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Trace elements analysis of *Biophytum sensitivum* (L.) DC. by EDXRF spectroscopy and its therapeutic role in diabetes mellitus

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Minerals and trace elements, as structural components of tissues, play vital role in cellular and basal metabolism. The Little Tree Plant, *Biophytum sensitivum* (L.) DC., locally called Lajalu, is a well-known medicinal herb, traditionally considered as one of the 10 flowers used since ancient time for healthy life. In Ayurveda, it is used to treat various ailments *viz*., asthma, insomnia, convulsions, cramps, stomach ache, chest complaints, inflammation, tumours and skin disorders. In this study, we analyzed the elemental contents of its leaves and roots using EDXRF spectroscopy, and tried to find possible correlation with its nutritional and curative effects. The elements Ca, K, Si, Cl, P, and S were found in the highest concentration in leaf, in the descending order, but in the root, K took the leading position. The results have been discussed with reference to the established role of these elements in physiology and pathology of human life with special focus on its role in bone building, controlling the sugar level and wound healing capacity of patients with diabetes mellitus.

Keywords: Ayurvedic, Lajalu, Little tree plant, Osteoporosis

The role of metabolic functions of trace elements in the human body, and exploring the medicinal plants as potential resource of such essential minerals, has been an interesting area of research since decades[1]. The human body needs certain minerals, such as Ca, Mg, K and Na at high levels, and are called macro-minerals and minerals which are required in much smaller levels are called micro-minerals, or more commonly trace minerals. Trace minerals are usually found at extraordinarily small levels in our bodies, in the range of parts per million. In spite of the relatively low concentrations of these minerals, they play an increasingly vital role in human health.

Either the deficiency or excess of trace elements (TE) in the human body causes a number of health disorders. Deficiencies of certain trace minerals, such as Cu and Mn, for example, have been connected to lower bone density and weaker bones[2]. It is well known that Calcium (Ca) is essential for bone health. Studies show that supplementing with Ca and trace minerals together increases bone density in postmenopausal women more than Ca alone. Effect of TEs on wound healing has also been well recognized[3].

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Also, toxic elements are harmful even at low concentrations. Hence, reliable analyses are required to define the toxicity for effective treatment to the human body. Many studies reported the presence of trace elements content of biological, chemical and geological materials including plants using different techniques[4-11].

TEs, such as Zn, Cr, Cu, Mn, Se, Fe, I, Co (only as a component of Vit. B_{12}), and Mo have been identified to be essential for humans, *i.e.*, they must be provided to the patient, parenterally or enterally. Generally, TE can be classified into three major groups such as; (i) Cationic elements (Zn, Fe, Mn and Cu which are absorbed from the gut with variable efficiency and whose homeostatic control is mediated by the liver and gastrointestinal (GI) tract); (ii) Anionic elements (Cr, Se, I and Mo which are absorbed efficiently by the gut and excreted mainly in the kidneys), and (iii) TE that exists as organic complexes which affect their metabolism, *e.g.*, selenoamino acids, Cr in glucose tolerance factor, Fe and Co in cobalamin. TEs are involved in cellular biochemistry, and the deficiencies of these can cause metabolic alterations which may lead to increased morbidity and mortality. However, in clinical practice, various conditions have been identified with specific TE deficiencies.

Biophytum sensitivum (L.) (Family: Oxalidaceae), is a well-known plant for its phytochemical and pharmaceutical characters[12-19]. The elemental analysis of plant parts, in general, gives a fundamental relation between herbal medicine and its role in curing illness effectively. The Ayurvedic system is a recognized Indian traditional medicinal practice for long healthy living. Actually, diseases are to be considered as symptoms of mineral imbalance in the body. Once it is balanced, the diseases get cured. Hence the elemental analysis of plants used in *Ayurveda* or herbal medicine in general is having much importance. Also, *B. sensitivum* is treated as one among the *dasapushpangal* (Ten Flowers) such as, Cheroola (*Aerva lanata* (L.), Mukkutty (*Biophytum sensitivum* (L.) DC), Uzhinja (*Cardiospermum halicacabum* L.), Nilappana (*Curculigo orchioides Gaertn.)*, Karuka (*Cynodon dactylon* (L.) *Pers.*), Kayyonni (*Eclipta alba* (L.) *Mant*), Muyalchevi (*Emilia sonchifolia* (L.) DC), Vishnukranthi (*Evolvulus alsinoides* (L.) *L. var. alsinoides*), Thiruthali (*Ipomea sepiaria Koen. ex Roxb.*), Puvamkurunnela (*Vernonia cinerea* (L.) Less), according to the Kerala traditional practice. It is said that worshiping these ten flowers will provide a long healthy life. All these plants are used in one or other way as home remedy till today.

In *Ayurveda* system, *B. sensitivum* is used for the treatment of stomach ache, asthma, treating insomnia, convulsions, cramps, chest complaints, inflammations, tumours, and remedying chronic skin diseases[15]. Commonly, the whole plant decoction is used for asthma and phthisis and the decoction of the root is used for gonorrhoea and lithiasis[19]. Specifically, the leaves are diuretic and relieve strangury and are used for Diabetes mellitus (*Madhumeha*), particularly in Eastern Nepal[20]. The powdered leaves and seeds were used to apply to wounds. The whole plant is used to counteract the snake venom activity both in *Sidha* and *Ayurveda* practice in India[21]. The photograph of the plant is given in (Fig. 1) for identification.

This paper concentrates on the curative effect of the herb *Biophytum sensitivum* used in *Keraleeya Ayurveda Chikitsa*, for treatment of Diabetes mellitus and the associated diseases. We analyzed the elemental constitution of *B. sensitivum* leaves and roots for its possible role in this activity.

Materials and Methods

The EDXRF technique was used to identify the elemental constitution of the leaves and roots of *B. sensitivum*. Compared to other elemental analysis techniques, such as Instrumental Neutron Activation Analysis (INAA)[7], X-Ray Fluorescence Analysis (XRF)[3], Atomic Absorption Spectrometry (AAS)[3,4], Particle-Induced X-ray Emission (PIXE)[5], and Inductively Coupled Plasma Spectrometry-Atomic Emission Spectroscopy (ICP-AES)[22], the Energy Dispersive X-ray Fluorescence (EDXRF)[9] technique has many advantages that it is multi-elemental, non-destructive and has better sensitivity and precision. Further, EDXRF is rapid and inexpensive method with simple sample preparation.

Fig. 1 — Aerial view of *Biophytum sensitivum* (L.) DC

Sufficient quantity of the plant *Biophytum sensitivum* (L.), was collected from Mahe region of the Union Territory of Puducherry, located in North Kerala (latitude: 11.3867 & longitude: 75.7358) during the month of August, since this seasonal plant grows only after the rainy season. The samples were washed well with distilled water and dried in shadow. The leaves and roots were separated from the dry plants. The samples were ground well-using mortar and pestle. 100 mg of the sample powder was pelletized into a thin pellet of uniform thickness having 10 mm in diameter under a pressure of 130 kg/cm^2 for 3 min using KBr press. The pellets of the samples were analyzed by the Jordan Valley Ex-3600 Energy-dispersive X-ray fluorescence (EDXRF) spectrometer, at UGC-DAE Consortium for Scientific Research, Kolkata Center, which works with an oil-cooled system Rh anode X-ray tube having maximum voltage 50 kV and a current of 1 mA. The measurements were carried out in a vacuum chamber with appropriate filters that were inserted in between the sample and source to find the optimum detection

of elements in the sample. For lower Z elements (Z< 19), no filter was used and the anode voltage was kept at 8 kV and current at 85 mA. A 0.05 mm thick Ti filter was used for elements whose Z = 20 - 30 with anode voltage 20 kV and current 400 mA. Again for higher Z elements, a Fe filter of 0.05 mm thickness was used at an anode voltage 35 kV and current 500 mA. The characteristic X-rays emitted from the sample were detected by the 12.5 mm^2 Si (Li) detector cooled at liquid nitrogen temperature (77 K). Four pellets were prepared for each sample and the elemental concentration is obtained through averaging.

Results and Discussion

The elemental constitution of *B. sensitivum* obtained from the EDXRF analysis is tabulated in (Table 1). In the leaf of *B. sensitivum*, the presence of Ca and K is very high, *i.e.*, around 20 mg/g, and Si is around 8.5 mg/g. The elements Fe, Cl, P, Al, and S content is found to be in the mg range, while Mg, Ti, Sr, and Mn are between 10-100 μg range. The content of Br is only 6 μg/g. The trace elements Co, Ni, Se, and Pb are not found in the leaf or less than 1 ppm level. In the root, K is available plenty compared to Ca, *i.e.*, in the ratio ≈ 2:1 (K=29 mg/g and Ca=13.5 mg/g). The next abundant element to K and Ca is Cl (≈4.7 mg/g), which is little higher than Si (≈4 mg/g). S, Al, P and Mg are in the mg level while Ti, Mn, Sr, and Zn are in the μg range (≈450 to 100 μg/g). The logarithmic concentration of the averaged value of leaf and root is plotted in (Fig. 2), against the atomic no., of the elements.

Glucose tolerance

All three major types of diabetes, such as, type 1, type 2, and gestational, have the problem of not make out or use of insulin, and thus reduction of glucose level is hardly possible. Oral intake of Chromium picolinate (a chemical compound that contains chromium) can lower the fasting blood sugar, lower insulin levels, and help insulin to work better for type-2 diabetes. Higher Chromium doses might be more effective and work more quickly. Higher doses might also lower the level of certain blood fats (cholesterol and triglycerides) in some people. However, researchers are looking carefully at the results about chromium which may be effective for treating diabetes. Some suspects that Chromium

Table 1 — Elemental composition of *Biophytum sensitivum* leaf and root: the values given in bracket are standard deviation

Element	At. No.	Concentration (ppm)			
		Leaf	% of STD	Root	% of STD
Mg	12	924.37(±42.13)	4.5577	1495.465(±200.50)	13.4072
Al	13	3397.6275(±229.70)	6.7606	3174.695(±393.07)	12.38135
Si	14	8621.3625(±429.41)	4.98077	6682.4025(±394.69)	5.90641
P	15	3552.1775(±84.34)	2.37432	2247.09(±181.49)	8.07667
S	16	2269.2725(±58.38)	2.57263	3201.0475(±302.46)	9.44878
Cl	17	3790.74(±153.39)	4.04644	4742.475(±481.90)	10.16136
K	19	20824.2025(±900.36)	4.32362	29241.075(±2391.32)	8.17795
Ca	20	22150.8775(±386.12)	1.74314	13512.895(±584.10)	4.32254
Ti	22	533.5725(±25.90)	4.85407	430.6575(±25.06)	5.81901
V	23	17(±1.62)	9.52941	15.34(±0.62)	4.04172
Cr	24	25.8275(±9.22)	35.69838	25.6075(±11.05)	43.15142
Mn	25	137.14(±3.22)	2.34797	243.495(±11.94)	4.90359
Fe	26	4448.4325(±79.32)	1.7831	4104.36(±201.14)	4.90064
Co	27	0	--	0	--
Ni	28	0	--	0	--
Cu	29	28.3825(±1.88)	6.6238	17.6975(±0.84)	4.74643
Zn	30	80.6525(±2.72)	3.37249	108.3675(±0.88)	0.81205
As	33	14.55(±3.46)	23.78007	8.96(±2.50)	27.90179
Se	34	0	--	0	--
Br	35	6.26(±0.30)	4.79233	6.76(±0.27)	3.99408
Rb	37	67.5725(±1.32)	1.95346	120.63(±6.68)	5.53759
Sr	38	184.1725(±4.05)	2.19903	163.285(±10.47)	6.4121
Pb	82	0	--	0	--

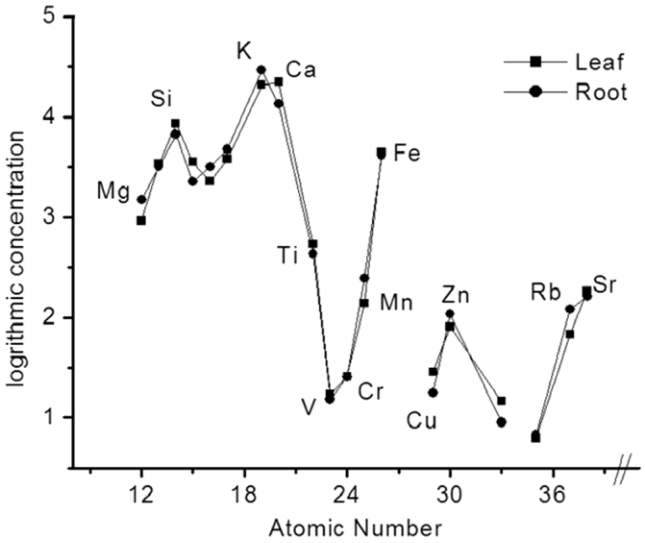

Fig. 2 — Logarithmic concentration of elements in the leaf and root *of Biophytum sensitivum* (L.) DC

supplements might primarily benefit patients with poor nutrition or low Chromium levels. Chromium levels can be below normal in patients with diabetes. Insulin is also used to decrease blood sugar. Taking chromium along with insulin might cause the blood sugar to drop too low. Chromium can make it hard for the body to use Iron. This could lead to iron deficiency and so an iron supplement is prescribed along with Cr content drug. But this is unlikely to happen when people take chromium supplements at the usual doses. Chromium is likely safe during pregnancy and breast-feeding when using orally equal to or less than "adequate intake" (AI) levels. The studied plant contains ≈25 μg/g of Cr in both its leaf and root and hence it is strongly evidenced that this plant may have a potential curative effect on diabetics.

Bharathi and Sahu[18] reviewed that *B. sensitivum* shows hypoglycemic activity. In their dose- response study, they reported that 16.1% fall in fasting plasma glucose (FPG) level for 16 h fasted non-diabetic rabbits at the end of 1 h and 2 h, the hypoglycemic effect persisted at the end of 6 h (13.8% fall), but in diabetic rabbits (subdiabetic and mild diabetic) the fall in glucose value is 25.9% & 27.4% and 36.9% & 37.7% after 1 and 2.5 h of administration. Also, they reported that there is a rise in serum insulin levels in the treated animals which suggests a pancreatic mode of action (*i.e.* insulinotropic effect) of *B. sensitivum*. This is in good correlation with its elemental composition, *i.e.*, the role of the presence of Cr in this plant is well revealed.

As a good antioxidant and essential for the functioning of the central nervous system, Mn (B.S. leaf: 0.137 mg/g & root: 0.243 mg/g) is known to facilitate insulin production[23]. The presence of Zn, Cr, Rb, Cu, and V in μg level (10-100 μg/g) also ensures that this plant can have a good effect on the diabetic patients since the said elements have a critical role in insulin production[24,25]. Also, it facilitates a good immune system (Zn), good brain health (Rb)[26], good cardiac function (Cu)[27] and good glucose tolerance (Cr and V)[24].

Bone Building

The Ca content of this plant (leaf: 22.15 mg/g & root: 13.51 mg/g) (Table 1) pronounces its effectiveness in strengthening of bone. The presence of Mg (≈1 mg/g) is another advantage of this plant since it decreases Ca uptake and hence it protects the skeleton from the excess deposition of Ca[28]. Strontium (Sr) content of this plant (leaf: 184 μg/g & root: 163 μg/g) is very essential for the treatment of Osteoporosis. Silicon promotes Ca absorption and it interacts with Co, Zn and Ge. Since the presence of Si is around 8.6 mg/g in leaf and 6.7 mg/g in root, this plant helps much in curing bone problems and also helps in strengthening of bones. Even though the Ca:P ratio in this plant is around 7:1 it will not produce any harmful effect since less quantity of P will not cause any damage to Ca storage. The presence of Cu in leaf and root (28 & 17 μg/g) may protect the human being from the Cu deficiency caused by anemia and bone demineralisation. It is said that the menopausal women experience a sudden bone loss and hence this plant may be a supplement to eradicate such loss[29].

Physical and Mental Strength

The haemoglobin level in RBC and Myoglobin are important factors which determine the reaction which produces energy in the human body; this is due to the presence of Iron and is available plenty in the plant *B. sensitivum*, *i.e.*, in the mg range (≈4 mg/g in both leaf & root). The presence of Cl (3.7 mg/g in leaf & 4.7 mg/g in root) in this plant ensures the digestion through the breakdown of proteins by activating the pepsinogen into pepsin.

However, the dosage of minerals is very important and which should not exceed the AI levels. The presence of Rb in leaf (≈68 μg/g) and root (≈120 μg/g) made this plant a good medicine for depression, since Rb is a natural serotinin booster[25], and it provides good health by supporting better Iron absorption. The K

content (leaf: 21 mg/g & root: 29 mg/g) and Rb (leaf: 67 μg/g & root: 120 μg/g), both in leaf and root of the studied plant ensures its importance in brain development, which is the root cause for physical strength. K with Ca regulates neuromuscular activity and heartbeat rate and activate enzymes[30,31].

Mass spectrographic and isotope studies have shown that K, Rb, and especially Cs are most efficiently taken up by cancer cells. It is likely that Rb and Mn, in addition to Zn, are preferentially taken up by tumours in the brain[32]. Hence, it is suggested here that this plant may be potentially used for both diagnosis and treatment of brain tumour[33].

Rb acts at the level of the central nervous system (CNS) by increasing synaptic neuro-transmitter levels and it has antidepressant activity. The presence of Rb may be the cause for the usage of this plant in *Ayurvedic* practice, in the treatment of depression.

This plant may be an effective remedy for hyperthyroidism, a symptom of Zn deficiency, since this plant contains a remarkable quantity of Zn (≈80 μg/g in leaf & ≈108 μg/g in root). The presence of a comparatively high quantity of Sulphur (≈2 mg/g) may be the major cause for its high topical usage such as acne ointments, antidandruff shampoos, the antidote for radioactive exposure, and treatment for psoriasis, rheumatic pain , and infections[34].

Wound Healing

Wound healing is a biochemical process in which vitamins, micronutrients , and trace minerals play crucial roles. Patients with chronic or non-healing or slow healing wounds and experiencing nutrition deficiency often require special nutrients. Energy, carbohydrate, protein, fat, vitamin, and mineral metabolism can affect healing process[35]. Vitamin C (L-ascorbic acid), Vitamin A (retinol), and vitamin E (tocopherol) show antioxidant and anti-inflammatory effects. Vitamin C deficiency leads to an impaired immune response and increased susceptibility to wound infection[35,36]. Similarly, vitamin A deficiency also leads to impaired wound healing. Vitamin E maintains and stabilizes cellular membrane integrity by protecting against destruction by oxidation. It has also been suggested to have a role in decreasing excess scar formation in chronic wounds[37].

Carbohydrates and fats are the primary sources of energy in the wound-healing process. Glucose is the major source of fuel used to create the cellular ATP that provides energy for angiogenesis and deposition of the new tissues[38]. The wound healing process depends upon many factors, such as oxygenation, infection, age and sex hormones, stress, diabetes, obesity, medication, alcoholism, smoking and nutrition[39].

Simple abrasions and scrapes can typically be managed by most people in the home using basic wound care. In this context, the fresh leaf juice of the studied plant *B. sensitivum* is applied on the just formed scrapes of a man with diabetics, as a rural practice got cured within two days, and which raised curiosity in us over this plant to study about its wound healing capacity of a diabetic patient[39].

According to Major Gooyit *et al*.[40], there are currently no therapeutics for diabetic wound healing. Acute wounds are initially treated with standard first aid. Immediately, there is a need to stop bleeding by applying direct pressure to the wound and elevating the affected limb. The Calcium content of the body supports to clot the blood in the wound. Usually, open wounds are subject to contamination and infection. Poor blood circulation can limit the amount of oxygen and healing nutrients that reach the wound. Haemoglobin in RBC transport oxygen to tissues in the body and Iron plays a major role in oxygen transport.

To heal a wound, the body needs to clear away dead and damaged tissues and build new skin cells. To develop new skin cells the mixture of enzymes and hormones that are made by the immune system should function well and hence Copper and Zinc are crucially important in new cell building[41,42]. , The formation of new blood vessels is a crucial step in wound healing: the newly-formed vessels allow anti-inflammatory proteins to reach the wound site, improve oxygenation of the damaged tissue and carry essential nutrients for the re-structuring of the tissue. In addition, hyperglycaemia caused by decreased insulin availability and increased resistance to insulin can affect the cellular response to tissue injury. The supplementation of P, Mn, and Si to the patient will enhance the process of tissue restructuring[43].

Every growing cell in the body is provided with oxygen and nutrients *via* blood vessels. Blood vessels are formed by endothelial cells which line the inside wall of the vessel, require energy to be able to form new blood vessels. The chronic skin wound is an increasing medical problem since it is commonly found in diabetic patients and those suffering from morbid obesity[44]. Skin shields against bacteria on the injury, and helps to prevent the loss of water, electrolytes (minerals), proteins and other vital

substances. It also controls temperature and helps to sense heat, cold, pressure and pain. It is common for a wound to develop an infection, particularly in a person with poorly controlled diabetes. Phosphate has a role in intermediary metabolism and cellular/ skeletal structure as phospholipids and hydroxyapatite. The deficiency of phosphate is associated with impaired glycolysis, impaired phagocyte function, hemolysis, and congestive cardiomyopathy[45]. The P content of the plant gives strong support to the claim of wound healing in diabetic patients.

Zinc is a co-factor for RNA and DNA and is involved in the production of alkaline phosphatase and other enzymes in protein synthesis. Low level of Zinc is one of the reasons for poor wound healing. Copper is essential for proper healing as it is a co-factor for the action of lysyl amine oxidase (LAO) in the aldehyde reactions, which generate strong covalent bonds in collagen. Manganese is necessary for the glycosylation of hydroxyproline residues in the formation of collagen[46]. The said elements such as, Zn, P, and Mn are present in these plant parts for the essential healing process. Magnesium functions as a co-factor for many enzymes involved in protein and collagen synthesis. Iron is required for the hydroxylation of proline and lysine, and, severe iron deficiency can result in impaired collagen production[35,36,38]. The abundance of Fe (4.45 mg/g in leaf and 4.1 mg/g in root) in this plant is critical evidence for its curative nature of wounds.

Another type of wound which is common to people with diabetes is the arterial ulcer, caused by the poor blood circulation to the tissues. Sulphur supplementation increases blood circulation to tissues, hence wound healing is accelerated. 2.2 mg/g of S content may considerably enhance the blood circulation. The higher concentration of K in this plant reduces heavily the possibility of wound infection. Patients suffering from dehydration, a cause of potassium deficiency, are considered to be more susceptible to infection.

Hence, for controlling diabetics, the elements Cr, Zn, Rb , and Cu are necessary and Mg, Cu, Zn and Fe play a significant role in wound healing, and Ca, Mg, Sr, Si and Cu supports bone health, the plant *Biophytum sensitivum* (L.) is ,therefore, a potential Ayurvedic medicinal plant.

Conclusion

Even though many studies on *Biophytum sensitivum* are available, elemental analysis of trace minerals of this plant has not been studied, and hence, this work is possibly the first report of this nature. The clinical importance of nutrition, minerals and trace elements is addressed with a vision of correlating the bio-elements and the elemental content of *B. sensitivum*. The presence of Rb helps Fe absorption and excess Sr replaces Ca. Its role in diabetes treatment, particularly the post diabetic ailments, is substantiated with its trace elemental composition in both leaves and roots as revealed in this study. The results show the potential usage of this plant in bone building, strength, wound healing of diabetic patients. However, assessing the medicinal value of a plant does not depend only on its elemental contents and hence a complete review of the plant is required in the context of pharmaceutical and nutritional value and claims.

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Conflict of Interest

All authors declare no conflict of interest.

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