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World Applied Sciences Journal 12 (9): 1464-1468, 2011
ISSN 1818-4952

Evaluation of Inorganic Profile of Selected Medicinal Plants of Khyber Pakhtunkhwa Pakistan

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Abstract: Medicinal plants play an important role in the maintenance of human health as they are always with no side effects. Keeping in view the importance and vital role of medicinal plants, *Acorus calamus*, *Artemisia annua*, *Chenopodium foliosum*, *Cupressus sempervirens*, *Euphorbia helioscopia*, *Lipedium sativum*, *Nerium oleander*, *Rananculas ripens*, *Tecoma stans* and *Urtica dioca* have been selected to carry out their inorganic analysis using the literature methods. The study is of particular importance as to know the amount of different inorganic profile and to provide a scientific data base.

Key words: Analysis % Medicinal Plants % *Artemesia annua* % *Lipedium sativum*

INTRODUCTION

The discovery of elements in living organisms dates back to a century [1-4]. It was in fact the discovery by Raulin [5] of the essentiality of zinc. The existence of the link between Iodine content and human Goiter due to inadequate intake of food and water [6] along with the discloser of iron as a blood constituents [7] that stimulated investigations on the importance of elements in human body, nutrition, disease, health as well as the environment surrounding human life.

The attention for the investigation of elements was drawn by Hakim Abdul Hamid originator of the discipline "Elementology" [8]. According to him health depends upon the organized state of elements in the body and their imbalance causes diseases [9]. He believed that the restoration of balance by drug can cure diseases. The remarkable progress that has been made in the science of Medical Elementology during the past few decades has not only opened avenues for research on human health related aspects but also aroused the interest of the pharmaceutical industries to reap the benefits of this research by formulations containing elements reported to be essential for human health. A variety of such formulations are available world wide.

It has been reported that out of 110 known elements, 81 were present in living organism which were then biologically classified [10]. Medicinal uses, excess or deficiency of 81 elements [11] was reported to affect at least 235 diseases or functions of the body.

A Varity of such formulations are available world wide. In Pakistan over twenty formulations manufactured from expensive imported raw materials are available exorbitant varying prices [12, 13]. These general tonics are beyond the reach of the major indigent segment of the 117.32 million populations having a per capita income of only Rupees 10,358 [14]. Though expensive medicines cannot be made available to the entire population, but efforts could be directed towards exploring the possibilities, within economic provisions, for the development of economical and efficacious substitutes from within the available natural resources. Medicinal properties have been attributed to a large verities of plants cultivated in different parts of Pakistan. The active constituents, especially inorganic elements present in plants are very variable in quantity if grown under favorable or unfavorable conditions and different type of varieties used for cultivation [15]. Keeping these factors in view, standardization of medicinal plants is essential to provide drugs of good average quality for obtaining desired results. The present work was therefore an attempt to evaluate the Inorganic profile of selected medicinal plants.

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Table 1: List of Some Highlighted Essential or Possibly Essential Elements Their Medicinal Uses and Pharmacological action by their Deficiency and Excess

Element	Medicinal uses	Deficiency	Excess
Calcium	Osteoporosis	Menopausal problem, Cardio vascular disease	Hypercalcemia,
Kidney stone			
Bromine	Epilepsy	Insomnia Osteoporosis	Not known
Carbon	Atelectasis	Not known	Not known
Chlorine	Antiseptic and profuse sweating	Maintenance of pH and osmotic equilibrium	Maintain PH and
Osmotic equilibrium			
Fluorine	Dental caries	Dental caries	Convulsion,
Hypoclcaemia			
Hydrogen	Not known	Maintenance of optimum pH of body fluids	Maintain pH of body Fluids
Iodine	Cuts and burn	Goiter	Thyrotoxicosis
Magnesium	Antacid, Cathartic	Pancreatitis, convulsion	CNS and GIT
Disturbance			
Phosphorus	Reduction of urinary calcium	Disturbance of growth and repair,	
		Metabolism of CHO, Protein, Fats	Coma, GIT disturbance
Potassium	Hypercalcemia, Myasthema gravis	Anorexia, Lung disease	Acidosis
Sodium	Replacement Therapy	Dehydration, decrease blood volume	Hypertention, Cardiac failure

MATERIAL AND METHODS

Sample Preparation: 2 g of powdered plant materials (whole plant) of each species was taken in a china dish. The samples was then heated in an electric oven at 110 °C to remove moisture and then charred by heating in a furnace for 4 h at 550 °C. The content of china dish was cooled in desiccator and added to it 2.5 mL of distilled water to dissolve the contents. The suspension was then filtered and the filtrate was transferred to 100 mL flask and diluted to the mark with distilled water [16, 17]. The inorganic constituents in the samples were determined as per literature method.

Determination of Calcium: EDTA titrimetric method was used for determination of Ca [18, 19]. Briefly 10 mL of sample was taken in a 100 mL titration flask and 10 mL of distilled water was added to it. Added 0.4 mL of 2 M NaOH to the diluted sample with the help of micropipette, followed by the addition of 0.04 g of murexide indicator. The color of the solution turned pink. The sample was titrated against 0.1 M EDTA solution with constant stirring till the appearance of the light purple color as end point. Blank determination was carried out by repeating the same procedure using distilled water instead of sample. The concentration of the sample was determined using the following formula. Triplicate readings were taken for each sample and reported as mean of three readings.

Calculation:

$$Ca\ (mg/L) = A.B.400.8 / Volume\ of\ sample\ (mL)$$

Where

A= Volume of titrant (EDTA) used (mL)

B= Weight of $CaCO_3$ equivalent to 1.00 mL.

Determination of Magnesium: Calculation method was used for the determination of Mg [18, 19]. Concentration of Mg in the sample is actually equal to the "difference between Ca and total hardness as $CaCO_3$, if interfering metals are present in non-interfering concentrations in the Ca titration and suitable inhibitors are used in the hardness titration. The following equation was used to calculate Mg from the values of total hardness and Ca in the samples. The method was repeated as triplicate and data was recorded as mean value of triplicate.

Calculation:

$$Mg\ mg/L = (Total\ Hardness - 2.5)\ Ca.\ 0.243$$

Determination of Bicarbonate: 10 mL of Sample was taken in 100 mL beaker and added to it 2 drops of methyl orange [18, 19].The appearance of light yellow color indicates the presence of bicarbonate. The solution was titrated against 0.02N HCl. The orange color appearance shown the end point and the volume of HCl used was noted from the burette. The bicarbonate concentration was determined as follow. The method was repeated in triplicate and data was recorded as mean value of triplicate.

$$Bicarbonate\ (mg/L) = Volume\ of\ HCl\ used\ (mL).\ 100$$

Determination of Sulphate: Sulphate was determined by Spectrophotometer at 420 nm wave length using turbidimetric method. Standard solution (1 to 35 mg/ L) of 10 mL was taken in 100 mL beaker [18, 19].

Table 2: Analysis of Inorganic Constituents in Ten Selected Medicinal Plants (ppm)

S No.	Sample Code	Calcium	Chloride	HCO_3	NO_3	SO_4	Fluoride	Mg
1.	*Acorus calamus*	32	35.5	100	1.3	202	0.05	0
2.	*Artemisia annua*	16	92.3	330	1.9	111	7.0	4.86
3.	*Chenopodium foliosum*	59	68	86	2.63	75.9	0.16	5.3
4.	*Cupressus sempervirens*	16	28.4	120	0.7	34	8.0	4.86
5.	*Euphorbia helioscopia*	40	134.9	260	0.95	158	7.2	6.86
6.	*Lipedium sativum*	16	77	103	6.28	72.5	0.26	1.2
7.	*Nerium oleander*	32	63.9	200	0.5	56	6.6	3.72
8.	*Rananculas ripens*	31	85	103	3.17	30.7	0.12	2.9
9.	*Tecoma stans*	24	42.6	200	0.6	49	5.4	0
10.	*Urtica dioca*	12.5	51	327	5.05	127	0.18	0

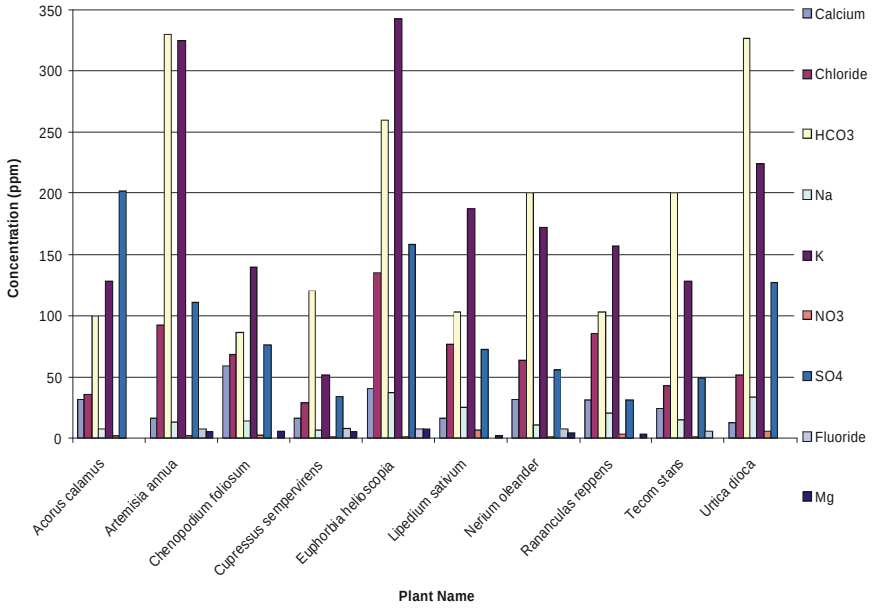

Fig. 7: Graphical Representation of Analysis of Inorganic Constituents in Ten Selected Medicinal Plants (ppm)

Then added 2 mL buffer solution and 0.1g $BaCl_2$ crystals to it. Then after 5 minutes exactly ± 0.5 minute the absorbance of each sample was taken at 420 nm. The standards calibration curve was plotted by taking absorbance of the standard solutions from 1 to 35 ppm at 420 nm. Then after the calibration curve made, it was used to find out the concentration of Samples. Now 10 mL sample was taken to which 2 mL buffer and 0.1 g Barium chloride was added and absorbance was taken after 5 min. The concentration of the sulphate in the sample was indicated by the calibration curve. The method was repeated as triplicate and data was recorded as mean value of triplicate.

Determination of Fluoride: The fluoride was determined by spectrophotometer at 580 nm wave length using Spand's method [18, 19]. Standard solution of 10 mL was taken in 100 mL beaker and added 2 mL of the Spand's reagent to it, then taken the solution in the covet and inserted in the sample holder of the spectrophotometer to determine its absorbance. Absorbance taken of all the standards at 580 nm and standard calibration curve was plotted thereof. At this moment, the absorbance of the sample was measured in the same way; the concentration of fluoride was indicated by the calibration curve. The method was repeated in triplicate and data was recorded as mean value of triplicate.

Determination of Nitrate: Spectrophotometer was used to determined nitrate at 420 nm wave length [18, 19]. The standard solution of 50 mL was taken in 100 mL beaker and added 1 mL of 1N HCl to it and measured its absorbance with spectrophotometer at 420 nm wave length. Calibration curve was plotted for the absorbance

of all the standard solutions. The sample was also treated such and its absorbance was determined. Then the concentration of nitrate in the sample was determined by the instrument from the absorbance of the sample. The method was repeated in triplicate and data was recorded as mean value of triplicate.

Determination of Chloride: Chloride was determined by Argentometric titration method. Briefly, 20 mL of sample was taken in 250 mL titration flask and added 3 drops of potassium dicromate to it [18, 19] and then titrated against standard silver nitrate solution. The titration was stopped at pinkish yellow end point. For blank determination, the same procedure was also repeated using distilled water. The chloride concentration was determined as following. The method was repeated in triplicate and data was recorded as mean value of triplicate.

Calculations:

$$\text{Chloride mg/ L} = \frac{(A-B)xNx35.450}{ml.of\ .Sample}$$

A = Volume of silver nitrate used of sample
B = Volume of silver nitrate used of blank
N = Normality of $AgNO_3$ solution.

RESULT AND DISCUSSION

The organized state of elements in the human body is considered as health and their unevenness causes diseases [20]. The reinstatement of balance by medicine can cure diseases.

Calcium: Calcium is the basic constituent used in the synthesis of new cell walls. The blood plasma of human contains 5ppm of calcium [21, 22]. Table-10 data shows high concentration of Calcium 59ppm was determined in *Chenopodium foliosum*. *Euphorbia helioscopia* contains 40ppm of Calcium while equal concentrations 32ppm was found in *Acorus calamus* and *Nerium oleander*. The concentration of Calcium 31ppm was determined in *Rananculas ripens* and 16ppm of Calcium was present in *Lipedium sativum*, *Cupressus sempervirens a*nd *Artemesia annua*. The low concentration 12.5ppm was noted in *Urtica dioca*.

Chloride and Bicarbonate: Concentration of Chloride was recorded high in *Euphorbia helioscopia* 134.9ppm. The low concentration 28.4ppm was found in *Cupressus sempervirens* while the concentration of Chloride in other plant samples are as follows: 92.3ppm in *Artemisia annua*, 85ppm in *Rananculas ripens*, 77ppm in *Lipedium sativum*, 68ppm in *Chenopodium foliosum*, 63.9ppm in *Nerium oleander*, 51 ppm in *Urtica dioca*, 42.6ppm in *Tacoma stans* and 35.5ppm in *Acorus calamus*. Chloride is important in osmotic pressure regulation, acid base equilibrium and as well as water balance. It is mostly essential for cell division of leaves and roots [23].

Concentration of Bicarbonate was also present high in *Urtica dioca* 327ppm followed by 330ppm in *Artemisia annua* where 260ppm in *Euphorbia helioscopia*. In other samples of plant the concentration of bicarbonate was 200ppm in *Acorus calamus* and *Nerium oleander*, in *Rananculas ripens* and *Lipedium sativum* it was recorded same as 103ppm, 120ppm in *Cupressus sempervirens*, 100ppm in *Acorus calamus* and low concentration was noted 86ppm in *Chenopodium foliosum*.

Sodium and Potassium: High concentration level of sodium was found in *Euphorbia helioscopia* 37ppm while *Urtica dioca* contains 33ppm, *Lipedium sativum* contain 25ppm, *Rananculas ripens* contain 20ppm, *Tecoma stans* contain 15ppm, *Chenopodium foliosum* contain 14ppm, *Artemesia annua* contain 13ppm and *Acorus calamus* contain 7ppm. High concentration of Potassium 342ppm was determined in *Euphorbia helioscopia* while the rest of plants sample covered in the range of 128ppm-325ppm. Sodium and Potassium are mainly electrolytes. The major component of the cation of extra cellular fluid is Sodium while the cation of intracellular fluid is Potassium. Potassium 5ppm is present in the blood plasma of human being while Sodium concentration is 139ppm. High concentration of Potassium leads to the dilation of arteries and normalize the blood pressure. Extensive level of Potassium leads to the failure of heart while high concentration of sodium leads to hypertension [21, 22].

Nitrate and Sulphate: Concentration of Nitrate was high in *Lipedium sativum* 6.28ppm followed by 5.05ppm in *Urtica dioca*, 2.63ppm in *Chenopodium foliosum*, 1.9ppm in *Artemesia annua*, 1.3ppm in *Acorus calamus*, 0.95ppm in *Euphorbia helioscopia*, 0.7ppm in *Cupressus sempervirens*, 0.6ppm in *Tecoma stans* and 0.5ppm in *Nerium oleander*. Concentration of sulphate 202ppm was recorded high in *Acorus calamus* while concentration in the samples of other plants was in-between 34ppm - 158ppm.

Fluoride: Fluoride mineral is naturally occurring in water sources, soils and plants. Its concentration plays vital role in decay of human teeth.Table-10 indicates high concentration level of fluoride 8ppm in *Cupressus sempervirens* followed by 7ppm in *Euphorbia helioscopia*, 7ppm in *Artemesia annua*, 6.6ppm in *Nerium oleander*, 5.4ppm in *Tecoma stans*, 0.26ppm in *Lipedium sativum*, 0.18ppm in *Urtica dioca*, 0.16ppm in *Chenopodium foliosum* and less concentration of fluoride 0.05ppm was present in *Acorus calamus*.

Magnesium: Table-10 shows high concentration of 6.68 ppm in *Euphorbia helioscopia*, 5.3ppm In *Chenopodium foliosum*. *Cupressus sempervirens* and *Urtica dioca*, equal concentration 4.86ppm was detected while concentration of magnesium in other samples of plants are in descending order, 3.72ppm in *Nerium oleander*, 2.9ppm in *Rananculas ripens* and in samples of *Acorus calamus*, *Tecoma stans* and *Urtica dioca* magnesium was not detected.

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