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Short Communication

Detection and Measurement of Zinc Concentrations in Selected Indigenous Medicinal Plants of Pakistan

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ABSTRACT

Importance of trace elements has been documented over many years in human health and disease cure. A number of pharmaceutical formulations are commercially available containing combinations of different trace elements. The importance of herbal medicines in the health care system of the larger section of the world's population, the developing countries, is also an undeniable fact. In this study Twenty indigenous medicinal plants belonging to eight families, which are used in the traditional system of medicine (Unani) practiced in Pakistan, were investigated and analyzed by using Atomic Absorption Spectrophotometery as a potential natural sources of zinc. Calculated on the basis of gm dry plant sample, zinc concentrations ranged between 0.0005 mg/gm to 0.0317 mg/gm. Maximum concentrations of zinc were present in the washed and unwashed seeds of *Argeria speciosa* of family Convolvulaceae (0.0317mg/gm & 0.0310mg/gm), whereas minimum concentration of zinc was present in *Butea Monosperma* of family Leguminoseae (0.0057mg/gm).

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Knowledge about the healing properties of plants dates back to many centuries. People from all over the world have been applying poultices and imbibing infusions of hundreds, if not thousands, of indigenous plants dating back to prehistory. The use of medicinal herbs is increasing not only in Asia but also in other parts of the world because of their low side effects. However a substantial number of the medicinal herbs are yet to be evaluated for their efficacy, safety and quality. Some of the modern medications are actually originated from the plant kingdom, examples are those of aspirin from white willow bark, digitalis from foxglove, morphine from poppies, warfarin (Coumadin) from sweet clover, and taxol from the yew tree.

A large number of elements are now known to be present in the human body in minute quantities. Nutrition in the form of food and fluids mainly supply the intake of these trace elements to the human body. There are a number of situations in which deficiency or excess of specific elements are a primary cause of disease and this has brought with it the recognition of a new medical speciality termed as "Medical Elementology". These elements, in one form or the other, play an important role in the field of medicine for combating disease and serve as a curative or preventive agent.

Epidemiological studies over the past decades have documented the importance of trace elements in human health and disease. Zinc is an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. It is a mineral that is found naturally in sulfur compounds. As a trace element, it plays a significant role in boosting human metabolism. As a homeopathic remedy, it is considered a power source for the nervous system. The homeopathic preparation is used to treat anxiety and the headaches that accompany it, as well as mental and physical weakness and exhaustion. Zinc plays a central role in the immune system, affecting a number of aspects of cellular and Humoral immunity. A normal human body contains 2–4 grams of zinc (Rink, L and Gabriel P, 2000) which is mostly present in the brain, muscle, bones, kidney, and liver, with the highest concentrations in the prostate and parts of the eye (Wapnir and Raul A, 1990). Semen is particularly rich in zinc, which is a key factor in prostate gland function and reproductive organ growth (Berdanier *et al*, 2007.

Zinc deficiency usually occurs due to insufficient dietary intake, but can be associated with malabsorption, chronic liver disease, chronic renal disease, sickle cell disease, diabetes, malignancy, and other chronic illnesses (Prasad A.S, 2003). Clinical picture of zinc deficiency includes depressed growth, diarrhoea, impotence and delayed sexual maturation, alopecia, eye and skin lesions, impaired appetite, altered cognition, impaired host defense properties, defects in carbohydrate utilization, and reproductive teratogenesis. In case of mild deficiencies immune system is depressed (Ibs and Rink, 2003).

The Recommended Dietary Allowance (RDA) for zinc is 8 mg/day for women and 11 mg/day for men (Institute of Medicine, Food and Nutrition Board, 2001). Keeping in view the importance of zinc, the present study was conducted for the detection and measurement of its levels in twenty selected indigenous medicinal plants of Pakistan

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Table 1: Family wise detail of selected indigenous medicinal plants along with their zinc contents (mg/gm)

Sr. No.	Name of Plant	Family	Parts used	Weight on dry basis (mg)	Weight of ash (mg)	Zinc Concentrations (mg/g) ±SD
1	Argyeria speciosa	Convolvulaceae	Unwashed seeds	5006.5	683.5	0.0310±0
2	Argyeria speciosa	Convolvulaceae	Washed seeds	5005.8	698.4	0.0317±0.0005
3	Butea Monosperma	Leguminoseae	Gum /Resin	5003.1	181.5	0.0057±0.0009
4	Cassia Fistula	Leguminoseae	Fruit pulp	5000.8	228.4	0.0197±0.0009
5	Glycyrrhiza glabra	Leguminoseae	Root	5017.3	327.3	0.0100±0
6	Tamarindus indica	Leguminoseae	Fruit pulp	5007.3	224.1	0.0103±0.0005
7	Moringa oleifera	Moringaceae	Old roots	3004.1	226	0.0277±0.0047
8	Moringa oleifera	Moringaceae	Young roots	2508.9	76.9	0.0025±0.0002
9	Moringa oleifera	Moringaceae	Flowers	1947	167.5	0.0233±0.0017
10	Carum bulbocastanum	Umbelliferae	Fruit	4713.8	287.2	0.0317±0.0005
11	Carum Copticum	Umbelliferae	Fruit	5001.2	321.6	0.0280±0
12	Coriandrum Sativum	Umbelliferae	Seeds	5018.9	308.3	0.0250±0
13	Cuminum cyminum	Umbelliferae	Fruit	5031.2	301.3	0.0200±0
14	Foeniculum vulgare	Umbelliferae	Seeds	5011.8	398.9	0.0247±0.0005
15	Ferula foetida	Umbelliferae	Resin	5004.4	1479.1	0.0080±0.0008
16	Peucedanum graveolens	Umbelliferae	Seeds	5003	315.4	0.0310±0
17	Citrullus colocynphis	Cucurbitaceae	Seeds	5011.4	204.3	0.0190±0
18	Citrullus colocynphis	Cucurbitaceae	whole Fruit	5002.9	658.5	0.0183±0.0024
19	Citrullus colocynphis	Cucurbitaceae	Fruit Skin	4336.1	479.5	0.0113±0
20	Cordia latifolia	Boraginaceae	Fruit	4843.4	475.5	0.0177±0.0009
21	Onosma bracteatum	Boraginaceae	Leaves	4091.2	993	0.0217±0.0034
22	Onosma bracteatum	Boraginaceae	Flowers	4507.3	468.5	0.0215±0.0011
23	Onosma echioides	Boraginaceae	Roots	4845	555.1	0.0073±0.0005
24	Gossypium herbaceum	Malvaceae	Seeds kernels	5009.3	225.9	0.0257±0.0005
25	Hibiscus rosa/ sinensis	Malvaceae	Flower	4007	239.9	0.0270±0
26	Melia azadirachta	Meliaceae	Leaves	3005.2	223.4	0.0260±0
27	Melia azadirach	Meliaceae	Fruit	5023.5	230.1	0.0130±0.0007

belonging to eight families. These are commonly used in the indigenous system of medicine.

The plants were selected on the basis of their wide use in the Unani system of medicine prevailing in Pakistan. The selected medicinal plants were purchased from the reputed crude drug dealers of the country. The authenticity of plant materials was ascertained by various renowned drug dealers and practitioners of the Unani system of medicine. Their identity was confirmed by comparing the specimens with the herbarium samples at the National Agriculture Research Council (NARC), Islamabad, Pakistan and from the taxonomist of Pakistan Council of Scientific and Industrial Research (PCSIR), Lahore, Pakistan.

The selected plant material was freed from the twigs and extraneous matter. The soil, grit, sand and dirt were removed by sifting through a stainless steel sieve. To remove remnants of adhering foreign matter the samples were rapidly and thoroughly washed with distilled water and then immediately dried in a hot air oven. The dried plant samples were pulverized using a porcelain pestle and mortar, packed and sealed in polyethylene bags and stored in plastic bottles with tightly fitting lids. Plants of mucilaginous character were cleaned by initial rubbing with a clean cloth to loosen the adhering contamination, which was sifted out, and then samples were quickly rinsed with distilled water before drying.

For determination of zinc contents, the samples of plant materials, in triplicate, were subjected to the dry ashing procedure described in AOAC, 1984, official methods of analysis. Accurately weighed quantities of dried, pulverized plant material were taken in tared porcelain crucibles which were pre-cleaned by igniting in a furance, then soaked overnight in concentrated nitric acid, washed with glass distilled water, dried in an oven at 130 ^{0}C and finally cooled in a desiccator. The samples were incinerated initially on a low flame and then in a covered muffle furnace with temperature maintained at 5000 ^{0}C for 5–6 hours. To completely oxidize the mass and free it from carbon, the cooled crucible contents were moistened with few drops of water and 2 – 3 ml of concentrated nitric acid were added with caution. The crucibles were heated in a fume cupboard at 110 ^{0}C on a hot plate to evaporate excess of nitric acid and then returned to the furnace to complete the ashing. These were then cooled in a desiccator and weighed. Known quantities of ash were digested with 5ml of hydrochloric acid (6N) by heating gently on a hot plate. On cooling the solution was diluted with 5ml distilled water and filtered into a 50ml volumetric flask, previously washed thoroughly with nitric acid, tap water and then with distilled water. The excess portion of filter paper was cut off to fit into a small glass funnel before starting the filtration. Finally, solution was eluted with a small portion of distilled water and volume of solution was made up to 50ml with constant shaking. The solutions prepared by this technique were transferred to pre-cleaned tightly stoppered polyethylene bottles till final analysis.

Flame atomic absorption spectrophotometry was used as the analytical method for the detection and measurement of zinc in the pretreated plant sample solutions (Hussain et al., 2006). The absorption readings were taken in Flame Atomic

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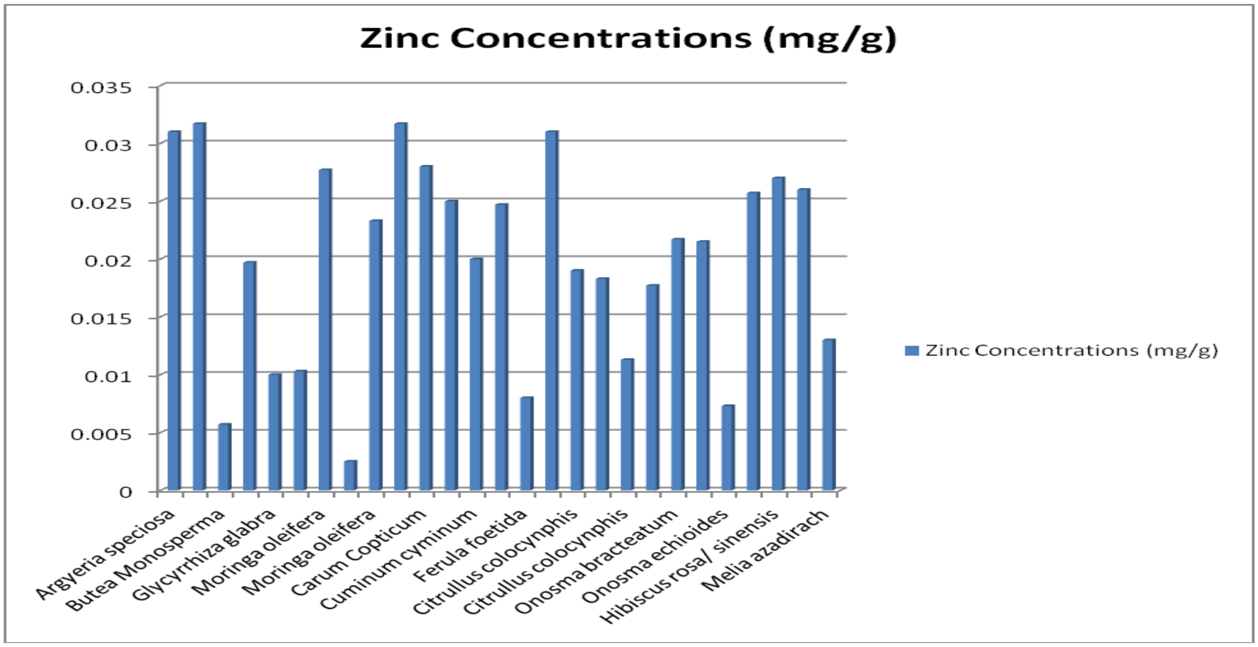

Figure 1: Plant wise distribution of zinc levels (mg/gm) in selected medicinal plants of Pakistan

Absorption Spectrophotometer (Polarized Zeeman Hitachi-2000) at 213.8 nm wavelength and 10mA lamp current using 1.3 nm slits. The standard curve was drawn using the know reference solution of zinc. The sample stock solutions were diluted further to obtain appropriate concentrations that fell within the range of analytical concentrations of the standard solution of zinc. The zinc levels investigated in the selected medicinal plants were quantified in mg/gm of the plant materials on dry weight basis.

From this study level of zinc (mg /gm) in twenty plants of eight families, indigenous to Pakistan was determined by using atomic absorption spectrophotometry. Zinc was found in all the plant species evaluated and was generally relatively high (0.0025 – 0.0317 mg/gm). Three highest concentrations were found in *Argyeria speciosa*, *Carum bulbocastanum* and *Carum copticum*, respectively, whereas young roots of *Moringa oleifera* had the lowest (0.0025 mg/g).

Recommended Dietary Allowance (RDA) of zinc is 2 – 11 mg per day. Zinc is a main component of many metallo-enzymes, especially those enzymes which play a key role in nucleic acid metabolism. It is also a membrane stabilizer and a stimulator of the immune response (Das KK, 2002). The estimated safe and adequate daily intake of zinc is between 10,000 and 20,000 µg/day (NRC Recommended Dietary Allowances, 1980).

Zinc plays a vital role in functioning of immune system, enzyme triggering, semen production, DNA and protein synthesis. Its deficiency can result in growth retardation, increased rate of infections due to impaired immune functioning, impotence, diarrhoea, skin and eye lesions, and, impaired appetite. Its deficiency is 5th major risk factor causing diarrhoea and pneumonia in children resulting in high mortality rates especially in underdeveloped countries. It was concluded from the present study that the selected indigenous medicinal plants commonly used in the Alternative System of Medicine (Unani) had fair concentrations of zinc. The zinc contents were more in family Convolvulaceae followed by Umbelliferae, Malvaceae, Boraginaceae, Meliaceae, Moringaceae, Cucurbitaceae and Leguminoseae respectively. *Argyreia speciosa* washed seeds and *Carum bulbocastanum* fruit had the highest (0.0317mg/g) whereas *Moringa oleifera* (young roots) had the lowest (0.0025mg/g) zinc contents. Glycyrrhiza glabra roots are being used extensively in Unani system of medicine for example Sharbat Aijaz and Syrup Laooq Sapistan contain glycerrhiza root indicated for viral flu and cough. It was observed from our study that Glycyrrhiza glabra roots contained appreciable quantity of zinc (as shown in figure 1). Glycerrhiza is reported to have immunomodulatory potential in combination with zinc (Mazumder PM et al., 2012). Coriandrum Sativum is used in many Unani formulations for its aphrodiasic potential, which could be due zinc present in it (as shown in figure 1).

These plants could be of significant therapeutic importance in various ailments due to high zinc contents naturally present in them in appreciable quantities. Moreover, these medicinal plants could serve as a natural and cost-effective source of zinc instead of using some costly multi-mineral allopathic formulations.

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