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Minor and Trace Elemental Determination in the Indian Herbal and Other Medicinal Preparations

DHANANJAY L. SAMUDRALWAR*[1,2] AND AMAR N. GARG[1]

[1]*Department of Chemistry, Nagpur University, Nagpur-440010, India; and* [2]*Livestock Disease Diagnosis Center, 1429, Newton Pike, Lexington, KY 40503*

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ABSTRACT

Medicinal plants described in the Indian "Ayurvedic" literature viz. Tulsi *(Ocimum sanctum)*, Gulvel *(Tinospora cardifolia)*, bitter Neem *(Azadirachta indica)*, Kanher *(Nerium Åndicum)*, Vekhand *(Acorus calamus)*, and Peacock's feather (ash) were analyzed for minor and trace elements by instrumental neutron activation analysis. The samples and the standards from the National Institute of Standards and Technology, USA and IAEA, Vienna were irradiated for 5 min, 1 h, 5 h, and 10 h with thermal neutrons at a flux of 10^{12} – 10^{13} n cm^{-2} s^{-1} in APSARA and CIRUS reactor at BARC, Bombay. High resolution γ ray spectrometry was performed using a 45 cm^3 HPGe detector and a 4096 MCA system. Concentrations of 13 elements were determined. Zinc, manganese, and sodium were significantly higher in Tulsi leaves while zinc is higher in Neem leaves. Peacock's feathers were found to be rich in manganese, iron, copper, and zinc. A high concentration of mercury was also found in the peacock's feather ash. The therapeutic significance in restoring ionic balance is discussed.

Index Entries: Indian medicinal plants; INAA; trace elements; peacock feathers.

INTRODUCTION

The ancient medical philosophy in India is known as "Ayurveda," and should have been developed thousands of years ago in the Indian subcontinent. There are several references about the herbal medicines in

*Author to whom all correspondence and reprint requests should be addressed.

ancient Hindu epics like *"Ramayan"* and *"Mahabharat,"* which are considered to be from about 4000–3000 BC. The knowledge of herbal and other natural medications has been passed over generations and preserved in well-written documents. There are two volumes of approx 4000–6000 pages written by the Indian sages "Charak" and "Sushruta." The former discusses in his treatise the medicinal plants, their characteristics, biomedical usages, formulations and dosages, and so on, whereas the latter treatise mainly deals with the anatomy, physiology, medical surgeries, tools to be used, and their design in details. In modern India, "Ayurvedic medicine" is still well and alive. The majority of the 900 million people in India and in Bangladesh, Sri-Lanka, Indonesia, Malaysia, Myanmar, and Tibet are aware of these natural medicines. Physicians formally trained in Ayurvedic medicine ("Vaidya") are common in modern India. However, people prefer to seek these ancient medicines for chronic diseases like eczema and other skin diseases, diabetes, epileptic seizures, constipation, fertility and antifertility, muscular strength and vitality, memory loss, and so on, for which there are no definitive and easy solutions available in the allopathy.

Although the concept of trace elements being "the inorganic switches" has evolved during last 30–50 yr, its origin could be traced back to the ancient civilizations all over the world *(1,2)*. ***"Ayurveda,"*** the traditional Indian medicinal system (which has its origin in prehistoric civilization), also emphasizes the importance of minor and trace elements in human health and disease *(1,3)*. Many drugs and pharmaceutical preparations derived from specific plants and animal products are widely used in Asia, particularly in India *(2–6)*. Numerous plants and derived medications are used for common as well as chronic diseases. Tulsi (*Ocimum sanctum*) and bitter Neem *(Azadirachta Indica)* are the most analyzed plants and several drugs have been identified from different parts of these plants. They are used extensively in treating inflammation and fever, malaria *(1–5)*, ulcer *(7)*, reproductive behavior *(8)*, cardiac arrest *(9)*, and so on. Huperzine A, an acetylcholinesterase inhibitor extracted from a Chinese herbal tea and used traditionally for the prevention of memory loss in elderly is now under clinical trials for Alzheimer's disease in the United States *(10)*. However, the data on the trace element availability and their reactivity in such herbal medications are scanty.

In recent years, with the awareness about the importance of trace elements in health and disease *(11)*, an increasing number of reports on the role of trace elements in traditional medicines are being published. Rao *(12)* had analyzed the neem cake for its mineral elements as a feed stock. Rajurkar and Winchurkar *(13)* analyzed four popular *Ayurvedic* medications in India for concentrations of manganese, sodium, potassium, chlorine, copper, gold, and tin by NAA using a ^{252}cf source. A few reports of trace element analysis of traditional medicines have also been published in China *(6,14)*, Nigeria *(15)*, and Greece *(16)*. Mumba et al.

Table 1
List of Medicinal Plants Analyzed and their Therapeutic Uses

Sample	Part Analyzed	Uses (for the treatment of..)	Reference #
Tulsi (O.sanctum)	Leaves	Fever, headache, periodic, antifertility, ozena	1,2,5,26-28
Bitter Neem (A.indica)	Leaves	Malaria, low fever, eczema, antifertility, chewing sticks,	1-5,7-9,12,29
Gulvel (T. cardifolia)	Leaves	Common cold, cough	1,3,5
Kanher (A. thevetica)	Bark	Heart burns, severe heart pain	1,3,5
Vekhand (A.columus)	Roots	Bronchitis, asthma, gastric troubles, headache, epileptic seizers	2, 3
Peacock's feathers	Feathers	Bronchitis, anti-convulsions antidote (snake bite)	3, 5
Orange peel (C. sinensis)	Peelings	Source of:vitamins, flavoring agents tooth powder, shampoo	2 - 4

(17) from Hungary have reported the concentrations of magnesium, aluminum, silicon, chlorine, and potassium in some herbs.

Most of the natural medications are dispensed traditionally in the form of paste, slurry, extract, or decoction from raw plant leaves, bark, roots, and so on. These (along with their specific uses and relevent references from modern literature) are listed in Table 1. Instrumental Neutron activation analysis (INAA), which is multi-elemental, accurate, fast, and needs the least sample preparation, was employed for these analyses *(18)*. The instrumental NAA also minimizes the sample contamination.

METHODS

Tulsi *(O. sanctum)*, Neem *(A. indica)*, and Gulvel *(T. cardifolia)* leaves were hand plucked and washed with 0.1*M* HCl and then with double-distilled deionized water. Kanher *(N. indicum)* bark was peeled with a stainless steel blade while Vekhand *(A. calamus)* tubers were purchased from the local market and cleaned. After drying at ≈65°C in a glove box, the samples were powdered and irradiated with ^{60}Co gamma rays at a dose of 2.5 Mrads to prevent biological degradation (Gamma Chamber 900, BARC Bombay). The central eye and peripheral wig of the peacock's feathers were cut separately and ashed in silica crucible initially at 200°C (30 min) and later at 400°C for about 2 h. The weight ratio of the raw sample/ash was determined. The NIST SRM Spinach 1570 was used as the primary standard while Bowen's Kale, a widely analyzed international standard and IAEA CRM Hay Powder V-10, was used as a sec-

Table 2
Concentration of Minor and Trace Elements in IAEA CRM Hay Powder, V-10, Bowen's Kale, and Some Medicinal Plant Leaf Samples
(NIST SRM Spinach 1570 as Primary Standard)

Element	Hay Powder IAEA V-10	Bowen's Kale (B.oleracea)	Tulsi leaves (O.sanctum)	Neem leaves (A.indica)	Gulvel leaves (T.cardifolia)
Na (%)	0.07±0.005(0.055)	0.23±0.03(0.25)	1.46±0.07	0.02±0.001	0.24±0.02
K (%)	2.17±0.12(2.07)	2.38±0.20(2.46)	1.10±0.10	1.57±0.20	2.55±0.20
Mg (%)	0.19±0.01(0.14)	-	0.42±04	1.52±0.12	-
P (%)	0.26±0.03(0.23)	0.42±0.05(0.45)	0.43±0.04	0.20±0.01	0.26±0.02
Cl (%)	0.79±0.10(0.66)	0.39±0.05(0.34)	0.86±0.03	-	-
Fe (μg/g)	154±14(185)	113±10(120)	104±10	72±5	72±8
Zn (μg/g)	30.1±2.2(24)	29.4±2.0(32.7)	140±13	10.1±0.8	8.94±0.81
Mn (μg/g)	42.6±2.2(47)	12.9±1.5(14.9)	153±13	56.8±4.6	15.1±1.2
Br (μg/g)	9.32±0.61(8.34)	25.7±1.5(25)	47.9±2.8	7.31±0.21	4.10±0.30
Cu (μg/g)	8.31±1.12(9.27)	4.91±0.60(4.89)	12.8±1.5	-	4.52±0.50
Cd (μg/g)	-	1.09±0.31(0.85)	-	-	0.52±0.14
Co (ng/g)	-	48±8(63.2)	-	510±80	70±17
Hg (ng/g)	-	139±30(166)	-	180±33	27±7

Values in parentheses are the certified concentrations in standards.

ondary standard to check the accuracy of the method. These were used without any further processing. The moisture content in each sample was determined on separate aliquot.

Approximately 25–35 mg each of the samples and standards (NIST SRM 1570 Spinach, IAEA CRM Hay Powder V-10, and Bowen's Kale) were packed in polyethylene bags of 2.5 × 1.0 cm and thermally sealed. The samples and standards were grouped into batches and irradiated for 5 min, 1 h, 5 h, and 10 h with thermal neutron flux of $\approx 10^{12}$n cm^{-2} s^{-1} in APSARA reactor at Bhabha Atomic Research Center, (BARC) Bombay. After suitable delay, these were counted on a 45 cm^3 HPGe detector (EG & G ORTEC) coupled with a 4096 multichannel analyzer (Tracor Northern 1700). The resolution (FWHM) of the detector was 1.99 keV at 1332 keV of ^{60}Co. The nuclides were identified with the gamma ray photopeaks and the half-life follow-up whenever possible *(19)*. Phosphorus was determined by β^- particle counting (^{32}P, β_{max} 1.71 MeV; half-life, 14.3 d) on an end window gas flow proportional counter (ECIL, Hyderabad) using a 27 $mgcm^{-2}$ aluminum filter after a delay of 3 wk, as described elsewhere *(20)*.

Table 3
Concentration of Minor and Trace Elements in Medicinal Samples

Element	Kanher bark (A.thevetia)	Vekhand root (A.columus)	Orange peel	Peacock s feather ash	
				Eye	Periferal
Na (%)	0.03±0.004	0.10±0.01	0.08±0.01	5.81±0.40	2.50±0.15
K (%)	2.27±0.20	1.48±0.30	1.41±0.11	3.20±0.31	3.20±0.20
Mg (%)	0.30±0.04	1.32±0.13	0.35±0.05	0.18±0.02	0.12±0.02
P (%)	0.08±0.01	0.18±0.02	-	-	-
Cl (%)	0.92±0.10	0.60±0.10	0.85±0.10	7.20±0.69	2.52±0.32
Fe (μg/g)	129±11	355±25	142±17	613±35	393±17
Zn (μg/g)	110±10	12.2±1.1	9.5±0.5	730±16	1300±80
Mn (μg/g)	-	-	11.8±1.1	1830±40	505±20
Br (μg/g)	1.72±0.15	20.2±1.5	9.22±0.52	1250±140	569±41
Cu (μg/g)	-	-	4.2±0.5	77±2	57±5
Cd (μg/g)	-	-	1.05±0.21	3.12±0.71	< 1.0
Co (ng/g)	680±85	710±57	500±60	-	-
Hg (ng/g)	12.5±2.3	11.7±2.2	< 10	-	-

RESULTS

The concentrations of elements in samples and secondary standards were calculated by using NIST SRM Spinach (1570) as the primary standard. The concentrations from different irradiation batches were compiled. The mean and standard deviations for secondary standards along with the certified or literature values are given in Table 2. It is observed from Table 2 that the concentrations of most of the elements in Bowen's Kale and Hay Powder V-10 are within 10–12% of the certified or literature values *(21–23)*. The concentrations in medicinal samples are given in Tables 2 and 3. The concentrations of zinc and manganese are higher (5–6 times) in Tulsi leaves as compared to other medicinal plants and leafy vegetables that we analyzed previously *(24)*. Neem leaves had significantly lower concentrations of sodium and iron, but a higher concentration (180 ng/g) of mercury. The sodium, bromine, and phosphorus concentrations in Kanher bark are 2–3 times lower, whereas zinc concentration is higher by an order when compared to the Neem, Gulvel, Vekhand, and Orange peelings.

DISCUSSION

Tulsi is the most analyzed plant and known for its numerous medicinal uses in Ayurvedic and modern literature. It is shown in Table 2 that concentrations of zinc and manganese are higher than the concentration ranges reported in several other plant leaves by Kabata-Pendias and Pendias *(25)*. Copper is also higher in Tulsi leaves as compared to the other samples analyzed. It is well known that zinc, manganese, copper, and iron exist mostly in a combined state with large protein or enzyme molecules in important subcellular organelles like mitochondria and chloroplasts or as free ions involved in various catalytic and transportation processes across the cell membrane. The medicinal preparations from Tulsi, therefore, provide supplementary zinc and manganese in diseased states and may help restore the ion balance or accelerate the metabolism process. Chattopadhyay et al. *(26)* have reported that extract of Tulsi leaves provides hepatic damage protection after using the paracetamol. Khanna et al. *(27)* have observed that the higher concentration of zinc in Tulsi leaves and the antifertility effect caused owing to its extract may be correlated. The role of zinc in spermatogenesis is well documented *(27,28)*. Although zinc is one of the most essential elements, its very high localized cellular concentration may affect the activity of the sperms and hinder the fertilization process.

Neem is the most useful medicinal plant; each of its parts (leaf, bark, fruit, and root) has been prescribed in several medicinal preparations as curative for a number of ailments as listed in Table 1. Aromatic oils such as margonsin, nimbidin, and nimbin ($C_{28}H_{48}O_8$) are extracted mainly from its fruits and used as astringent, antiperiodic, and antimalarial *(29)*. A paste of its bark and leaves is applied over boils, skin allergies, yeast infections, and eczema *(29)*. Particularly, its yellow leaves are known to help wound healing. The decoction of its leaves is recommended for ulcers and long standing fever. Neem leaves that we analyzed had significantly lower concentrations of sodium and iron, but a higher concentration of mercury as compared to other edible plant leaves. The concentration of mercury (180 ng/g) is significantly high compared to the reported range of 2.6–86 ng/g (dry wt) concentration in plants *(25)*. We had previously reported *(24)* a concentration of 70 ± 6 ng/g of mercury (3–4 times higher than in other vegetables) in sweet Neem *(Muraya exotica)*. The sweet neem is used as spicy flavoring agent in many Indian recipes. The tender branchlets of bitter neem *(A. indica)* are used as tooth brush in the rural India. These are similar to the Nigerian chewing sticks reported by Osubiojo *(30)*. The higher concentration of mercury in both the varieties of Neem *(A. Indica* and *M. exotica*) should further be investigated for the speciation of mercury in it.

Kanher bark extract is mainly used to treat heart troubles *(3)*, whereas Vekhand is used as antiphlegm, and antiepileptic. It can be seen in Table 3 that sodium, bromine and phosphorus concentrations in Kanher bark are 2–3 times lower, while zinc concentration is higher by an order when compared to the Neem, Gulvel, Vekhand, and Orange peelings. Vekhand root *(A. calamus)* is marked by its highest iron content (355 ± 25 μg/g) among the plant samples analyzed. Bromine content is also significantly higher than in the neem, gulvel, kanher, and the orange peelings. Vekhand root extract or dry powder is used for severe headache, gastric troubles, heart burns, and epileptic seizers *(2,3)*. It is also used for treating chronic diseases like bronchitis and asthma, mostly owing to its capacity to remove phlegm. It is a strong tongue stimulant and may help in the digestion process owing to intense salivary stimulations.

Gulvel and Neem have comparable concentrations of most elements. However, Gulvel has been documented for its use in the treatment of common cold and cough while Neem has been a versatile source of several Ayurvedic drugs. Orange peelings are a rich source for volatile oils, hesperic acid, and vitamins A, B, and C *(4)*. Many flavoring agents are extracted from peelings and also used as tooth powder and natural shampoo. It has been observed that the concentrations of several elements in peelings are comparable to the orange leaves we analyzed earlier *(24)*. We could not find any literature references on the trace element analysis of Kanher bark, gulvel leaves, and orange peelings.

The concentrations of most elements in Peacock's feather ash are much higher than other samples analyzed in this study. The weight ratio of raw feathers/ash was found to be ≈10. Since the medications are prepared using the feather ash, we have reported the concentrations in ash (Table 2). Even after normalizing the concentrations to the raw weight, it is observed that the concentrations of sodium, zinc, manganese, bromine, and copper are much higher than other medicinal samples analyzed in this study. The central "eye" is richer in manganese, bromine, iron, and copper as compared to the peripheral feathers. The abnormally higher concentration of zinc in the peripheral feathers is significant. The copper concentrations of 57 and 77 ppm in peripheral and central eye portions, respectively, are 5–6 times higher than a normal concentration of 10–15 ppm in poultry *(31)*. In the Indian medicinal system, the feather ash is used to treat recurring convulsions, bronchitis, and respiratory disorders *(5)*. It has been mentioned as an antidote for snake bites *(5)*. The action of copper through cytochrome oxidase (last reaction in the mitochondrial respiratory chain) in the respiratory process is well known. It also helps in the maturation and survival of red blood cells (RBC) in the circulation. Copper also can act as a substitute for iron in several biochemical reactions and, therefore, supplement through such medication may restore ionic balance in diseased and anemic conditions.

The concentrations of zinc, manganese, copper and iron are significantly higher in some of the medicinal samples analyzed. 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. The high concentration of mercury in Neem leaves warrants speciation studies as it is the most versatile source of traditional as well as modern drugs in India *(4)*. The elucidation of elemental speciation in these herbal drugs would help interpret the therapeutic actions and may help in designing chemically pure medications.

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