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Identification of Metals Found in the Leaves of *Tabernaemontana alternifolia*

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Abstract: *Tabernaemontana alternifolia*, an Ayurvedic medicinal plant is found in the western ghats of Maharashtra. The leaves, roots, bark-stem are used in Ayurvedic system of medicines. Leaves are used in the form of churna/extract or in combination with the other plant materials in their formulations. It is administered orally. Metal analysis of the leaves of *Tabernaemontana alternifolia* shows a high percentage of metals like magnesium, calcium, iron, copper and manganese. The present results will prove to be useful in understanding the role of metal ions in the biogenesis of the secondary metabolites of the medicinally important plant.
Key words: *Tabernaemontana alternifolia*, iron, atomic absorption spectroscopy.

Introduction

Some medicinally important plants are a rich source of essential elements including metals in trace amounts. These metals can help to restore and maintain the uptake of the essential nutrients in the body. Medicinally important plants like *T. alternifolia* commonly known as *Naagakuda* are found in the western ghats of Maharashtra, India. It is a shrub or deciduous tree. All parts of *T. alternifolia* are used as local medicines. Whole plant including roots and leaves contains alkaloids which are found to be active against lymphocytic leukemia[1]. A compound like camptothecin is isolated from the plant. This compound is widely used against various forms of cancer[2]. The leaves of *T. alternifolia* are used in the treatment of various diseases like cancer[1], diarrhea, syphilis etc. The metals like potassium, calcium, iron etc. play a vital role in the human body metabolism. Metals are of very much importance as they are required to initiate and carry out different enzymatic activities in the body. Consequently, various disorders are developed in the body due to deficiency and overdose of metals like Cu, Na, Fe, Mg, Mn etc. Iron is an essential nutrient required for well functioning of the brain and other parts of body. It carries oxygen from lungs to every organ and cell of the body. The metal deficiencies and imbalances are known to be involved in disorders of the cardiovascular system, gastrointestinal, muscular, skeletal, neurological, immune and endocrine systems. The perfect balance of concentration of metals in the body is the key for good health of human beings.

Experimental

The taxonomic identification was done with the help of Flora of Bombay Presidency[3] (Cooke 1958) and Flora of Maharashtra[4] (Singh et. al. 2000) and herbaria were prepared. The specimens were also compared with the authentic herbaria of Botanical Survey of India, Western Circle, Pune (M. S.) for identification and conformity.

Preparation of ash:

Powdered dried leaves (1gm) were kept in the muffle furnace at 550^0C till constant weight was obtained. The major constituents of ash were determined qualitatively and quantitatively by employing respective techniques (Table 1). For the detection of metals, the ash was converted to chlorides and the solution was tested for metals by applying standard procedures. Initially, the ash was dissolved in 10% HCl (5.0ml) and evaporated to dryness on water bath. It was then treated with 10% HCl (5.0ml) for completion of chloride formation. The material was digested with 25% HCl (5.0ml) on water bath for 30 minutes. The resulting solution was filtered through Whatman paper (No. 40). The residue was made chloride free (tested with silver nitrate solution) by washing repeatedly with hot water. The filtrate was

diluted to 100 ml and used to estimate metal contents by using standard methods as illustrated in Table 1.

Results and Discussion

The presence of different metals in the body in proper concentrations is of prime importance as trace elements are spark plugs of life. Metals present in the body in trace concentrations carry out a large number of enzymatic reactions. Thus their deficiency or overdose can cause severe disorders in different metabolic pathways. The leaves of *T. alternifolia* are being used in ancient Ayurvedic systems for curing various diseases such as cancer, diarrhea, therefore its metal analysis also becomes important.

Metal analysis of acid soluble ash of leaves of *T. alternifolia* shows the presence of potassium, phosphorous, calcium, magnesium, zinc, copper, iron and manganese as the metals. Out of these, potassium, calcium and magnesium are found as major constituents where as iron, copper, zinc and manganese are as minor constituents.

Phosphorous is required in the human body to carry out many complex reactions in the body. There is over 1 lb (464gm) of phosphorous found in the body. It is a component of adenosine tri phosphate (ATP) a fundamental energy source in the living beings. It is a principle material of teeth and bones and is found in many organic compounds in blood, muscles and nerves as such the amount of phosphorous in the body becomes a matter of great importance. Inadequate intake of phosphorous results in abnormally low serum phosphate levels (hypophosphatemia). The symptoms are anorexia, muscle weakness and osteomalacia. Phosphorous deficiencies can be caused by excessive intake of aluminum containing agents as aluminum can bind to phosphorous. Symptoms of phosphate deficiency include loss of appetite, anxiety, bone pain, bone fragility, stiffness in the joints, fatigue, irregular breathing, irritability, numbness and many more. In order to overcome the phosphorous deficiency, phosphorous compounds need to be included in the diet or taken as dietary suppliments. Ayurvedic preparations and formulations of leaves would be a good source of naturally occurring phosphorous**.** Phosphorous is present in moderate quantity in the leaves of *Tabernaemontana alternifolia*. **Table-1**

Copper [5] is a sedative mineral. A too high or too low concentration of copper may affect central nervous system. Copper, after ingestion, gets absorbed in the stomach and small intestine from where it is absorbed in the blood stream. In the blood stream it is bound to carrier proteins and is delivered to the liver. From the liver it is distributed throughout the body to places where it is needed. Copper is crucial for the normal functioning of brain and nervous system. It also plays an important role in making neurotransmitters and the movement of electrical impulses along the nerves. Copper helps to sustain elasticity of blood vessels which allows maintenance of proper blood pressure. Copper plays a vital role in collagen formation which is crucial for bone formation, health and repair and also a connective tissue in the skin. Copper is necessary for the maintenance of a healthy immune system. Copper is required for the normal myelination of nerves. Myelination is a fatty substance that acts as insulation around nerves the absence of which can lead to spasticity, tremors and paralysis. Copper deficiency can cause a syndrome of anemia or pancytopenia. The deficiency can also cause hypertension, antibiotic sensitivity, hyperactivity and many more.

T. alternifolia leaves contain an adequate amount copper. **Table-1**

Magnesium is a key metal in cellular metabolism. A balance of magnesium is vital to the well being of all living organisms. Mg^{2+} ions have been evolved for signaling, enzyme activation and catalysis. In presence of higher percentage of potassium and phosphorous, absorption of magnesium increases. Magnesium can affect nerve relaxation through direct action on the cell membrane. The adult human daily requirement of magnesium is 300-400 mg/day. Inadequate intake of magnesium frequently causes muscle spasms, cardiovascular disorders, diabetes, hypertension, anxiety disorders, migraines etc.

Leaves of *T. alternifolia* are having a high accumulation of magnesium metal. **Table-1**

Calcium plays an important role in building of bones and teeth in our body. Besides it, calcium is also important for nerves and muscles. Plasma calcium has a role in blood coagulation. Calcium is present in three forms in plasma and serum. About 50% is in ionized form, 40% bound to proteins and 10% complexed with substances like citrate. Normal value of serum or plasma calcium is 8.5-10.5 mg/100ml. Calcium plays a major role in the Central Nervous System function. Calcium is seen as a major factor in neurotransmission and required in the synaptic discharge of neurotransmitter.

Calcium is essential for nerve impulse conduction and activates some enzymes such as protein kinases[6], which generate neurotransmitters. A long term deficiency of calcium may result in weak and fractured bones, cramp pains in legs, poor sleep disorder, extremely irritable nerves etc. In leaves calcium percentage is moderate **Table-1**.

Potassium plays an important role in protein synthesis, activating many enzymes such as pyruvic phosphoferase, adenosine triphosphatase[7], stimulation of movement of intestinal track etc. It helps to regulate body's fluid level. It also regulates the blood pressure. It is essential for the nervous system and also for maintaining normal heart beats. Potassium helps to promote the proper functioning of the tissue that makes up the nervous system. It also serves to enhance muscle control plus the health and growth of cells particularly through its importance in

waste product removal. This metal is also vital to kidneys for their waste removal tasks. It also plays an important role in mental as well as physiological functions. It helps to promote efficient cognitive functioning by playing a significant role in getting oxygen to brain. Potassium deficiency can cause symptoms of fatigue, weakness, depression, abnormal heartbeat and irregularities in the ECG, dry skin, glucose intolerance, low blood pressure, muscle cramps and many more.

Zinc is extremely important to numerous body functions. Deficiency of zinc may be associated with mental lethargy, emotional disorders etc.

Without iron no world can live. The human body reuses or conserves approximately 90% of its iron each day. The remaining 10% is eliminated and this 10% must be replenished otherwise the body can develop iron deficiency. The major role of iron is in the oxygen transport to the cells. It is found in a protein called hemoglobin which carries oxygen from lungs to all over the body, to each and every cell. It has an ability to donate and accept electrons. Iron present in the free state, will catalyze the conversion of hydrogen peroxide to free radicals which cause damage to the cells and kill them. Thus iron is bound to different proteins like hemoglobin, transferring etc. One of the most noted symptoms of iron deficiency is anemia. Its symptoms include fatigue, insomnia, and reduced mental capacity, loss of appetite, headaches and incapability of body to maintain the body temperature. Iron toxicity results when the amount of circulating iron exceeds the amount of transferring available to bind it, but the body is able to vigorously regulate its iron uptake. People with increased amount of iron are more susceptible to bacterial infections. The human body prevents iron overload solely by regulating its absorption, but in some cases where there is iron tablet overdose, the toxicity of iron starts overwhelming body's ability to bind and store it[8]. In adequate concentration, iron is present in the leaves of *T. alternifolia.* **Table-1**

The human body contains 10-20 mg of manganese and is widely distributed in body especially in intestinal track, liver[9] and reproductive organs. Its role is vital in defense mechanism of body. It helps the synthesis of hemoglobin. A combination of iron, copper and manganese is given in case of hypochronic anemia. Manganese ions activate many enzymes and help in proper utilization of vitamin B and E. Manganese as a component of different enzymes helps to metabolize carbohydrates, proteins and fats. Manganese helps and nourishes brain and nerves to maintain a better co-ordination between brain, nerves and muscles in every part of the body. The deficiency of manganese can result in bone malformation, infertility, weakness, seizures, convulsions, eye problem, hearing problem and many more. A moderate amount of manganese is present in the leaves as can be seen in **Table-1**.

Table-1: Metals analysis of *Tabernaemontana alternifolia* (Leaves)

Metals	Method used	Amount
Total Phosphorous(P_2O_5) %	Flame photometer	0.06
Total Potash (K_2O) %	Flame photometer	0.15
Total Calcium (Ca) %	Flame photometer	0.03
Total Magnesium (Mg) %	Atomic absorption	0.60
Total Zinc (Zn) ppm	Atomic absorption	34
Total Iron (Fe) ppm	Atomic absorption	1969
Total Manganese (Mn) ppm	Atomic absorption	728
Total Copper (Cu) ppm	Atomic absorption	49

Conclusion

The results indicate that the leaves are a good source of essential metals required for the well being of human body. Although there are not many reports on the administration of the leaves, excepting for the treatment in certain diseases, the presence of the biologically important metals in the plant point towards the possibility of their use to restore the different imbalances caused in the body.

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