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Determination of Macronutrients, Micronutrients and Heavy Metals Present in *Spilanthes acmella* Hutch and Dalz: Possible Health Effects

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Abstract – The study was conducted for quantitive determination of macronutrients, micronutrients and heavy metals present in *Spilanthes acmella* Hutch and Dalz, a traditionally used important medicinal plant. The results illustrated the presence of substantial amounts of essential nutrient elements in different parts of the plant. K and Mg were detected in the range between 36.35 ± 1.01 to 67.78 ± 1.33 g/kg and 2.17 ± 0.13 to 7.02 ± 0.84 g/kg of DWS respectively. While the essential micronutrients Fe, Na, Mn, Zn and Cu were detected in the range between 62.62 ± 12.72 to 856.95 ± 76.61 mg/kg, 365.47 ± 23.84 to 633.03 ± 38.12 mg/kg, 51.66 ± 7.77 to 186.33 ± 13.92 mg/kg, 41.87 ± 2.85 to 53.89 ± 3.79 mg/kg and 18.49 ± 2.07 to 48.71 ± 4.89 mg/kg of DWS respectively. Besides, heavy metals (Cd, Ni and Pb) detected in some of the plant samples were beyond the maximum permissible limit (MPL) of FAO/WHO for herbal medicines. The concentrations of the essential nutrient elements except zinc were significantly different in different parts of the plant ($p < 0.001$). Further variation in the concentrations of the elements in the plant was observed with respect to seasonal changes and geographical conditions. Nevertheless the findings corroborate ethnomedicinal significance of the plant and signify the possibilibity of utilizing its standardized products for preparation of food supplements as well as multidimensional therapeutic herbal medicines.

Keywords – Herbal medicine, Macronutrients, Micronutrients, Heavy metals, Oxidative stress, Health effects

Introduction

Herbal medicines constitute a major part of traditional therapeutic medication system around the world. Plants contain numerous pharmaceutically important bioactive compounds, many of which have found applications in the formulation of drugs for the treatment of numerous illness and diseases.[1] The remedial effects of medicinal plants are further enhanced by essential nutrient elements (NE) present. About 70-80% of the world population in both industrialized and developing countries are estimated to rely on herbal medicines and their derivatives for primary healthcare because of its effectiveness, economical and minimal side effects.[2] NE are divided into macronutrients and micronutrients based on requirements and relative amounts of the elements present. These elements play vital roles in various structural, physiological, biochemical and metabolical functions in living organisms. Plants use NE for the regulation of various metabolic processes including absorption, assimilation, photosynthesis, respiration, transpiration, translocation and storage of metabolites. Deficiency of these elements compromises nutritional value and productivity of the plants. Besides, these elements are involved in the regulation of metabolism, biosynthesis of metabolites and hormones, nerve transmission, skeletal activities etc in animals and human beings.[3] Earlier studies have also reported the involvement of NE in various defence mechanisms against biotic and abiotic stress conditions.[4] However, discrepancy between the need and availability of NE may produce harmful effects on both plants and animals. Billions of people around the world are malnourished and suffered from various diseases primarily due to deficiency of essential elements in foods.[5] Yet, excessive intake of NE may produce toxic effects and affects physiological and metabolic activities. Recently, studies have been extended towards understanding the etiology of diseases with respect to the status of NE present.

In parallel to the beneficial effects of herbal medicines, assessment of safety and toxicity of the plant products to

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the consumers is also equally important. Contamination of heavy metals (HM) in foods and herbal medicines has been a huge challenge due to its harmful effects. Increasing environmental pollution due to natural and anthropogenic activities are the main causes of HM accumulations in the ecosystem. The toxic HM such as Pb, Hg, As, Ni, Cd etc present in the air, water and soil enter into the biological systems either directly or through food chains. These toxic HM are not required in essential biological reactions. Moreover, they are not easily biodegradable, metabolized or excreted from the body. Excessive accumulation of the HM in plants and animals may result in oxidative stress conditions and increased production of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS/RNS further causes lipid peroxidation and damage intracellular organelles and other important biomolecules including nucleic acids and proteins.[6] This in turn affects cellular functions leading to various metabolic dysfunctions. Toxic HM accumulation in plants disrupts vital physiological functions including photosynthesis, respiration, transpiration, gaseous exchange, water retention, nutrient absorption etc.[7] Thus compromise their growth, productivity, quality and safety for use as food or herbal medicine. Excess accumulation of HM in human beings damages skeletal, neurological, cardiovascular, renal, pulmonary and hepatic systems. This may further cause various slowly progressive degenerative diseases such as Alzheimer's disease, Parkinson's disease, sclerosis, muscular dystrophy etc.[8] Hence it is imperative to ensure edible plants, herbal medicines and their products free from toxic HM contaminations for the safety of the consumers.

Spilanthes acmella is an important plant that has been using as a vegetative food or spice as well as ethnomedicine for the treatment of several ailments and diseases such as toothache, throat infections, stomatitis, fever etc primarily in developing countries. The plant has been reported to contain some of the important bioactive compounds such as spilanthol, *β*-caryophyllene, vanillic acid, scopoletin etc.[9] Pain reducing activities of *S. acmella* is attributed to the local anaesthetic effect of the alkylamides present. The plant has also been illustrated to possess high antioxidant, anti-inflammatory, antimicrobial, insecticidal, vasorelaxant, diuretic effects etc.[10,11] Raw and processed products of the plant are available in the market for use as food or herbal medicines in many parts of the world including India and Brazil. Considering its nutritional and multidimensional therapeutic uses, the study is being conducted for quantitative determination of macronutrients and micronutrients present in different parts of the plant thereby collecting the samples from different geographical locations. This might enable to enhance our knowledge on the nutritional and medicinal significance of the plant in addition to its traditional therapeutic understanding. Further determination of toxic HM contamination in the plant was also performed to substantiate its safety and possible detrimental effects on the consumers.

Experimental

Sample collection and preparations – The plant samples were collected during pre-monsoon (March-May)

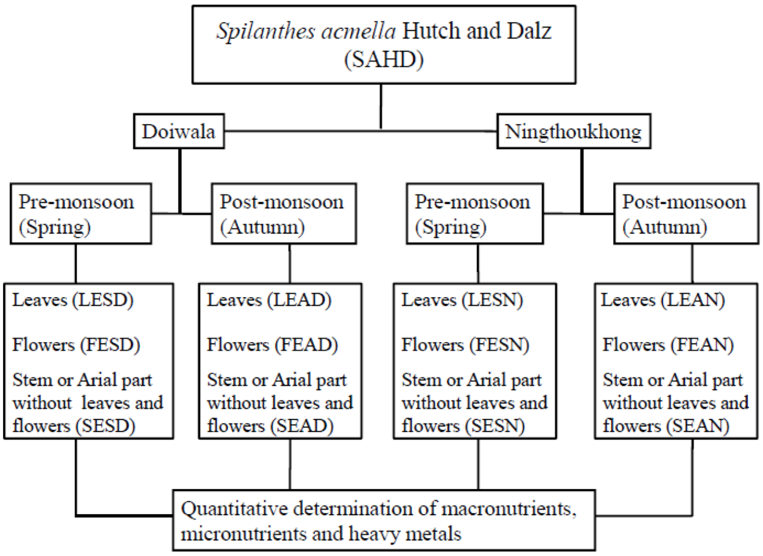

Fig. 1. Sample collection and segregation of different parts of *S. acmella* Hutch and Dalz for quantitative determination of macronutrients, micronutrients and heavy metals present.

and post-monsoon (October-December) periods from two regions of different climatic conditions- (a) Doiwala, Uttarakhand (Latitude: 30°09'23.1"N; Longitude: 078°07' 01.0"E) in the North-Western part and (b) Ningthoukhong, Manipur (Latitude: 24°34'31.2"N; Longitude: 093°46' 03.8"E) in the North-Eastern part of India. The study was conducted to examine NE and HM present in the plant with respect to seasonal variation and geographical locations. Therefore the plants were separated into- Leaves, Flowers and Stem or Arial parts without leaf and flower (Fig. 1), then washed and dried at 50 °C in a hot air oven. Later the samples were ground into fined powders and stored in airtight bottles for analysis. Systematic investigations of the samples were performed using reagents of analytical grades.

Microwave digestion of the samples – The plant samples were digested using a microwave digestor for quantitative analysis of NE and HM present. Digestion of plant samples using microwave digestion system is efficient and advantageous because it digests samples rapidly at a comparatively lower temperature in closed vessels thereby preventing cross-contamination and loss of volatile elements from the samples. The powdered samples (0.5 g) were digested with a mixture of nitric acid (HNO_3), hydrogen peroxide (H_2O_2) and Milli-Q Water (6:2:2) in 75 mL TFM vessels using microwave digester (MARS-Express) based on digestion program (Table 1). The digestion method adopted is simple, fast and reliable for quantitive determination of various elements present in plant samples. A blank was also prepared in the same way. The clear digested solutions of the samples were filtered using filter paper (Whatman no. 42) and made up to 50 ml with Milli-Q Water and stored at 4 °C for analysis. All the glassware, plastic ware and TFM vessels used in the procedure were soaked in 10% HNO_3 (v/v) solution overnight and rinsed multiple times with Milli-Q water before used.

Assessment of nutrient elements and heavy metals present in the samples – The concentrations of Mg, Fe, Mn, Zn, Cu, Ni, Cd and Pb present in different parts of the plant were determined by using Atomic Absorption Spectroscopy (AAS) (Thermoscientific-iCE3000) based on instrumental operational condition (Table 2). Further determination of the concentrations of K and Na was performed by using Flame photometer (Systronic-126) and N by using Kjeldahl apparatus (Pelican- Classic DX).

For determination of N, 1 g of each sample was digested with 10 ml of conc. sulphuric acid (H_2SO_4) in the presence of copper sulfate ($CuSO_4$) and potassium sulfate (K_2SO_4) (1:5) in Kjeldahl digestion unit at 350 °C for 30 min followed by 420 °C for 60 min. K_2SO_4 increases the boiling point of sulfuric acid and copper sulfate acts as a catalyst for increasing the speed and efficiency of the digestion procedure. After digestion, the clear sample solutions were cooled at room temperature and transferred to the auto distillation unit supplemented with 40% sodium hydroxide (NaOH) and 4% Boric acid (H_3BO_3). During the distillation process, NaOH converts ammonium

Table 1. Operatioanal conditions of microwave digestion system for digestion of the plant samples

Sl No	Target Temp (°C)	Ramp Time (min)	Hold Time (min)	Power (W)
1	150	10	10	250
2	50	10	10	0
3	200	10	20	600
4	0	30	30	0

Table 2. Operational conditions of Atomic Absorption Spectroscopy (AAS) for analysis of elements present in *S. acmella* Hutch and Dalz

Elements	Wave length (nm)	Lamp current (mA)	Slit width (nm)	Flame	Fuel flow (L/min)	Read Time (sec)	Washing Time (sec)
K	766.5	8	0.5	Air-C_2H_2	1	4	4
Mg	285.2	4	0.5	Air-C_2H_2	1	4	4
Fe	248.3	15	0.2	Air-C_2H_2	1	4	4
Mn	279.5	12	0.2	Air-C_2H_2	1	4	4
Zn	213.9	10	0.2	Air-C_2H_2	1	4	4
Cu	324.8	5	0.5	Air-C_2H_2	1	4	4
Pb	217	10	0.5	Air-C_2H_2	1	4	4
Cd	228.8	8	0.5	Air-C_2H_2	1	4	4
Ni	232	15	0.2	Air-C_2H_2	1	4	4

ions (NH_4^+) into ammonia (NH_3). The ammonia formed is captured by boric acid solution (absorbing solution) forming solvated ammonium ions. Methyl red was used as indicator. The distillates were then titrated with 0.1N HCl and N content in the samples were calculated using the formula-

$$\text{Nitrogen } \% = 1.401 \times (TV - B) \times N \times Df / \text{Weight of sample (g)}$$

where TV is titrant value, B is blank, N is normality of acid and Df is dilution factor. Calibration curves for the elements were constructed using a multi-element standard solution (Merck) and the regression coefficient (r^2) values ranged from 0.989 to 0.999. Concentrations of the elements were expressed in mg/kg or g/kg of dry weight of samples (DWS) and percentage (%).

Statistical Analysis – All the experiments were conducted in triplicate and the concentrations of the elements in the samples were expressed as mean ± standard deviation or percentage. Statistical analysis of the elements present in the samples was performed by using one way analysis of variance (ANOVA) and student t-test. A statistical probability (p value) of less than 0.05 was considered significant.

Results and discussion

In this study, a total of 11 elements including macronutrients (K, Mg and N), micronutrients (Zn, Cu, Fe, Mn and Na) and heavy metals (Cd, Ni and Pb) present in different parts of *S. acmella* Hutch and Dalz were analyzed. The concentrations of all the NE except Zn present in different parts of the plant were significantly different ($p < 0.001$).

Potassium content in the plant samples was in the range between 36.35 ± 1.01 to 67.78 ± 1.33 g/kg of DWS. The stems of the plant contained utmost K primarily during pre-monsoon period ($p < 0.01$). While K content in the leaves was significantly higher during post-monsoon period ($p < 0.05$). The highest concentration of K was observed in SESN and the lowest was observed in FEAD. Significant variation in the concentration of the element in different parts of the plant with respect to seasonal changes and geographical locations was observed (Fig. 2(a)). K contents in different parts of the plant collected from Ningthoukhong were significantly higher than those collected from Doiwala during the respective seasons ($p < 0.05$). K is abundantly found in plant cells and involved in many biological functions including protein and starch synthesis. Plants absorb K as K^+ from the soil. It moves across the plasma membrane through K ion channels and helps to maintain membrane potential. This facilitates turgor generation, electron transport, translocation of photosynthates and phytohormones. Deficiency of K decreases ribulose bisphosphate carboxylase (RuBisCO) enzyme content that is essential for CO_2 fixation during photosynthesis. Besides, K plays vital roles in the regulation of various other cellular and physiological processes including enzyme reactions, osmotic potential of cells, stomatal movement, gaseous exchange etc.[12] Some studies have reported an increased in the oil content of plants with the application of potassium humate.[13]

Magnesium (Mg) content in the plant samples was in the range between 2.17 ± 0.13 to 7.02 ± 0.84 g/kg of DWS. Leaves of the plant contained Mg the highest ($p < 0.001$) and the proportion was significantly higher during pre-monsoon period ($p < 0.05$) (Fig. 2(b)). The highest Mg content was observed in LESD and the lowest in FEAN. Mg constitutes the central atom in the porphyrin structure of chlorophyll molecule and takes part in photosynthesis. Mg-protein complexes facilitate the liberation of oxygen molecules through the splitting of water during photosynthesis. It also takes part in many enzymatic reactions being a cofactor of numerous enzymes such as adenosine triphosphatases (ATPases), superoxide dismutase (SOD), catalase (CAT), Peroxidase (POD), decarboxylases, dehydrogenases etc.[14] Moreover, Mg acts as bridging ion for association, configuration and stabilization of ribosome subunits during protein synthesis.[15] Deficiency of Mg may cause chlorosis and oxidative stress effects in plants thereby increasing the production of reactive oxygen species (ROS). Proper availability of the element has also been illustrated to alleviate oxidative stress effects in plants caused by HM. In animals and human beings, Mg is involved in many biochemical and physiological functions of the body including synthesis of DNA, RNA and protein, cardio-vascular activities, glycolysis and energy production, insulin secretion, neuro-muscular activities, cellular homeostasis etc. Its deficiency may cause several physiological complications and diseases including cardiovascular diseases, hypertension, muscle spasms, diabetes, hypocalcemia, hypokalemia, osteoporosis, atherosclerosis, cerebral infarction etc.[16,17]

Further, comparatively higher N content was observed in the leaves and flowers of the plant collected during pre-monsoon period (Fig. 2(c)). The flowers collected during pre-monsoon period from Ningthoukhong (FESN) possessed the highest N (2.82 ± 0.21%) and the stems collected during post-monsoon period from Ningthoukhong (SEAN) contained the lowest N (0.51 ± 0.16%). Plants

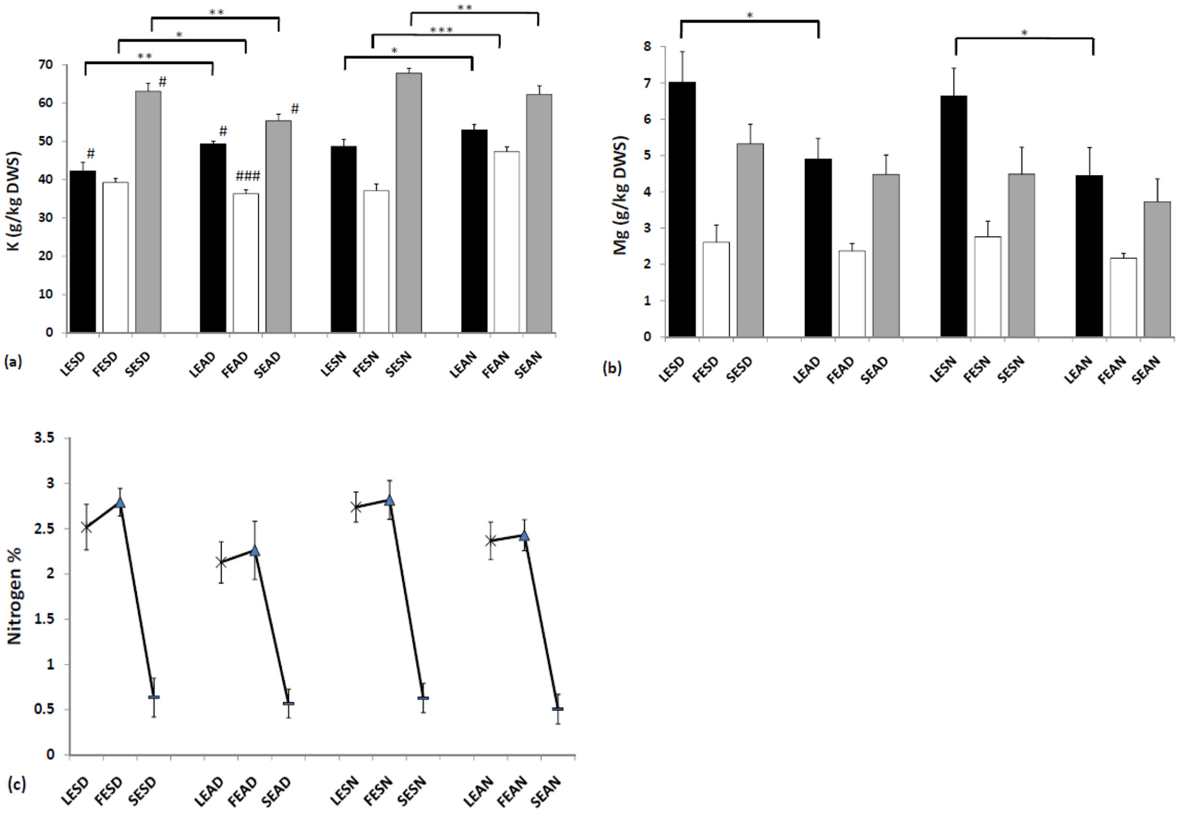

Fig. 2. Determination of concentrations of (a) K (b) Mg and (c) N present in different parts of *S. acmella* Hutch and Dalz. Asterisks (*) indicate level of significant difference of the elements present in the plant samples with respect to seasonal variation (*$p < 0.05$; **$p < 0.01$ and ***$p < 0.001$); Hash marks(#) indicate level of significant difference of the elements present in the same parts of the plant collected from the two regions (#$p < 0.05$; ##$p < 0.01$ and ###$p < 0.001$).

absorb N either from the soil through roots in the form of ammonium (NH_4^+) or nitrate (NO_3^-) or from the atmosphere through nitrogen fixation. N is a crucial element required for the structural organizations and functional aspects of most cellular mechanisms in all living organisms. It is a constituent of proteins (including enzymes), coenzymes, nucleic acids- Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) etc. that are vital components for cellular activities and cell survival. Besides, it is involved in the regulation of metabolic processes including translocation of NE, cytokinins and metabolites further influencing the growth and productivity of the plants.[18]

Significant variation in the concentration Fe in different parts of the plant was also observed with respect to seasonal variation and geographical locations. The highest concentration of Fe was recorded in the leaves and the lowest in the stems of the plant ($p < 0.001$). Fe content in different parts of the plant collected during pre-monsoon was significantly higher than those collected during post-monsoon period (Fig. 3(a)). Further, the plants collected from Ningthoukhong possessed comparatively higher Fe during the respective seasons. The highest Fe content was observed in LESN (856.95+76.61 mg/kg of DWS) and the lowest in SEAD (62.62 ± 12.72 mg/kg of DWS). Fe can exist in different oxidation states Fe^{2+} or $Fe^{3+}$and form complexes with haemoglobin, myoglobin, cytochromes, ferredoxin and many other enzymes (lipoxygenase, peroxidases, SOD, aconitase etc). Thus it is involved in several biological reactions such as biosynthesis of chlorophyll, electron transport chain (ETC) of photosynthesis and respiration etc.[19] Pande et al. (2011) reported increased in biomass content, oil yield and chlorophyll content in *Mentha spicata* with an optimal supply of iron.[20] In animals, Fe is required for various biological processes including biosynthesis of hormones, immunity, hemopoiesis, growth and development.[21] WHO have reported Fe deficiency to be the reason for more than half of the total anaemia cases worldwide.[22]

Sodium (Na) content in different parts of the plant was significantly different and the leaves contained the highest ($p < 0.001$). It was found distributed in the ranged between 365.47 ± 23.84 to 633.03 ± 38.12 mg/kg of DWS. The

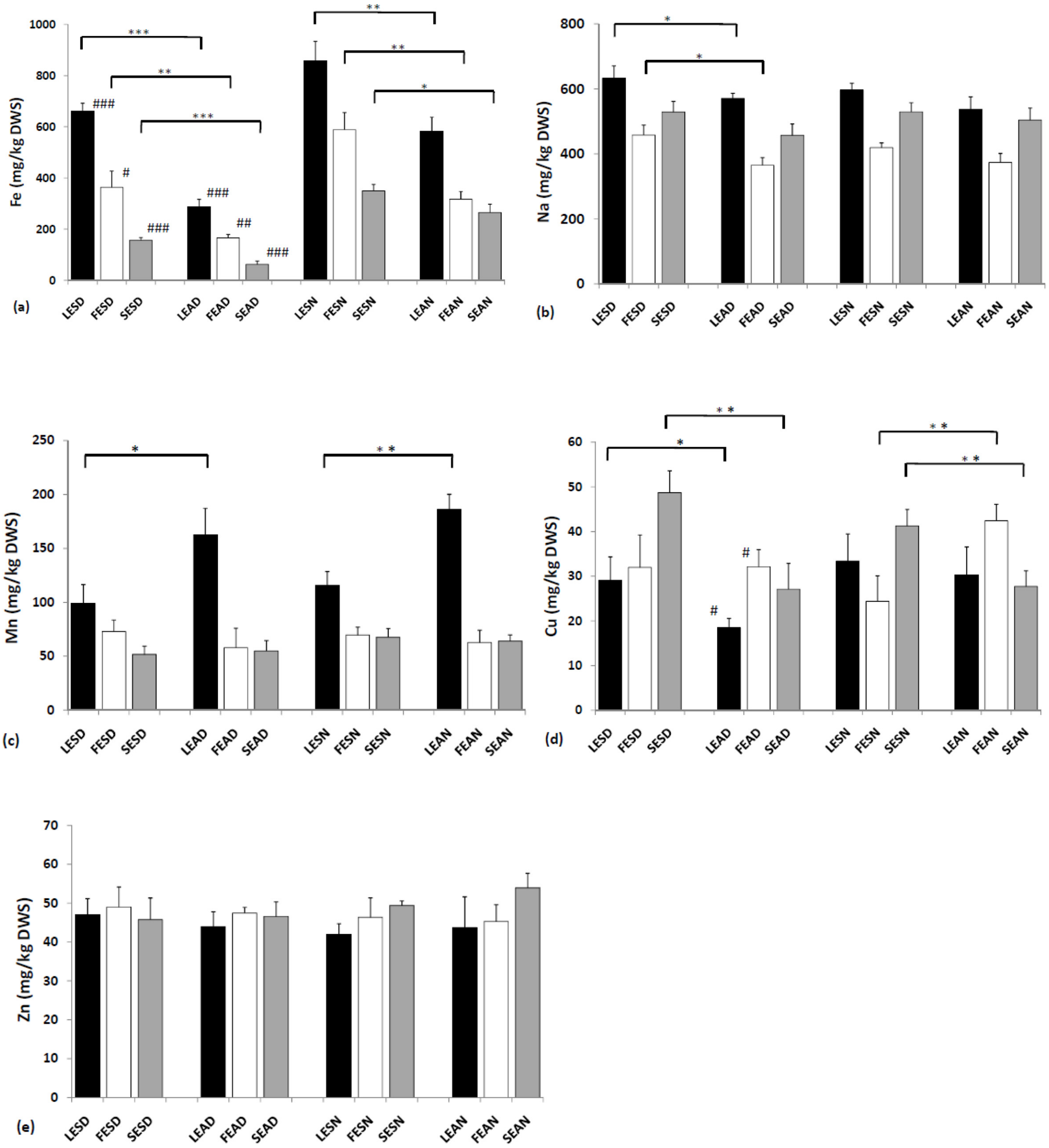

Fig. 3. Determination of concentrations of (a) Fe (b) Na (c) Mn (d) Cu and (e) Zn present in different parts of *S. acmella* Hutch and Dalz. Asterisks (*) indicate level of significant difference of the elements present in the plant samples with respect to seasonal variation (*$p < 0.05$; **$p < 0.01$ and ***$p < 0.001$); Hash marks(#) indicate level of significant difference of the elements present in the same parts of the plant collected from the two regions (#$p < 0.05$; ##$p < 0.01$ and ###$p < 0.001$).

leaves and flowers of the plant collected from Doiwala contained Na significantly higher during pre-monsoon period ($p < 0.05$) (Fig. 3(b)). The highest concentration of Na was observed in LESD and the lowest in FEAD. Sodium is also required for the synthesis of chlorophyll and proper growth of plants. Deficiency of Na compromises chlorophyll content, photosynthetic rate and biomass contents. Na enhances nitrate uptake and assimilation thereby increasing the activities of nitrate reductase enzyme. It can substitute K in non-specific functions including osmoregulatory processes and long-distance transport of nutrients.[23] Besides, electrolyte balance and fluid contents in animals are determined by Na levels in the body. Na helps in maintaining membrane potential and its depolarization processes regulate nervous and muscular functions. Control of Na level is equally important concerning genesis and treatment of hypertension.[24]

Mn content in the samples was in the range between

51.66 ± 7.77 to 186.33 ± 13.92 mg/kg of DWS and leaves of the plant contained significantly higher Mn ($p < 0.001$). The highest Mn content was observed in LEAN and the lowest in SESD. The leaves of the plant collected during post-monsoon period consisted of significantly higher Mn than those collected during pre-monsoon period ($p < 0.05$) (Fig. 3(c)). Manganese has been reported to be involved in the regulation of several important biological functions including enzymatic activities, hormonal functions, ROS scavenging, cellular energy production, immune system, formation of tissues, growth and development etc.[25] Deficiency of Mn may alter metabolism, impair blood glucose level or affect blood clotting factors.[3]

Cu content in the plant samples was in the range between 18.49 ± 2.07 to 48.71 ± 4.89 mg/kg of DWS. Leaves and stems of the plant collected during pre-monsoon period from Doiwala contained significantly higher Cu than those collected during post-monsoon period (Fig. 3(d)). While flowers of the plant collected from Ningthoukhong consisted of Cu significantly higher during post-monsoon period ($p < 0.01$). The highest Cu content was observed in SESD and the lowest in LEAD. Besides, leaves and flowers of the plant collected during post monsoon period from Ningthoukhong contained significantly higher Cu than those collected during the same season from Doiwala ($p < 0.05$). Copper in reduced (Cu^+) and oxidized (Cu^{2+}) forms act as a catalyst in many enzymatic reactions involved in various metabolic processes including RuBP carboxylation, CO_2 assimilation and ATP synthesis during photosynthesis, peroxidation, antioxidation, electron transport chain (ETC) in mitochondria etc. Besides, Cu is required for hematologic and neurologic functions, blood clotting, melanin production, development of bone and connective tissues in animals.[26] Its deficiency may lead to osteoporosis, nerve damage, anaemia, cardiac hypertrophy, achromotrichia etc.

Zn was observed almost uniformly distributed in the range between 41.87 ± 2.85 to 53.89 ± 3.79 mg/kg of DWS in different parts of the plant (Fig. 3(e)). No significant change in the concentration of the element was detected under the influence of seasonal variation and geographical differences. Zinc acts as a cofactor and activates many enzymes including superoxide dismutase (SOD), RNA polymerase, carbonic anhydrase, enolase, lyases, ligases, alcohol dehydrogenase etc. which are involved in various biological processes.[23] Thus, it constitutes an important component in many metabolic and physiological functions of plants (carbon assimilation, terpene biosynthesis and activation of antioxidant enzymes) and animals (immunity, muscular activity, liver and thyroid function, insulin function and genetic expression). Deficiency of Zn may lead to impairment of the immune system, memory loss, thymic atrophy and growth retardation in human beings.[27]

Uptake of NE and HM from the soil, their distribution and storage in plants are governed by various biotic and abiotic factors including plant species, the microbial community in the soil, temperature, pH, moisture content, aeration, organic and inorganic matter contents etc.[28] Excessive accumulation of toxic HM in both plants and animals results in various oxidative stress-related effects such as interruption to electron transport chains (ETC) in mitochondria and chloroplast, inhibition of antioxidant enzymes, overproduction of reactive oxygen species (ROS) and its harmful consequences. In response, plants produce secondary metabolites such as proline, ascorbic acid, glutathione, Camptothecin, Quercetin, Catechins, Catharanthine Morphine and other polyamines that act as osmolytes or plant growth regulators and help to neutralize free radicals, counteract oxidative stress effects, maintain intracellular homeostasis and improve plant growth.[29] Moreover, HM mediated genotoxic effects may lead to mutations and carcinogenesis through chromosomal aberrations, DNA modifications, DNA cross-linkage and DNA strand breaks.[30]

The concentrations of three toxic HM (Cd, Ni and Pb) present in the plant samples were determined (Table 3). Cadmium was detected in the leaves and stems of the plants collected from both the regions in the range between 0.14 ± 0.03 to 0.46 ± 0.08 mg/kg of DWS. The highest concentration of Cd detected in SEAN was above the maximum permissible limit (MPL) of 0.30 mg/kg of FAO/WHO for herbal medicines.[31] Cd is toxic to both plants and animals. It hinders nutrient uptake and photosynthetic rate in plants and also disturbs enzymatic activities involved in the biosynthesis of metabolites.[7] Excessive Cd exposure may cause several harmful effects on human beings such as organelle damage, immune impairment, osteoporosis etc.[32] Besides, Cd has been classified as a potential carcinogen as it can induce carcinogenesis in various organs through DNA hypermethylation and inhibition of expression tumour suppressor genes (*RASSF1A* and *p16*).[33]

Similarly, the presence of Ni was detected in the range between 0.29 ± 0.08 to 2.85 ± 0.73 mg/kg of DWS. The highest concentration of Ni was observed in SESN and the lowest in SESD. However, Pb was detected only in the leaves and stems of the plants collected from Doiwala in the range between 1.09 ± 0.26 to 2.06 ± 0.48 mg/kg. The concentrations of Pb in the samples were below the

Table 3. Heavy metal contents (mg/kg of DWS) in different parts of *S. acmella* Hutch and Dalz collected during pre-monsoon and post-monsoon periods from Doiwala and Ningthoukhong. ND denotes not detected

	LESD	FESD	SESD	LEAD	FEAD	SEAD	LESN	FESN	SESN	LEAN	FEAN	SEAN
Cd	ND	ND	0.19 ± 0.02	0.14 ± 0.03	ND	0.15 ± 0.04	ND	ND	0.25 ± 0.08	0.19 ± 0.04	ND	0.46 ± 0.08
Ni	ND	1.24 ± 0.40	0.29 ± 0.08	ND	ND	ND	1.97 ± 0.41	ND	2.85 ± 0.73	ND	ND	ND
Pb	1.12 ± 0.05	ND	2.06 ± 0.48	ND	ND	1.09 ± 0.26	ND	ND	ND	ND	ND	ND

prescribed MPL (10 mg/kg) of FAO/WHO.[31] Even though Ni is required for activation of urease enzyme during urea metabolism in plants, excessive accumulation of the element adversely affects nutrient absorption, metabolic processes, seed germination, growth and yield. Acute Ni exposure may lead to oxidative stress conditions and induce mutagenic and carcinogenic effects via hydroxylation and deglycosylation of DNA, inhibiting DNA repair mechanisms, intrastrand DNA cross-links, double strand breaks etc.[34] Besides, the replacement of Mg^{2+} in the phosphate backbone of DNA by Ni^{2+} may result in structural modification of chromatin or chromatin condensation leading to silencing of vital genes including tumour suppressor genes.[35] Ni has also been reported to induce hypoxia inducible factor-1α (HIF-1α) that can promote carcinogenesis via activation of anti-apoptotic (Bcl2) gene.[36] Earlier studies have reported a close association between the consistent inhalation of Ni compounds and the development of lung cancer.[37]

Moreover, environmental contamination of Pb has also been increasing as a result of incessant anthropogenic activities such as industrial effluents, cosmetic products, gasoline, paints, batteries etc. Excessive accumulation of Pb in plants may cause stunted growth, chlorosis, inhibition of enzymatic activities and seed germination, hormonal imbalance, alteration of membrane structure and permeability, damage ETC etc.[7] Pb can also act as a pseudo-element and substitutes other essential elements such as Ca^{2+}, Mg^{2+}, Na^{+} and Fe^{2+} during metabolic processes. Further, excessive exposure of Pb to human beings adversely affects biological processes including neuro-muscular activities, hematopoietic system, Cardiovascular functions and results in mental retardation, psychosis, autism, dyslexia, weight loss, paralysis or even death.[38] Contaminations of toxic HM in many other herbal medicinal products have also been reported earlier.[39,40]

The results illustrated that *S. acmella* Hutch and Dalz contains substantial amounts of essential macronutrients and micronutrients that play vital roles in the regulation and maintenance of biological functions. The presence of essential NE abundantly along with other potential bioactive compounds in the plant substantiates the significance of its use as food item as well as multidimensional therapeutic medicines. This hints the possibility of application of the plant products in the preparation of food supplements or therapeutic items for prevention or treatment of other related infirmity or diseases. Moreover, varying concentrations of the elements in different parts of the plant under the influence of seasonal variations and geographical conditions were also observed. This could be due to the influence of different environmental conditions on the plant metabolism and growth. *S. acmella* flowers and grows copiously during pre-monsoon period and the growth declines during post-monsoon period with the advent of winter season. The presence of higher concentration of Mg in the leaves primarily during pre-monsoon period may be because it constitutes a primary component of chlorophyll molecules. Comparatively higher proportion of N present in the flowers and leaves illustrates possibility of higher protein contents. Variation in the concentration of other elements in different parts of the plant collected from the two regions may be related to different soil conditions or soil fertility, efficiency of mineral uptake and different climatic conditions. Similarly accumulation of different concentrations of the elements in plants collected during different seasons may be due to differences in their growth and maturity levels, the influence of other environmental conditions including temperature, pH, moisture, humidity etc. However, the presence of toxic HM such as Pb, Ni and Cd above MPL in some of the samples suggests possible hazardous effects of the plant products on the consumers thereby adversely affecting the physiological, biochemical and cellular functions. Hence it is imperative to screen and standardize the quality of the plant and its products prior to use as food as well as medicines.

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