The Biomineral Analysis of Five Indian Ayurvedic Polyherbal Formulations by Instrumental Neutron Activation Analysis (INAA) and Atomic Absorption Spectroscopy (AAS)

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Abstract
Minerals and trace minerals are essential to human health for maintaining adequate levels in every tissue, fluid, cell and organ in the body may be the key considerations. Medicinal plants are essential natural resource of new bioactive products for drug development. Present study was investigated to the elemental analysis of polyherbal medicinal formulations by Instrumental Neutron activation Analysis and Atomic Absorption Spectroscopy. Medicinal herals formulations from India, prescribed for specific treatment purposes were analyzed by Instrumental Neutron Activation Analysis using $^{252}$Cf, Californium spontaneous fission neutron source (flux $10^9$ n/s) and the induced activities were counted by $\gamma$-ray spectrometry and Atomic Absorption Spectroscopy techniques (Perkin Elmer 3100 Model) available at University of Pune, India was used for the measurement of major, minor and trace elements from five polyherbal Ayurvedic formulations. 15 elements viz. Al, K, Cl, Na, Mn by Instrumental neutron activation analysis and Cu, Co, Pb Ni, Cr, Ca, Fe, Zn, Hg and Cd by Atomic Absorption Spectroscopy were analyzed from different Ayurvedic herbal formulations. A critical examination of the data shows that the elements Ca, K, Cl, Al and Fe are found to be present at major levels in most of the samples while the other elements are present in minor or trace levels. The elemental concentration in different medicinal formulations is discussed.

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1. Introduction

India possesses an unmatched heritage represented by its ancient systems of medicine which are a treasure house of knowledge for both preventive and curative healthcare. The positive features of the Indian Systems of Medicine, namely, their diversity and flexibility; accessibility; affordability; a broad acceptance by a section of the general public; comparatively low cost; a low level of technological input and growing economic value have great potentials to make them providers of health care that the larger sections of our people need. Fairly comprehensive information about herbs has been recorded in two treatises Charak Samhita and Shusruta Samhita—a base for Ayurvedic system of medicine (Parchure S.N., 1983). These herbs are now being increasingly used in cosmetics, food as well as alternative medicine (Bakhru H.K., 1998). The Ayurvedic system of medicine has been prevalent in India since the Vedic period, and still remains the mainstay of medical relief to over 60 per cent of the population of the nation. Ayurvedic materia medica supports a successful industry in India with annual production worth more than Rs. 5000 crores and an export market of 500 crores. It is estimated that approximately 1000 herbal formulations prepared from around 750 plants are in regular use at the present time. Ayurveda is ancient science of life according to Indian philosophy (Hansche et al., Nitya Anand in Comprehensive Medicinal Chemistry. Pergamon Press. Oxford. 1990, 1,113). Preference to the Ayurvedic medicines due to cost
Effective and have less/no side effects. Poly-herbal Ayurvedic formulations are based on plants, animal’s extracts & minerals both in single ingredient drugs and compound formulations.

Literature survey shows that the Ayurvedic formulations are derived from vegetable sources from the various parts of plants like root, leaf, flower, fruit or plant as a whole. Now a day’s scientists all over the world are discovering the new secondary substances in plants (Lawrence P. et al., 1973, W.H.O., 1998, Mulzer et al., 2000). In recent years, the use of herbs as medicines is increasing therefore the importance of scientific knowledge about the herbs used in traditional medicines become essential. Ayurvedic compound formulations are mainly divided into two groups, Kasthausadhi (predominantly plant drugs) and Rasausadhi (predominantly metals and minerals). Traditional Indian medical herbs used for strengthening the body immune system are known to have many essential and nutritional elements. Their excess or deficiency may disturb normal biochemical functions of the body (Lyengar et al., 1989). Some western scholars have pursued the analysis of various Indian plants and herbs for their medicinal properties (Ambasta et al., 1986). Besides, several organic compounds, it is now well established that many trace elements play a vital role in general well-being as well as in the cure of diseases (Underwood et.al.,1977 and Prasad.,1993). Several studies have reported elemental contents in plant extracts, which are consumed by us either as an herbal health drink or medicine (Powel et al., 1998). These elements are presented at varying concentrations in different parts of the plants, especially in roots, seeds and leaves which are used as a dietary item as well as ingredient in the Ayurvedic medicinal preparation. Since these trace elements constitute a minute fraction in different parts of the medicinal plants, a sensitive and reliable analytical technique is a prerequisite for obtaining precise and accurate data.

Considering the importance of trace elements in various human metabolic processes and also considering their curative properties, in the present investigation we have applied one of the sensitive analytical techniques like by Instrumental neutron activation analysis (INAA) and Atomic Absorption Spectroscopy (AAS) to study the essential elemental content in different parts of Indian medicinal plants and herbs. The overall impact of these essential trace elements on human health is also discussed. Due to increasing industrialization and medicinal herbs not only provide us chemicals of medicinal value but also provide us nutrition and trace mineral elements in natural form.

1.1 Minerals and Their Functions and Sources
Minerals and trace minerals do not exist by themselves but in relationships to one another. Minerals and trace elements are chemical elements required by our bodies for numerous biological and physiological processes that are necessary for the maintenance of health. Human body cannot produce minerals within our bodies, so we must obtain them through our food viz. plants in natural form. There are 103 known minerals; at least 18 of these are necessary for good health. Enzymes do not work without minerals. All cells require enzymes to work & function. They give us our vitality, maintain the pH balance within the body, facilitate the transfer of nutrients across cell membranes, help to contract and relax muscles, help to regulate our bodies tissue growth. Two categories of minerals essential within the body, macro-minerals & micro-minerals listed in Table1. (WebMD Medical Reference from Health wise).

By the literature survey taking into consideration of the medicinal importance of five Ayurvedic formulations was not undertaken previously. It is important to investigate the elemental contents present in different Ayurvedic formulations. In the experimental work five Ayurvedic formulations have been discussed for their elemental content and could be carried out in the Laboratory.

Quality Control and Standards- At present there is no pharmacopical standard on each of the active ingredients of Ayurvedic medicine like allopathic medicine. For standardization and quality control of Ayurvedic drugs, various steps can be followed like physical description, physical tests, pharmacognised techniques, their mineral contents, toxicity etc, to ascertain the species of plant and study their pharmacognostic character for the purpose of identification detection and analyzing the crude drug. Generally quality of Ayurvedic products is fully dependent on the
quality of raw materials and process of manufacture. The products are to be manufactured as per Indian system of medicines of Ministry of Health. Present work will be beneficial for the determination of their mineral contents and toxicity of the formulations under consideration (Table 1).

Deficiency or excess of one or more mineral causes the metabolic imbalance so diseases. Recent research indicates that adequate dietary intake of essential minerals and trace minerals may prevent and reduce affects of poisoning by environmental pollutants and enhance the ability to work. Medicinal Plants are important sources of protective foods, which are highly beneficial for the maintenance of good health and prevention of diseases (Sheela et al., 2004). Much effort has been concentrated on seeds while leafy vegetables have to large extent been ignored. They are known as potential sources of minerals and vitamins (Ifon et al., 1979). Several studies have reported elemental contents in plant extracts, which are consumed by us either as a herbal health drink or medicine (Powel et al., 1998). Abou-Arab and Abou Donia investigated the heavy metals content in spices and vegetables. They found excessive amounts of lead, cadmium and zinc in the examined mixed spices. Similarly, analytical profile of Pb, Cd, Cr, Ni, Sn, Zn, Mn, Cu and Fe exhibited concentrations at levels higher than permissible limits in various spice and medicinal plant species collected from exportation areas of Egypt (Abou-Arab and Abou Donia., 2000). Some western scholars have pursued the analysis of various Indian plants and herbs for their medicinal properties (Ambasta S., 1986).

Lot of scope to do the research as very less research work is conducted in this area using INAA and AAS techniques.

2. Objective of Research

Literature survey of Ayurvedic medicinal formulations from India and selection of poly-herbal Ayurvedic formulation.

Determination of the elemental content of five polyherbal Ayurvedic medicinal formulations, Alsarex, Eugynin Forte, Hawaban harade, Lukol and Madhumehari by Instrumental Neutron activation Analysis (INAA) and Atomic Absorption Spectroscopy (AAS) techniques.

To find the elemental concentration and their correlations in different medicinal formulations.

3. Material and Methods

3.1. Chemicals:
All chemicals used were analytical grade.

3.2. Sampling
The various medicinal Ayurvedic Formulations for the specific treatment purpose (Table 2) were purchased from Ayurvedic medicine shops and Ayurvedic Rasshala Pune, Maharashtra, India. The solid samples were first powdered and passed through 100-mesh sieve. Sampling was done from this powder. The method in detail is discussed in the previous paper (Rajurkar and Pardeshi., 1997).
### Table 2: Ayurvedic formulation with its main ingredients and uses

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Ayurvedic formulation</th>
<th>Main ingredients in the formulations (Indian/botanical name)</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alsaex</td>
<td>Bhamas viz Kamadudha rasa , Sutashekhara rasa , Pravala pishati , Aparanga kshara, Jaharmohar pishati, Jatamansi , Jeeraka, Kapardika bhasma, Mukta shukthi bhasma, Shankha bhasma, Yashada bhasma (calx of zinc)</td>
<td>Ulcer, hyper acidity, inflammations, reduces nausea gastric ulcers, duodenal ulcers, heartburn and dyspepsia hyperacidity, burning feet syndrome, headache and fever</td>
</tr>
<tr>
<td>2</td>
<td>Eugynin Forte</td>
<td>(Dashamool) Ten roots, (Lodhra) Symlocos racemosus, (Guduchi) Tinospora cordifolia, (Saariva) Hemidesmus indicus , (Deodara) Cedrus deodara, (Nagkesar) Glycyrrhiza</td>
<td>Relieves uterine spasms and pain regulates menstrual flow , helps maintain balance of female reproductive hormones, improves complete reproductive system for better performance lessens the chances of dysmenorrhea, normalizes hormonal balance, controls white discharge (Leucorrhea) by astringent action., controls local itching, pain, inflammation. Controls infection, anti-fungal, anti-bacterial properties , restores tissue damage.</td>
</tr>
<tr>
<td>3</td>
<td>Hawaban Harade</td>
<td>(Amila) Emblica-officinalis, (Harad) Terminalia chebula, (Behda) (Termilly bellirica), Black salt, Hing(Asafoetida)</td>
<td>Digestive system disorders, it eases condition of flatulence - controls Dyspepsia - Increases appetite - Helps in proper digestion n relieves indigestion</td>
</tr>
<tr>
<td>4</td>
<td>Lukol</td>
<td>(Shatavari) Asparagus, (Dhataki) Fire Flame Bush Spreading, (Panarnava) Hogweed</td>
<td>Anti-fungal, antioxidant ,anti-inflammatory, analgesic and antispasmodic pain and leucorrhea.</td>
</tr>
</tbody>
</table>

### 3.3. Instrumental Neutron Activation Analysis (INAA) Irradiation and Counting

About 50-100 mg of each sample was sealed in a polyethylene cover. Samples, reference standard were packed together and irradiated in the E-8 position of the. The samples were irradiated for 24 hours using \(^{252}\text{Cf}\) spontaneous fission neutron source and the induced activities were counted by \(\gamma\)-ray spectrometry using efficiency calibrated high resolution High Purity Germanium (HPGe) detector connected with 8K multichannel analyzer and, which was connected, to an IBM PC XT computer system) and the activity of the radioisotopes formed, viz., \(^{28}\text{Al}\), \(^{42}\text{K}\), \(^{38}\text{Cl}\), \(^{24}\text{Na}\), \(^{56}\text{Mn}\) at the end of irradiation was measured and finally the concentration of the elements was calculated using the calibration curves. The presence of these elements was confirmed by measuring their half and gamma energies. The elements \(^{28}\text{Al}\), \(^{42}\text{K}\), \(^{38}\text{Cl}\), \(^{24}\text{Na}\), and \(^{56}\text{Mn}\) were analyzed by (flux~10\(^9\)ns\(^{-1}\)) available at Department of Chemistry, University of Pune, India. Elemental concentrations of various Ayurvedic medicinal formulations were calculated by relative method using control and reference multi elemental standard as comparators. The values listed in Table 3.

### Table 3: Radionuclide used for the analysis and their \(\gamma\)-energies for INAA

<table>
<thead>
<tr>
<th>Nuclide</th>
<th>(\gamma)-ray energy in keV</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{56}\text{Mn})</td>
<td>847.0</td>
</tr>
<tr>
<td>(^{28}\text{Al})</td>
<td>1524.0</td>
</tr>
<tr>
<td>(^{38}\text{Na})</td>
<td>1368.0</td>
</tr>
<tr>
<td>(^{38}\text{Cl})</td>
<td>1642.0</td>
</tr>
<tr>
<td>(^{42}\text{K})</td>
<td>1779.0</td>
</tr>
</tbody>
</table>
3.4. Atomic Absorption Spectrometer (AAS) Measurement

The samples in powdered form were accurately weighed and digested in (5:1) mixture of nitric acid and after digestion few drops of concentrated HCl were added. The solution was heated gently and then filtered. The residue was again subjected to digestion and filtrate was collected. The entire filtrate was diluted suitably with distilled de-ionized water. The dilute filtrate solution was used for analysis of elements of interest (elements Cu, Co, Pb, Ni, Cr, Cd, Fe, Ca, Hg and Zn) by AAS (Perkin Elmer 3100 model) for the measurement of minor and trace elements. The values listed in Table 4 and Table 5 (which are averages of three independent measurements) are having the precision of ±2 to 10%.

Table 4: Elemental analysis of some Ayurvedic Formulations by INAA technique

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Ayurvedic Formulation</th>
<th>Concentration of elements (%)</th>
<th>Cu/10³</th>
<th>Co/10³</th>
<th>Pb/10³</th>
<th>Ni/10³</th>
<th>Cr/10³</th>
<th>Cd/10³</th>
<th>Fe/10²</th>
<th>Zn/10²</th>
<th>Hg/10²</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alsarex</td>
<td></td>
<td>3.87</td>
<td>2.00</td>
<td>9.90</td>
<td>2.73</td>
<td>0.88</td>
<td>0.69</td>
<td>123</td>
<td>19.7</td>
<td>39.4</td>
<td>7.8</td>
</tr>
<tr>
<td>2</td>
<td>Eugynin Forte</td>
<td></td>
<td>5.80</td>
<td>0.46</td>
<td>4.80</td>
<td>3.88</td>
<td>0.47</td>
<td>0.46</td>
<td>3.00</td>
<td>8.9</td>
<td>65.4</td>
<td>4.2</td>
</tr>
<tr>
<td>3</td>
<td>Hawaban Harade</td>
<td></td>
<td>5.17</td>
<td>0.49</td>
<td>10.8</td>
<td>0.75</td>
<td>1.95</td>
<td>0.59</td>
<td>5.17</td>
<td>3.82</td>
<td>0.00</td>
<td>3.16</td>
</tr>
<tr>
<td>4</td>
<td>Lukol</td>
<td></td>
<td>2.49</td>
<td>1.30</td>
<td>1.40</td>
<td>4.32</td>
<td>0.53</td>
<td>0.44</td>
<td>33.2</td>
<td>64.3</td>
<td>5.2</td>
<td>6.1</td>
</tr>
<tr>
<td>5</td>
<td>Madhumehari</td>
<td></td>
<td>2.85</td>
<td>1.45</td>
<td>1.51</td>
<td>2.03</td>
<td>0.71</td>
<td>0.54</td>
<td>34.4</td>
<td>47.3</td>
<td>25.9</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Table 5: Elemental analysis of some Ayurvedic Formulations by AAS technique

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Ayurvedic formulation</th>
<th>Concentration of elements (%)</th>
<th>Al/10³</th>
<th>K/10³</th>
<th>Cl/10³</th>
<th>Na/10³</th>
<th>Mn/10³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alsarex</td>
<td></td>
<td>2.040</td>
<td>0.424</td>
<td>0.000</td>
<td>163.40</td>
<td>2.130</td>
</tr>
<tr>
<td>2</td>
<td>Eugynin Forte</td>
<td></td>
<td>1.166</td>
<td>0.270</td>
<td>0.925</td>
<td>25.72</td>
<td>4.764</td>
</tr>
<tr>
<td>3</td>
<td>Hawaban Harade</td>
<td></td>
<td>1.083</td>
<td>0.480</td>
<td>15.817</td>
<td>190.52</td>
<td>5.432</td>
</tr>
<tr>
<td>4</td>
<td>Lukol</td>
<td></td>
<td>1.165</td>
<td>1.414</td>
<td>0.588</td>
<td>31.87</td>
<td>4.237</td>
</tr>
<tr>
<td>5</td>
<td>Madhumehari</td>
<td></td>
<td>0.000</td>
<td>0.773</td>
<td>0.925</td>
<td>22.11</td>
<td>3.381</td>
</tr>
</tbody>
</table>

4. Results, Discussion and Conclusion

An examination of the data showed that the different medicinal plants formulations contain elements K, Mn, Na, Fe, Zn, Cu, Co, Cl, Al, Cr, Ca, Cd, Ni, Pb and Hg in various proportions (Table 4 and Table 5). The variation in elemental concentration is mainly attributed to the differences in botanical structure, as well as in the mineral composition of the soil in which the plants are cultivated. An examination of the data from Table 4 and Table 5 indicates that the highest Cl content was found to be rich (15.817), Na (190.52 x 10⁻³), Cr (1.95x 10⁻³), and Mn in Hawaban Harade (5.432 x 10⁻³), Hg (65.4 x 10⁻²) and Cu in Eugynin forte (5.80 x 10⁻³), Pb in Madhumehari (151 x 10⁻³), K (1.414%), Ni (4.32 x 10⁻³) and Zn in Lukol (64.3 x 10⁻²), Al (2.040%), Co (2.00 x 10⁻³), Cd (0.69 x 10⁻³), Fe (123 x 10⁻²) and Ca (7.80 x 10⁻²) in Alsarex. Hawaban Harade is found to be rich in Cl, Na, Mn and Cr while minimum Ni and Zn, Hg. It is important here to note that the regulation of potassium is intimately involved with that of sodium and the two are largely interdependent. Potassium is accumulated within human cells by the action of the Na⁺, K⁺-AT Phase (sodium pump) and it is an activator of some enzymes; in particular co-enzyme for normal growth and muscle function (Birch N. et al.,1994). Eugynin Forte is rich in Cu and Hg while minimum Co and Cr. Alsarex is found to be rich in Al, Co, Cd, Fe and Ca while minimum Cl and Mn. Lukol contain high K, Ni and Zn while minimum Cu, Pb and Cd is high and Al, Na and minimum in Madhumehari. Cu, Hg rich Eugynin forte and Co, Cr and Fe is minimum in Eugynin forte. The Cl is low and Co, Cd, Fe and Ca content is to the maximum in Alsarex, Madhumehari contain minimum Na and maximum Hg but under permissible level as compared to all the formulations. Ni and Zn, Hg is minimum in Hawaban Harade. Ca content in various medicinal plants formulations analyzed varies from 7.80 x 10⁻² % to 2.80 x 10⁻²%. Calcium is essential for healthy bones, teeth and blood (Charles P. et al., 1992). The elements like Zn (64.3 x 10⁻² to 3.82 x 10⁻²), Fe (123.0 x 10⁻² to 5.0 x 10⁻²) and Cr (1.95x 10⁻³ to 0.47 x 10⁻³) are essential trace elements (micro nutrients) for living organisms. Zinc is relatively non-toxic (Prasad., 1982). Zinc is necessary for the growth and multiplication of, for skin integrity, bone metabolism and functioning of taste and eye sight.
(Thunus L. and Lejeune R, 1994). The high concentration of zinc in Lukol suggests its possible use in sex tonic, treatment of gynecological problems and leukemia. Different formulations are rich in one or more elements. In all five formulations, concentration of Al, K, Ca and Cl is found to be more as compared to the concentration Fe and Zn. Other elements are present at trace or minor level. Iron occupies a unique role in the metabolic process. The role of iron in the body is clearly associated with hemoglobin and the transfer of oxygen from lungs to the tissue cells (Sigel., 1978). Iron deficiency is the most prevalent nutritional deficiency in humans. (Reddy et al., 1987).

In various medicinal plants samples analyzed, the Fe content was observed maximum in Alsarex (123.0 x 10^{-3}), Lukol (33.2x 10^{-3}), Madhumehari (34.4 x 10^{-3}), and minimum in Eugynin forte (5.00x 10^{-2}), and Hawaban Harade (5.17x 10^{-3}). Hence the use of Alsarex, Lukol and Madhumehari in general tonic preparation may be advised to compensate for treatment of anemia. Chromium plays an important role in diabetes treatment. The function of chromium is directly related to the function of insulin, which plays a very important role in diabetes. Chromium is found in the pancreas, which produces insulin. One usable form of chromium is the Glucose Tolerance Factor (GTF), an inorganic compound containing glutamic acid, cysteine and niacin. Chromium deficiency can cause an insulin resistance, impair in glucose tolerance and may be a risk factor in atherosclerotic disease (Zetic et al., 2001). From the results obtained, it is observed that Cr content is high in Hawaban Harade (1.95 x 10^{-3}), Alsarex (0.88 x 10^{-3}) and Madhumehari (0.71 x 10^{-3}). Hence the use of this medicinal formulation may be advised for the treatment and control of diabetics.

The higher Mn content was observed in Hawaban harade (5.432 x 10^{-3}), Eugynin Forte (4.764 x 10^{-3}), Madhumehari (3.381 x 10^{-3}). It is important here to note that Mn is an essential element required for various biochemical processes. Mn is also important for several enzymatic processes. It helps in eliminating fatigue and reduces nervous irritability (Guenther et al., 2003; Hamilton et al., 1994). Hence use of these medicinal preparations may help to supplement Mn for various body functions. The Co content was observed to be high in Alsarex (2.00 x 10^{-3}), Madhumehari (1.45 x 10^{-3}) and Lukol (1.30 x 10^{-3}).

Animals are able to synthesize vitamin B12, which is the main source of Co in animal foods. In humans, deficiency of vitamin B12 leads to anemia. The higher Co and Fe content in in Alsarex, Madhumehari and Lukol suggests there use in medicinal preparation for treatment of anaemia. The elements like Hg, Pd, Cd and Ni are supposed to be toxic in nature and their presence in trace amount in various medicinal plant sample analyzed is due to the pollution arising from automobile and industrial activities. The transition elements Fe, Zn and Co are well known for their role in biochemical processes (Kumar et al., 2005). Iron deficiency is common in uremic patients, it causes substantial blood losses. Deficiency of zinc causes diabetic hyposima, hypogeusia or coma. The availability of Zn in the range of 14.8-8.4 µg/g may be beneficial for diabetic patients as its deficiency has been correlated with acute and chronic mal absorption states (Hamilton et al., 1994). The requirement of Fe for an adult is 20 mg/day and for a child is 10 mg/day. It is very essential to provide 3 µg per day in the form of Vitamin B-12 for a diabetic individual. A plot of Fe versus Cr shows linear relationship (Fig. 22) which represents somewhat poor relationship. It is possible due to the fact that all parts of the medicinal plants are different i.e. seeds and fruits used in different formulations as reported by Razic et al. (Razic et al., 2003).

In general, it may be mentioned that interrelationship of several elements in medicinal herbs suggest synergistic or antagonistic effects, thus providing various elements to the body in bio-available form in a balanced manner with almost no harmful effects except some environmental contaminants. These, however, should be avoided by collecting herbs grown in a clean and well controlled environment (Kumar A. et al., 2005).

A critical examination of the data shows that the elements Ca, K, Cl, Al and Fe are found to be present at major levels in most of the samples while the other elements Cu, Co, Pb, Ni, Cr, Cd, Fe, Hg and Zn are present in minor or trace levels. Most of the Ayurvedic formulations containing medicinal plants, vitamins and some minerals were found to be rich in one or more of the elements under study. The concentration of Ca, Al,Cl and K was found to be present more as compared to Fe, Zn, Hg, Pb, Ni, Cr, Cd, Na, Mn, Cu and Co which are present at minor or trace level. Pb and Hg were detected in some samples with good precision and accuracy. The concentration of elements determined was found to vary from major levels to minor or trace levels. This study showed that the toxic elements Pb, Cd and Hg found in the samples were below the detection limits prescribed by World Health Organization (WHO) regulations. As all the formulations under study contain different plant combination for the ailments of different diseases, no trend or sequence in their elemental contents observes with respect to each other.
Such data are important to understand the pharmacological action and formation of active constituents for each medicinal plant and to decide the dosage of herbs used in the formulations. Medicinal formulations containing medicinal herbs play a significant role especially in modern times when side effect of synthetic drugs and the damaging effect of food processing and over medication have assumed alarming proportions. The data obtained in the present work will be of immense importance to the research workers in the field of Ayurveda as well as in the synthesis of new Ayurvedic drugs with various proportions of medicinal plants in the treatment of various diseases.

The Elemental analysis of five Ayurvedic polyherbal Formulations by INAA and AAS techniques listed in Table 4 and Table 5 respectively.

The graphical representation of variation of % concentration of different elements in five formulation viz. Alsarex, Eugynin Fort, Hawaban Harade, Lucol and Madhumehari are shown in Fig. 16-20 respectively and % Concentration of 15 elements in all five formulations and Fe/Co are shown in Fig 1 to 15 and Fig. 21 and Fig. 22 respectively.
Graphs
The comparative graphical representation of concentration of each of 15 elements viz. Al, K, Na, Cl, Mn, Cu, Co, Pb, Ni, Cr, Cd, Fe, Ca, Zn and Hg in 5 Ayurvedic formulations Alsarex, Eugynin Fort, Hawaiyan harade, Lucol and Madhumehari are given below (Fig 1-15) and The comparative graphical representation of all 15 elements in each of the formulations are represented in Fig.16- Fig.20 and Fig 21. Fig.22 represent the ration of Fe/Co.

The study conducted in year 2009-10 in Pune, Maharashtra, India

Research Highlights
Simultaneous Quantitative Elemental analysis of 15 useful Ca, Fe etc. as well as hazardous elements viz. Pb, Hg, Al and Cd from polyherbal formulations was performed. It is very good method for multi-elemental analysis were performed. Use of fast and nondestructive analytical method like Instrumental Neutron Activation Multi-elemental analysis of medicines with green origin, herbs combination. Presence of different mineral content in major, minor and trace quantities will be useful to other scientist to understand the mechanism. Quantitative presence of bioinorganic minerals will be very much useful in the future to know the inter relationship with the ailment of diseases.

Such data are important to understand the pharmacological action and formation of active constituents for each medicinal plant and to decide the dosage of herbs used in the formulations as well as to the research workers in the field of Ayurveda and the synthesis of new Ayurvedic drugs with various proportions of medicinal plants in the treatment of various diseases Medicinal formulations containing medicinal herbs play a significant role especially in modern times when side effect of synthetic drugs and the damaging effect of food processing and over medication have assumed alarming proportions.

Limitations
Quality Control and Standards- At present there is no pharmacopeia standard on each of the active ingredients of Ayurvedic medicine like allopathic medicine. Inter relationship of elemental content with the particular diseases were not done in the present study. Evaluation of antioxidant, phytochemicals and bioactive compounds were not done in the present study.

Recommendations
For standardization and quality control of Ayurvedic drugs, various steps can be followed like physical description, physical tests, pharmacognised techniques, their mineral contents, toxicity etc, to ascertain the species of plant and study their pharmacognostic character for the purpose of identification detection and analyzing the crude drug.

Funding and Policy Aspects
The bioactive compounds from these medicinal plants formulations can be a lead molecule for many pharmaceutical preparations and in turn it can cure many disorders. If private funding is provided the isolation and evaluation for the bioactive lead molecule can be performed for the betterment of society and for the local poor population in the world.
Justification of Research

In the present work, it will be of immense importance to the research workers in the field of Ayurveda as well as in the synthesis of new Ayurvedic drugs with various proportions of medicinal plants in the treatment of various diseases. The data of elements in the formulations is of great importance to understand the pharmacological actions of these formulations containing medicinal plants.

By the literature survey taking into consideration of the medicinal importance of five Ayurvedic formulations was not undertaken previously. It is important to investigate the elemental contents present in different Ayurvedic formulations. In the experimental work five Ayurvedic formulations have been discussed for their elemental content and could be carried out in the Laboratory. The biomineral quantitative estimation of these polyherbal formulations was not published in any article.

Medicinal plants are the natural source of minerals which are useful for the treatment of many disorders, so quantitatively; elemental content in the five polyherbal formulations will be carried out not only for standardization and consumer protection, but also because it is a vital factor in advancing drug research, enhancing the effectiveness of these drugs and improving their effects.

Conclusion

Concentrations of various elements in different formulations estimated in the present studies are at major, minor and trace levels. The data obtained in the present work will be of immense importance to the research workers in the field of Ayurveda as well as in the synthesis of new Ayurvedic drugs with various proportions of medicinal plants in the treatment of various diseases. Most of the Ayurvedic formulations containing medicinal plants, vitamins and some minerals were found to be rich in one or more of the elements under study. Trace element concentrations plants used in the formulations vary widely with the soil type, pH, fertilizer and organic content, climate, species, etc. Although there appears to be little knowledge of the precise molecular mechanisms, many Ayurvedic preparations nevertheless appear to demonstrate significant success in treatment of complex diseases. Presumably Ayurvedic medicines contain trace elements in a bio-available form and their impact on the overall pharmacological action cannot be ruled out. Although the direct link between elemental content and curative capability is yet to be established, such studies are vital to understanding the pharmacological action of herbs. In order to develop a stronger basis for appreciating the curative effects of medicinal plants and their formulations, there is a need to investigate, their elemental composition. The polyherbal Ayurvedic formulations showed the presence of important mineral constituents which may be useful to cure much disease by providing new leads and clues for modern drug design. Due to many medicinal properties further pharmacological and clinical studies can be conducted to investigate the invisible potential of these polyherbal combinations of different herbas.

It has been demonstrated that INAA, with multi-elemental characterization over a wide range of concentration, its blank free-nature and minimum sample preparation is ideal for such studies in INAA and AAS.

Author’s Contribution and Competing Interests

We are working in this field since last fifteen years and published number of papers in various national and international conferences and journals, which shows that our work is widely accepted in the scientific community worked in the similar field. Moreover, as a reviewer, I have reviewed many papers of same kind in well-reputed journals which shows my contribution in the field of medicinal plants, Ayurveda, and natural product.

Present works is concentrated in minor, major and trace elemental contents of the biominerals present in the medicinal formulations. Myself the sole Author is interested to conduct the research work of antioxidants, phytochemical content and their metabolic activities which play very important role in curing the different diseases.

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