cough, heart diseases, stomach, liver, intestine
3. Praval Pishti (Ca)	Pearls	Antacid, used in cough, phthisis, scrofulous, affections, spermatorrhoes, pulmonary hemorrhage and calcium deficiency.
4. Shankh (Ca)	Conch shell	Antiperodic, carminative and analgesic, used in colic flatulence and tympanites.
5. Vanaspati yog Lauh (Fe)	Magnetic iron (purified)	Sprue, stomach disorders, anaemia, diabetes, blood disorders, restorative, haematinic, astringent, jaundice, disorders of liver and spleen
6. Kant Lauh (Fe)	Magnetic iron (purified), Ash of incinerated magnetic iron	Ant rheumatic, haematinic and used in anaemia
7. Mandoor (Fe)	Ash of incinerated purified ferric oxide	Alterative, haematinic, diuretic, used in anaemia, oedema, chlorosis, rickets and jaundice.
8. Trifala Yog Lauh (Fe)	Ferrum (purified), incinerated and potentiated, rubbed with Trifala decoction	Strengthening the body, deficiency of iron, anaemia, indigestion
9. Yashad (Zn) (a) Baidyanath (b) Deshrakshak	Zinc/ Shudh Yashad	Dysentery, sweating, phthisis, tuberculosis, diabetes, hypoglycemic, astringent, used in urinary disorders
10. Kharpar (Zn)	Zinc carbonate/ash of incinerated purified zinc carbonate	Antacid, Bone strengthening,
11. Sidhmakardhwaja (Hg)	Mercury	Physical disorders, strengthening the body, fever, malaria, asthma
12. Parad (Hg)	Mercury	Syphilis, genital disorders, rejuvenation
13. Rajat (Ag)	Silver	Wasting, nerve disorders, brain functions, eye disorders, tuberculosis
14. Swet Parpati (K)	Potassium nitrate, alum, ammonium nitrate (crystal powder)	Acidity, calculi, Urinary tract infection, Enlargement of prostate.
15. Khushta khas (As)	Arsenic	Nervine tonic, Asthma, leucoderma, paralysis and impotency
16. Tamra (Cu)	Ash of incinerated purified copper	Acidity, ascites, jaundice, piles, leprosy, leucoderma, asthma, tuberculosis, cough, skin diseases, obesity, chronic bloating, spleen and liver enlargement, cirrhosis.
17. Vanga (Sn)	Tin	Asthma, cough, sweating, blood disorders, diabetes, diuretic and urinary antiseptic, semen disorder, syphilis and gonorrhea
18. Jahar Mohra Khatai Pishti (Stone)	Ash of incinerated purified Serpentine Orephite	Heart related disorders, blood pressure, vomiting, burning sensation (pitta), cholera, antidote to poison, provides strength, potency and vigour
19. Vaikrant (Stone)	Ash of incinerated purified Tourmaline	Anaemia, ascites, asthma, tuberculosis, diabetes & cancer, substitute of diamond bhasma

Details of procurement, batch no. etc. are listed in Table V.2. A photograph of some *bhasma* bottles as procured is shown in Fig. V.3. It may be emphasized that each *bhasma* has specific use depending on its method of preparation and the way it is prescribed by the physician. A mono standard of gold for k_0-method was used as described earlier [37]. Also three RMs, one each of Peach Leaves (SRM–1547) from the NIST (USA), Mixed Polish Herbs (MPH-2) from the INCT (Poland) and Cabbage (IAEA-331) from the IAEA (Vienna) were analyzed for quality control/data validation.

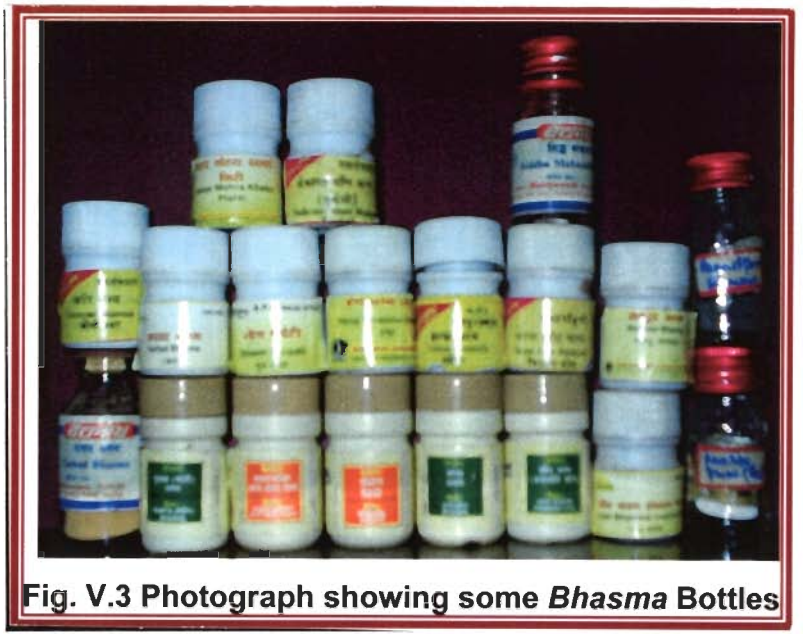

Fig. V.3 Photograph showing some *Bhasma* Bottles

20 g each of two bottles of Marine sediment (IAEA-433) # 2002/187 & 208 and Estuarine sediment (IAEA-405) [38] were sent to us by Dr. S. J. de Mora of Marine Environment Laboratory (MES), Monaco as a part of the preparation of sediment standard by IAEA, Vienna. Its moisture content was determined as per recommended procedure by oven drying at 105 ^{0}C for 1 d and found to be 1.44%. Synthetic elemental standards of As, Hg, Fe and Zn were used as comparators. Three other RMs including Pond Sediment (NIES, Japan), W-1 (USGS) and Soil-5 (IAEA) [39-41] were used for quality assurance and data validation.

(ii) Irradiation and Counting: 30-50 mg each of samples and RMs were weighed accurately and packed in alkathene/ aluminium foil (Supra pure) for short (5 min.) and long (7 h) irradiation respectively in APSARA reactor at thermal neutron flux of ~6×10^{11} $cm^{-2}.s^{-1}$ the BARC. Irradiations were carried out in the E8 position where thermal to fast neutron flux ratio is 52:1. Irradiated samples were delayed for appropriate period before measurement of γ-activity as mentioned in Chapter II.4.

Irradiated samples after decontamination were mounted on standard Perspex plate and assayed by high-resolution γ-ray spectrometry using a 40% relative efficiency HPGe

Table V.2 Details of *Bhasmas* analysed.

Bhasma (Colour)	Firm/Source of Procurement	Batch No./Date of Manufacture
Ca based		
1. Mukta Moti (White)	Dabur India Ltd., Delhi	9064/Mar., 2000
2. Mukta Shukti (White)	"	1039/Jan., 2002
3. Praval Pishti (Pink)	"	25014/Dec., 2000
4. Shankh (White)	"	1121/Oct., 2001
Fe based		
5. Vanaspati yog Lauh (Dark brown)	"	2790/Jan., 2000
6. Kant Lauh (Dark brown)	Deshrakshak Aushdhalya, Haridwar	-
7. Mandoor (Dark brown)	"	-
8. Trifala Yog Lauh (Dark brown))	"	-
Zn based		
9. Yashad		
(a) # 1 (Pale brown)	Baidyanath Ayurved Bhawan Ltd., Jhansi	08/Feb., 2001
(b) # 2 (Cream White)	Deshrakshak Aushdhalya, Haridwar	-
10. Kharpar (Cream White)	"	-
Hg based		
11. Sidhmakardhwaja (Red brown)	Baidyanath Ayurved Bhawan Ltd., Jhansi	01/Aug., 2002
12. Parad (Black)	Local Physician	-
13. Rajat (Black), ***Ag***	Yogi Pharmacy Ltd., Haridwar	22/Nov., 93
14. Swet parpati (White crystals), ***K***	Deshrakshak Aushdhalya, Haridwar	-
15. Kushta khas (White), ***As***	Local Physician	-
16. Tamra (Black), ***Cu***	Deshrakshak Aushdhalya, Haridwar	-
17. Vanga (Grey), ***Sn***	"	-
Gemstone based		
18. Jahar Mohra Khatai Pishti (Grey)	"	-
19. Vaikrant (Off white)	"	-

detector coupled to 4k-channel analyzer at the reactor site or Radiochemistry Division of BARC. Long irradiated samples were brought to Roorkee and counted on our experimental set up. Elemental contents were calculated using different RMs as comparators as well as by k_o based NAA for short lived nuclides.

(iii) Chemical and Instrumental Methods: For this purpose weighed amounts of the *bhasmas* were dissolved in HCl/HNO_3 /aqua regia to make up to 50 mL solution and then appropriately diluted. Ca in all the four Ca based *bhasmas* and K in *Swet parpati* were determined by flame photometry (ELICO model CL-361, Hyderabad). Ca, Fe, Cu and Zn were also determined by complexometric EDTA titration method [42]. Ag in *Rajat bhasma* was determined gravimetrically as AgCl. Atomic Absorption Spectrophotometer (GBC, Avanta) was used for the determination of Fe, Hg, Zn, Ag, As, Cu and Sn as described in Ch.II.6. C, H, N and S were also determined as mentioned in Ch II.7.

V.6 RESULTS

Typical gamma ray spectra of Ca and Fe based *bhasmas* for short and long irradiated samples are illustrated in Fig. V. 4 A & B respectively. Elemental concentrations in

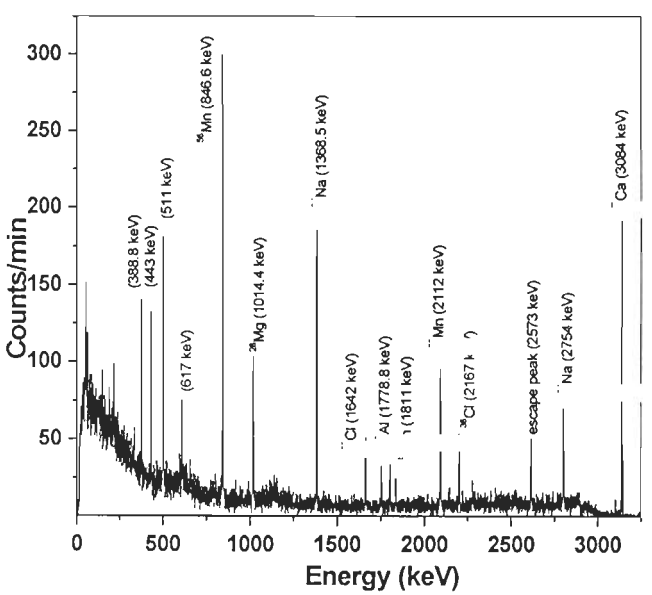

Fig. V.4A Gamma-ray Spectrum of *Praval Pishti Bhasma* (t_i=5 min, t_d=3 min, t_c= 2 min)

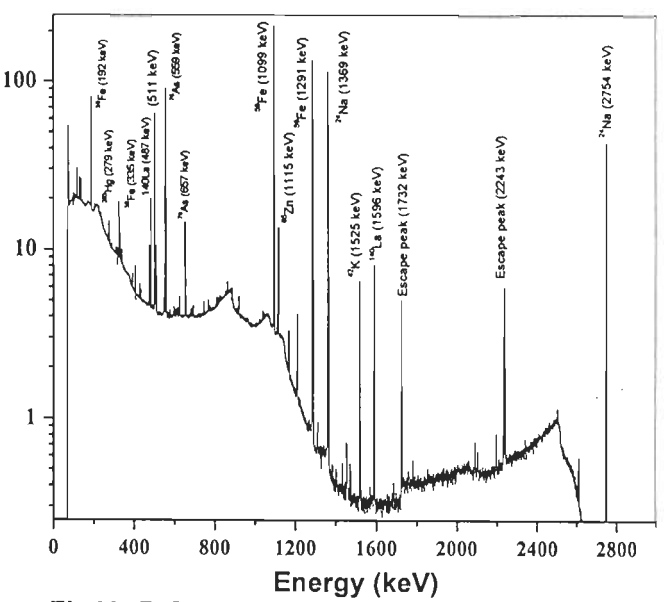

Fig.V.4B Gamma-ray Spectrum of *Kant Lauh Bhasma* (t_i=7 h, t_d=20 h, t_c=1h)

all the *bhasmas* are listed in Tables V.3A & B. Data on elemental contents of the main constituents as analysed by other methods including a comparison with literature values are summarized in Table V.4. C, H, N and S data with major constituents are listed in Table V.5.

Table V.3A: Elemental concentrations in various *Bhasmas*

Element	CALCIUM				IRON				Mercury	
	Mukta Moti	*Mukta Shukti*	*Praval Pishti*	*Shankh*	*Vanasp-ati Yog Lauh*	*Kant Lauh*	*Triphala yog Lauh*	*Mandoor*	*Siddha Makardh waja*	*Parad*
Al (mg/g)	0.13±0.01	0.70±0.03	1.62±0.15	0.30±0.04	2.44±0.05	2.50±0.27	3.52±0.04	ND	ND	0.53±0.12
As (µg/g)	1.29±0.18	0.82±0.04	3.61±0.03	3.67±0.40	1.39±0.15	10.6±1.0	97.3±16.8	9.59±1.0	ND	ND
Au (ng/g)	68±3	ND	5.6±0.2	59±2	ND	ND	ND	ND	ND	ND
Br (µg/g)	138±25	0.64±0.12	112±9	8.16±1.41	0.58±0.05	ND	ND	0.82±0.08	ND	ND
Ca (mg/g)	**31.0±2.6%**	**33.6±2.4%**	**29.5±2.0%**	**41.9±4.0%**	ND	2.37±0.25	ND	ND	ND	247±5
Cl (mg/g)	2.66±0.35	0.40±0.06	2.08±0.30	2.27±0.18	ND	0.24±0.03	0.22±0.03	0.21±0.03	ND	ND
Co (ng/g)	123±12	27±2	147±15	910±100	416±40	340±25	610±45	510±40	ND	ND
Cu (µg/g)	ND	ND	1.52±0.15	ND	1.08±0.12	15.0±0.3	16.9±0.5	ND	ND	ND
Fe (mg/g)	ND	1.28±0.12	2.83±0.19	ND	**56.9±5.6%**	**42.9±0.5%**	**5.31±0.53%**	**35.1±5.4%**	ND	ND
Hg (µg/g)	19.0±2.0	4.75±0.55	627±20	14.3±1.2	ND	0.49±0.04	0.42±0.03	0.45±0.04	**85.3±7.0%**	**0.018±0.002%**
K (mg/g)	0.22±0.02	0.59±0.01	1.26±0.07	0.35±0.01	1.83±0.07	1.18±0.11	0.93±0.04	10.3±1.6	ND	ND
La (µg/g)	ND	0.63±0.06	0.96±0.01	0.87±0.02	0.53±0.13	2.35±0.26	1.79±0.22	16.4±1.8	ND	ND
Mg(mg/g)	3.45±0.29	ND	22.1±4.3	2.73±0.21	ND	1.26±0.11	0.96±0.05	ND	ND	65.8±7.2
Mn (µg/g)	28.0±0.6	445±35	49.4±3.8	9.35±1.25	596±47	467±25	465±30	**11.4±0.4***	ND	252±23
Na(mg/g)	3.80±0.39	3.13±0.29	5.91±0.51	3.11±0.03	0.41±0.04	0.11±0.01	0.21±0.02	2.33±0.11	ND	ND
P (mg/g)	0.96±0.03	0.29±0.01	0.77±0.02	0.38±0.01	0.62±0.02	0.96±.01	0.86±0.04	3.26±.04	ND	0.97±0.04
V (µg/g)	2.55±0.01	ND	3.89±0.50	ND	10.1±1.0	219±20	ND	ND	ND	ND
Zn (µg/g)	176±20	ND	125±10	ND	ND	61.2±4.5	79.9±1.0	135±24	ND	ND

ND= Not detected, * = Concentration in mg/g

Table V.3B: Elemental concentrations in various *Bhasmas*

Element	ZINC			SILVER	POTA-SSIUM	ARSENIC	COPPER	TIN	STONE	
	Yashad #1	*Yashad #2*	*Kharphar*	*Rajat*	*Shwet Parpati*	*Kushta Khas*	*Tamra*	*Vanga*	*Jahar Mohra Khatai*	*Vaikrant Mani*
Ag (%)	ND	ND	ND	**23.4±2.5**	ND	ND	ND	ND	ND	ND
Al (mg/g)	3.03±0.14	1.02±0.05	1.70±0.02	7.08±0.5	0.02±0.01	0.71±0.08	ND	0.13±0.01	3.17±0.04	0.79±0.01
As (µg/g)	15.3±1.4	89.6±13.5	269±30	142±19*	ND	**3.65±0.6%**	19.7±1.0	24.1±6.4	53.9±5.5	231±44
Au (ng/g)	ND	ND	139±10	140±10	ND	ND	ND	ND	ND	153±15
Br (µg/g)	ND	7.13±0.18	8.33±0.65	ND	0.37±0.03	ND	ND	ND	0.35±0.02	0.27±0.02
Ca (mg/g)	ND	193±5.5	239±20	ND	ND	301±2	ND	73.5±2.9	ND	15.8±1.3
Cl (mg/g)	ND	0.21±0.03	0.59±0.15	ND	4.09±0.66	0.20±0.02	ND	0.24±0.03	0.45±0.01	0.13±0.01
Co (ng/g)	188±10	205±20	310±25	387±24	ND	ND	810±55	880±40	335±25	285±10
Cu (µg/g)	5.05±0.8	9.03±0.78	23.7±1.8	ND	12.3±0.9	ND	**44.1±3.0%**	ND	10.6±0.3	21.5±0.6
Fe (mg/g)	22.8±2.4	3.87±0.24	7.14±0.45	ND	ND	ND	71.2±3.1	1.34±0.06	32.6±0.2	2.95±0.18
Hg (µg/g)	ND	0.75±0.03	0.17±0.01	ND	0.07±0.01	ND	0.23±0.01	0.97±0.08	1.01±50	0.18±0.02
K (mg/g)	0.59±0.02	2.22±0.16	3.59±0.09	ND	**39.3±3.5%**	2.42±0.05	23.7±2.0	0.26±0.03	0.33±0.02	8.79±0.82
La (µg/g)	0.74±0.08	0.99±0.12	3.29±0.41	113±10	ND	18.1±1.0	ND	ND	ND	6.65±0.83
Mg(mg/g)	ND	67.2±5.6	ND	ND	ND	ND	ND	ND	3.25±0.24	1.06±0.09
Mn (µg/g)	116±13	334±13	288±12	183±18	2.70±0.01	84.0±1.0	134±12	257±25	319±33	89.2±6.7
Na (mg/g)	0.21±0.02	0.57±0.02	0.37±0.08	12.8±0.1	0.08±0.01	0.83±0.08	0.25±0.01	0.47±0.01	0.36±0.02	3.75±0.42
P (mg/g)	2.19±0.06	0.92±0.04	0.65±0.03	51.4±1.5	0.09±0.01	1.06±0.04	10.9±0.2	0.72±0.01	0.21±0.01	0.73±0.03
Sn (%)	ND	ND	ND	ND	ND	ND	ND	**43.8±2.4**	ND	ND
Zn (µg/g)	**60.0±7.0%**	**13.4±2.4%**	**0.012%**	ND	54.3±4.0	ND	358±26	67.0±4.9	308±4	94.8±1.2

ND= Not detected, In Vanga *bhasma*, Indium (In) was found to be 17.1±0.7µg/g.

Table V.4: Comparison of major constituent content (in %) in various *bhasmas* by INAA with literature values

Bhasma	Major Constituent	This Work	Literature Values
Mukta Moti (Pearls)	Ca	31.0±2.6 *30.7* [30.9]	40.0 (*Ref. 44*) 38.55 (*Ref.46*) 32.58 (*Ref. 26*)
Shankh (Conch)	Ca	41.9±4.0 *40.1* [40.1]	55.5 (*Ref. 44*) 41.0 (*Ref. 30*) 38.6 (*Ref. 46*)
Mukta Shukti	Ca	33.6±2.4 *30.2* [31.1]	38.13 (*Ref. 44*) 48.6 (*Ref. 30*)
Praval Pishti (Coral)	Ca	29.5±2.0 *30.3* [31.1]	39.19 (*Ref. 46*) 30.62 (*Ref. 30*) 36.6 (*Ref. 44*)
Vanaspati Yog Lauh	Fe	56.9±5.6 *57.3*	
Kant Lauh	Fe	42.9±0.5 *42.3*	42.55 (*Ref. 31*)
Trifala Yog Lauh	Fe	5.31±0.53 *4.79*	
Mandoor	Fe	35.1±5.4 *31.7*	
Siddh Makardhwaja	Hg	85.3±7.0 *82.0*	80-86 (*Ref. 19*)
Parad	Hg	0.018±0.002 *0.014*	
Yashad # 1	Zn	60.0±7.0 *63.0*	69.0 (*Ref. 30*)
Yashad # 1	Zn	13.4±2.4 *13.0*	
Kharpar	Zn	0.012 *0.010*	
Rajat	Ag	23.4±2.5 *23.4* 22.0*	69.67 (*Ref. 24*) 38.06 (*Ref. 31*)
Swet parpati	K	39.3±3.5 *39.1*	
Kushta khas	As	3.65±0.60 *3.15*	
Tamra	Cu	44.1±3.0 *42.4*	
Vanga	Sn	43.8±2.4 *47.7*	

NOTE; Values in *italics* are by AAS/Flame photometry, In [ ] are by EDTA titrimetry, * by gravimetry

Table V.5 Concentrations of major constituents (in %) in various *Bhasmas*

Bhasma	C	H	N	S	Major Constituent(s), (>1%)	Total (%)
Mukta Moti (Pearls)	11.8	0.36	ND	0.39	Ca (31.0),	43.6
Mukta Shukti	12.1	0.25	0.01	0.12	Ca (33.6)	46.1
Praval Pishti (Coral)	11.9	0.30	0.04	0.38	Ca (29.5), Mg (2.21)	44.3
Shankh	15.1	0.81	ND	0.81	Ca (41.9)	58.6
Vanaspati Yog Lauh	1.22	0.01	ND	0.12	Fe (56.9)	58.3
Kant Lauh	0.11	0.04	ND	0.05	Fe (42.9)	43.1
Trifala Yog Lauh	0.12	0.02	ND	0.03	Fe (5.31)	5.48
Mandoor	0.07	0.01	ND	0.16	Fe (35.1), K (1.03), Mn (1.14)	37.5
Siddh Makardhwaja	0.06	0.03	ND	14.2	Hg (85.3)	99.6
Parad	13.4	0.03	ND	0.04	Hg (0.018), Ca (24.7), Mg (6.58)	44.8
Yashad # 1 (Baidyanath)	0.09	0.04	ND	0.44	Zn (60.0), Fe (2.28)	62.8
Yashad # 2 (Deshrakshak)	7.13	0.42	0.03	0.26	Zn (13.4), Ca (19.3), Mg (6.72)	42.3
Kharpar	8.37	0.21	ND	0.38	Zn (0.012), Ca (23.9)	32.9
Rajat	0.63	0.25	ND	19.9	Ag (23.4), As (14.2), Na (1.28), P (5.14)	64.8
Swet Parpati	0.02	0.03	13.7	ND	K (39.3)	53.1
Kushta Khas	0.24	0.05	ND	21.1	As (3.65), Ca (30.1)	55.1
Tamra	0.09	0.51	ND	22.0	Cu (44.1), Fe (7.12), K (2.37), P (1.09)	77.3
Vanga	4.15	0.64	ND	0.15	Sn (43.8), Ca (7.35)	56.1
Jahar Mohra Khatai	0.68	1.30	ND	0.56	Fe (3.26)	5.80
Vaikrant	0.06	0.03	ND	0.13	Ca (1.58)	1.80

Elemental concentrations in IAEA-433 (Marine Sediment) and IAEA-405 (Estuarine Sediment) along with three SRMs of similar matrices are given in Table V.6. The errors quoted are the standard deviations derived from the mean of replicate measurements/ different irradiations and countings.

Table V.6. Elemental concentrations in Marine Sediment and other reference materials

Element	Marine Sediment (IAEA-433) [36]	Estuarine Sediment (IAEA-405) [38]	Soil-5 (IAEA) [39]	W-1 (USGS) [41]	Pond Sediment (NIES, Japan) [40]
As	19.8±1.3	24.6±1.7	100±4	1.14±0.13	12.5±2.3
(μg/g)	(18.9±1.8)	(23.6±0.7)	(93.9±7.5)	(1.9)	(12.0±2.0)
Ba	313±5	450±8	583±23	154±10	298±13
(μg/g)	(268±32)	{-}	(562±53)	(160)	{-}
Br	83.9±8.8	104±13	6.26±0.67	ND	14.7±1.6
(μg/g)	(67.0±16.0)	[85±25]	(5.40±1.0)		[17]
Ce	71.5±0.4	80.2±0.5	60.4±1.0	22.7±1.2	34.3±0.2
(μg/g)	[64.5±2.8]	{-}	(59.7±3,0)	(23)	{-}
Co	14.5±0.7	15.0±0.6	16.0±0.4	47.1±2.4	25.0±0.6
(μg/g)	(12.9±1.2)	(13.7±0.7)	(14.8±0.8)	(47)	(27.0±3.0)
Cr	137±2	85.0±1.3	32.3±2.5	122±11	63.5±1.0
(μg/g)	(136±10)	(84.0±4.0)	(28.9±2.8)	(114)	(75.0±5.0)
Cs	6.32±0.65	11.7±1.1	47.3±0.6	1.08±0.02	3.06±0.33
(μg/g)	(6.40±0.44)	[12.5±2.1]	(56.7±3.3)	(0.9)	{-}
Eu	1.24±0.02	1.15±0.15	1.20±0.05	ND	ND
(μg/g)	[1.18±0.07]	[1.25±0.36]	(1.18±0.08)		
Fe	42.9±1.5	40.8±2.6	49.9±1.4	76.2±5.9	58.8±2.9
(mg/g)	(40.8±1.9)	(37.4±0.7)	[44.0]	(77.6)	(65.3±3.5)
Ga	25.9±0.9	25.1±1.7	20.1±2.4	13.1±1.2	20.0±2.6
(μg/g)	{-}	{-}	(18.4±1.6)	(16)	{-}
Hf	4.42±0.04	6.07±0.05	6.20±0.10	2.72	3.10±0.03
(μg/g)	[3.66±0.18]	[5.80±0.87]	(6.30±0.30)	(2.67)	{-}
K	16.4±1.9	26.9±1.0	21.3±1.5	4.68±0.29	6.66±0.26
(mg/g)	(16.6±3.2)	[24.9±7.2]	(18.6±1.5)	(5.31)	(6.80±0.60)
La	36.9±0.4	38.9±5.6	30.6±2.6	10.7±0.9	14.4±0.3
(μg/g)	(33.7±2.7)	[40.4±7.3]	(28.1±1.5)	(9.8)	[17]
Lu	0.41±0.04	0.47±0.05	0.40±0.02	0.29	0.38±0.04
(μg/g)	[0.36±0.04]	[0.47±0.19]	(0.34±0.04)	(0.35)	{-}
Mn	318±18	476±33	855±75	1250±50	792±46
(μg/g)	(316±16)	[495±11]	(852±37)	(1278)	[770]
Na	13.0±1.1	20.0±1.9	22.2±1.3	15.0±1.2	5.99±0.70
(μg/g)	[13.5±1.5]	{-}	(19.2±1.1)	(15.9)	(5.70±0.40)
P	1.22±0.09	1.55±0.08	1.19±0.06	0.599±0.05	1.34±0.07
(mg/g)	{-}	{-}	[1.10]	(0.611)	[1.40]
Rb	120±1.5	167±2	134±5	25.5±0.3	39.1±4.0
(μg/g)	(99.9±14.2)	{-}	(138±7)	(21)	[42]
Sb	2.15±0.09	2.14±0.38	19.1±2.6	0.83±0.15	1.91±0.44
(μg/g)	(1.96±0.18)	(1.81±0.19)	(14.3±2.2)	(1.0)	[2.0]
Sc	15.8±0.8	13.9±0.6	16.1±0.5	35.2±2.0	25.7±0.7
(μg/g)	[14.6±1.1]	[13.5±2.0]	(14.8±0.7)	(35.1)	[28]
Sr	320±24	120±10	310±20	200±25	115±12
(μg/g)	(302±20)	[118±14]	[330]	(190)	[110]
Tb	0.71±0.06	1.07±0.09	0.78±0.02	0.55	0.54±0.04
(μg/g)	[0.70±0.09]	[0.93±0.43]	(0.665±0.075)	(0.65)	{-}
Th	10.4±0.57	14.0±0.5	10.9±0.4	2.59±0.01	5.42±0.18
(μg/g)	(9.78±0.57)	[14.3±2.1]	(11.3±0.7)	(2.42)	{-}

In parentheses ( ) are Recommended values, in [ ] are Information values, in {-} where no data available, ND = Not detected

V.7 DISCUSSION

We have analysed several *bhasmas* of the same element and each one of these has its own medicinal importance depending on the preparation methodology, dose and prescription method along with dietary restrictions. These are described in following lines.

(i) Calcium based: It is an essential constituent of all the living cells and is present in the body to a larger extent (>1%) than any other mineral element. Ca along with P is considered as structural elements both of which play an important role in the correction of bone metabolic disorders associated with calcium deficiency [9]. With increasing urbanization, osteoporosis, a skeletal disease characterized by low bone mass, micro structural deterioration of bone tissue leading to enhanced bone fragility, has become a major public health problem resulting in increasing tendency of fractures. Reddy et al. [43] have found effectiveness of *Praval bhasma* in the prevention of calcium and estrogen deficient bone loss and justified its continued use for the management of bone metabolic disorders such as osteoporosis. In *Ayurveda*, several calcium preparations from natural sources are widely recommended for supplementing calcium deficiency to growing children and ladies especially after the age of 50 yrs. Some workers have studied antacid activity of calcium preparations [44] and in enhancing effectiveness of antibiotics though no antibacterial activity was observed for any of the pearl preparations [45]. Several workers have reported analytical studies on Ca based *bhasmas* primarily derived from *pearls/conch/coral* because of their use as supplement in the treatment of bone metabolic disorders associated with calcium deficiency [26-31]. Motlag and Nath [46] studied metabolic role of calcium *bhasmas* derived from *praval, mukta* and *shankh* compared with $CaCO_3$ and calcium lactate and correlated its intake with growth in weight in rats.

All the *bhasmas* analysed in this study were prepared by repeated incineration/ calcination of pearls, oyster shell or outer shell of conch in a covered vessel and reducing it to powder. These contained Ca in a wide range of 29.5-41.9 % with maximum amount in *shankh bhasma.* Besides, significant amounts of Na (3.11-5.91 mg/g), K (0.22-1.26 mg/g) and P (0.29-0.96 mg/g) were also observed along with 12 trace elements including Co and Au in < 1 μg/g. Surprisingly all the Ca based *bhasmas* also show C (11.8-15.1%), H (0.25-0.81%) and S (0.12-0.81%) but no N suggesting the presence of carbonate and/or some organic compounds as suggested by some workers [26]. The dosage prescribed is of the

order of 65-500 mg/d. These *bhasmas* are well known to increase the intestinal absorption of calcium thus correcting metabolic conditions beneficial for bone mineralisation. Pandit et al. [47] have found *shankh bhasma* to be active against gastric ulcers. Dietary habits play a major role in the development and prevention of postmenopausal bone loss recommended for women with high risk of osteoporosis. It has been suggested that calcium supplementation when given with hormone replacement therapy (HRT) has an additive effect in the prevention of postmenopausal bone loss [48]. Chauhan et al. [49] have found *mukta shukti bhasma* to be one third to half as potent anti-inflammatory compared to acetylsalicylic acid and attributed this to the inhibition of prostaglandin, histamine and by stabilization of the lysosomal membranes. The constituent of *shankh bhasma* is mainly silicate of magnesia widely used in the treatment of ulcers, dysentery, dyspepsia, indigestion and jaundice [47]. Histographic variations in elemental contents of As, Cl, Co, K, Mg, Mn, Na and P in four Ca-based *bhasmas* is shown in Figs. V.5 & 6. It is observed that these *bhasmas* are most enriched in Ca and Mg followed by Na, K and P. Further Mn content is also higher but *mukta shukti* is most enriched in Mn (445± 35 µg/g) and so is *shankh* in Co (910 ng/g). However, *praval pishti* has higher contents of Na (5.91 mg/g), K (1.26mg/g), P (0.77 mg/g) and Mg

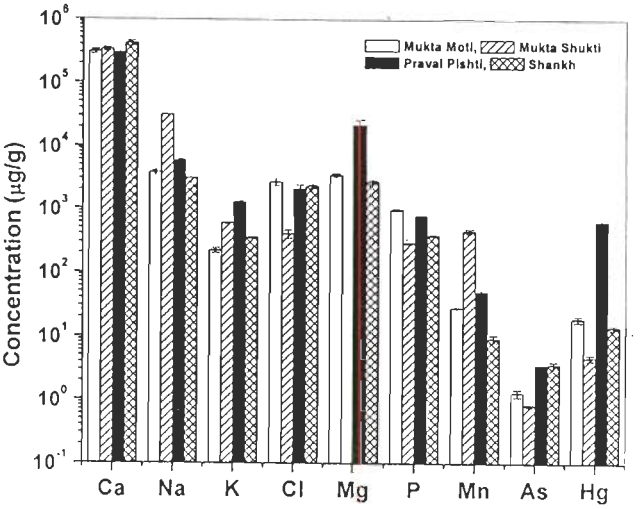

Fig. V.5 Variation in Elemental Contents in Ca based *Bhasmas* (n=4)

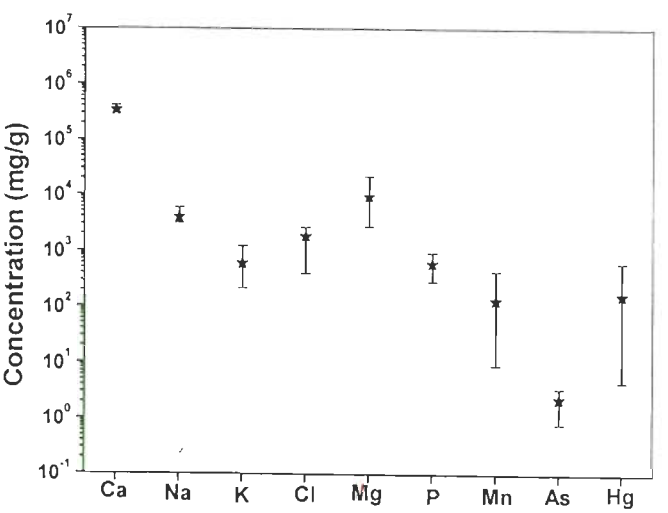

Fig. V.6 Range and Mean Elemental Content in Ca based *Bhasmas* (n=4)

(22.1mg/g). Interestingly all the four *bhasmas* contain toxic elements As and Hg in much higher amounts than permissible limits [50]. This may perhaps be attributed to the polluted of marine environment wherefrom pearls/conch must have been collected.

(ii) Iron based: Fe nourishes blood, enhances vigour and its astringency prevents blood from becoming too hot or too fluid [6]. It is an essential trace element, a part of haemoglobin, which plays an important role in oxygen transport [7,9,10]. Pandit et al. [32] evaluated chemical and pharmacological action of different *Ayurvedic* preparations in iron deficiency anaemia. They observed varying concentrations of iron and other metals depending on the number of *Putas*. Out of four *bhasmas* viz. *vanaspatiyog lauh, kant lauh, trifalayog lauh* and *mandoor* analysed in this study, Fe content varies in a much wider range of 5.31 to 56.9% with least amount in *trifalayog lauh* and highest in *vanaspatiyog lauh*. Other minor constituents are Na, K, Mn and P besides trace amounts of As, Co, Cu and Zn besides toxic elements As and Hg. Histograms showing elemental variations are depicted in Fig. V.7 and ranges with mean in Fig.V.8. *lauh bhasma* (Calxes of Fe) is used in anaemia similar to iron tablets/capsules/syrup prescribed as iron supplementation in allopathy. These *bhasmas* are prepared from purified iron filings/ferric oxide or magnetic iron

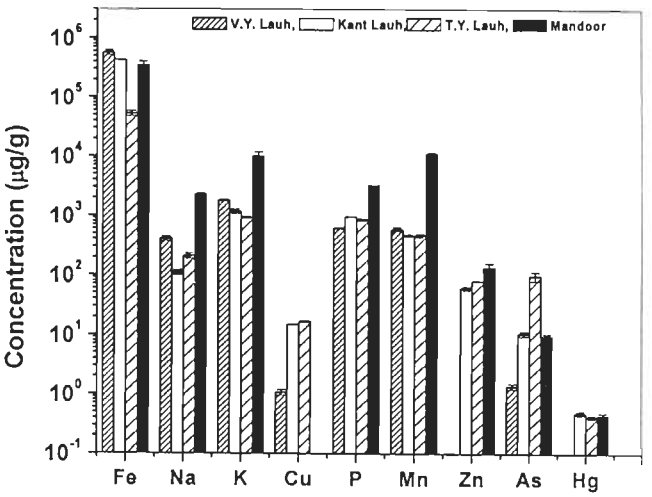

Fig. V.7. Variation in Elemental Contents in Fe based *Bhasmas* (n=4)

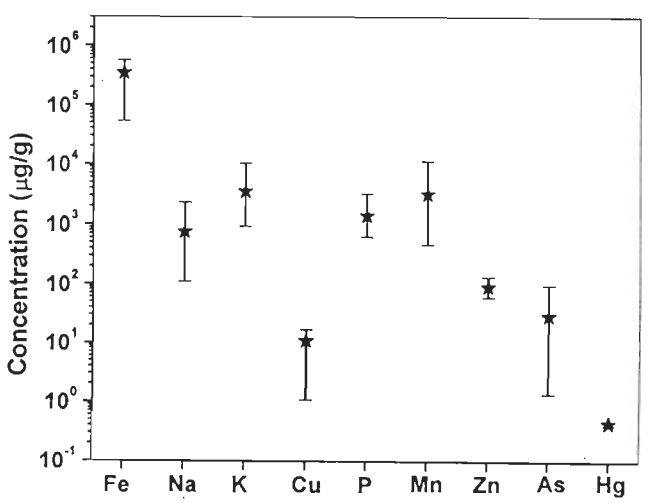

Fig. V.8 Range and Mean Elemental content in Fe based *Bhasmas* (n=4)

incinerated with decoction of *Trifala*, *ghritkumari ras*, vinegar and sesame oil. *Lauh bhasma* is believed to improve strength, vitality, complexion and life span. *mandoor bhasma* is especially useful in the enlargement of liver, spleen, jaundice, hepatitis, blood loss, leucoderma, obesity and high blood pressure. Kanase et al. [51] have studied curative effects of *mandoor bhasma* on liver and kidney of albino rats where a total recovery was noticed in two weeks. Our studies have shown that four iron containing *bhasmas* also contain mg/g amounts of Mn, P, K and traces of As, Co, Cu, Zn, and Hg which are all likely to be derived from herbs. Reddy et al. [52] screened *lauh bhasma* for its antihepatotoxic activity. Iron *bhasmas* are prescribed in the dosage of 125 to 750 mg/day and taken along with honey. The treatment of iron deficiency anaemia with iron salt preparation like ferrous sulfate results in several adverse effects, viz. severe gastrointestinal irritation and cardio vascular collapse, damage to brain and liver [10]. *vanaspatiyog lauh bhasma* contains highest amount of Fe (56.9%) along with Al, K, Mn, Na, and V at minor and trace level. Though *trifalayog lauh,* contains least amount of iron (5.31%) but it is described to be more potent because of reduction of oxide to fine particles of iron (may be nanoparticles) by carbon from *Trifala* extract [1]. It is also found to contain Mg, K, P, Al in mg/g amounts besides As, Cu, Mn and Zn at μg/g level. Fig. V.7 shows that *mandoor* contains highest amounts of Na (2.33 mg/g), K (10.3 mg/g), P (3.26 mg/g), Mn (11.4 mg/g) and Zn (135 μg/g). A perusal of data in Table V.5 shows that *lauh bhasma* contains a bit higher amounts of C (1.22%) and S (0.12%) whereas other three *bhasmas* contain much smaller amounts of C and S (~0.1%) with very little H (0.1-0.4%) suggesting the presence of some organic compounds as impurities or ligands. Jani et al. [53] have detected ppm level polycyclic aromatic hydrocarbons (PAH) by HPLC in traditional Ayurvedic medicinal preparations.

(iii) Zinc based: Zn plays a vital role as a constituent of many enzymes in the human body. More than a hundred zinc metalloenzymes are needed in almost all stages of both nucleic acids and protein synthesis. Its deficiency may cause reduction in cell division resulting in growth failure, weight loss and impairment of tissue repair [9,10]. Total dietary intake of zinc is 10-15 mg/d, which primarily comes through diet. Out of three zinc based *bhasmas* analysed in this study *yashad* #1 and #2, procured from two different pharmaceutical firms, contained widely different amounts of Zn, 60.0 and 13.4% respectively whereas *kharpar* contained 123 μg/g Zn only. All three Zn based *bhasmas*

contain higher amounts of Fe besides K, P and Al whereas As, Al, Mn and Cu were found at trace level as illustrated in bar and range plots in Fig. V.9 & 10. However, *yashad* #1 from Baidyanath showed Al, Fe and P but no Ca whereas #2 from Deshrakshak Aushdhalaya contained significant amounts of Mg (6.7%), Ca (19.3%), K (0.22%) and Mn (0.03%). *kharpar* is described primarily as $ZnCO_3$ [54] though it also has a major component of Ca (23.9%) and C (8.4%) suggesting $CaCO_3$ as well. Besides mg/g amounts of Na, K, P & Fe and traces of Au (139 ng/g) were also observed. It helps in eye disease, diabetes, skin disease, fever, memory enhancer, chronic pyrexia, cough, asthma, boils, and urinary tract infections [54]. Khosa and Dixit [55] have shown *yashad bhasma* to increase, virility and intelligence whereas Puri et al. [56] have shown its role in the treatment of myopia. There are several eye drops in the Indian market such as Zincoren, Occulus and Phenolsulf all containing ~0.1% $ZnSO_4$ as are skin ointments which contain zinc salts as essential ingredient. Thus zinc is useful internally as well as externally for skin and eyes.

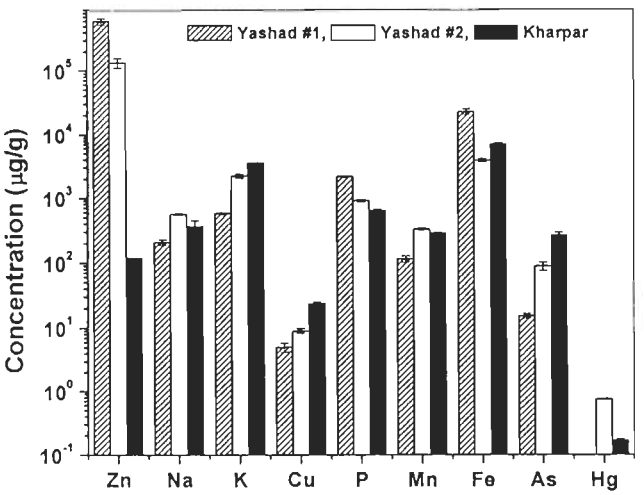

Fig. V.9. Variation in Elemental Contents in Zn based *Bhasmas* (n=3)

(iv) Mercury based: Hg is primarily an environmental contaminant and industrial hazard known for its toxicity causing *Minimata* disease [22, 57]. It is said to be toxic in any form, the difference lying only in how it is absorbed, the clinical signs and symptoms, and the response to treat modalities. It has

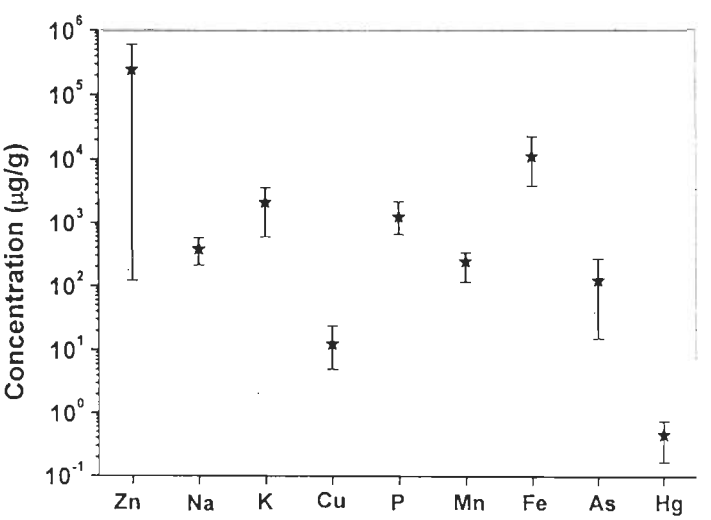

Fig. V.10 Range and Mean Elemental Contents in Zn based *Bhasmas* (n=3)

been suggested that mercury can cure all diseases if it is properly prepared and used. However, if improperly prepared then it may cause all kinds of diseases [6]. It is considered as a marvel drug in *Ayurveda* with a long history of being used as a nervine tonic and for restoring normalcy to collapsing patients [58]. Mercury toxicity leads to Alzheimer disease, Parkinson disease, gastrointestinal symptoms, renal dysfunction and neuro-psychiatric abnormalities. In a pharmaco-kinetic and bio-distribution study of Ayurvedic formulation *kajali* by using ^{203}Hg tracer, Subramanian et al. [59] did not observe any ill effects including brain in Wistar rats. According to the *Ayurvedic* literature, the toxic effects of Hg are neutralized in presence of sulfur [60]. Vohora et al. [19] have studied its CNS and adaptogenic effects and found it to be growth promoting, rejuvenating and facilitating learning process in small doses of 15 mg/kg. The most renowned of all mercury preparations is *Makaradhwaja* which acts as rejuvenator. Our sample of *Siddha Makaradhwaja* procured from Baidyanath, a reputed firm for *Ayurvedic* preparations contained 85.3 % Hg and 14.2 % S totaling 99.5 % corresponding to almost perfect stoichiometry of HgS. Surprisingly no other element could be detected in this *bhasma,* except small amounts of C (0.064%) and H (0.034%) which may be attributed to some organic impurities. No traces of Au could be detected as reported in *Ayurvedic* literature [6, 61]. On the other hand, *parad*, another Hg based *bhasma* procured from a local physician showed only 0.02% Hg and 0.04% S with major constituents of Ca (24.7 %) and Mg (6.58%) besides Mn, Al and P in few hundred μg/g amounts. Presumably, some $CaCO_3$ and $MgCO_3$ must have been added by the physician to make it cheaper. This is a typical case which calls for stringent quality check of the product not only for its toxicity but also for its trace impurities, which may be playing a role in disease curing.

(v) Gemstone based: Gemstones have long been used in *Ayurveda* since alchemy days. Most minerals require 5, 7 or 11 incinerations for purification (*shodhan* and *maran*). Mica is considered as the most powerful when it is incinerated 100 or even 1000 times. Many companies tout their diamond *bhasma* as a panacea for all ills including cancer [6]. We have analysed two *bhasmas*, *vaikrant* and *jahar mohara khatai pishti*, which are tourmaline (black stone with hexagonal crystal) and serpentine orephite (green) based respectively as illustrated in Fig V.11. Both were procured from Deshrakshak Aushdhalaya, and contain many other nutrient elements including K, Mg, P, Mn, Fe, Cu, and Zn in

significant amounts. *Bhasmas* based on gemstones, mica and diamond find much importance in *Ayurveda* but their incineration process is very important. For example, mica (called *abhraka*) is believed to be an excellent rejuvenator for lungs and for Rasa Dhatu. *Vaikrant* alleviates excess *Vata-Pitta-Kapha,* increases vitality and can be used as a substitute of diamond *bhasma*. *Jahar Mohara* is a mineral stone, also called magnesium silicate, green in color [54]. It is commonly used to neutralize poisonous effects of snakebite causing vomiting and is most recommended by *Unani* physicians. Some physicians have used it for heart palpitation, nervousness, depression and irregular heartbeats [54].

Fig. V.11 Minerals showing natural *Tourmaline* (1,2 &3) and *Serpentine orephite* (4 & 5)

(vi) Other Bhasmas: Gold and silver have long been used in India either as water utensils by the upper class society or as tiny particles/thin foils for covering the eatables including sweets. There are several wines widely used in western countries where fine gold particles remain suspended. Vohora and coworkers [61] have investigated silver preparations for analgesic activity, neuropsychobehavioral effects and attributed its therapeutic ability in CNS diseases including epilepsy. It has been shown that *rajat bhasma* based on Ag acts on the brain and nervous system through nutritive mechanism [58]. In lower doses it has anxiolytic effect but in higher doses (10-20 mg/kg) it induces behavioural despair. It is also recommended for eye disorder and tuberculosis [4]. Our sample of *rajat bhasma* contained 23.4 % Ag besides As (14.2 %), P (5.14%) and Na (1.28%) with Mn (183 μg/g) and Au (140 ng/g) in trace amounts. It also showed 19.9% S suggesting the possibility of silver sulfide (Ag_2S) or other sulfides (possibly As_2S_5) besides small amounts of C (0.63%) and H (0.25%) due to some minor organic constituents such as PAH [53].

Though arsenic is considered as the king of poisons, it is now known to be a possible essential element in *Unani* medicine with analgesic activity and proconvulsant effects [62]. Siddiqui et al. [62] have shown that calcined As preparations showed no acute toxicity and

apparently had a wide therapeutic index. Arsenic based *kushta khas* shows 3.65 % As along with a major components of Ca (30.1 %) and S (21.1%) besides minor constituents of Na (0.83 mg/g), K (2.42 mg/g), P (1.06 mg/g) & Cl (0.20 mg/g) and trace amounts of Mn and La. Higher amount of Ca may be due to CaO or $CaSO_4$. As may be present as arsenic sulfide (As_2S_3 or As_2S_5) besides some organic impurities as evidenced by small amounts of C (0.24%) and H (0.05%) as minor constituents.

Copper is an integral part of several enzymes as it influences the immune system. Its vessels are still considered as auspicious in Indian household and water boiled or just kept in copper vessel is prescribed to ladies after delivery and for treating for many ailments [6]. It is reported to act as antioxidant and plays an important role in scavenging superoxides [63]. Tripathi and Singh [64] investigated its role in lipid peroxidation with no detectable adverse effects. Our sample of *tamra bhasma* contained 44.1% Cu besides Fe (7.12%), P (1.09%), K (2.37%) and µg/g amounts of As (~20), Mn (134) and Zn (358). Higher amount of S (22.0%) may be indicative of $CuSO_4$. It is useful in jaundice, piles, leprosy, leukoderma, asthma, tuberculosis, sluggish and fatty liver, obesity, weight loss, parasitic infestation and skin disease [64].

Chopra et al. [24] have described the preparation of *vanga bhasma.* Our sample was prepared by incineration of tin in an iron pot till red hot and mixed with Aparmaga (*Achyranthes aspera*) and Ghritkumari (*Aloe barbadensis)* [54]. It contained 43.8% Sn besides significant amounts of Ca (7.35%), Fe (0.3%), and K (0.88%) besides µg/g amounts of P (720), Mn (257), Zn (67) including In (17.1). It may be mentioned that indium is a rare element, which is frequently present in stannite and other complex sulfides of tin because of similarities in properties [65]. It also contained C (4.2%), H (0.64%) and S (0.15%) suggesting the presence of some sulfides of tin or organo-sulfur compounds, which may remain chelated with various metals. In Ayurvedic literature it has been recommended in diabetes, semen disorder, impotency, skin disease, syphilis and gonorrhea [54]. It is also prescribed for asthma, cough and blood disorders [4].

Potassium based *swet parpati*, containing 39.3% K and 13.7% N, corresponds to the stoichiometry of KNO_3 (>99%) and is commonly known as *kalami sora* a local name for KNO_3. Besides some KCl may also be present in <1% amount. We have also observed Na (80), P (90), Cu (12.3), Mn (2.7) and Zn (54.3) in µg/g amounts. It is prepared by melting in

an earthen pot and then in a ceramic pot till flakes are formed and its powder with cold water or coconut water is recommended for urinary tract infection, enlargement of prostate gland and acidity [54].

(vii) Verification of Particle Size: A *bhasma* is suitable for drug use if it has no metallic luster. It is extremely fine powder such that the particles are smaller than skin ridges of the index finger and the thumb, float on cold water, does not revert to metallic form, maintains its potency indefinitely and manifests no toxicity [4]. The processing technology during the preparation of *bhasmas* is very complex and specific according to ancient literature. It has been observed that micro cracks are developed at the grain boundaries during thermal cycling. It is supposed that microfine medicinal product has easy digestive power and quick reaction with the bile juices. Some workers have suggested *bhasmas* as biologically produced nano particles. Prakash [1] has reproduced some photographs showing microstructures of gold, zinc and lead *bhasmas*. However, no experimental measurements have ever been reported confirming the nano-sized particles. In order to confirm these literature claims, we recorded TEM photographs (Fig V.12), a few *bhasmas* of *tamra, yashad, shankh, lauh* and *vanga*, corresponding to Cu, Zn, Ca, Fe and Sn respectively. As can be seen from the photographs (Fig. V.12), the particle size of *yashad* (Zn) was found to be 520 nm and those for *shankh* (Ca) and Fe these correspond to 260 nm. *Vanga* containing Sn showed a much bigger particle size of 2 μm. Surprisingly the *tamra bhasma* showed only a diffused picture, which did not allow us to draw any conclusion what so ever. Thus, our experimental observations clearly show that the *bhasmas* are not exactly of nano particle size but somewhat of larger size. It may be mentioned that all the *bhasmas* analysed in this study

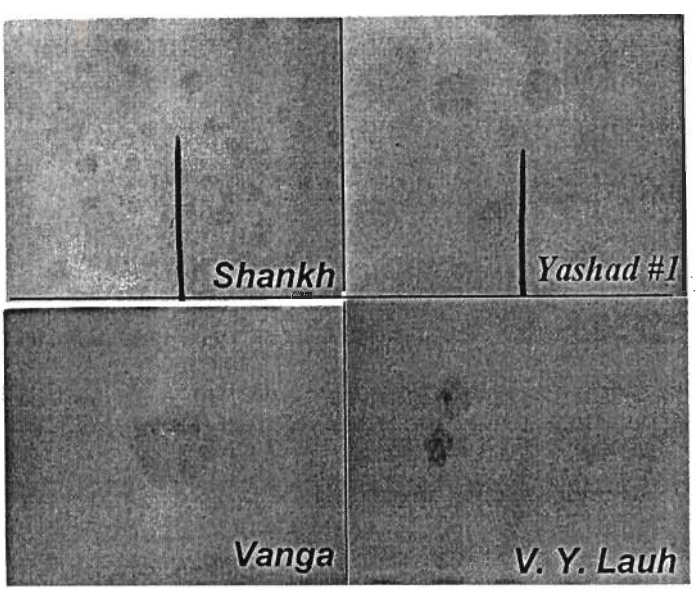

Fig.V.12 Transmission Electron Micrographs of some *Bhasmas*

were old samples where some aggregation might have occurred. It is suggested that more thorough experimental evidence using high resolution TEM are required to confirm the nano particle size of *bhasmas* where freshly prepared samples may be tested.

(viii) Honey as a carrier of *bhasmas:* As already stated *bhasmas* are highly inert in nature because of insoluble nature. Mostly the *bhasmas* are mixed with cardamom, cinnamon, ghee and honey and are taken orally. Although honey is one of the most frequently suggested vehicles in *Ayurvedic* texts, royal jelly, another honeybee product, is apparently not mentioned. Honey is considered as highly nutritious with several nutrient elements (Na, K, Rb, Ca, Mg, Fe, Mn, Cu, P and Zn) besides toxins (As, Cd, Pb) reported in literature [66-68] where metal profiles have been used for its classification and source of origin. It is widely used in western market to apply on a toast in the morning. Natural food users claim that royal jelly very quickly lowers blood sugar when taken orally by the diabetic patients. In fact, it contains a polypeptide that is similar to bovine insulin [69]. The crude royal jelly and a fraction that co-migrates chromatographically metabolize [^{14}C] glucose in *invitro* incubation with rat adipose fat tissues. The molecule is about 5000-6000 Daltons, contains disulfide bonds, and has an amino acid composition similar to that of bovine insulin [4]. Looking to its importance as a diet in recent years, a new reference material of honey has been developed [70].

(ix) Comparison with Literature Studies: In order to confirm the quality of our analytical data, main constituent elements in various *bhasmas* were further analysed by other analytical techniques such as flame photometry, EDTA titrimetry and atomic absorption spectrophotometry (AAS). A comparison of our data as obtained by INAA and other analytical methods with those reported in literature are summarized in Table V.4. It is observed that our NAA data not only compare well with those obtained by other methods but also our overall data are in comparable range with those reported in literature (Table V.4). Of course small differences exist in some cases and these may be attributed to the fact that the samples analysed in this study are not exactly similar.

(x) Toxicity of *Bhasmas*: Risk assessment of essential trace elements examines high intake resulting in toxicity and low intake resulting in nutritional deficiency of the element. Most of the elements are present in low concentrations well within recommended dietary allowances. It has been observed that most *bhasmas* analysed in this study contain

significant amounts of As and Hg both of which are highly toxic. In clinical practice *bhasma* is not reported to have any serious untoward effects [1]. Our compositional data, in general, show the need for strict quality control to provide quality Indian *Ayurvedic* preparations now manufactured by pharmaceutical companies, which need to have R&D laboratory. We attempted to analyze the water extract of some of the above *bhasmas* but observed negligible solubility. Use of metals in medicine is often associated with the question of toxicity [71]. Many studies have clearly shown that these are non-toxic but exhibit free radical scavenging activity due to their antioxidant property [4,6]. The *bhasmas* are associated with organic compounds, show significant increased superoxide dismutase and catalase activity, two enzymes that reduce free radical concentration in the body. The preparation and purification of *bhasmas* undergo elaborate traditional purification procedures and are well mixed with extracts of herbs, fruits and juices etc. The presence of extraneous elements present at minor or trace level is due to the medium in which they are prepared and thus may perhaps help in enhancing their potentiation.

It has been suggested that insoluble salts present in *bhasmas* are less toxic as these are likely to be less absorbed or if at all absorbed, these should not have any adverse effects [72]. However, it may be emphasized that presence of toxic metals in these bhasmas is quite controversial and a matter of worry. As already mentioned many arsenic and mercury preparations have been considered as medicinally important in various therapies [19,62]. It seems all the pharmaceutical firms are not following the procedures laid down by *Charak Samhita* and there is a strict need for standardization of procedures while preparing these *bhasmas* or other medicinal formulations.

(xi) Inter-elemental Correlations: Despite the fact that one element in all the *bhasmas* is the main constituent and all others may be derived from the herbs or may have been due to contamination. According to Ayurvedic literature each constituent is added in a definite proportion. Our studies have shown that the concentration level of many elements in various herbs is strongly affected by the plant characteristics as well as soil and climatic conditions. Therefore, it is quite obvious that some inter-elemental correlations may be observed. The most important of these is K/P ratio in 16 *bhasmas*. Histographic representation of these ratios is shown in Fig. V.13. It is observed that K/P ratio varies in a wide range of 0.23 (*mukta moti*) to 12 (*Vaikrant*). In three *bhasmas* (*mukta moti, yashad* #1

and *vanga)*, it is < 0.5 whereas for majority of *bhasmas* (n=9), it is in a close range of 0.9-2.4 with only two *bhasmas* (*kharpar* and *vaikrant)* showing K/P > 5.5. For 2 out of 3 iron based *bhasmas*, (*mandoor* and *vanaspati yog lauh*) K/P is ~3.

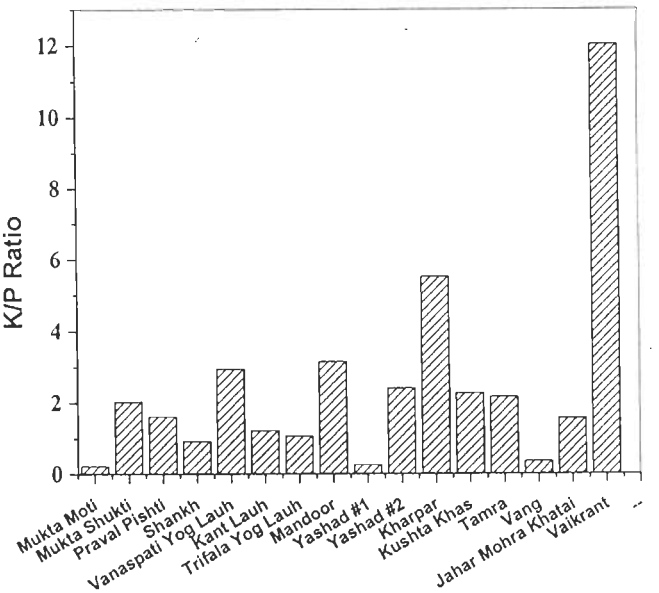

Fig. V.13 Variation in K/P in various *Bhasmas* (n=16)

Na and K, both derived from herbs only, are primarily electrolytic elements responsible for maintaining fluid balance [7]. Therefore, K/Na ratio should also be a factor of importance. Four Ca-based *bhasmas* show lowest K/Na ≤0.2 whereas for *tamra bhasma* it is highest (95). For most other *bhasmas,* K/Na (n = 9) is in a small range of 2 to 10.

Further, Fe/Mn ratio in nine non-iron containing *bhasmas* also varies in a large range of 2.9 to 530, but it is linearly correlated (r = 0.96) with Fe content as shown in Fig. V.14. It may be noted that in all these *bhasmas*, Fe and Mn are likely to be derived primarily from herbs only. Thus, elements that are not primary constituents, but having been derived from herbs seem to be somewhat correlated.

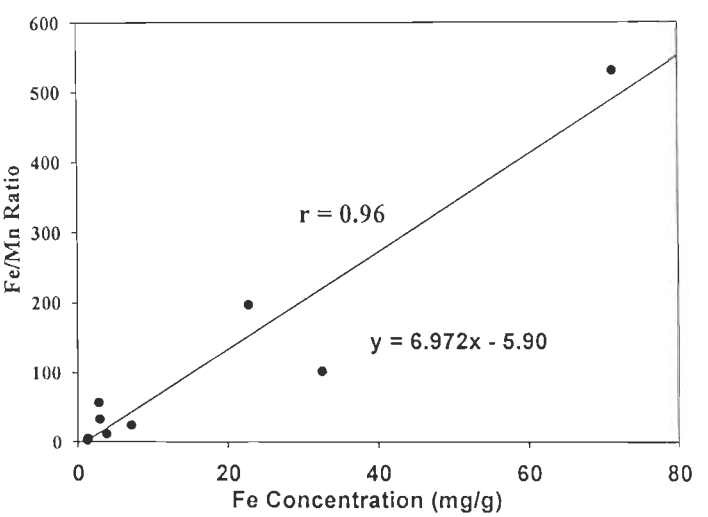

Fig. V.14 Variation of Fe/Mn with Fe Content in non-iron containing *Bhasmas* (n = 9)

In the preceding Ch. III, IV and the present V involving the analysis of 30 herbs and 20 bhasmas, K/P values have been calculated for leaves (n = 13), fruits (n = 8), and 3 each of seeds, bark and roots including 16 bhasmas. Fig. V.15 shows a plot of ranges and mean K/P values, which also show

variations. It is observed that for *bhasmas*, range of K/P is minimum (0.23-12.0) with a mean value of 2.49±2.87. For fruits and roots, maximum ranges observed were, 2.07 – 20.7 and 6.56 –24.2 with their mean values being comparable (13.0±6.4 and 14.1±7.4). On the contrary, mean K/P values for leaves, bark and seeds are comparable (9.25±4.6, 8.86±2.56, 10.1±6.3 respectively) though these lie in between those for *bhasmas* and roots/fruits. It may be noted that both the elements in *bhasmas* are derived from herbs only whereas in other plant parts these represent their inherent concentrations. Thus, K/P seem to be characteristic of plant part being maximum for root/fruits and lesser for leaves/bark/seeds.

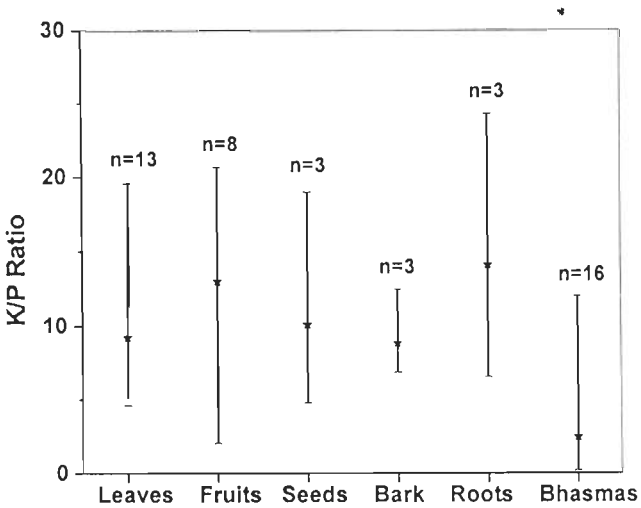

Fig. V.15 Correlation of K and P in various parts of Plants used as Herbal Medicines

(xii) Inter laboratory Comparison Study: We participated in the intercomparison study of Marine sediment (IAEA-433) sent by the MES, Monaco and submitted our data for 23 elements (As, Ba, Br, Ce, Co, Cr, Cs, Eu, Fe, Ga, Hf, K, La, Lu, Mn, Na, P, Rb, Sb, Sc, Sr, Tb and Th) by INAA. Also Estuarine sediment (IAEA-405) was sent as control whereas three RMs Pond Sediment (NIES, Japan) [40], W-1 (USGS) [41] and Soil-5 (IAEA, Vienna) [39] having similar matrices were analysed used as comparators and for quality assurance. The data are listed in Table V.6 where certified/literature values are also included. A perusal of our data for various RMs, shows good agreement (within ±10%) with the certified values. Now we have received the Final Report [36] with recommended and information values and the certified data are listed in the same Table. Comparison of our analytical data for both samples of Marine Sediment, IAEA-433 as submitted agrees with the certified values in the Final report. This is further illustrated in Fig V.16. It is observed that;

- Our data for As, Cr, Cs, Eu, Fe, Na, K, Mn, and Tb are in agreement within ±5% of the certified values.

- Similarly our data for Ce, La, Sb, Sc, Th, Co and Sr agree within ±10% of the certified means.
- Our data for P obtained by neutron irradiation followed by β^- counting in all three RMs are in excellent agreement (±5%) with the certified values. Though no certified or information value is available but our method seems to provide good quality data for geological samples. Hence, future workers could use this as reference value.
- However, our data for Ba, Br, Hf, Lu, and Rb do not agree so well as these are within ±10-20 % of the certified values.

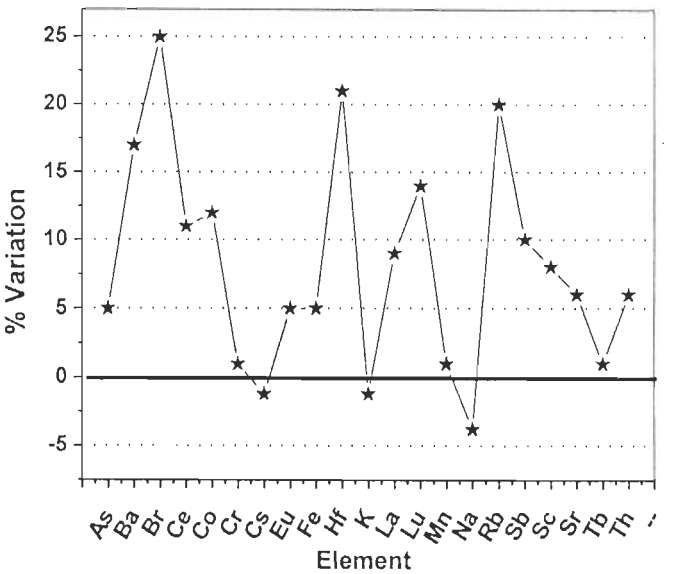

Fig. V.16 Comparison of Elemental Contents in Marine Sediment with Certified values

It may be visualized from Fig. V.16 that our most analytical data are on the positive side of the recommended values except in a few cases where negative deviations are also observed. Despite these observations, it may be concluded that elemental data provided by our laboratory are of reasonably good quality and it provides further confidence and quality control for our analytical data.

V.8 CONCLUSION

On the basis of analysis of twenty *bhasmas* following conclusions may be drawn;

- Hg based *Siddhmakardhwaj*, a Hg based *bhasma* from Baidyanath containing 85.3 % Hg and 14.2 % S corresponds to the stoichiometry of HgS. *Parad*, another Hg based *bhasma* procured from a local physician showed only 0.02% Hg and 0.04% S with major constituents of Ca (24.7 %), C (13.4%) and Mg (6.58%) suggesting the

possibility of $CaCO_3$ and $MgCO_3$ being mixed. This is a typical case which calls for stringent quality check of the product.

- Similarly *swet parpati* containing K (39.3%) and N (13.7%) may correspond to almost 99% KNO_3 with small amounts of KCl. Also in the cases of Ag and As based bhasmas corresponding sulfides may be present.
- Besides main constituent, several nutrient elements such as Mn, Fe, Co and P are also present. However, As and Hg the two toxic elements are also present in significant amounts (beyond permissible limits) in several cases.
- Several clinical studies of calcium and iron based *bhasmas* have shown their wide use for supplementation or treatment of osteoporosis and iron deficiency.
- Such studies play a vital role in providing scientific evidence to Ayurvedic physicians. Better standardization for indigenous metallic prepartions is required by the Research & Development of pharmaceutical firms.
- Some organic macromolecules acting as ligands may be derived from herbs but a more thorough investigation is needed with regard to their elemental contents, speciation and organic constituents including clinical studies so as to understand their therapeutic effects.
- Toxicity part of the *bhasmas* is quite controversial and a cause of worry though it is supposedly neutralized by the medium of honey/butter/milk/ghee.
- Our participation in the Intercomparison study of Marine sediment (IAEA-433) has resulted in the good quality data for many elements providing us confidence in the analytical data from our laboratory.

Metallic preparations offered some advantages over plant drugs by virtue of their stability over a long period, lower doses, easy storability and sustained availability. These have been in use since ancient times and are still considered as useful. However, strict quality control by using contamination free raw materials is essential.

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CHAPTER VI

BIOASSAY OF *BRAHMI*

VI.1 ANTIOXIDANTS

In recent years, there has been increasing awareness about the possible role of nutrients and dietary supplements in the prevention of diseases. Antioxidants neutralise the toxic and volatile free radicals, which are defined as atoms or group of atoms having an unpaired electron [1-3]. Antioxidant compounds in food play an important role as a health-protecting factor [4,5]. Scientific evidences suggest that antioxidants reduce risk of chronic diseases including cancer and heart disease [3]. Therefore, antioxidants specially derived from natural sources such as dietary constituents or herbal products have created a lot of interest and require special attention. It is now well recognized that Indian medicinal plants are a rich source of many beneficial compounds including vitamins [6,7]. In view of this a thorough investigation, especially in terms of the antioxidant activity of various medicinal herbs is required.

Free radicals are highly reactive species because of the unpaired electrons. Free radicals also include related reactive species such as 'excited states' which lead to the generation of free radicals or which result from free radical reactions. In general, free radicals are very short lived, with half-lives in the range of ms to ns [8]. Fortunately, nature has provided a solution to treat free radical damage. Antioxidants are stable molecules having spare electrons, which on contact with free radicals, share their electrons and stop the degenerative chain reaction of free radical oxidation [9]. A study of free radicals as well as antioxidants has attracted the attention of health professionals as well as basic scientists interested in biomedical research. Biology of free radicals is an emerging area of biomedical research with many potential applications, especially in relation to human health, both in terms of prevention of disease and therapy [9,10].

(i) Free Radicals in Biological Systems: Most of the free radicals in biological systems are derived from oxygen. It gives rise to a large number of free radicals and other reactive species collectively known as *Reactive Oxygen Species* (ROS). Oxygen, being paramagnetic, has spin restriction that forbids it from reacting freely with other molecules [11]. During cellular respiration oxygen undergoes incomplete reduction producing super oxide ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$). Many nitrogen containing reactive species called *Reactive Nitrogen Species* (RNS), have both

nitrogen and oxygen and include physiologically important nitric oxide ($NO^{\bullet}$) and toxic peroxynitrite ($ONOO^{\bullet}$). Biologically important ROS/RNS are listed in Table VI.1. Apart from these, there are other biologically relevant reactive species derived from sulphur (thiyl radical, $RS^{\bullet}$), carbon etc. that are being formed in tissues [3]. All these reactive species have important biological implications.

Table VI.1 Reactive oxygen and nitrogen species of significance in human health

Reactive species	Symbol	Half life (in s)	Reactivity/ Remarks
Reactive Oxygen Species			
Superoxide	$O_2^{\bullet -}$	10^{-6}	Generated in mitochondria and cardiovascular system
Hydroxyl radical	$^{\bullet}OH$	10^{-9}	Highly reactive and generated during iron overload and such conditions in our body
Hydrogen peroxide	H_2O_2	Stable	Formed in various biochemical reactions in the body and yields potent species like $^{\bullet}OH$
Peroxyl radical	$ROO^{\bullet}$	1s	Formed from lipids, proteins, DNA, sugar etc. during oxidative damage
Singlet oxygen	$^1O_2$	10^{-6}	Formed from photosensitization
Ozone	O_3	1s	Present as an atmospheric pollutant, can react with various molecules, yielding $^1O_2$
Reactive Nitrogen Species			
Nitric oxide	$NO^{\bullet}$	1s	Neurotransmitter and blood pressure regulator can yield potent oxidants during pathological states.
Peroxynitrite	$ONOO^-$	10^{-3}	Formed from $NO^{\bullet}$ and super oxide
Peroxynitrous acid	ONOOH	Fairly stable	Protonated form of $ONOO^-$
Nitrogen dioxide	NO_2	1s	Formed during atmospheric pollution

(ii) Generation of Free Radicals: These are being constantly generated in our body by a large number of reactions involving either endogenous system, exogenous xenobiotics, during exposure to physicochemical agents or under varying pathological conditions [11,12]. The endogenous system includes;

a) Cellular respiration in mitochondria that involves the reduction of molecular oxygen (O_2) to water in the electron transport chain;
b) During functioning of the microsomal electron transport involving cytochrome P450;
c) Iron and copper salts that promote the generation of oxidizing radicals from peroxides;
d) Peroxisomes, which are orgamelles responsible for degrading fatty acids and other molecules that produce H_2O_2 as a by product and under certain conditions, $O_2^{\bullet -}$ is produced along with to form more potent $^{\bullet}OH$;
e) Auto-oxidation of natural compounds such as catecholamines, coenzymes Q_{10} and epinephrine (generates $O_2^{\bullet -}$ in blood vessels and catecholamine). Autooxidation is an important source of ROS which is released under certain pathophysiological conditions;
f) Oxidizing enzymes that produce ROS including diamine oxidase, tryptophan dioxygenase, xanthine oxidase, lipoxygenase, cycloxygenase, nitric oxide synthase (under low arginine conditions); guanyl cyclase, glucose oxidase, myeloperoxidase, lactoperoxidase and chloroperoxidase.

Other sources of free radicals are redox cycling of xenobiotics, exposure to physiochemical agents, ionizing radiations such as X- and γ-rays, UV in presence of oxygen and photosensitizer. Cigarette smoke contains a large amount of reactive species including several oxides of nitrogen and stable free radicals such as semiquinone radicals. Smokes from other sources are also potent generator of peroxidants [11,12].

VI.2 OXIDATIVE STRESS

Apple turns brown, butter turns rancid, iron rusts; all are everyday signs of oxidative stress -destruction caused by free radical molecules. But none of these nuisances compare to what these unstable molecules can do inside the body, especially to brain cells. Free radicals, products of normal cell processes, wreck havoc during their hunt for a mate. The source of their devastating action is the oxygen molecule's unpaired electron, which makes it unstable and electrically charged. It becomes stable by

interacting with the nearest available molecule. Having no prejudices, it targets lipids, proteins and DNA. Scientists have discovered that the free radical's action can damage molecules they react with and sometimes cause the cell's demise. In a normal healthy organism or human body, the generation of pro-oxidants in the form of ROS and RNS are effectively kept in check by the various levels of antioxidant defense. However, when the humans are being exposed to adverse physicochemical environmental or pathological agents this balance is shifted in favour of pro-oxidants resulting in oxidative stress [11-13]. Cellular damage induced by oxidative stress has been implicated in the etiology of a large number (> 100) of human diseases as well as the process of ageing. The degenerative diseases associated with ageing where free radicals have been implicated include:

(a) Cardiovascular ailments like coronary heart disease and cardiomyopathy
(b) Neurodegenerative disease like Parkinson's disease, Alzheimer's disease, brain dysfunction and lateral sclerosis
(c) Multistage process of carcinogenesis
(d) Immune system decline during aging
(e) Cataract formation

The pathological conditions implicating ROS/RNS are diabetes, rheumatoid arthritis, cystic fibrosis, hemorrhagic shock, gastrointestinal ulcerogenesis, AIDS, metabolic disorders like erythropoietic porphyria, lung disease like adult respiratory distress syndrome, chronic obstructive pulmonary diseases, etc.

All aerobic organisms, including human beings, have antioxidant defense that protects against oxidative damage, numerous damage enzymes to repair or remove damaged molecules [14]. However, this natural antioxidant mechanism can be inefficient, and hence dietary intake of antioxidant compounds is important [15]. There are some synthetic antioxidant compounds, such as butylated hydroxy toluene (BHT) and butylated hydroxyanisole (BHA), commonly used in processed foods. However, it has been suggested that these compounds have side effects [16]. In addition it has been suggested that there is an inverse relationship between dietary intake of antioxidant rich food and the incidence of human disease. Therefore, investigation of the natural antioxidant sources is important [17-19].

VI.3 MEDICINAL PLANTS AS A SOURCE OF ACTIVE CONSTITUENTS

A large portion of the world population, especially in developing countries depends on the traditional system of medicine for a variety of diseases. Several hundred genera used medicinally, mainly as herbal preparations in the indigenous systems of medicine in different countries, are sources of very potent and powerful drugs. These have stood the test of time and modern chemistry has not been able to replace most of them. The World Health Organization (WHO) reported that 80% of the world's population relies chiefly on traditional medicines and a major part of the traditional therapies involve the use of plant extracts for their active constituents [6,7]. Herbal medicinal plants are rich source of substances that have several therapeutic properties like cardioprotective, chemoprotective and other effects. Supplementation of our diet with antioxidants, especially from natural sources like medicinal plants, also greatly help in prevention of diseases. Hence there is a need to review antioxidant and therapeutic activities of medicinal plants. Natural compounds from medicinal plants having antioxidant activities have potential as therapeutic agents.

Brahmi (*Bacopa monnieri)*, family scrophulariaceae is a reputed nerve tonic used for enhancing memory, curing epilepsy and insomnia and as a mild sedative in Ayurvedic literature and benefits both the mind and spirit, and improves the intellect and consciousness; the cognitive disorders of aging [20, 21]. It assists in heightening mental acuity and supports the physiological processes involved in relaxation. *Brahmi* is also reported to possess hepatoprotective [22] and anti-ulcer properties [23]. Due to these properties, *brahmi* is considered to have curative potential towards Alzheimer and Parkinson diseases, both being neurodegenarative under clinical conditions.

VI.4 LITERATURE SURVEY

The antioxidants can prevent ROS-mediated damages and thus, may have potential application in prevention and/or curing of the disease [16-19]. Hence, there is a growing interest on new antioxidants especially from natural sources for use in preventive medicine.

Several workers from different countries have analysed medicinal plants for their various biological activities and active constituents e.g. from Bangladesh [24], China [25], France [26], Hungary [27], Italy [28], Japan [29], Korea [30], Mexico [31], Nepal [32], Nigeria [33], Norway [34], Pakistan [35], Russia [36], Switzerland [37], Tanzania [38], Turkey [39], UAE [40], USA [41], Ukrain [42] and U.K. [43].

Shukla et al. [44] studied *brahmi* rasayana and Ayurvedic preparations for its effect on central nervous system (CNS) at oral dose ranging between 1 and 30 g/kg in mice and rat. Jain et al. [45] reported the anti-inflammatory effect of an Ayurvedic preparation of *brahmi* in rodents. Tripathi et al. [46] determined the effect of alcoholic and hexane extracts of *brahmi* on lipid peroxidation. Renukappa et al. [47] applied HPLC coupled to NMR, MS and bioassay for the determination of active saponins from *Bacopa monnieri*. Rao et al. [48] studied extracts of some medicinal plants and shrubs including *brahmi* leaves as inhibitors in the mineralization of urinary stone forming minerals, viz., calcium phosphate, oxalate or carbonate. Stough et al. [49] and Roodenrys et al. [21] studied the chronic effects of an extract of *Bacopa monnnieri* on cognitive function in healthy human subjects. Chowdhuri et al. [50] studied antistress effects of bacosides of *Bacopa monnieri*; modulation $H_{sp}70$ expression, superoxide dismutase and cytochrome P450 activity in rat brain. Sumathy et al. [22] reported protective role of *Bacopa monnieri* on morphine induced brain mitochondrial enzyme activity in rats. Krishnaraju et al. [51] reported some antioxidant activities in various extracts of *Bacopa monnieri*. Bhattacharya et al. [52] reported antioxidant effect of *brahmi* in the rat frontal cortex, striatum and hippocampus.

VI.5 PRESENT STUDY

Since *brahmi* is neuroprotective and has been demonstrated to enhance memory we envisaged that its beneficial properties could at least in part be attributed to its antioxidant behaviour. In the present study we evaluated different extracts of *brahmi* towards the radical scavenging properties such as DPPH radical scavenging, anti-lipid peroxidation, H_2O_2 scavenging, super oxide anion scavenging and target protection abilities such as DNA strand break. Antioxidant activity is correlated significantly and positively with the total phenolic content of individual extract. The antioxidant activity of

phenolics is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors and metal chelators. In view of this, total phenolics were determined using the Follin's reagent. In order to understand the role of essential elements in respective extracts, these were also analysed for their elemental contents by NAA.

VI.6 EXPERIMENTAL

(i) Preparation of Extracts from *Brahmi:* 50g commercial *brahmi* powder (Yogi pharmacy, Haridwar, India) was excessively extracted twice with methanol (250 mL). The residue was extracted similarly with aqueous methanol (methanol:water = 50:50). The plant residue was further extracted with water. The individual extract was evaporated to dryness. The dry extracts thus obtained were designated as methanol extract (**BM**), aqueous methanol extract (**BAM**) and aqueous extract (**BA**) respectively.

(ii) 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Scavenging Assay: An ethanolic solution of DPPH (100 μM) was incubated with an aqueous solution of the test compound (0.1-1.0 μM) and the absorbance monitored spectrophotometrically at 517 nm. The concentration or inhibition capacity ($IC_{0.2000}$) of the test compound that induced a decrease of 0.20 in absorbance during a 30 min observation was taken as the free radical scavenging potency [53].

(iii) Superoxide Anion Scavenging Assay: Test solutions were prepared by adding xanthine (final concentration 50 μM), hydroxylamine (final concentration 0.2 mM), EDTA (final concentration 0.1 mM) and the test compound in various concentrations (0.1-1.0 μM). The reaction was initiated by adding 0.2 mL xanthine oxidase (6.25 mU/mL) in a 200 mM pH 7.5 phosphate buffer solution. After incubating the mixture (total volume 1 mL) for 30 min at 37°C, 0.1 mL of 0.58 M HCl was added to stop the reaction. The amount of uric acid produced was measured spectrophotometrically at 295 nm [54]. A test mixture without TSP gave the total uric acid produced. The concentration of uric acid produced was calculated from the differential absorbance with a blank solution in which xanthine oxidase was replaced by the buffer solution. The superoxide was assayed by adding the colour reagent (final

concentration of 300 μg/mL sulfanilic acid, 5 μg/mL of N-(1-naphthyl)-ethylenediaminedihydrochloride and 16.7% (v/v) acetic acid) after the incubation and measuring the absorbance at 550 nm after 30 min [55]. Superoxide dismutase was used as a reference inhibitor and BHA as a positive control in these experiments.

(iv) Hydrogen Peroxide Scavenging Assay: Following the reported procedure [56], different concentrations of TSP and 1 mM H_2O_2 were incubated at 25°C for 30 min and the concentrations of H_2O_2 remaining in each case was assayed by the addition of buffered phenol red solution (PRS) and measuring the absorbance at 610 nm. The PRS used in all the assays contained: 140 mM sodium chloride, 10mM pH 7.0 potassium phosphate buffer, 5.5 mM dextrose, 0.28 mM phenol red and 8.5 U/mL of horse radish peroxidase (HRPO). Catalase was used as reference inhibitor.

(v) Anti lipid peroxidation assay: Small unilamellar vesicles (SUV) were prepared from phosphotidyl choline as reported by Patro et al. [57] and peroxidation was initiated either by 50 μM ferrous ammonium sulfate and 500 μM ascorbic acid or by 50 mM AAPH. In both the cases the volume of the reaction was 0.5 mL in a 10mM potassium phosphate buffer (pH 7.4). The test extracts were added at the stated concentrations before the initiation of lipid peroxidation. The peroxidation was initiated by addition of ferrous and ascorbic acid and incubated at 37°C for 30 min in the case of former and by addition of AAPH followed by incubation at 37°C for 60 min in the latter case. In both the cases the peroxidation was terminated by addition of 1 mL TBA-TCA-HCl (0.375 w/v-15% w/v-0.25N) solution followed by incubation at 100°C for 15 min. The colour developed was extracted in equal volume of water-saturated n-butanol and read spectrophotometrically at 532 nm.

(vi) Gamma-ray Induced DNA Strand Break Assay: This assay was carried out as described elsewhere [58] using the *brahmi* extracts. The DNA samples in presence or absence of the test samples were prepared in a final volume of 20 μL and irradiated at 25°C for 3 min (dose rate 6.64 Gy/min) using a ^{60}Co source. In all experiments, the concentration of super-coiled pBR322 DNA was 10 mg/L in a 10 m*M* potassium phosphate buffer (pH 7.4). The test compounds were added as aqueous solutions to achieve the final concentration. After irradiation, the resulting forms of the

plasmid, Form I (super-coiled) and Form II (open circular) were separated by electrophoresis, stained with ethidium bromide and visualized under UV light. The relative intensities of the bands were determined with a Bio-Rad gel documentation system. A typical chromatogram illustrating the damaged and undamaged forms of DNA are shown in Fig. VI.1.

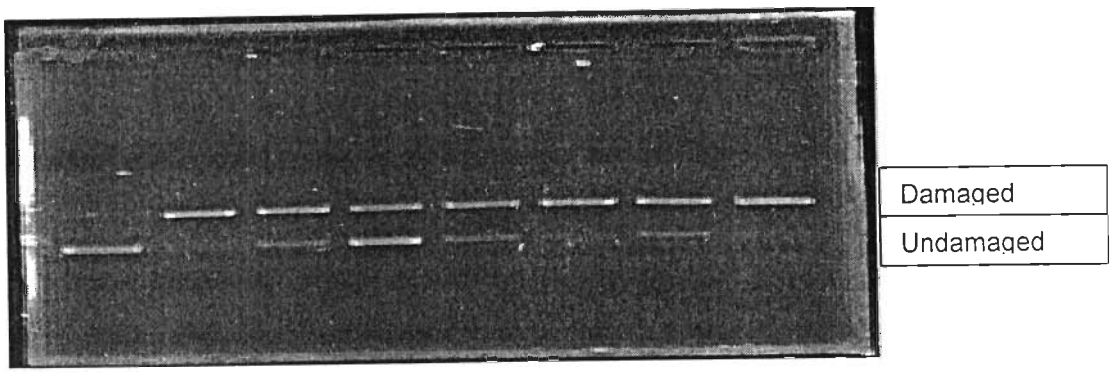

Fig.VI.1. Chromatogram of Damage and Undamaged DNA

(vii) Estimation of Total Phenolic Content: It was measured spectrophotometrically, based on a colorimetric oxidation/reduction reaction using Folin-Ciocalteu as the oxidizing reagent [59]. To 500 µL of extract, 2.5 mL Folin-Ciocalteu reagent (10 times diluted) was added followed with 2 mL of Na_2CO_3 (75 g/L) within a time interval of 0.5 to 8 min. The sample was incubated for 5 min at 50 ^{0}C and then cooled. As a control sample, 500 µL of distilled water was used. The absorbance was measured at $\lambda_{max.}$ = 760 nm and the results were expressed as equivalent of gallic acid (in %). A calibration plot is shown in Fig. VI.2.

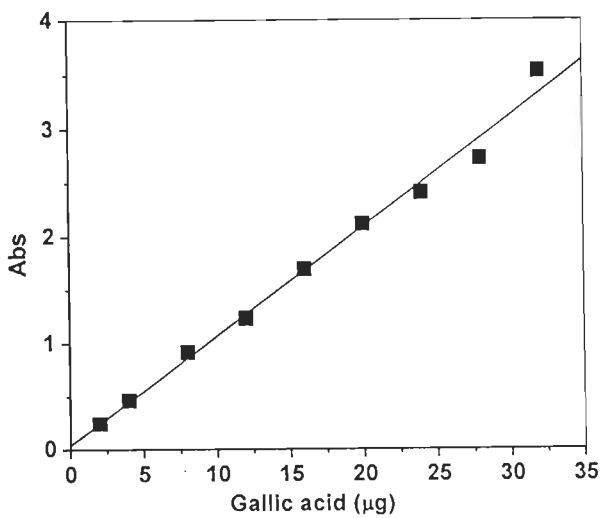

Fig. VI.2. Calibration plot for Gallic acid

VI.7 RESULTS AND DISSCUSSION

In order to understand the antioxidant behaviour and radioprotective properties of three extracts, various parameters were studied and compared to decide its effectiveness. These are described in following lines.

(i) 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Scavenging Assay: Scavenging potential of the nitrogen-centered stable DPPH radical is a useful measure [53] of the antioxidant properties of test compounds. Evaluation of the different extracts of the medicinally important plant *Bacopa monnieri* revealed that all the extracts exhibited DPPH scavenging properties. **BAM** was found to be most effective in scavenging of the DPPH radicals followed by **BA** and **BM** as shown in Fig. VI.3. It is observed that methanolic extract (BM) showed minimum activity (<20% scavenging) in the entire concentration range.

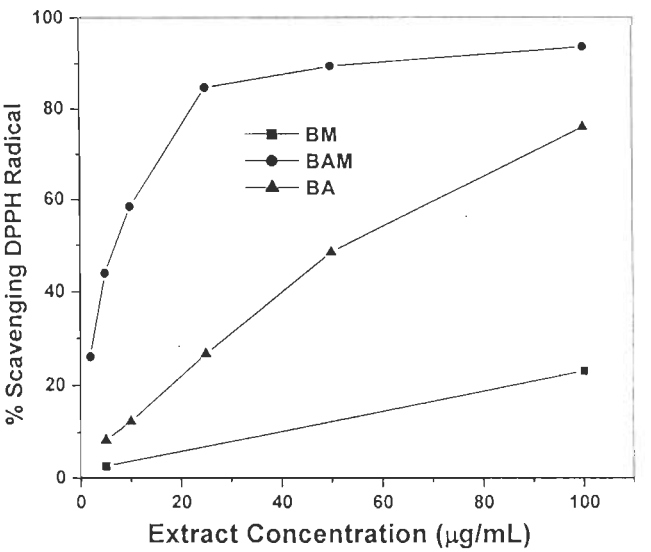

Fig. VI.3. Scavenging Activities of *Brahmi* Extracts for DPPH Radical

(ii) Hydrogen peroxide scavenging assay: Hydrogen peroxide has an important physiological role as it is a relatively stable molecule, non-toxic and has an essential role in the oxygen-radical-generating system as a precursor of hydroxyl radical, which oxidizes all substances close to it. The generation of hydroxyl radical from H_2O_2 is fully dependent upon the availability of iron, which is always present in tissues [60]. More importantly redox potential of Fe^{2+}/Fe^{3+} couple is favourable (+0.77 V). H_2O_2 plays a crucial role since it diffuses freely across cellular membranes [61] and perpetuates the oxidative damage from the site of its generation. *brahmi* extracts (**BM**, **BAM** and **BA**) showed a concentration dependent H_2O_2 scavenging activity as shown in

Fig. VI.4. It is observed that H_2O_2 scavenging increases with concentration in all the three cases though for BAM it reaches maximum at a much lower concentration of <10 μg/mL whereas BM extract shows up to ~60% scavenging at 400 μg/mL. The order of effectiveness, however, remains similar (**BAM> BA> BM)** to DPPH radical scavenging.

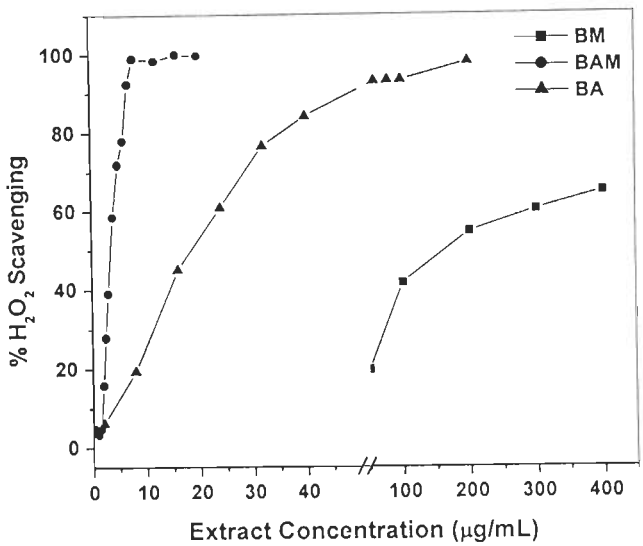

Fig. VI.4. Effect of *Brahmi* Extract against H_2O_2 Scavenging Activity

(iii) Superoxide Scavenging Assay: The superoxide radical, despite being less reactive, can furnish more toxic hydroxyl radicals [62] and this has been the cause for many pathological processes including ischaemia-reperfusion injury [63]. Besides, its generation in living systems during xanthine oxidase (XOD) mediated oxidation of hypoxanthine or xanthine leads to accumulation of uric acid, which plays a crucial role in gout [64]. Consequently, scavenging of the radical and/ or inhibition of XOD would be a promising remedy for these diseases. Superoxide scavenging

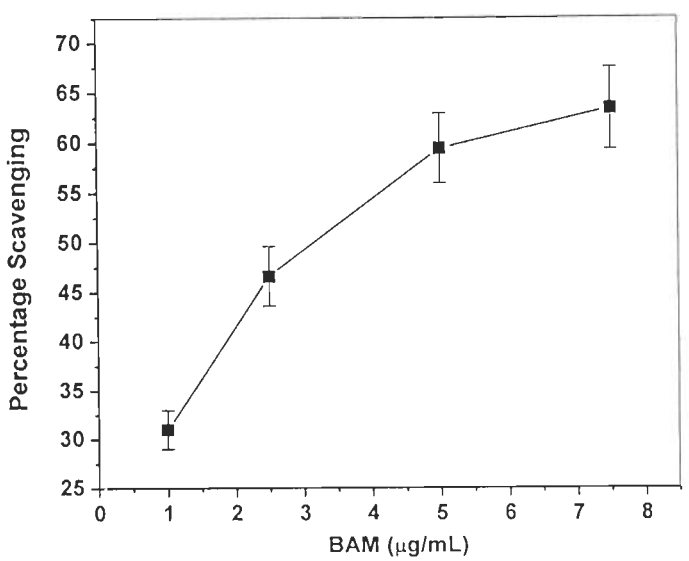

Fig. VI.5 Superoxide Scavenging Properties of BAM

properties of **BAM** was investigated and the results are presented in Fig. VI.5. It is observed that a maximum of ~60% activity is observed for BAM extract. For other extracts it was not studied.

(iv) Anti Lipid Peroxidation Assay: Lipid peroxidation is a free radical-related process in biological systems that may occur (i) under enzymatic control, e.g., for the generation of lipid-derived inflammatory mediators, or (ii) non-enzymatically. This latter form is mostly associated with cellular damage as a result of oxidative stress. Oxygen radicals and lipid peroxidation play a pivotal role in the observed damage during central nervous system trauma and stroke [65], and more generally in neurotoxicity [66]. Two different peroxidation inducers viz. Fe^{2+}/ascorbate and AAPH were used for studying the anti-lipid peroxidation activity of *brahmi* extracts in liposomes. AAPH is a radical generator itself and iron does not play any role in the peroxidation induced by it. On the contrary Fe^{2+} is known to react with lipid hydroperoxides to yield highly reactive alkoxyl radical. It was observed that *brahmi* prevents lipid peroxidation induced by Fe^{2+}/ascorbate (Fig.

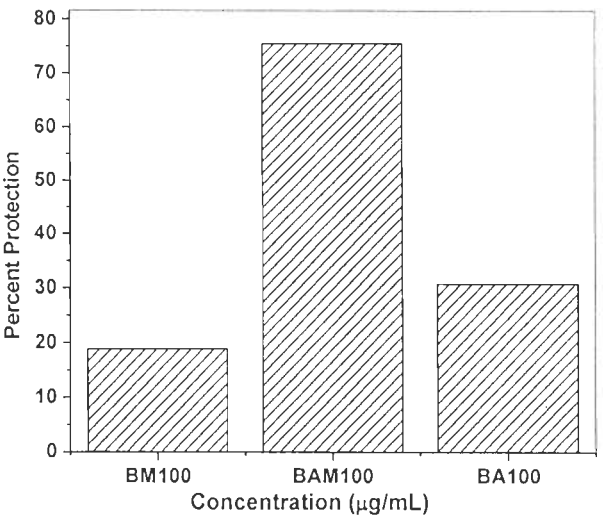

Fig. VI.6. Anti-lipid Peroxidation Activity of *Brahmi* Extracts in Liposomes: Fe(II) + Ascorbic acid

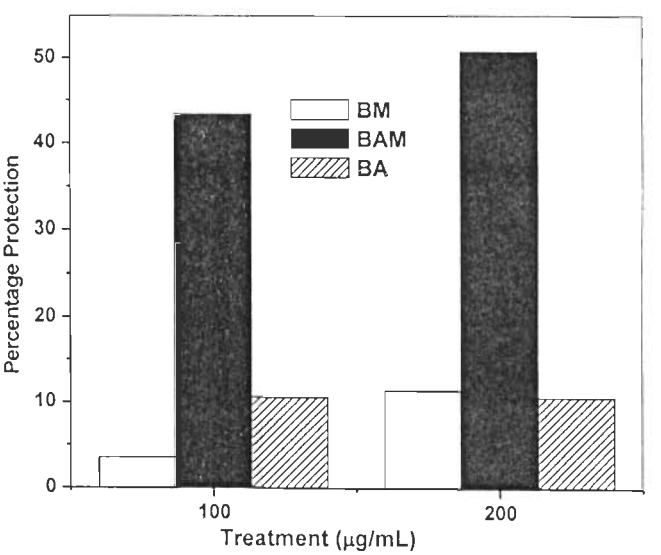

Fig.VI.7. Anti-lipid Peroxidation Activity of Brahmi Extracts in Liposomes: AAPH

VI.6) as well as AAPH in a concentration dependent manner (Fig. VI.7). In both the cases, **BAM** showed superior effectiveness to prevent lipid peroxidation than **BM** and **BA**. In the case of **BAM,** the IC_{50} values were found to be 8.57±0.93μg/mL and 144±29.7μg/mL respectively as shown in the Figs. VI.8 & 9 with Fe^{2+}/ascorbate and AAPH systems This indicates that **BAM** exhibits its effects by chelating iron apart from scavenging radicals.

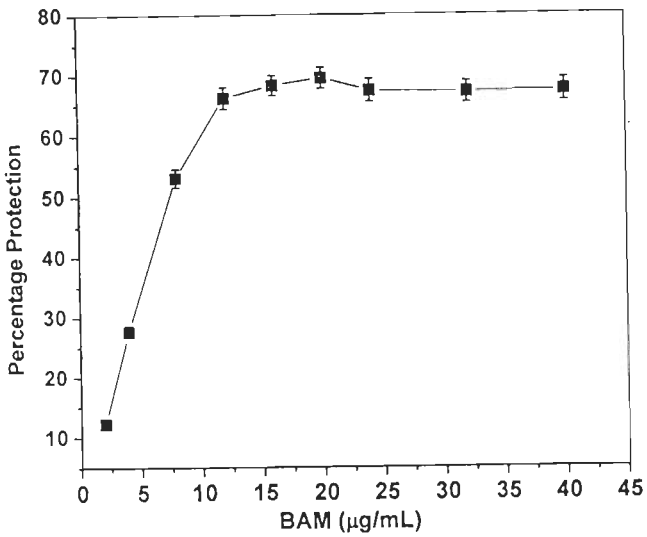

Fig.VI.8. Anti-lipid Peroxidation Activity of BAM in Fe(II) + Ascorbic acid System

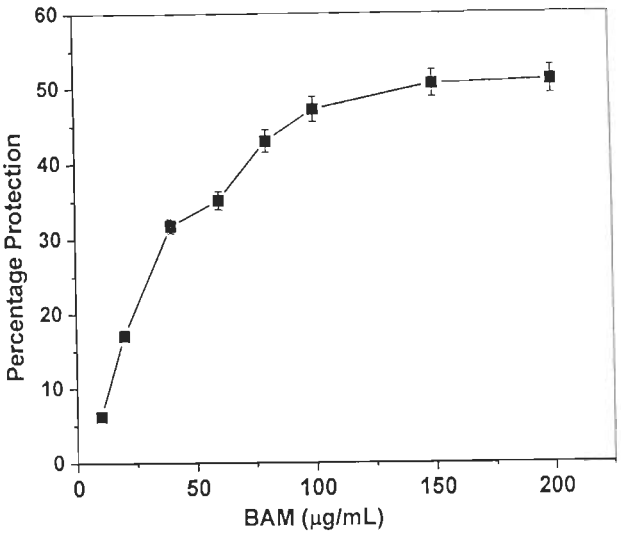

Fig.VI.9. Anti-lipid Peroxidation Activity of BAM in AAPH system.

(v) DNA Strand Break Assay: Due to the polyanionic nature of DNA it binds transition metal ions that mediate hydroxyl radical generation. Free radical damage to DNA has been proposed to lead to several types of cancers [67,68]. The deleterious biological consequences of both ionizing and non-ionizing radiations especially with respect to causing mutation and carcinogenesis are well documented [69,70]. The main cause of these effects is believed to be scission of DNA molecules by radiation. A pre-requisite of radical-mediated DNA strand break is the degradation of its sugar moiety via a direct or indirect process. Due to the high concentration of water in metabolizing cells, radiation exposure of biological systems primarily leads to its radiolysis furnishing e^-_{aq}, $^\bullet OH$ and $H^\bullet$. These primary radicals can directly abstract hydrogen atoms at the C(4′) position of deoxyribose or generate DNA base radicals which subsequently damage the sugar moiety finally leading to DNA strand breakage. In aerobic cells, the electron is readily accepted by the easily reducible oxygen molecule generating various reactive

oxygen species (ROS), all of which can damage DNA. *Brahmi* extracts could effectively prevent γ-ray induced damage to plasmid DNA in a concentration-dependent manner (Fig. VI.10). It is observed that maximum undamaged % DNA occurs at 200 μg/mL and at still higher concentration it exhibits a little decrease. Thus, similar to other cases, BAM extract of *brahmi* is most radioprotective (~70%) compared to other extracts.

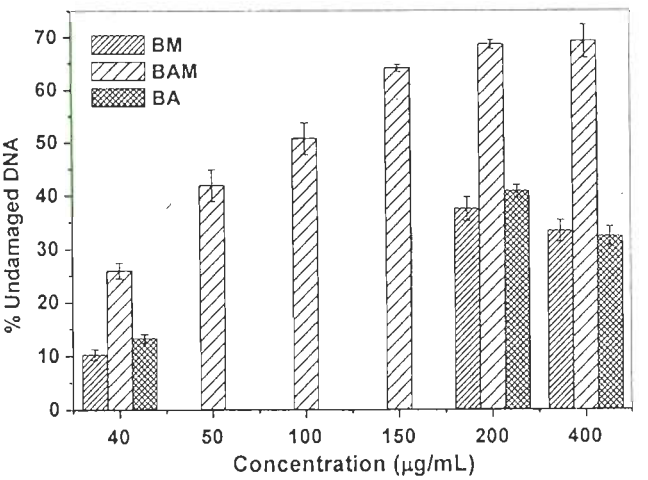

Fig. VI.10. Protective Effect of *Brahmi* Extracts against γ-ray induced Damage of DNA

(vi) Total Phenolics: The antioxidant properties of plant extracts are largely due to the presence of different phenolics present in them [71,72]. In this context total phenolic content of the different fractions of *brahmi* was determined and expressed in terms of the standard compound gallic acid equivalent. The total phenol contents of the three extracts **BM**, **BAM** and **BA** were found to be 3.56, 33.5 and 4.12% equivalent of gallic acid respectively. Thus, the **BAM** extract showed maximum antioxidant activity. It is now amply clear that the antioxidant preventive role of plant products is due to their constituent chemicals, especially the polyphenolic compounds. Hence, it is concluded that antioxidant activity of these extracts could be correlate with their respective phenol content.

(vii) Elemental Concentrations in Raw Materials and their Extracts: As already emphasized in earlier chapters many elements in trace amounts play a vital role in various biochemical processes and these are crucial for many body functions including transport of oxygen, normalizing the nervous system and stimulating the growth, maintain and repair of tissues and bones. Perfect balance of these elements is important to all parts of our lives and nutrition, but it is particularly crucial to have them in optimum concentration range and bioavailable form. Even though minerals and trace elements comprise only a fraction of our total body weight, these are most beneficial if they are in balanced amount and interact with each other. In addition, there are

hundreds of isotopes of the elements, some of which may play an important as yet undiscovered role in human health. It is becoming increasingly important to study the relationship of minerals to the human health that keep the level in balance in every tissue, fluid cell and organ in the human body, a key to maintaining good health. Several studies have reported elemental contents in plant extracts, which are taken by us either as tea or medicine [73,74]. In order to understand the role of elemental contents in respective extracts and raw *brahmi*, INAA data are listed in Table VI.1. A perusal of data shows that only a fraction of a few elements except Na, K and Cl, the electrolytic

Table VI.2. Elemental contents in raw and different extracts of *brahmi*

Element	Raw Sample (50 g)	BM (2.35 g)	BAM (4.99 g)	BA (2.77 g)	Residue (39.1 g)
Al (µg/g)	4389±439	147±16	143±14	2004±212	4211±424
(mg)	(219±22)	(0.35±0.04)	(0.714±0.070)	(5.55±0.59)	(165±16)
As (µg/g)	0.709±0.001	-	-	-	0.794±0.007
(µg)	(35.4±2.0)				(31.0±0.24)
Au (ng/g)	12.4	24.5	18.1	24.2	8.4
(ng)	(620)	(57.6)	(90.3)	(67.0)	(328)
Ba (µg/g)	66.7±0.7	-	-	-	99.8±1.2
(µg)	(3.34±0.04)				(3.90±0.05)
Br (µg/g)	7.56±0.88	34.1±4.0	21.8±2.55	25.6±2.8	2.94±0.34
(µg)	(378±44)	(80.1±9.4)	(109±13)	(70.9±7.8)	(115±13)
Ca (mg/g)	11.2±1.5	-	-	3.50±0.36	12.3±1.3
(mg)	(560±75)			(9.70±1.0)	(481±51)
Cl (µg/g)	1965±221	15587±1746	6001±671	4903±459	146±14
(mg)	(98.3±11.1)	(36.6±4.1)	(29.9±3.3)	(13.6±1.3)	(5.71±0.55)
Co (µg/g)	0.641±0.123	0.824±0.158	0.535±0.102	0.585±0.123	0.496±0.095
(µg)	(32.1±2.0)	(1.94±0.37)	(2.67±0.51)	(1.62±0.34)	(19.4±3.7)
Cr (µg/g)	5.91±0.04	2.74±0.01	2.53±0.01	5.27±0.03	5.69±0.03
(µg)	(296±2)	(6.43±0.02)	(12.6±0.05)	(14.6±0.1)	(222±1.17)
Fe (µg/g)	3427±204	-	-	1526±82	3271±126
(mg)	(171±10)			(4.23±0.23)	(128±5)
Hf (µg/g)	0.304	-	-	-	0.370
(µg)	(15.2)				(14.5)
Hg (ng/g)	34.0±1.6	-	3.79±0.28	31.1±1.5	40.7±2.0
((µg)	(1.70±0.08)		(0.02±0.01)	(0.086±0.004)	(1.59±0.08)
K (mg/g)	16.0±1.3	25.1±2.1	35.7±3.3	76.2±2.9	11.4±1.1
(mg)	(800±65)	(59.0±4.9)	(178±16)	(211±8)	(446±43)
La (µg/g)	5.68±0.16	-	-	1.36±0.04	5.73±0.16
(µg)	(284±8)			(3.77±0.11)	(224±6)
Mn (µg/g)	123±10	4.56±0.42	15.2±1.6	82.2±10.3	117±11
(mg)	(6.15±0.50)	(0.011±0.001)	(0.076±0.008)	(0.23±0.03)	(4.57±0.43)
Na (µg/g)	1050±180	1986±294	1332±195	3870±365	697±71
(mg)	(52.5±9.0)	(4.67±0.69)	(6.65±0.97)	(10.7±1.0)	(27.2±2.8)
P (mg/g)	2.92±0.24	1.31±0.11	2.17±0.18	9.86±0.81	2.26±0.19
(mg)	(146±12)	(3.08±0.26)	(10.8±0.9)	(27.3±2.2)	(88.4±7.4)
Se (ng/g)	195±4	-	-	152±3	255±4
(µg)	(9.75±0.20)			(0.42±0.01)	(9.97±0.20)
Sm (µg/g)	1.18±0.23	-	-	0.305±0.059	1.26±0.24
(µg)	(59.0±11.5)			(0.844±0.163)	(49.2±9.4)
Zn (µg/g)	69.2±2.4	69.1±2.4	34.5±1.2	52.5±1.8	56.6±2.0
(mg)	(3.46±0.12)	(0.162±0.006)	(0.172±0.006)	(0.145±0.005)	(2.21±0.08)

The values in parenthesis are obtained by material balance

elements are extracted and major amounts of Ca, As, Ba and Se are left behind in the residue. In order to further compare the elemental contents, bar plots of elemental concentrations are shown in Fig. VI.11. It is observed that ~42 % Na, 56% K and 81% Cl are extracted. Interestingly, BM extracts showed minimum amounts of Na (8.9%) and K (7.4%) whereas a maximum of 20.4% and 26.4% are extracted in BA. However, in the case of Cl an opposite trend is observed with a maximum of 37.2% in BM and a minimum of 13.8% in BA. This may be due to easy solubility of some chloro compounds in methanol. It means that BAM extracts moderate amounts of these elements along with Mn, Co and Zn exhibiting maximum activity due to DPPH radical scavenging, H_2O_2 scavenging, superoxide anion scavenging and anti-lipid peroxidation including DNA strand break. Since residue itself is about 78%, it is obvious that many elements go along with it and are not able to play any role even if these are beneficial for biological functions in the body.

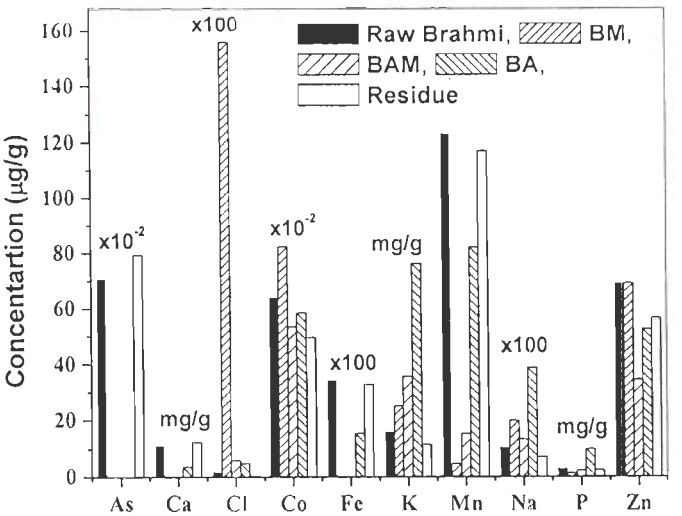

Fig. VI.11. Elemental Contents in Raw and different Extracts of *Brahmi*

VI.8 CONCLUSION

On the basis of various bioassay activities in different extracts and the determination of elemental contents, following conclusions may be drawn;

- Percent scavenging of DPPH radical and H_2O_2 are maximum in aq.-methanol (BAM) extract at 30 and 10 µg/mL respectively and remain constant at higher concentrations. However, BA extract also exhibits significantly higher activity.

- Protective effect of γ-ray induced damage of DNA is also maximum in aq.-methanol (BAM) extract at 150-200 μg/mL.
- Anti lipid peroxidase activity of aq.-methanol (BAM) extract is maximum and for methanol (BM) extract it is minimum.
- Total phenol content in BAM extract is higher by an order of magnitude compared to BM or BA extracts.
- Present studies suggest relationship between phenolic content and antioxidant activity.
- BAM extract contains maximum amounts of Na, K, Cl including significant amounts of Zn, Mn and other nutrients.

It is suggested that *in vivo* studies should be carried out to confirm the role of different extracts and nutrient elements present therein. Perhaps radiotracer studies along with bio-distribution in different body parts will be still more useful.

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CONCLUSIONS

Trace elements play a vital role in the human metabolism and their excess or deficiency may cause many diseases. Its main source is the diet and in case of deficiency, these may have to be supplied through dietary supplements. *Ayurveda*, the Indian traditional system of medicine, was the first to recognize the importance of essentiality of elements and their supply through natural herbs. Though many studies on herbs have reported organic constituents but only scanty reports are available on trace element contents. In recent years, many researchers around the world have found the medicinal herbs as panacea for the treatment of chronic ailments including diabetes, blood pressure and cancer. As a result many pharmaceutical firms have sprung up with branded products that follow their own standardization procedure but without any set guidelines. In order to know the availability of essential trace elements and also determine toxic elements, if any, we have analysed 30 medicinal herbs, 2 herbal formulations and 20 *bhasmas* (metallic - herbal formulations) for 25-30 elements by instrumental neutron activation analysis (INAA) and atomic absorption spectrophotometry (AAS). Also bioassay including antioxidant activity (DPPH radical scavenging, antilipid peroxidation, H_2O_2 scavenging, superoxide anion scavenging) and radioprotective activity as DNA strand break of aqueous-methanol (50:50) extracts of *brahmi* was studied. On the basis of our analytical data obtained by NAA and AAS following general conclusions can be drawn;

- Many herbs are enriched in essential elements such as Na, K, Ca, Mg, Fe, Mn, Cu, Zn, Se etc., which are in bioavailable/biocompatible form and hence are likely to be easily assimilable by our body system.
- No single herb is enriched in all the essential elements though some herbs may be enriched in several elements. The herbs, in general, do not have any harmful/side effects.
- It is supposed that essential nutrient elements may remain complexed with organic constituents/macromolecules acting as ligands from herbs, thus making these available to our body system.
- Some herbs are active ingredients of Indian diet, used as a spice or mouth freshener and have been in use as medicine since ages.

- Use of many herbs depends on the way these are prescribed and have dietary restrictions. Thus, herbal medicines act in a synergistic/antagonistic way with the diet.
- Herbal formulations where several herbs are mixed in a definite proportion have been considered more useful so as to improve the efficacy of these medicines.
- Metallic-herbal preparations called *bhasmas* prepared by repeated incineration process are unique and taken along with honey/butter/ghee/milk. These are considered as more potent and are taken in small doses. Some *bhasmas* based on Ca and Fe are widely used as supplements.
- It is believed that in *bhasmas* metals may remain bound with organic macromolecules derived from herbs thus affecting the nervous system and enhance their shelf life compared to those of herbs.
- There are several *bhasmas* containing elements such as As, Hg and Pb, which are known for their toxic effects. However, their use as medicine is matter of controversy and needs further investigation especially with regard to their speciation.
- Present study is an attempt to provide base line data on elemental contents of some medicinal herbs, herbal formulations and *bhasmas* to correlate these with their pharmacological action.
- Instrumental neutron activation analysis (INAA) using short and long irradiation with high flux of thermal neutrons in Dhruva reactor followed by high resolution gamma ray spectrometry is ideal for the determination of 25-30 elements. However, use of atomic absorption spectrometry (AAS) is complementary for the determination of Ni, Cu, Cd and Pb, difficult to be determined by INAA. Counting at successive intervals up to 2 months is especially useful for the determination of up to 15-20 elements.
- Bioassay of aqueous-methanol (BAM) extract of a typical herb *brahmi (Bacopa monnieri)* has shown it to be a potential antioxidant and radioprotective.
- Several elements such as Na vs. K, K vs. P, Fe vs. Mn, Fe vs. Co are correlated with each other in various herbs. However, strong correlations are observed if similar plant parts such as leaves, fruit or seeds are considered. K/P in various herbs/herbal formulations and metallic-herbal preparations are of special interest.

FUTURE SCOPE OF WORK

India is one of the richest biodiversity countries with oldest civilization. *Ayurveda* the traditional system of medicine based on treating diseases using herbs offers a holistic approach for long, healthy and happy life. It is an alternative natural medication without no side effects. However, modern technological developments require strict quality control and assurance. In order to have uniformity in standardization, a data base is essential for trace and toxic elements in medicinal herbs/ herbal preparations marketed by pharmaceutical manufactures. It is all the more essential because the plants take up nutrient elements from the soil and the environment where they are grown.

It would be proper to take up an intensive project involving the elemental analysis of medicinal herbs specially used for anemia, arthritis, acidity, asthma, cancer of various kinds, cataract, cholera, conjunctivitis, constipation, dandruff, diarrhea, diabetes, eczema, glaucoma, hearth disorders, blood pressure, intestinal worms, menstrual problems, rheumatism, syphilis, toothache, ulcer etc. Many herbs have been analysed for organic constituents such as alkaloids, polyphenols, glycosides, essential oils etc. However, their characterization/identification using modern instrumental methods (ICP-AES and MS, TXRF, EDXRF, PIXE including NAA) and spectral (IR, ^{1}H and $^{13}CNMR$, GC-MS) characterization have not been carried out in a systematic manner. Since many elements are present in combination with organic macromolecules acting as ligands derived from herbs it is essential to establish the bonding or speciation whether a particular metal exists in ionic or organic form. It may provide a clue to its pharmacological action.

Bhasmas are unique in Indian subcontinent only. It is essential to systematically analyze these for various essential trace elements as well as investigate their toxic effects, a matter of controversy. The belief that bhasmas are biologically produced nano materials needs experimental confirmation by using scanning electron microscopy (SEM) and high resolution transmission electron microscopy (HR-TEM). In order to have international recognition for herbal medicines, a detailed and systematic analysis is very essential.

LIST OF PAPERS PUBLISHED/ACCEPTED/COMMUNICATED

1. Availability of Essential Elements in Indian and US Tea Brands by **A. Kumar**, A.G.C. Nair, A.V.R. Reddy and A.N. Garg, *Food Chemistry,* **89** (2005) 441-448.

2. Isotope Dilution Analysis Method for the Determination of Zinc in Blood of Diabetic Patients by A.N. Garg, **A. Kumar**, G. Maheshwari and S. Sharma, *J. Radioanal. Nucl. Chem.,* **263** (2005) 39-43

3. Determination of Minor and Trace Elements in *Trifala*-a Herbal Preparation by A.N. Garg, **A. Kumar**, A.G.C. Nair and A.V.R. Reddy, *J. Radioanal. Nucl. Chem.,* **263** (2005) 751-758.

4. Analysis of Essential Elements in *Pragya-peya* – A Herbal Drink and its Constituents by Neutron Activation by **A. Kumar**, A.G.C. Nair, A.V.R. Reddy and A.N. Garg, Accepted to *J. Pharmaceut. Biomed. Anal.*, 37 (2005) 631-638.

5. Phosphorus Content in Biological Standards and Samples by Thermal Neutron Irradiation and β^- Counting by A.N. Garg, **A. Kumar** and R. Paul Choudhury in *J. Radioanal. Nucl. Chem.,* Under consideration

6. INAA of Some Indian Medicinal Herbs by A.N. Garg, **A. Kumar**, A.G.C. Nair and A.V.R. Reddy in *J. Radioanal. Nucl. Chem.,* Accepted.

7. Chemical Characterisation of *BHASMAS; AYURVEDIC* Metallic Preparations by Instrumental Neutron Activation Analysis by **A. Kumar**, A.G.C. Nair and A.V.R. Reddy and A.N. Garg, *Biol. Trace Elem. Res.,* Accepted.

8. Availability of Essential Elements in *Bhasmas*: The Unique Ayurvedic Metallic Preparations by INAA, by **A. Kumar**, A.G.C. Nair, A.V.R. Reddy and A.N. Garg, NAMLS8, Rio de Janeiro, April 2005, To appear in *J. Radioanal. Nucl. Chem.*.

9. Analysis of Trifala Powder and Tablets for Essential and Trace Elements by INAA and Spectral Identification of Gallic acid by R. Paul Choudhury, **A. Kumar** and A.N. Garg, under preparation.

10. Antioxidant and Radioprotective Activities of *Brahmi*, **A. Kumar**, A.N. Garg, S.C. Chattopadhyay and M. Subrmanian, under preparation.

PAPERS PRESENTED IN INTERNATIONAL / NATIONAL CONFERENCES /SYMPOSIA

1. Availability of Manganese in Tea Leaves by AAS and Spectrophotometric Methods by **A. Kumar**, D.K. Chauhan and A.N. Garg in 89th Indian Science Congress, Lucknow University, Lucknow, Jan 3-7, 2002, Abstract. No. 77

2. Determination of Na, K, Mn and Cu in Tea Leaves and medicinal herbs by Instrumental Neutron Activation Analysis by **A. Kumar** and A.N. Garg in 4th National Symposium in Chemistry, National Chemical Laboratory, Pune, Feb. 1-3, 2002, Abstract No. P14

3. Multielemental Neutron Activation Analysis of Essential Elements in *Pragya Peya* – a Herbal Drink and its Constituents by **A. Kumar**, A.G.C. Nair, A.V.R. Reddy and A.N. Garg, Nuclear and Radiochemistry Symposium-2003, Bhabha Atomic Research Centre, Mumbai, Feb. 10-13, 2003, Abstract No. RA-2

4. Interlaboratory Comparison Study in the Determination of Elemental Composition of SRM-1575a, Pine Needles by A.N. Garg and **A. Kumar**, Nuclear and Radiochemistry Symposium-2003, Bhabha Atomic Research Centre, Mumbai, Feb. 10-13, 2003, Abstract No. RA-3

5. Determination of Minor and Trace Elements in Trifala-a Herbal Preparation by A.N. Garg, **A. Kumar**, A.G.C. Nair and A.V.R. Reddy, Sixth International Conference on Methods and Applications of Radioanalytical Chemistry (MARC-VI), Hawaii (USA), April 7-11, 2003, Abstract No. 183

6. Isotope Dilution Analysis Method for the Determination of Zinc in Blood of Diabetic Patients by A.N. Garg, **A. Kumar**, G. Maheshwari and S. Sharma, Sixth International Conference on Methods and Applications of Radioanalytical Chemistry (MARC-VI), Hawaii (USA), April 7-11, 2003, Abstract No. 257

7. TLC Separation, Spectral Identification of Organic Components and Availability of Essential Elements in *Trifala* by R. Paul Choudhury, **A. Kumar** and A.N. Garg in 91st Indian Science Congress, Punjab University, Chandigarh, Jan. 2-7, 2004, Abstract No. 162.

8. Analysis of Chewing Tobacco for Essential and toxic Elements by NAA and AAS by A.N. Garg, **A. Kumar**, R. Paul Choudhury and S. Kar, ICOB-4 & ISCNP-24 IUPAC International Conference on Biodiversity And natural Products: Chemistry and Medical Applications, New Delhi, India, January 26-31, 2004 Abstract No.P-99.

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10. Phosphorus Content in Biological Standards and Samples by Thermal Neutron Irradiation and β^- Counting by A.N. Garg, **A. Kumar** and R. Paul Choudhury,

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