



Trace elements analysis in some medicinal plants using graphite furnace - Atomic absorption spectroscopy

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Abstract

Ten trace elements (Mg, Ca, V, Cr, Mn, Fe, Cu, Zn, Mo, and Cd) were determined in seven traditionally used wild medicinal plants of the Nagas in the North-East India viz *Cynoglossum furcatum* Wallich, *Elsholtzia blanda* Benth, *Lycopodium cernuum* Linnaeus, *Potentilla fulgens* Wallich ex Hooker, *Swertia macrosperma* C.B. Clarke, *Thalictrum foliolosum* DC and *Valeriana jatamansi* Jones. Plant samples were dried, weighed, digested and analyzed for their mineral distribution level ranging from trace to major elements by GF-AAS. All the medicinal herbs studied were found to contain the ten elements analyzed. The elemental concentration is given in mg/kg. Mg and Ca are present in high concentrations in both the roots and leaves of the plant samples. Among trace transition metals Fe had the highest concentration, followed by Mn, Cr, Cu, V, Zn, and Mo. Analysis showed that the toxic Cd element was at a lower concentration and is within the permissible limit of FAO/WHO, California standards and United States Pharmacopeia (USP) Limit for Nutritional Supplements.

Keywords: Environment and human health effects, Ethnomedicine, Trace elements, Wild medicinal plants,



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1. Introduction

Medicinal plants play an important role in traditional medicine and are widely consumed as home remedies. Recent study by the World Health Organization (WHO) has shown that about 80% of the world's population still relies on traditional medicine for their primary healthcare needs. Thus it can safely be presumed that a major part of traditional therapy involves the use of plant extracts or their active principles [1]. The past decade has seen a significant increase in the use of herbal medicine due to their minimal side effects, availability and acceptability to the majority of the populace especially in third world countries. Consumption of these plants contributes to the intake of minerals -essential and non essential by the people including infants and the elderly [2]. Many metabolic disorders resulting in human ailments have experimentally been shown to be managed by traditionally used medicinal plants. Among the factors attributing to the healing potential of these medicinal plants, are the trace elements present in them. The study of such elements with respect to traditional medicinal plants reveal that major and trace elements have significant roles in combating a variety of human ailments and diseases [3]. However, it is widely known that in higher concentration, trace elements in medicinal plants are responsible for their toxicity. Due to their potential impact on human health, the pharmacological properties of these medicinal plants must be studied.

The knowledge on concentrations of these trace elements is important for determining the effectiveness of the plants in treating various ailments so as to understand their pharmacological actions. It also helps to develop a stronger basis for appreciating the traditional knowledge on the therapeutic potential of these plants. In the present study, elemental analysis of some selected medicinal plants was carried out using Graphite Furnace-Atomic Absorption Spectroscopy (GF-AAS). Seven ethnomedicinal plants viz *Cynoglossum furcatum* Wallich, *Elsholtzia blanda* Bentham, *Lycopodium cernuum* Linnaeus, *Potentilla fulgens* Wallich ex Hooker, *Swertia macrosperma* C.B. Clarke, *Thalictrum foliolosum* DC and *Valeriana jatamansi* Jones used in the traditional medicine system of Nagas (Table 1) in the North East India were

analyzed for trace elements content. The ten medicinal plants were chosen for the present study because they are economically important, have high therapeutic values (Table 2) and are found in abundance in a clean and pollution free environment in the study area. The benefits of many medicinal plants are profitably harnessed across the foothills of the Himalayas, where they are successfully domesticated and traded in a large scale. However such initiatives are yet to be developed in most parts of North-East India. Most of the plants selected for the study were wild relatives of more popularly known medicinal plant varieties. The possibility of economic opportunities that these medicinal plants can provide if these plants are domesticated also calls for its popularization. Biologically important trace elements are selected for analysis since they are important for normal growth and development of the human body and further have more specific properties that improve the health of the human body in certain dosage and are highly toxic at a higher concentration. The analysis of the concentration of these trace elements in these medicinal plants thus plays a vital role so as to avoid endangering the users of toxic effects on overuse and to educate the users of its optimum usage.

Traditionally, these medicinal herbs are collected fresh from the wild, washed and either the decoction or infusion is taken for immediate use [4]. For use latter on, plant material is collected from the wild; air dried, powdered and are stored in air tight containers either one species alone or in combination with other herbs. The method of preparation of *Cynoglossum furcatum*, *Thalictrum foliolosum*, *Potentilla fulgens* and *Valeriana jatamansi* is the same. The outer covering of the roots are scraped off lightly, then crushed with mortar and pestle and boiled in water for ten minutes. The plant material is strained and the decoction is taken orally. The rest of the plant parts of *Lycopodium cernuum* and *Swertia macrosperma*, including roots are used medicinally in the same way. *Elsholtzia blanda* is used externally by crushing the leaves into a paste and applying on the skin due to its repellent properties to insects such as flies, mosquitoes and leeches. This simple treatment also cures sores, cuts, rashes and wounds. Boiling water is poured into a bowl of cleaned fresh leaves and allowed to cool down. The resulting infusion is taken orally for kidney

and urinary trouble and choleric diarrhoea. The information on the seven medicinal plants, including the traditional method of use and ailments cured is discussed briefly in Table 1. In Table 2, the pharmacological constituents and activities from studies conducted around the world is given. The dosage of these medicinal plants are taken as directed by the traditional healers only during a bout of illness and are not consumed on a daily basis.

2. Methods and experiments

2.1. Reagents and chemicals

All chemicals used were of Analytical Grade purchased from S.D. fine-Chem. Ltd. and Qualigens, Fischer Scientific, India. The ultra pure deionized water (Millipore S.A., France) was used for the preparation of standards, and modifier solutions. The stock standard solutions were purchased from Sigma Aldrich chemical company for calibration by preparing standard solutions.

2.2. Plant Sample collection

Based on the traditional medicine system widely used by the Nagas of North East India, seven of the relatively rare medicinal herbs were chosen for analysis of its trace elements content. Voucher specimens of the medicinal herbs were prepared and identified at the Botanical Survey of India, Eastern Regional Circle, Shillong, Meghalaya, India. The herbariums were deposited in the Department of Environmental Studies, North Eastern Hill University, Shillong, for future reference (see Table 1). Specimens of the plants selected for the study were collected from their wild habitat from Kohima district of Nagaland in March 2014 (Figure 1). They were washed, dried in the shade, powdered and then subjected for analysis.

2.3. Plant Sample digestion

For the digestion of plant samples, 0.5 g sample(s) of powdered roots and leaves of the plants under study were weighed into the Teflon PFA vessels and digested for 3 hours at 85°C with conc. HNO₃: HCl

(3:1) mixture. Then conc. HClO_4 (1.0 ml) was added to enhance the oxidation process in the digestion. The resulting solutions were filtered and diluted to 50.0 ml with distilled water. The blank solution was taken as the same procedure without addition of the sample [5].

2.4. Instrumentation

An Analytik jena AAS vario 6 Graphite furnace (Analytik Jena, Germany) spectrometer equipped with PC-controlled 6-piece lamp turret was used for all of the absorption measurements. Hollow cathode lamps were mounted as line radiator along with a deuterium hollow cathode lamp for compensation of the background absorption and argon gas supply. The hollow cathode lamps fitted for specific element that has to be analyzed with their respective wavelength and the slit width were adjusted accordingly. Signal measurements were done in peak area/peak height and calibration was in linear mode. The sample injection volume is $20\mu\text{L}$. The typical heating program of GF-AAS is drying (injection of the sample into the filter furnace), pyrolysis, atomization and cleansing. The details of the instrumental conditions are given in Table 3.

2.5 Data Analysis

Calculation of each heavy metal depends on the laboratory procedure and WinAAS® Version 4.2.0 software (Analytik Jena, Germany). Calibration curves were prepared using a linear curve. Data analysis was also performed using SPSS data editor 16.0 and Microsoft Office Excel 2007.

3. Results and Discussion

Analysis of the ten elements namely Mg, Ca, V, Cr, Mn, Fe, Cu, Zn, Mo, and Cd was performed in seven traditionally used wild medicinal plants of Nagaland. The distribution levels of the plant samples resulting from the analysis of the ten trace elements *viz.*, Mg, Ca, V, Cr, Mn, Fe, Cu, Zn, Mo, and Cd are given in Table 4. The concentration of the metals and elements analyzed were compared with the permissible limit/tolerable upper levels given by the FAO/WHO (Table 5). The toxic Cd metal is also found to be below

permissible levels of FAO/WHO, California standards and United States Pharmacopeia (USP) Limit for Nutritional Supplements.

All the leaves and roots of the plants analyzed contain all the ten heavy metals and trace elements studied with the exception of non detection of Cd in the roots of *Swertia macrosperma*. The distribution levels of the trace elements analyzed in the present study follows the order Fe > Mg > Mn > Ca > Cr > V > Cu > Zn > Mo > Cd (Figure 2). In no single plants analyzed, were all the metals and elements concentrated neither there was any observable trend of concentration of the metals and elements studied. Except for *Elsholtzia blanda* and *Lycopodium cernuum*, where only leaf samples were analysed, the concentrations of the analyzed trace elements were appreciably higher in leaf samples of *Swertia macrosperma*, *Potentilla fulgens* and *Thalictrum foliolosum* than root samples. However, *Cynoglossum furcatum* and *Valeriana jatamansi* exhibited higher concentrations of these trace elements in their roots. The highest distribution levels of trace elements found amongst the seven medicinal plants are in the following order: Mg, Cr & Cu in *Valeriana jatamansi*; Ca and V in *Cynoglossum furcatum*; Mn & Fe in *Swertia macrosperma*; Zn & Mo in *Potentilla fulgens* and Cd in *Elsholtzia blanda*. The concentration of Mg was similar in almost in all the plants analyzed while there were much variation in the concentrations of Cu and Cd. Variations in the metallic and elemental content among the plants maybe due to the differences in the plants' physical structures, responsible for their preferential absorbability and the property of the soil on which they grow. The soil properties inturn are directly related to climatic condition of the place in which the plants grow. The lesser concentration of the studied parameters as compared to other studies is attributed to the unpolluted environment from which the plant materials were collected.

3.1. Magnesium

Magnesium content in the all the 7 selected plant leaves range from is 4.57-4.97 mg/kg whereas in root it varies from 4.60-4.69 mg/kg. *Swertia macrosperma* with 4.97 mg/kg of Mg contained the highest amount of Mg in its leaves among the plants analyzed. Mg content of 4.68 mg/kg in *Potentilla fulgens* was

reported in the present study, a plant that is traditionally used in treating diabetes. Magnesium functions as a cofactor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes [6]. In addition, low serum and dietary Mg maybe related to the etiologies of cardiovascular problems, hypertension, diabetes, and atherosclerosis in humans [7].

3.2. Calcium

Calcium content in the plant leaves range from is 2.38-4.01 mg/kg whereas in root 2.87-3.29 mg/kg. The highest value of Ca was found in leaves of *Cynoglossum furcatum*. Analysis showed that leaves (4.01 ± 0.059 mg/kg) of this plant contained more Ca than its roots (3.29 ± 0.002 mg/kg). The root of *C. furcatum* is used in treatment of womb ailments and urinary problems in women, while the leaves are not used in traditional medicine system. Calcium is an essential nutrient that plays a vital role in neuromuscular function, many enzyme-mediated processes, blood clotting, metabolic processes as well as providing rigidity to the skeleton. Calcium fluxes are also important mediators of hormonal effects on target organs through several intracellular signalling pathways [8]. Where calcium intake is low, calcium supplement as part of the antenatal care is recommended for the prevention of preeclampsia (high blood pressure, sometimes with fluid retention and proteinuria) in pregnant women [9].

3.3. Vanadium

Vanadium content in the plant leaves range from is 0.598-1.40 mg/kg whereas in root 0.686-1.460 mg/kg. Roots of *Cynoglossum furcatum* contained the highest amount of vanadium at 1.46 mg/kg, followed closely by 1.40 mg/kg in leaves of *Thalictrum foliolosum*. *Potentilla fulgens* known to have anticancer properties in Naga traditional medicine was found to have a concentration of 1.169 mg/kg of vanadium. Vanadium is a potent inhibitor of many enzymes, lower plasma cholesterol levels, directly influence glucose metabolism *in vitro*, suggesting a role in its regulation and supposedly play a physiological role on levels of the endogenous antioxidant- glutathione indicating its importance with respect to toxic

interactions of chemicals [10]. Signs of excessive vanadium intake in humans include gastrointestinal disturbances [11]. Vanadium was identified in almost all the anti-cancer medicinal plants that are analyzed [12].

3.4. Chromium

Chromium content in the plant leaves range from is 0.67-3.07 mg/kg whereas in root 0.910-2.38 mg/kg. Leaf of *Valeriana jatamansi* was found to contain the highest amount of Chromium. Cr is an essential nutrient that potentiates insulin action and thus influences carbohydrate, lipid and protein metabolism [12]. However, the role of Chromium as cofactor for insulin action is not fully understood as studies from several *in vivo* and *in vitro* studies at the molecular level are ongoing [13].

3.5. Manganese

Manganese content in the plant leaves range from is 1.81-5.25 mg/kg whereas in root 1.79-4.31 mg/kg. The highest amount of Mn was reported in leaf of *Swertia macrosperma*. Mn is a ubiquitous trace element required for normal growth, development and cellular homeostasis [14]. Manganese can be correlated with therapeutic properties against diabetic and cardiovascular diseases [15]. In addition, it is reported to have a role in neurodegenerative diseases [16].

3.6. Iron

Iron content in the plant leaves range from is 7.146-16.34 mg/kg whereas in root 11.70-12.81 mg/kg, with *Swertia macrosperma* exhibiting the highest concentration of Fe in leaves. In roots, a concentration of 12.81 mg/kg was found to be highest in *Valeriana jatamansi*. Fe in human body has three main functions. It is a part of haemoglobin and is responsible for oxygen transport, maintains a healthy immune system and being a constituent of several enzymes, is responsible for energy production. It is also an active site for several enzymes. Fe deficiency is probably the most common nutritional deficiency in the world though it performs the most vital functions in the body. An estimate based on WHO criteria indicated that

around 600–700 million people worldwide have marked iron deficiency anaemia especially in developing countries. In developed countries, the prevalence of iron deficiency anaemia is between 2% and 8% [17].

3.7. Copper

Copper content in the plant leaves range from is 0.41-0.84 mg/kg whereas in root 0.50-0.94 mg/kg. Roots of *Valeriana jatamansi* contained the highest Cu content among the plants studied. Functional roles for Cu is one of the most important in human's health like Fe and are found in erythropoietin's, myelin formation, modulation of catecholamine metabolism and antioxidant protection, and in the regulation of immune functions, cholesterol and glucose metabolism. Genetic diseases are caused by the body's inability to utilize copper properly, if it is present in excess. For medicinal plants, the WHO limits have not yet been established for copper [18].

3.8. Zinc

Zinc content in the plant leaves ranged from is 0.32-0.51 mg/kg whereas in root 0.35-0.45 mg/kg. Leaves of *Potentilla fulgens* had the highest content of Zn among the plants analyzed. It is interesting to note that Zn content in leaf was more than the tap root (0.39 mg/kg), since the tap root is medicinally used but the leaves are usually discarded. Zn is an essential component of many enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. It stabilizes the molecular structure of cellular components and membranes and in this way contributes to the maintenance of cell and organ integrity. Furthermore, Zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression. Its involvement in such fundamental activities probably accounts for the essentiality of Zinc for all life forms [19]. It plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity. Zinc is an extremely important part of insulin and it is known to improve the sensitivity of insulin in the management of diabetes [20].

3.9. Molybdenum

Molybdenum content in the medicinal plant leaves range from is 0.072-0.419 mg/kg whereas in root 0.035-0.302 mg/kg. Leaves of *Potentilla fulgens* has the most Mo content, followed by *Lycopodium cernuum* (0.40 mg/kg). Molybdenum is an essential element in human nutrition necessary for processing amino acids. Ingestion of 10-15 (mg/day) of molybdenum, for prolonged periods of time, may lead to an increase of uric acid in the blood. Lower-limb osteoporosis may be associated with ingestion of cereals with high molybdenum content [21].

3.10. Cadmium

Cadmium was detected in the plant leaves ranging from 0.004-0.039 mg/kg and in root its content was 0.002-0.015 mg/kg. Leaves of *Cynoglossum furcatum* had the most Cd content. The level of Cd in the different plant species is lower than tolerable daily intake of heavy metals in ingested products as compared to California standards and United States Pharmacopeia (USP) Limit for Nutritional Supplements [22]. Once absorbed, Cd is efficiently retained in the human body and it accumulates throughout life. Cd is primarily toxic to the kidney and cause bone demineralization, either through direct bone damage or indirectly as a result of renal dysfunction [23]. Cadmium in food has inherent toxicity with a tendency to accumulate in the food chain and a particularly low removal rate through excretion [24].

3.11. Biological importance of Metals

There is increasing evidence that much of the inorganic material on the surface of the earth has undergone transformations during long-term contact with organisms and their metabolic products. Trace metals plays a very important biological role in the human systems especially in its catalytic and hormonal or regulatory functions. They act as metalloenzymes, hydrolases, nonenzymatic metalloproteins, low-molecular-weight natural products, coenzymes, vitamins, nucleic acids. Some examples include $\text{DNA}n-(\text{M}^+)n$, $\text{M} = \text{Na}, \text{K}$); hormones (e.g. thyroxine, triiodothyronine: I); antibiotics (e.g. ionophores: valinomycin/K) and biominerals (e.g. bones, teeth, shells, coral, pearls: Ca, Si, etc.) [25]. Evidence

abounds on the deficiency of these metals and elements in human beings that lead to various metabolic malfunctions or orientations. Morawiec (1991) reported that deficiency of Ca, Fe, Zn and Cu results in increased Pb toxicity through considerable enhancement of Pb absorption from intestinal tract, producing greater degree of anemia. Deficiency of Fe leads to an increased Cd absorption in addition to Pb. Also a decrease of metalloenzymes activity though the addition of Fe, Zn and Cu to the diet prevents Lead accumulation within the tissues [26]. Interference with the metabolism of other trace elements had been reported on long-term exposure to zinc in excess of requirements, with copper utilization especially sensitive to an excess of zinc. Also the utilization of copper may be impaired as a consequence of high intakes of molybdenum; manifestations are the induction of anaemia, cardiac hypertrophy, and achromotrichia (loss of hair pigment) arising from the development of defects in melanin synthesis in hair [11]. Molybdenum toxicity is directly related to the amount of copper stored in the body. If someone does not have an adequate amount of copper in their bodies that person could be at greater risk of molybdenum toxicity. In some instances it has been noted that ingesting an excessive amount of molybdenum could cause a copper deficiency making the person more susceptible to molybdenum toxicity. Molybdenum prevents plasma proteins from binding to copper, and it also increases the amount of copper that is excreted in urine. Ruminants that consume high amounts of molybdenum develop symptoms including diarrhea, stunted growth, anemia and achromotrichia. These symptoms can be alleviated by the administration of more copper into the system, both in dietary form and by injection [27]. It is also used to treat rare inherited metabolic diseases, such as Wilson's disease, which prohibits the body from eliminating copper. Iron supplements should be used cautiously as they may interfere with the absorption of other nutrients such as zinc and calcium. The health effects of toxic-essential metal interactions have very critical clinical effects at the cellular and molecular level in the organ system of the human body. For instance, Lead toxicity would lead to exchange of calcium, iron and zinc causing impairment of cognitive and behavioral effects in children. Toxic Cadmium interacts with the metabolism of three essential metals- calcium, zinc, and iron which is responsible in causing nephrotoxicity in humans [28].

4. Conclusion

Plants may be passive receptors of trace elements but they also exert control over uptake or rejection of some elements by appropriate physiological reactions [29]. One of the basic problems is regarding the quantities of accumulated metals in plant parts used as food or herbal components. Special attention should be given to the form of metals distributed within the plant tissues, because metal forms in plants seems to have a decisive role in metal transfer to other organisms. Our investigation revealed that heavy metals and trace elements present in the analyzed medicinal plants were within permissible limits of FAO & WHO. Generally most of the herbal plants available in Nagaland, India are safe for human consumption as far as trace metal levels are concerned as the level of pollution is almost nonexistent due to the absence of polluting industries. The analyzed medicinal plants can be considered as potential sources for providing a reasonable amount of the required elements in diet and establish its nutraceutical value for the traditional healers and herbal remedy users.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Figures

Figure 1: Medicinal plants analyzed for trace and heavy metals a) *Elsholtzia blanda* Bentham b) *Cynoglossum furcatum* Wallich ex Roxburgh c) *Lycopodium cernuum* Linnaeus d) *Potentilla fulgens* Wallich ex hooker e) *Swertia macrosperma* Wallich f) *Thalictrum foliolosum* DC & g) *Valeriana jatamansi* Jones.

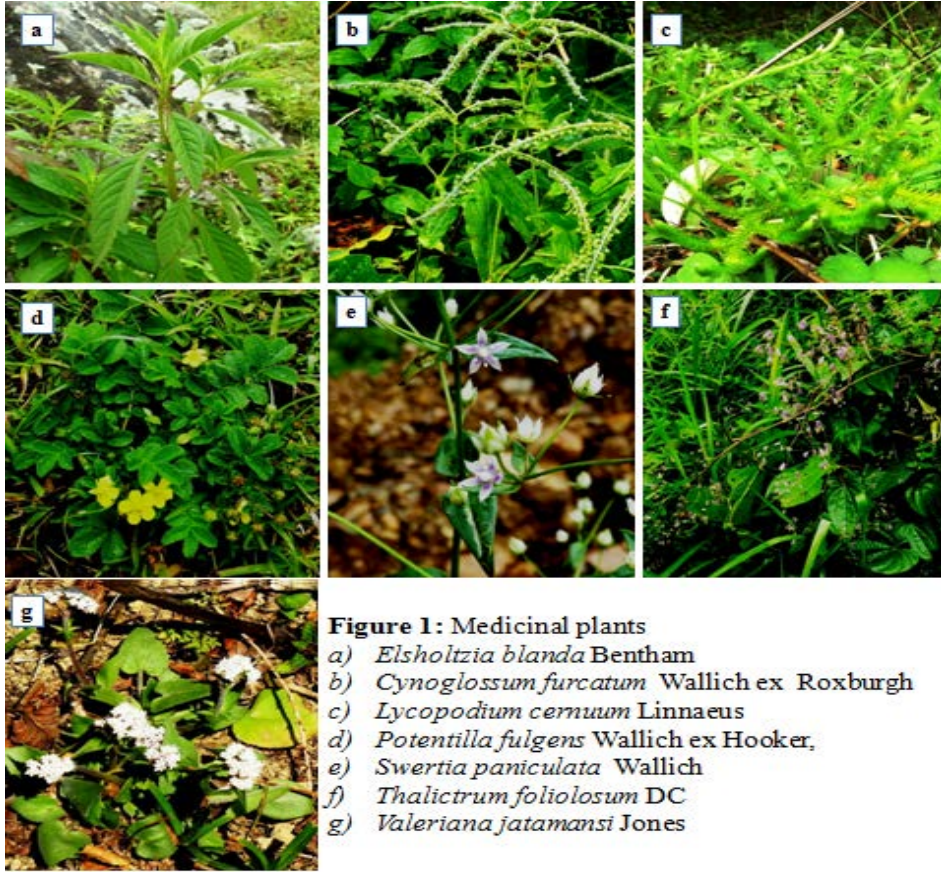
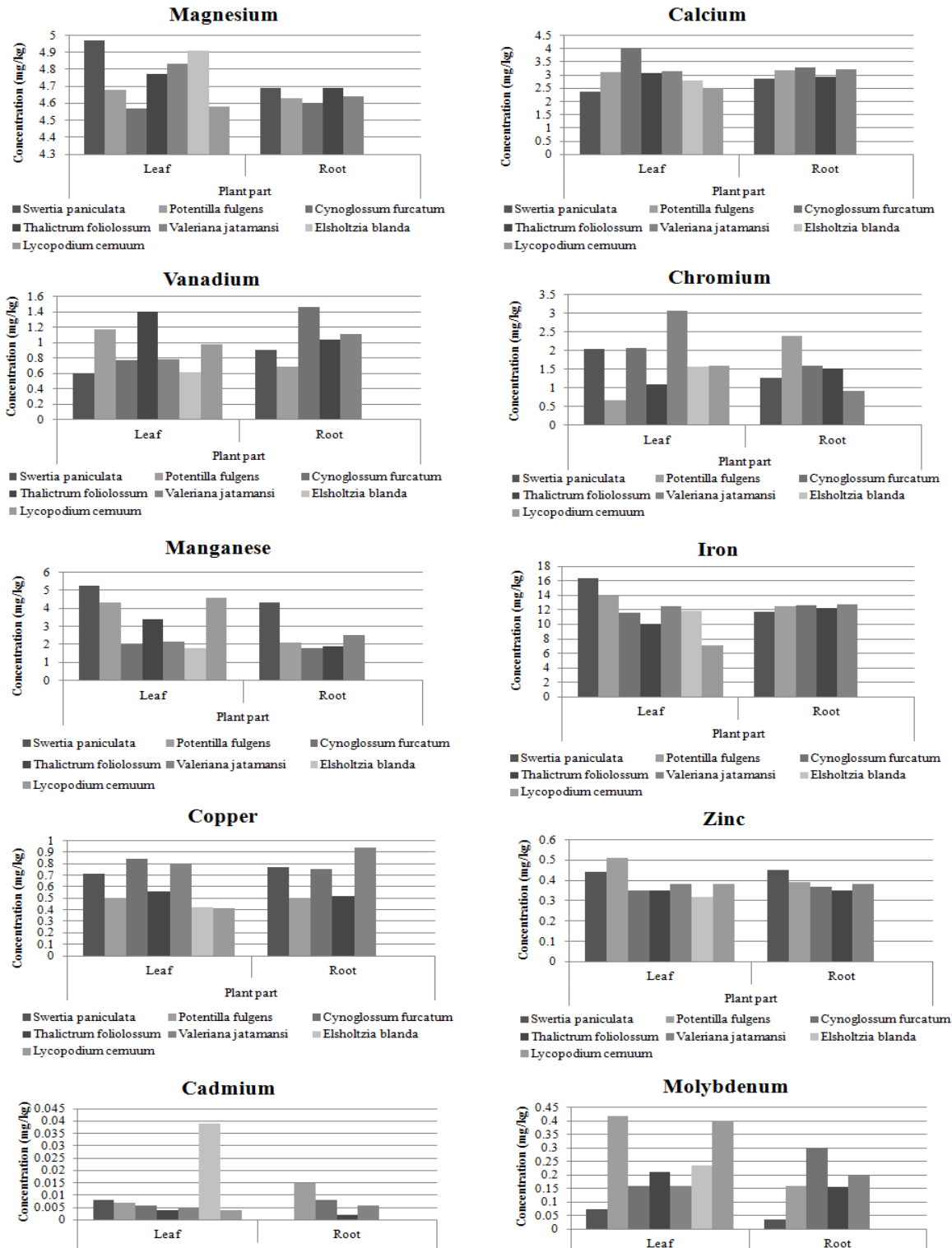


Figure 2: Distribution levels (mg/kg) of trace and heavy elements in leaves and roots of *Swertia macrosperma*, *Potentilla fulgens*, *Cynoglossum furcatum*, *Thalictrum foliolosum*, *Valeriana jatamansi*, *Elsholtzia blanda* & *Lycopodium cernuum*.



Tables

Table 1: Selected traditionally used medicinal herbs of the Nagas in North East India

Biological name [Voucher number]	Family	Vernacular name [Common name]	Part used	Traditional medicinal use
<i>Cynoglossum furcatum</i> Wallich ex Roxburgh [PCMPH30]	Boraginaceae	Thezumhiku [Ceylon Forget Me Not]	Roots	Womb and urinary problems
<i>Elsholtzia blanda</i> Benthham [PCMPH37]	Lamiaceae	Riinu [-]	Leaf	Cuts, wounds, sores, bee sting; skin diseases; insect repellent; headache; cold and fever; sinusitis; kidney and urinary trouble; choleric diarrhoea & dysentery
<i>Lycopodium cernuum</i> Linnaeus [PCMPH212]	Lycopodiaceae	Riipi [Arching club moss]	Whole plant	Spores are used as antiseptic for wounds and injuries; Other plant parts for rheumatism; cramps; varices; digestive disorders; constipation with flatulence; & urinary problems
<i>Potentilla fulgens</i> Wallich ex Hooker [PCMPH71]	Rosaceae	Kijiichiini [Silver weed]	Roots	Toothache; muscle spasms; stomachache; gastritis; diarrhoea; diabetes & cancer
<i>Swertia macrosperma</i> C.B.Clarke [PCMPH216]	Gentianaceae	Shushunokeciiu [Chiretta]	Whole plant	Fever; stomachache; digestive troubles; appetizer; gastritis; laxative; hypertension; tuberculosis; malaria; skin diseases & asthma
<i>Thalictrum foliolosum</i> DC [PCMPH96]	Ranunculaceae	Nhasiki [Meadow Rue]	Roots	Fever; malarial fever; high blood pressure; eye problems; stomachache; toothache; urinary problems; piles; indigestion; jaundice & diarrhoea
<i>Valeriana jatamansi</i> Jones [PCMPH104]	Valerianaceae	Valeriana [Indian valerian or Spikenard]	Roots	Liver function; urinary problems & indigestion

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Table 2: Pharmacological properties of the selected medicinal plants from other studies

Medicinal plants	Pharmacological properties	
	Constituents	Activity
<i>C. furcatum</i>	alkaloids - coromandaline, cynaustaline, heliotrine, echinatine, and isoechinatine	Antiseptic for cuts, wounds, boil, sores & swelling; fractured bone; ringworm; conjunctivitis; dissolve uterine tumours and draws out lymph fluids [30].
<i>E. blanda</i>	luteolin and its derivatives, flavinoides, flavones & tannins	Antibacterial [35]; antiviral [32, 34]; antioxidant [31] and Myocardial ischemia protection [33].
<i>L. cernuum</i>	alkaloids and serratene triterpenes	Antimicrobial [36] and Antibacterial activity [37].
<i>P. fulgens</i>	flavinoides, polyphenol, sterol, carotene, coumarin, tannin	Stomach disorders, certain forms of cancer, diabetes mellitus [38,39,40]; gastro-intestinal disorders [41]; high blood pressure [42].
<i>S. macrosperma</i>	Xanthones – Decussatin, swertiaperennin, swertianol, swertianin, etc.; Flavonoids-homo-orientin and Isoswertisin; terpenoids – Hederagenin, β -Amyrin & glycosides- mangiferin	Hypoglycaemic, anti-inflammatory, antimalarial, antihepatotoxic, anticarcinogenic, antioxidant and antimicrobial activities mainly due to the presence of xanthones [43]; As tonic and treatment of some mental disorders [44].
<i>T. foliolosum</i>	alkaloid	Diuretic, antiperiodic, purgative [45]; stomach pain, gastric trouble [46].
<i>V. jatamansi</i>	irridoid- veloptriates	Tranquilizer, nervous tension, insomnia, headache, antispasmodic, acute stomachache, menstrual cramps, carminative [47]; hysteria, nervous unrest, emotional troubles & as a sedative [48] curing Lewybody dementia [49] and Antioxidant activity [50].

Table 3: AAS Vario-6 Graphite Furnace Elements Instrumental Analytical Conditions

Elements	Wavelength (nm)	Slit width (nm)	Atomization temp(0°C)	Matrix Modifiers	Interference wavelength (nm)	Detection limit ($\mu\text{g L}^{-1}$)
Mg	285.2	0.8	1500-1650	-	-	0.0025
Ca	422.7	1.2	2350-2500	-	-	0.007
V	318.4	0.8	2550-2600	Mg(NO ₃) ₂	Ru 318.4	0.5
Cr	357.9	0.8	2100-2200	NH ₄ H ₂ PO ₄	Fe 358.1, Nb 358.0	0.1
Mn	279.5	0.2	1600-1650	Mg(NO ₃) ₂ + Pd(NO ₃) ₂	Mg 279.5, Fe279.5 Pb 280.2	0.014
Fe	248.3	0.2	1850-2050	Mg(NO ₃) ₂	-	0.100
Cu	324.8	0.8	1800-1900	--	Ni 324.3, Mn 324.9, Pd 324.3, Ag 324.8 Eu 324.8	0.19
Zn	213.9	0.8	1000-1100	Pd(NO ₃) ₂	Cu 213.9, Te 214.3 As 214.4, Fe 213.6 Fe 213.9	0.002

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Table 4: Distribution levels (mg/Kg \pm SD) of elemental contents in the medicinal plants (mean values \pm SD)

Medicinal plants	Part	Mg	Ca	V	Cr	Mn	Fe	Cu	Zn	Mo	Cd* $\mu\text{g/Kg}$
<i>Swertia macrosperma</i>	L	4.97 \pm 0.011	2.38 \pm 0 .023	0.598 \pm 0.018	2.03 \pm 0.361	5.25 \pm 0.076	16.34 \pm 0.250	0.71 \pm 0.241	0.44 \pm 0.017	0.072 \pm 0.003	0.008 \pm 0.002
	R	4.69 \pm 0.062	2.87 \pm 0 .012	0.902 \pm 0.013	1.26 \pm 0.016	4.31 \pm 0.016	11.70 \pm 0.895	0.77 \pm 0.229	0.45 \pm 0.047	0.035 \pm 0.004	ND
<i>Potentilla fulgens</i>	L	4.68 \pm 0.071	3.10 \pm 0 .019	1.169 \pm 0.033	0.67 \pm 0.076	4.32 \pm 0.009	13.95 \pm 0.005	0.50 \pm 0.041	0.51 \pm 0.002	0.419 \pm 0.015	0.007 \pm 0.001
	R	4.63 \pm 0.042	3.19 \pm 0 .044	0.686 \pm 0.026	2.38 \pm 0.039	2.12 \pm 0.016	12.53 \pm 0.014	0.50 \pm 0.035	0.39 \pm 0.014	0.158 \pm 0.001	0.015 \pm 0.004
<i>Cynoglossum furcatum</i>	L	4.57 \pm 0.066	4.01 \pm 0 .059	0.773 \pm 0.018	2.06 \pm 0.247	1.99 \pm 0.017	11.62 \pm 0.014	0.84 \pm 0.013	0.35 \pm 0.013	0.158 \pm 0.001	0.006 \pm 0.002
	R	4.60 \pm 0.105	3.29 \pm 0 .002	1.460 \pm 0.013	1.60 \pm 0.002	1.79 \pm 0.016	12.57 \pm 0.069	0.75 \pm 0.001	0.37 \pm 0.005	0.302 \pm 0.017	0.008 \pm 0.001
<i>Thalictrum foliolosum</i>	L	4.77 \pm 0.045	3.07 \pm 0 .011	1.400 \pm 0.039	1.10 \pm 0.029	3.37 \pm 0.125	10.02 \pm 0.013	0.56 \pm 0.050	0.35 \pm 0.001	0.211 \pm 0.003	0.004 \pm 0.001
	R	4.69 \pm 0.008	2.95 \pm 0 .034	1.033 \pm 0.033	1.51 \pm 0.313	1.88 \pm 0.018	12.19 \pm 0.016	0.52 \pm 0.008	0.35 \pm 0.012	0.156 \pm 0.001	0.002 \pm 0.001
<i>Valeriana jatamansi</i>	L	4.83 \pm 0.091	3.14 \pm 0 .419	0.786 \pm 0.026	3.07 \pm 0.304	2.13 \pm 0.140	12.50 \pm 0.021	0.80 \pm 0.004	0.38 \pm 0.005	0.161 \pm 0.005	0.005 \pm 0.001
	R	4.64 \pm 0.014	3.23 \pm 0 .012	1.106 \pm 0.023	0.91 \pm 0.059	2.49 \pm 0.016	12.81 \pm 0.010	0.94 \pm 0.165	0.38 \pm 0.007	0.201 \pm 0.043	0.006 \pm 0.004
<i>Elsholtzia blanda</i>	L	4.91 \pm 0.019	2.81 \pm 0 .007	0.617 \pm 0.012	1.57 \pm 0.052	1.81 \pm 0.013	11.80 \pm 0.140	0.42 \pm 0.012	0.32 \pm 0.015	0.234 \pm 0.037	0.039 \pm 0.002
<i>Lycopodium cernuum</i>	L	4.58 \pm 0.041	2.523 \pm 0.003	0.976 \pm 0.039	1.60 \pm 0.011	4.57 \pm 0.380	7.146 \pm 0.018	0.41 \pm 0.022	0.38 \pm 0.004	0.400 \pm 0.037	0.004 \pm 0.001

* Notes: i) Except for Cd, concentration is reported in $\mu\text{g/kg}$, ii) L- leaf; R- root

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Table 5: Deficiency symptoms of trace elements in humans and recommended daily intake (RNI)/tolerable upper intake levels (ULs) for adults only.

Element	Conspicuous deficiency symptoms	RNI/ULs, FAO/WHO.
Mg	poor muscle co-ordination and cramps, Nerve problems	220.0 mg/d
Ca	retarded skeletal growth, twitches	750.0-800.0mg/d
V	cardiovascular disease, metabolic dysfunction, hypoglycemia	ne
Cr	diabetic symptoms, hyperinsulinemia	25.0 µg/d
Mn	infertility, impaired skeletal growth	11.0 mg
Fe	anemia, disorders of the immune system	9.1mg/d*, 26.0mg/d**
Cu	artery weakness, liver disorders, secondary anemia	10.0 mg
Zn	skin damage, stunted growth, retarded sexual maturation	4.2-14.0 mg/d
Mo	retardation of cellular growth, propensity for caries, allergies	2.0 mg
Cd	-	7.0 µg/kg

*for male ** for female, ne= not established, mg= milligram, mg/d = milligram per day, µg/d=microgram per day.

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