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EVALUATION OF TRACE ELEMENTS IN *KSHARA* (*ACHYRANTHES ASPERA*).

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ABSTRACT
Mineral elements have very important functions as they serve as structural components of tissues and also functions in cellular and basal metabolism. The plant *Achyranthes aspera*. is known for various medicinal properties and used widely for the treatment of different diseases in human. Elemental profile of *Achyranthes aspera Linn*. (Ten elements to be analyzed-Cu, Na, Ca, Cr, Mn, Fe, Ni, Cd, Zn and Pb) was determined so as to develop a stronger basis for appreciating the curative effects of the plant. Elements were analyzed from root, stem and leaves by AAS/flame techniques. Various elements of Biological importance for human metabolism were found to be present in varying concentrations. The results were discussed with reference to established role of elements in physiology and pathology of human life. Data obtained would serve as a tool for deciding dosage of Ayurvedic drug prepared from this plant.

KEYWORDS: *Achyranthes aspera* Elemental composition.

INTRODUCTION
Ayurveda medicine which includes herbal drug therapies has maintained its popularity in all regions of the developing world and its use is rapidly spreading in industrialized countries. Over one-third of the population in developing countries lack access to essential medicines. The provision of safe and effective herbal drug therapies could become a critical tool to increase access to health care (WHO 2003). Mixtures of medicinal plants are prescribed by the traditional healers for diseases ranging from common colds to malaria, arthritis, ulcers, etc. (Obiajunwa et al., 2002). Mineral elements have very important functions and it is believed to be key component of proteins such as haemoprotein and haemoglobin which play role in biochemical functions.

Kshara has its unique place in Ayurvedic Therapeutics. *Ksharas* are basically made from Ashes of the medicinal plants. The process of *Kshara* preparation, *Achyranthes aspera (Apamarga*) is burnt to get of ash. The ash is mixed with six times (6L) of water to form a solution, which is filtered using a sterile filter paper 12 size in (21 times). The filtrate is boiled and water evaporated to collect white powder called *Kshara*. This is collected and stored in dry glass bottle.

There is increasing interest in the importance of dietary minerals in the prevention of several diseases. Fortification refers to the addition of mineral nutrients to a commonly eaten food. Both iron fortification of wheat flour and iodine fortification of salt are examples of fortification strategies that have produced excellent results (Vitamin et al., 1998). Minerals are of critical importance.

However, lack of knowledge of the elemental constituents of these medicinal plants often poses danger to consumers as some may contain toxic elements. Also the dose rate of many of these medicinal plants is not well defined and left to the judgment of the users. This can sometimes cause problems to user as the probability of taking overdose to speed up healing is highly elevated, ignorant of the dangers in doing so (Mino y et al., 1994).

Each element has its individual impact in the structural in living cell and organism. The present study is to undertaken to detect and determine concentration level of 10 different mineral elements in *Achyranthes aspera*. *Achyranthes aspera* is an indigenous medicinal plant of Asia, South America and Africa, it is found throughout India belonging to the family Amaranthaceae. The plant is known for various medicinal properties and used widely for the treatment of different diseases in human. In the recent time, *Achyranthes aspera* reported to have

array of medicinal compounds and medicinal properties. The plant is astringent, digestive, diuretic, laxative, purgative and stomachic. The juice of the plant is used in the treatment of boil, diarrhoea, dysentery, haemorrhoids, rheumatic pains, itches and skin eruptions. The ash from the burnt plant, often mixed with mustard oil and a pinch of salt, and is used as a tooth powder for cleaning teeth. It is believed to relieve pyorrhoea and tooth ache. The leaf is emetic and a decoction is used in the treatment of diarrhoea and dysentery. A paste of the leaves is applied in the treatment of rabies, nervous disorders, hysteria, insect and snake bite (Londonkar et al., 2011). *Achyranthes aspera* reported to possess wound healing activity, immune stimulatory properties, larvicidal activity, antibacterial activity and antifungal activity. Roots of *Achyranthes aspera* reported to possess antioxidant activity and anti-inflammatory properties. (Edwin et al., 2008). But no work has been reported on

MATERIAL AND METHODS

Sample preparation

The plant parts were sorted out and surface contaminants of plant samples were removed by washing with running tap water. It was then dried and then subjected to grinding for powder formation prepared as 10 Kg of *Achyranthes aspera (Apamarga*) was burnt to get 1/2 kg of ash. One Kg of ash was then mixed with six times of water(6L) to form a suspension, which was filtered using a sterile cloth, by filtration (21times). The filtrate was, followed evaporation (boiling the *Ksarajala* still all water evaporates) collection (process called lixiviation) of white powder called *Kshara*. This is collected and stored in air tight glass bottle. The powder was stored in air tight glass containers and used for further analysis.

Analysis of metals

Kshara is the main components of *Ksharasutra*, it is used as a form of special ash in the method of preparation described above section (Su.ni.4). So it is the only available chemical components of inorganic compound. Therefore detection of metals was important in this study to identify the metal that promote the wound healing process. The requirements of trace elements for human such as, Ca, Mg, Cr, Na, Fe, Cu, Mn,,K Ni and Zn for good health and wound healing property.

On the other hand, several heavy metals such as Cd Ni, Cr and Pb are known to be potentially toxic.

Atomic Absorption Spectrophotometric (AAS) analysis of *Kshara*

In this study AAS (Flame) method was used to analyze the Zn, Cu, Ca, Fe, Mn, Na, Ni, Cr, Cd and Pd. Flame atomic absorption methods were used for metal analysis (Soufleros et al. 2004). All glassware was rinsed with acid, washed well, soaked in 10% HNO3 acid for 24 hours and rinsed with de ionized water. They were dried in hot air oven for 24 hours prior to analysis. Dry ashing method;

Digestion

Five grams of homogenize sample of *Kshara* powder of each plant part was heated at 480 °C for 3 hours and residue was dissolved in 10.0ml concentrated nitric acid and heated on hot plate until the reddish brown fumes disappear and until 2-3ml remain. Repeated process twice and then filtered it by using 542 whattman filter paper. The volume was then made up to 50.0 ml in a standard volumetric flask by adding de ionized water. Estimation of elements was carried out using Atomic absorption spectrophotometer (Model; Varian AA240 FS, Australia)

Then this solution was transferred to a polypropylene bottle and refrigerated until the analysis is carried out. Calculated volume of respective metal solution was pipetted out from a standard stock solution using micro pipettes, pours in to a 100.00 ml volumetric flask and mixed well with de ionized water to prepare the test *Kshara* solution. Flame atomic absorption spectrophotometer was operated according to the instruction provided by the manufacturer for maximum sensitivity with an air acetylene flame. All measurements were performed at prescribed wave length in nm, using the hollow cathode lamp with prescribed lamp current and slit width for each metal. Instrument was calibrated for each metal by introducing the prepared standard working solution series. All parameters were entered to the instrument through automated software and instrument was calibrated for individual analysis (Table 1).

RESULTS AND DISCUSSION

Literature survey revealed that optimal intakes of elements such as sodium, potassium, magnesium, calcium, manganese, copper, zinc and iodine could reduce individual risk factors, including those related to cardiovascular disease for both human beings and animals (Anke, et al., 1984). The various plant parts of *Achyranthes aspera*. are a good source of trace and major elements. Since these trace elements constitute a minute fraction in different parts of medicinal plants, a sensitive and reliable technique is a prerequisite for obtaining precise and accurate data. In the present investigation we have.

Table 1: Concentration of elements in roots stem and leaf of *Achyranthes aspera*

Sample	Cu ppm	Zn ppm	Mn ppm	Fe ppm	Cr ppm	Na ppm	Ni ppm	Pb ppm	Cd Ppm	Ca ppm
Root	1.42	53.30	3.61	194.66	0.53	16.21	0.32	N/D	N/D	849.44
Stem	0.39	15.34	3.33	33.55	0.37	50.01	0.23	N/D	N/D	588.19
Leaf	0.44	3.24	1.44	37.49	0.18	157.29	0.11	N/D	N/D	1363.77

N/D Not detected.

Copper, iron, magnesium, zinc and calcium are known in connection with its antibacterial effects, in addition to this copper, iron, magnesium, and zinc are renowned for the role they play in actively promoting wound healing. Copper plays an important role in the antibacterial activity of *Kshara*

Ksharas are basically made from metals and minerals, the process of *Ksharas* are an integral part of Ayurveda surgery and it's a main components of the *Ksharasutra* preparation

Copper

The content of Cu 0.39 ppm which was found in stem of *Achyranthes aspera* and maximum concentration 1.43 mg/kg was found in roots of *Achyranthes aspera* (Table-1). In leaves the Cu concentration was found to be 0.43 ppm. WHO limits for medicinal plants not yet been established for Cu. Permissible limits for Cu set by China and Singapore for medicinal plants, were 20 ppm and 150 ppm, respectively (Obi E, Akunyili et al.,2006). It is widely accepted that the mechanism of contact killing was involves the following key steps; damage of the outer and or inner bacterial membrane, accumulation of copper ions in the cell, and degradation of the bacterial DNA (Rensing, et al., 2003). Inhibition of the respiratory chain by copper has been proposed as the primary event in contact killing of *Staphylococcus aureus*

(Magnani et al., 2008) While this may be a factor in contact killing of respiring cells, the sequence of events leading to cell death is by DNA degradation The results of this study indicate that concentration of Cu was well below permissible limit has been pointed out Lansdown et al., 1999 metallo enzymes play a pivotal role in wound healing. Cu sensitive pathways which regulate key mediators in wound healing such as angiogenesis and extracellular matrix remodeling have been studied and copper-based therapies may represent a feasible approach to promote dermal wound healing (Sen et al., 2002)

Sodium

Na is of great importance for many regulatory systems in the body. Concentration of Sodium element was observed to be higher in leaves, 157.28 ppm the concentration in roots, 16.21 ppm and stem, 50.01 ppm of *Achyranthes aspera*. Sodium is essential to all living organisms. Sodium remains one of the major electrolytes in the blood. Without sodium the body cannot be hydrated and then, it would dry off. (Gbolahan et al., 2001).

Calcium

High concentration of Calcium identified in leaf (1363.77 ppm). So the Calcium inhibits bacterial reproduction on wound surfaces (Prudent et al., 1963). Topical application of calcium increases pro epithelialization of the wound in a rate that is directly proportional to the calcium concentration on the wound surface. It reacts with magnesium ions on the surface of the wound to form chelates which have antibiotic properties (Lansdown et al., 2002). It was observed that among all the elements studied in the analyzed samples, calcium accumulation is the highest in all parts of *Achyranthes aspera*, than the concentration of other metals. Soil could be a factor for accumulation of higher amount of Ca. Similar to Sodium it is found that calcium accumulation is more in leaves than in roots is 849.44 ppm and 588.20 ppm in the stem of *Achyranthes aspera* .

Manganese

Manganese is an essential element required for various biochemical processes (Guenther et al., 2003). It reacts with calcium ions on the surface of the wound to form chelates which have antibiotic properties. High concentration of manganese is found in root and stem of *Achyranthes aspera.* Results of this study indicate that the concentration of Mn in roots is 3.61 ppm, in stem 3.33 ppm, where as in leaves was found to be 1.44 mg/kg. The chromium concentration in all the parts studied of *Achyranthes aspera*, was below the permissible limit for chromium as set by FAO/WHO in edible plants which is 2 ppm. Our results indicate that the concentration in roots is 0.52 ppm, in stem it is 0.37 ppm and in leaves it is 0.17 ppm,

Iron

Iron is important for the formation of hemoglobin and also plays an important role in oxygen and electron transfer in human body. In all parts of the sample of *Achyranthes aspera* studied, the amount of iron accumulated is much higher than the permissible levels. In roots it is found to be 194.66 ppm in stem 33.55 ppm, in leaves 37.44 ppm. Iron deprivation reduces the activity of the ribonucleotide reductase necessary for DNA synthesis, decreases the expression of cyclones A, B, & D and results in hypophosphorylation of the retinoblastoma protein (Valentino, et al., 2006).

Nickel

Nickel is considered to be highly mobile element within a plant. Accumulation of Ni takes place only in the leaves (Mc Grath et al., 1995). Ni toxicity in human is not very common occurrence as its absorption by the body is very low but this results show that nickel has also accumulated in stem, 0.23 ppm and roots, 0.31 ppm.

Zinc

Zinc the highest concentration was found in roots of *Achyranthes aspera* was 53.30 ppm, where as in stem it was 15.34 ppm and in leaves 3.24 ppm. Zinc is essential to all organisms and has an important role in metabolism, growth, development and general wellbeing. It is an essential co-factor for a large number of enzymes in the

body. Moreover, zinc is responsible for wound healing. Topical administration of zinc appears to be superior to oral therapy due to its action in reducing super infections and necrotic material via enhanced local defense systems and collagenolytic activity

(Lassdown et al., 2007), and the sustained release of zinc ions that stimulates epithelialization of wounds in normozincemic individuals. Zinc oxide in paste bandages protects and soothes inflamed peri-ulcer skin. Zinc is transported through

The skin from these formulations, although the systemic effects seem insignificant.

(Lassdown et al., 2007). Zinc has been described as being very important for wound. Zinc produces a beneficial effect on the healing of ulcers by modulating the cutaneous inflammation and accelerates re epithelialization process it stimulates the proliferation of epidermal cells and plays an important role in skin physiology. Its deficiency results in impaired immune response and decreases protein and collagen synthesis (Brian et al., 2007) Zinc deficiency increases the time for wound closure and decreases wound strength. It has, thus, been used as a topical application to treat diaper rash, bedsores, ulcers, and Incision wounds. The metals and metal ions are found in the *Kshara* of *Acyranthes aspera* under discussion. Appreciable levels of zinc are found in the *Kshara*. (Table 1) The presence of these metals in these plants points out towards the value of the plants for wound healing intervention

CHROMIUM

Chromium is known to regulate carbohydrate, nucleic acid and lipoprotein metabolism and it also potentiates acts as an activator of several enzymes. Deficiency of chromium decreases the efficiency of insulin and increases sugar and cholesterol in the blood. Chromium deficiency can cause an insulin resistance, impair in glucose tolerance and may be a risk factor in atherosclerotic disease. The chromium concentration in all the parts studied *of Achyranthes aspera* . Was below the permissible limit for chromium as set by FAO/WHO in edible plants which is 2 ppm. Our results indicate that the low concentration less than 0.5ppm.

CONCLUSION

In view of above facts, the medicinal plant, *Achyranthes aspera.* studied in this work is a source of biologically important elements, and they may play a part in the observed therapeutic use of this plant. Hence, it could serve as supplement of macro and micro elements in the body. Ayurvedic formulations do demonstrate significant success in treatment of many diseases. The medicinal plants contain trace elements whose activity has an impact on its overall pharmacological action. The variation in elemental concentration is mainly attributed to the differences in botanical structure, as well as in the mineral composition of the soil in which the plants are cultivated. Other factors responsible for a variation in elemental content are preferential absorbability of the plant, use of fertilizers, irrigation and climatological conditions. There is no direct link established between elemental content and curative capability of the plant. But such studies will help us to understand the pharmacological action of the herb and thus provide the vital link between the two. The mode of application of these medicinal plants as a source of mineral supplements in the body has been traced to insufficient data on the mineral accumulation in such plant. The need to screen medicinal plants used in the elucidation of element specification of *Achyranthes aspera.* done in the present work will be helpful in the designing of new Ayurvedic drugs which can be used for the control and cure of various diseases.

Medicinal plant *Achyranthes aspera Kshara* is rich in metals Fe, Cu, Ca and Na and it is expected that plants with high contents of the above-mentioned macro and micronutrients might play an important role in maintenance of human health. Also, all of the detected values for elements in plant studied here are below the WHO permissible level for medicinal plants and may not contains health hazard for consumers

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