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Analysis of essential elements in *Pragya-peya*—a herbal drink and its constituents by neutron activation

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Abstract

Ayurvedic herbal formulations are a good source of several nutrient elements essential for metabolic processes. *Pragya-peya*, a herbal drink and its 12 herbal constituents have been analyzed for 7 minor (Al, Ca, Cl, Mg, Na, K, P) and 15 trace (Ba, Br, Co, Cr, Cs, Fe, Hg, La, Mn, Rb, Sc, Se, Th, V, Zn) elements by instrumental neutron activation analysis (INAA). The method involves thermal neutron irradiation in a reactor followed by counting at several intervals. Also Cd, Cu, Ni and Pb contents were determined by atomic absorption spectrometry (AAS). Elemental data were validated by simultaneously analysing reference materials (RMs) such as mixed Polish herbs (INCT-MPH-2) and peach leaves (SRM-1547). Sample homogeneity was tested by analysing samples from three different batches collected at different intervals. *Pragya-peya* has been found to be especially rich in several nutrient elements such as Ca, K, V, Fe, Mn, Se and Zn whereas no single constituent is enriched in all the nutrient elements. Concentrations of elements are discussed vis-á-vis their medicinal/therapeutic uses. Several elements such as Na, K, P, Ca, Fe, Co, and Zn seem to be well correlated in 12 constituent herbs.

Keywords: *Pragya-peya*; Thermal neutron activation analysis; Herbal drink; Medicinal herbs; AAS; Nutrient trace elements

1. Introduction

There are ample evidences of herbs being used in the treatment of diseases and for revitalising body system in almost all ancient civilizations [1–5]. Plants were the mainstay of many medicines having mystical and almost super natural healing power. There are many references to the curative properties of several herbs in the ancient Indian literature, *Rigveda*, though a more detailed account is found in the *Atharvaveda* from where *Ayurveda*, the Indian traditional health care system (*ayus* = life, *veda* = knowledge, meaning science of life) originated. Fairly comprehensive information about herbs has been recorded in two treatises *Charak Samhita* and *Shusruta Samhita*—a base for Ayurvedic system of medicine [6,7]. Looking to the importance of herbs, A Compendium of Indian Medicinal Plants in six volumes has been published [8]. Herbs play a significant role in modern times, when the damaging effects of food processing and over medication have assumed alarming proportions. These are now being increasingly used in cosmetics, foods as well as alternative medicine [9]. Besides lipids, proteins and carbohydrates required for human growth, supply of optimum quantities of inorganic micronutrients is also essential [10,11]. Several micronutrients such as Cr, Mn, Fe, Cu, Zn, etc. constitute a small fraction of our diet and play an important role in metabolic processes. Their excess or deficiency may disturb normal biochemical functions of the body [12]. Two main criteria considered for essentiality of elements are: first, its absence from diet results in departure from normal growth and metabolism; and second, the replacement of an element may suppress pathological symptoms [10].

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The composition of biological systems is so complex that the trace elements are totally masked by major constituents and hence difficult to determine [13]. Some techniques, widely used in trace element analysis are atomic absorption spectrophotometry (AAS) [14], energy dispersive X-ray fluorescence (EDXRF) [15], electro thermal atomic absorption spectrometry (ETAAS) [16], inductively coupled plasma-atomic emission spectrometry (ICP-AES) and mass spectrometry (ICP-MS) [17]. Each one of these has one's own advantages and limitations with regard to accuracy, precision, sensitivity and specificity. Instrumental neutron activation analysis (INAA), a versatile multielemental analysis technique is ideal for analysing complex biological samples, including medicinal herbs [12,18].

Several workers from different countries have reported the analysis of medicinal herbs of their respective countries. Obiajunwa et al. [19] and Fakankun et al. [20] determined essential and trace element contents of some Nigerian medicinal plants. Serfor-Armah et al. [21] determined concentrations of seventeen elements (Al, Ba, Br, Ca, Cl, Co, K, Mn, Mg, Na, Rb, Sb, Sc, Se, Ta, V and Zn) in five medicinal plants used in Ghana. Vega-Corrillo et al. [22] reported elemental distribution in medicinal plants used in folklore medicines in Mexico. Orlova et al. [23] of Russia determined Cu, Zn, Mn and Cr in raw medicinal plants materials by ICP-AES after autoclave preconcentration. In recent years, much attention has been paid to the analysis of traditional Chinese medicines by using a variety of analytical techniques [24–27]. Kanias et al. [4] of Greece determined Sb, Cs, Cr, Fe, Eu, Rb, Sc, Sr, Th and Zn, in trace amounts in the leaves of the medicinal plant *Euculyptus camaldulensis*. Soltyk et al. [28] determined macro- and microelements Ca, Cr, Cu, Fe, Mg, Mn, Mo, P, Se and Zn in multimineral and multivitamin preparations and in pharmaceutical raw materials. Razic et al. [29] from Yugoslavia analysed trace elements in *Echinacea purpurea*, herbal medicine. Kist et al. [30] determined 32 elements in five species of medicinal plants by NAA, proton activation analysis (PAA) and emission spectral analysis (ESA). In a recent study, Zaidi et al. [31] have reported trace element evaluation of some medicinal herbs whereby variation in trace element contents of same species from different origin were attributed to ecological and geographical variations. Singh and Garg [32] determined 20 essential and toxic elements (As, Ba, Br, Ca, Cl, Co, Cr, Cu, Fe, K, Mn, Mo, Na, P, Rb, Sb, Sc, Se, Sr and Zn) in Ayurvedic Indian medicinal herbs using INAA. Rajurkar and Damame [33] determined 14 elements in some medicinal plants used in the treatment of cardiovascular disease and urinary tract disorders. Balaji et al. [34] determined essential elements in Ayurvedic medicinal leaves by INAA.

In the present study, we have analysed *Pragya-peya*, a herbal drink, and its 12 herbal constituents for 26 essential and toxic elements by INAA and AAS. It is taken as tea or with milk. It is often recommended for cold and cough and widely used as nervine tonic and stimulant. It preserves energy, enhances vitality and memory, and also acts as safeguard against body disorders. Local, English and botanical names of all the constituents and their uses as described in literature [7,35] are mentioned in Table 1. In order to study variation in batch products, elemental contents were determined in three batches collected over 1-year period at different intervals. Some inter-elemental correlations were also attempted.

Table 1
Nomenclature and medicinal uses of constituents of *Pragya-peya*

General name	English name	Family	Botanical name	Uses [1,7,35]
Aagya-ghas	Rosha grass	Poaceae (Graminae)	*Cymbopogon schoenanthus*	Heart disease, cough, colic, rheumatism, cold, fever, indigestion
Arjuna	Arjuna bark	Combretaceae	*Terminalia arjuna*	Heart and liver disease, cardiovascular system, styptic, tuberculosis, cough, dyscrasia, fever, ulcer
Bay leaves	Bay leaves	Lauraceae	*Cinnamomum tamala*	Asthma, abdominal disorder, diarrhea, diabetes, dyspepsia, cough and anti-inflammatory
Brahmi	Bacopa	Scrophulariaceae	*Bacopa monnieri*	Skin disease, nervous disorder, memory enhancement, leprosy, tuberculosis, anemia, cough, increases blood protein and RBC, hepatoprotective
Dalchini	Cinnamon	Lauraceae	*Cinnamomum zeylanicum*	Flu, indigestion, mouth wash, cough, heart disease
Fennel	Fennel	Umbellifereae	*Foeniculum vulgare*	Fever, urine inflammation, thirst, dysentery, diarrhea, cholera, spleenomegaly, analgesic, brain tonic, antacid, kidney disease
Nagarmotha	Nutgrass	Cyperaceae	*Cyperus rotundus*	Dyspepsia, skin disease, diarrhea, thirst, fever, stimulant, wound healer, hair growth
Red sandal	Red sandal	Leguminosae	*Pterocarpus santolinus*	Cough, blood purifier, fever, headache, skin disease
Shankhpushpi	Shankhpushpi	Convolvulaceae	*Convolvulus pluricaulis*	Brain and heart tonic, reduces blood pressure and tension, effective on thyroid high secretion, epilepsy, insomnia
Sharpunkha	Wild indigo	Leguminosae	*Tephrosia purpurea*	Flatulence, indigestion, diarrhea, cough, asthma, liver-spleenomegaly, oedema, leprosy, dyspepsia
Tulsi	Sacred basil	Lamiaceae	*Ocimum sanctum*	Bronchitis, malarial fever, asthma, urinogenital disorder, vomiting, indigestion, ear ache, dyscracia, hepatoprotective, spermatopoitic, antibacterial, antituberculosis
Yastimadhu	Liquoric root	Leguminosae	*Glycyrrhiza glabra*	Cough, vomiting, wound healing, haematemesis, thirst cholera, skin disease, spermatopoitic, heart disease, epilepsy

2. Materials and methods

2.1. Sample collection and preparation

Pragya-peya, a product of Shantikunj, Haridwar (India) is a mixture of 12 herbs in different proportions. 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 (Haridwar), Yogi Pharmacy (Haridwar) and unbranded samples from the local market. Its surface contaminants were wiped with tissue paper and then useful parts were separated. All the samples were dried in an oven at ∼80 °C. The samples were powdered in agate mortar and passed through 100 mesh sieve. RMs such as peach leaves (SRM-1547) from the National Institute of Standards and Technology (NIST), USA [36] and Mixed Polished Herbs (MPH-2) from Institute of Nuclear Chemistry and Technology (INCT), Poland [37] were procured and used as such. However, these were dried as per recommended procedure before use.

2.2. Irradiation and counting

An amount of 30–50 mg each of powdered samples and RMs were weighed accurately and packed in alkathene and aluminium foil (super pure) for short (1 min) and long irradiation (3 days), respectively. Irradiations were carried out in Dhruva reactor at the Bhabha Atomic Research Centre (BARC), Trombay, Mumbai, India, at a neutron flux of $\sim 10^{13}\,\mathrm{n\,cm^{-2}\,s^{-1}}$. Short lived 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. It is observed that up to 11 elements (Al, Br, Ca, Cl, K, Mg, Mn, Na, P, Ti and V) could be determined in a single irradiation of 1 min. Long irradiated samples were brought to our laboratories in Roorkee, and γ-activity was measured using a 20% relative efficiency HPGe detector with 8 k MCA and GENIE-2000 software (Canberra, USA). Resolution of the system is 1.8 keV at 1332 keV of ^{60}Co. Counting was followed at different intervals up to 3 months. Irradiation and counting schedule followed and elements determined are the same as described earlier [38]. Elemental contents were calculated by relative method using RMs as comparators. Phosphorus was determined by measuring β^- activity due to ^{32}P in neutron irradiated samples on an end window G.M. Counter using 27 mg cm^{-2} aluminium filter after a delay period of ∼20 days [39].

2.3. AAS measurements

For the analysis of Cd, Cu, Ni and Pb by AAS method, about 2 g of each sample was accurately weighed and digested in 5:1 mixture of nitric acid and perchloric acid [40]. After digestion, two to three drops of HCl was added and the solution was made up to 25 mL. Ni, Cu, Cd and Pb were determined by Atomic Absorption Spectrophotometer (GBC Avanta, Australia) using a mixture of acetylene–air flame. The wavelength and the sensitivities for these elements were 232 nm and 0.04 μg/mL; 324.7 nm and 0.025 μg/mL; 228.8 nm and 0.009 μg/mL and 217 nm and 0.06 μg/mL, respectively. The instrument was precalibrated using high purity grade salts of respective elements.

3. Results and discussion

Mean elemental concentrations of 7 minor (Na, K, Ca, Cl, P, Mg and Al) and 15 trace (Ba, Br, Co, Cr, Cs, Fe, Hg, La, Mn, Rb, Sc, Se, Th, V and Zn) elements in *Pragya-peya* and its 12 constituents, as determined by INAA using short and long irradiations are listed in Table 2(A and B). Also included in the tables are data for two RMs, along with their certified values [36,37]. A comparison of our data with the certified values shows that most elemental contents in INCT-MPH-2 are in good agreement within ±5% though in some cases larger differences (up to ±15%) are also observed. On the other hand, for SRM-1547, most of our data agree within ±10% with a few exceptions. Similarly R.S.D. values for most elements in both the cases are in the range of ±5–10%. Therefore, it is presumed that the elemental data for the samples analysed here should be accurate and precise within ±5–10%. The concentrations of Ni, Cu, Cd and Pb as determined by AAS are listed in Table 3. In order to test homogeneity of *Pragya-peya*, three different batches were analysed. Though only mean values are reported in Tables 2(A and B) and 3 but individual values are plotted in Figs. 1 and 2. Also bar plots of inter-elemental correlations K/P, K/Na and Ca/P in *Pragya-peya* and its 12 herbal constituents are shown in Figs. 3, 4 and 5, respectively. Some prominent features of elemental contents and their variations are discussed here.

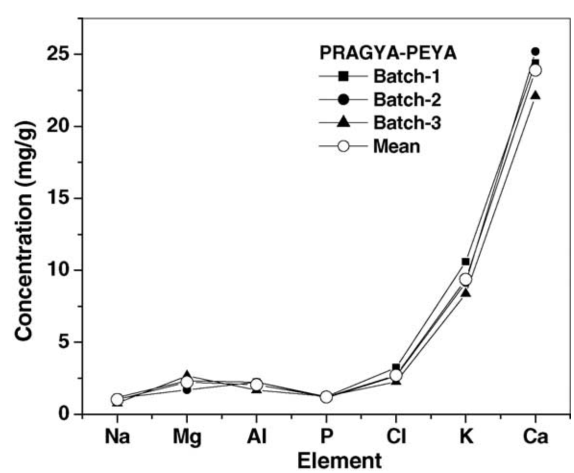

Fig. 1. Variation of elemental concentrations (Na, Mg, Al, P, Cl, K, Ca) in *Pragya-peya* from different batches.

Table 2
Concentration of (A) minor and (B) some 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)
(A) Minor elements										
Pragya-peya[a]	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
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
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
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
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
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
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
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
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
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
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
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
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
SRMs										
Mixed Polish herbs	0.64 ± 0.04	8.10 ± 0.45	11.2 ± 0.8	2.96 ± 0.32	515 ± 50	18.7 ± 0.6	2.88 ± 0.18	0.41 ± 0.02	2.39 ± 0.03	0.82 ± 0.08
(INCT-MPH-2)	(0.67 ± 0.11)	(7.71 ± 0.61)	(10.8 ± 0.7)	(2.84 ± 0.20)	{460}	(19.1 ± 1.2)	(2.92 ± 0.18)	{0.350}	{2.5}	(0.95 ± 0.16)
INCT, Poland [37]										
Peach leaves	0.28 ± 0.03	12.3 ± 0.3	17.5 ± 1.5	0.34 ± 0.02	192 ± 16	26.5 ± 2.0	4.15 ± 0.35	0.02 ± 0.01	1.32 ± 0.13	0.52 ± 0.05
(SRM-1547) NIST,	(0.25 ± 0.01)	{11}	(15.6 ± 0.2)	(0.36 ± 0.02)	(218 ± 14)	(24.3 ± 0.3)	(4.32 ± 0.08)	(0.02 ± 0.01)	(1.37 ± 0.07)	(0.37 ± 0.03)
USA [36]										

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)	Se (ng/g)	Th (ng/g)	Zn (µg/g)
(B) Trace element												
Pragya-peya[a]	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	126 ± 51	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	ND	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	96.4 ± 19.3	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	ND	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	512 ± 72	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	ND	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	602 ± 52	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	ND	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	ND	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	ND	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	ND	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	176 ± 10	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	ND	229 ± 37	31.1 ± 1.0
SRMs [36,37]												
Mixed Polish	32.6 ± 0.5	0.25 ± 0.03	1.78 ± 0.45	64.5 ± 5.4	ND	0.58 ± 0.02	185 ± 10	10.6 ± 0.7	146 ± 14	182 ± 16	171 ± 12	32.7 ± 2.3
herbs	(32.5 ± 2.5)	(0.21 ± 0.03)	(1.69 ± 0.13)	(76.0 ± 7.0)		(0.57 ± 0.05)	(191 ± 12)	(10.7 ± 0.7)	(123 ± 9)	(–)	(154 ± 13)	(33.5 ± 2.1)
(INCT-MPH-2)												
INCT, Poland												
Peach leaves	116 ± 4	0.05 ± 0.01	1.27 ± 0.19	ND	35.0 ± 5.0	9.37 ± 0.60	106 ± 6	19.2 ± 0.1	64.0 ± 5.0	ND	60.2 ± 5.0	20.5 ± 1.5
(SRM-1547)	(124 ± 4)	{0.07}	(–)		(31.0 ± 7.0)	{9}	(98 ± 3)	(19.7 ± 1.2)	{40}		{50}	(17.9 ± 0.4)
NIST, USA												

ND, not detected; ( ) certified value; { } information value.

[a] Mean of three different batches with SD of the mean.

Table 3
Concentration of some trace elements in *Pragya-peya* and its constituents analysed by AAS

Sample	Cd (ng/g)	Cu (μg/g)	Ni (μg/g)	Pb (μg/g)
Pragya-peya[a]	334 ± 34	14.7 ± 0.8	5.62 ± 3.43	3.79 ± 0.83
Aagya-ghas	184	15.0	2.78	1.52
Arjuna (bark)	195	3.80	0.61	0.79
Bay leaves	122	9.63	1.37	1.08
Brahmi	425	14.5	4.00	1.40
Cinnamon	240	4.46	ND	1.72
Fennel	86.1	10.9	1.46	0.95
Nagarmotha	ND	3.77	0.76	1.58
Red sandal	ND	6.85	1.54	1.71
Shankhpushpi	292	5.92	1.91	0.57
Sharpunkha	193	9.68	1.44	1.36
Tulsi	410	20.1	0.65	1.83
Yastimadhu	60.2	8.30	ND	1.70
SRMs				
Mixed Polish herbs (INCT-MPH-2) INCT, Poland [37]	188 (199 ± 15)	7.92 (7.77 ± 0.53)	1.59 (1.57 ± 0.16)	1.80 (2.16 ± 0.23)
Peach leaves (SRM-1547) NIST, USA [36]	27 (26 ± 3)	3.59 (3.7 ± 0.4)	0.75 (0.69 ± 0.09)	1.01 (0.87 ± 0.03)

ND, not detected; ( ) certified value.
[a] Mean of three different batches with S.D. of the mean.

3.1. Test for homogeneity

In order to test the homogeneity of the batch products of the herbal preparation, three different samples collected over 1-year period were analysed. A plot of minor constituents (Na, Mg, Al, P, Cl, K and Ca) at mg/g level in Fig. 1 shows very little variation with almost overlapping behaviour. 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, Cu, Zn, Se, Hg and Ba) in Fig. 2 also show little variation in elemental contents of three batches (small range of ±10%) except for Fe, Mn, Ni and Hg, which 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 [31,34]. Thus, our observations are in accordance with a recent study by Zaidi et al. [31]

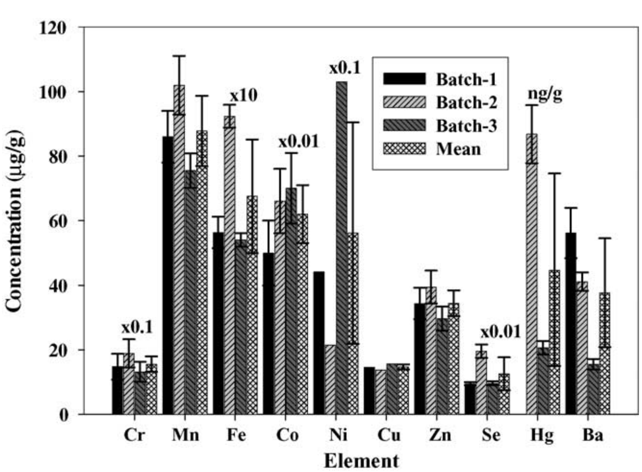

Fig. 2. Variation of elemental concentrations (Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Hg, Ba) in *Pragya-peya* from different batches.

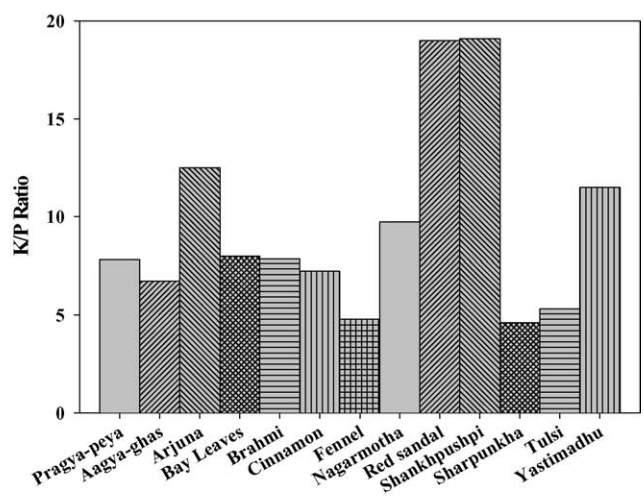

Fig. 3. Variation in K/P ratio in *Pragya-peya* and its 12 constituents.

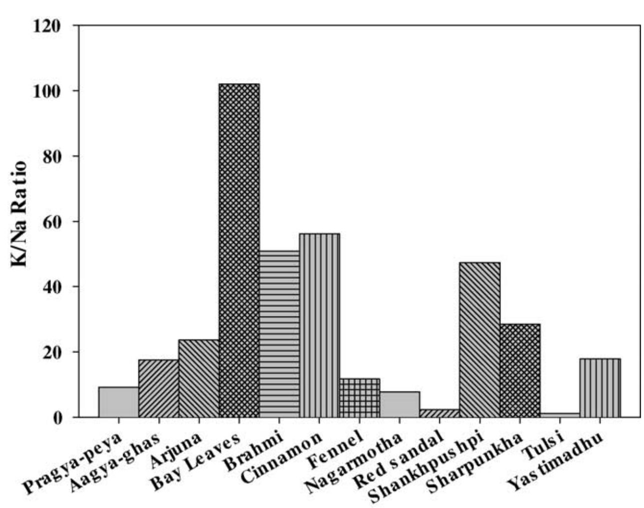

Fig. 4. Variation in K/Na ratio in *Pragya-peya* and its 12 constituents.

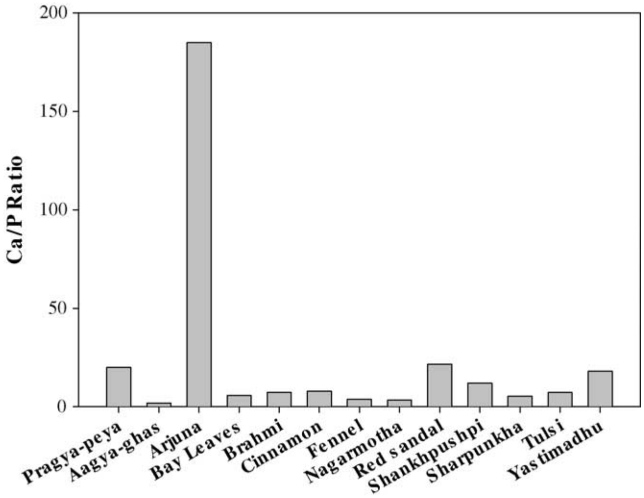

Fig. 5. Variation in Ca/P ratio in *Pragya-peya* and its 12 constituents.

where variation in 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 origin is not expected.

3.2. Elemental contents in the herbal preparation—Pragya-peya

It contains several nutrient elements in significant amounts and in bioavailable form, easily digestible by our body system. It is observed that Ca and K contents are higher (≥10 mg/g) whereas those of Na, Mg, Al and P contents are 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 [41]. 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 where trace elements such as Fe, Mn, Zn, Rb, V, Se, Cu, etc. may be responsible. 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 [10,11,41]. Of special importance are V and Se whose compounds have been found to be antidiabetic [42] and cancer protective [43]. Also, some toxic elements such as Cd, Hg, Th, Pb are environmental contaminants from soil and water [44]. However, the small amounts present are well within permissible limits [45].

3.3. Elemental concentrations in constituent herbs

Even though *Pragya-peya* is a preparation of 12 constituent herbs mixed in different proportions, each one of which has its own medicinal importance in day-to-day life (Table 1). Therefore, we have analysed each one of the herb for all the elements. It is observed from the elemental data in Tables 2(A and B) and 3 that no single constituent herb is enriched in all the elements. In general fennel seeds, most often used as a spice and mouth freshener [7,9] 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 [44]. Fennel seeds are available in different varieties (small, medium and, thick) and recommended as antacid, for treatment of urine inflammation, diarrhea and kidney disease. Its Se content (602 ± 52 ng/g) is of special interest because of cancer preventive properties [43]. Also *aagya-ghas* is most enriched in Fe (940 ± 103 (g/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 [10,11]. 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 [41]. *Arjuna* is most enriched in Ca content (∼10%) and used widely in many herbal preparations [9].

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 materials, proteins, immune reactions, wound healing, development of foetus and sperm production [46,47]. Our studies have shown that Zn level in blood of diabetic patients is lowered [48]. 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 being in *arjuna* and *aagya-ghas*, respectively. Mn is also an essential element required for various biochemical processes [47]. In most of the constituents, it is found to be <200 μg/g but in *bay leaves* and *cinnamon*, it is much higher, being highest in c*innamon* (631 ± 30 μg/g). In Ayurvedic literature, c*innamon* is recommended for flu, indigestion, cough and heart diseases [7,35]. 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 and found in the range of 18.8–168 ng/g with highest content being in *brahmi* [44,45]. This may be essentially due contamination from the soil. Many vanadium compounds have been described to possess therapeutic properties specially being used for the treatment of diabetes [49]. Vana-

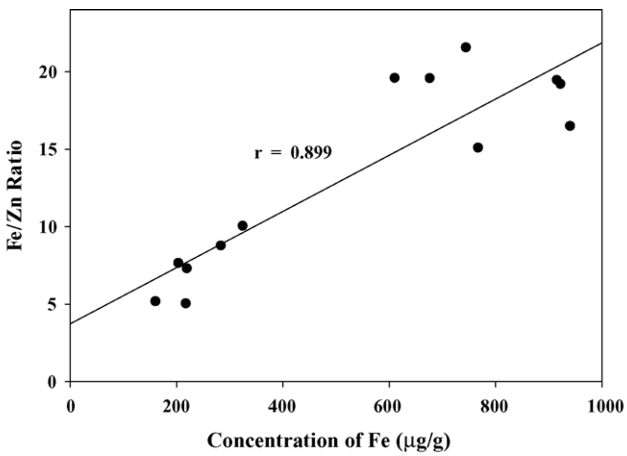

Fig. 6. Variation in Fe/Zn ratio vs. Fe concentration in *Pragya-peya* and its 12 constituents.

dium was also detected in all the constituents in the range of 0.5–5.2 μg/g with highest content in *nagarmotha*. The variation in elemental contents as observed in this study could be due to preferential uptake of the elements by a particular plant species from the soil. Therefore, soil characteristics together with environmental conditions play an important role in the nutrients contents [50].

3.4. Inter-elemental correlations

In general, all the constituent herbs are enriched in K, P, Na and Ca. Several literature reports suggest interrelationship of various elements [14,51]. We have plotted K/P ratio as shown in Fig. 3. 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 of P content. A plot of K/Na ratio in Fig. 4 was drawn with wide variation in the range of 1–100 being the highest in *bay leaves* (102). Only four constituent herbs exhibit this ratio in a comparable range of 35–55. A plot of Ca/P, both structural elements, is shown in Fig. 5. Most of the constituents exhibit Ca/P ratio in a narrow range (<10) except *arjuna* showing highest ratio (185). *Red sandal*, *shankhpushpi* and *yastimadhu* exhibit a ratio of ∼20.

Fe, Co and Zn, essential elements for biochemical processes, are well correlated in our study of *Pragya-peya* and its constituent herbs [29]. A plot of Fe versus Fe/Zn ratio shows linear relationship (Fig. 6) with $r = 0.899$, which is more like two clusters and represents somewhat poor 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. [29]. An excellent relationship is observed between Fe and Co ($r = 0.969$) in *Pragya-peya* and its 12 constituents as shown in Fig. 7. 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 bioavailable form in a balanced manner with almost no harmful effects [1,35] except some environmental contaminants. These, however, should be avoided by collecting herbs grown in a clean and well controlled environment.

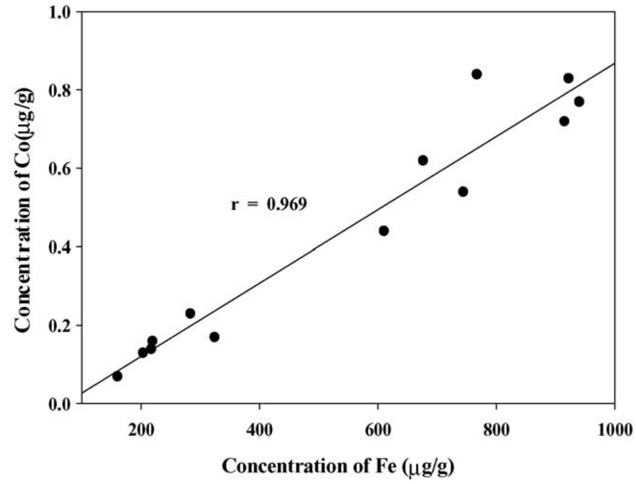

Fig. 7. Correlation between Fe and Co concentrations in *Pragya-peya* and its 12 constituents.

4. Conclusion

The concentrations of 26 minor and trace elements were determined in *Pragya-peya* and its 12 constituent herbs by INAA and AAS. The ranges of elemental concentrations have been found to vary in a wide range of mg/g to ng/g in all the samples. It has been observed that *Pragya-peya* is enriched in Ca, K, Mg, P, Mn, Fe, Cu, Cr, Co and Zn. Further, K/P ratio changes in a wide range of 4.6–19.1. Several interesting correlations have been observed between Fe versus Fe/Zn, Fe versus Co in all the herbs. It suggests that the concentration levels of many elements in the herbs are strongly affected by characteristics of plants as well as soil and climatic conditions. The content of Cd and Pb, two most toxic elements, in herbs may be attributed to their uptake from the soil polluted by industrial and automobile activities. Besides the essential elements, the concentrations of several other elements were also determined so as to obtain information about their intake from the soil and their possible role in the body system.

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