Evaluation of Mineral Contents in Medicinal Plant *Azadirachta indica* (Neem)

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Summary: Water extract and total contents of fifteen essential, trace and toxic elements such as Zn , Cr, K, Mg, Ca, Na, Cu, Fe, Pb, Al, Ba, Mn, Co, Ni and Cd, were determined, in different parts of *Azadirachta indica* (L) Neem using atomic absorption spectrophotometer with various flame absorption modes. The NBS spinach leaves (1570) reference materials were analyzed simultaneously with the plant extracts. Obtained results have shown a good accuracy and reproducibility of present method. The relative errors and the relative standard deviations were less than 10% for most of the elements.

It has been observed that, the level of essential elements was found high as compared to the trace and toxic elements. Although, considerable amounts of Zn, Fe, Cu was found in this plant. These all elements are biologically very much important for the skin and other diseases. The concentration of aluminum in all parts of plant i.e. leaves, fruit and stem was found to be high, ranging between (55.26-88.87), (66.51-99.29) and (48.35-58.72) mg/100g respectively.

Introduction

Medicinal plants are widely used for treatment of diseases all over the world. According to world health organization report about 80 % of the world population are taking interest in indigenous medicinal plants remedies. Herbal medicines have usually been used in the form of fruit and vegetable, drugs or their extract for the treatment of the disease and for maintaining health.

Azadirachta indica (L) is commonly known as "Neem" tree, and it is a member of the family Meliaceae. It is a common tree found in southern dry parts of Pakistan, Bangladesh and India. It is planted and in some cases self-grown, herb. The neem extracts applied to vegetable crops would repel locusts. Like most plants neem deploys internal chemical defenses to protect itself against leaf chewing insects and it is extensively used for remedies of skin diseases and as antidiabetic [1,2].

Plant minerals can be divided in to macro, micro and trace minerals based on the requirements of metals for the normal functioning of human body [3,4]. As the components of enzyme related with antioxidation, the importance of trace minerals i.e. Se, Mn, Cu, Zn, Fe [5], is being gradually emphasized with relation to their participation in maintaining normal cell metabolism, delaying of aging, and preventing cardiovascular, diabetes and immune diseases [6,7]. Metals and their compounds have been used since ancient times for their therapeutic as well as cosmetic effects on skin. Aluminum acetate solution is used as skin disinfectant and cleansing agent. Copper sulfate is probably still used in some countries as a mild astringent and antiseptic preparation to treat vitiligenous skin [8]. In leprosy seven elements are found to have therapeutic effect such as the use of Zn tape for the treatment of leprosy wounds [9- 11].

The present studies were conducted to analysis fifteen mineral contents in different parts of *Azadirachta indica* with the possibility of being used for the treatment of skin diseases and other ailments [12, 13].

Pharmacological importance

Azadirachta indica has many pharmacological actions such as the bark is refrigerant, pectoral, and useful in fever, thirst and bad odour in mouth, effective against ulcers and inflammation. Leaves are useful in biliousness and skin diseases. Fruit are also used as purgative, help treating urinary discharges, skin diseases such as eczema, ringworm infection and scabies. The leaves are carminative and expectorant,

lessen inflammation, and are useful in syphilitic sores and in blood impurities. Decoction of leaves helps healing of wounds, antiseptic and good as gargle for bad gums, the twig of this plant is used for centuries as a tooth cleaner. Seeds are good in treatment of leprosy . The bark, root and young fruits are regarded as tonic and antiperiodic, they are useful in some mild cases of intermittent fevers. Flowers are useful in dyspepsia. Sap is considered as refrigerant, nutrient and alterative tonic. The aqueous extract of tender *Azadirachta indica* leaves has shown hypoglycemic effect [14-19].

Results and Discussion

Analysis results of fifteen elements, total as well as in decoction are summarized in table 1and 2 of different parts of plant showed that many essential and important elements are present in considerable amounts hence they may provides direct or indirect remedies to many diseases.

Digestion of plant samples was carried out by using different mineral acids; they are widely used for sample digestion and preservation. Two methods were adopted for the digestion of plant samples. It was observed that sample digested with nitric acid and 30% hydrogen peroxide (2:1) as mentioned below found superior as compared to the sample digested with sulfuric acid and 30 % hydrogen peroxide (1:1) II. The decomposition of sample using method (I) found more rapid and % recovery of all elements is better as compared to the method (II).

Table 1. Determination of total metals in *azadirachta indica* (neem) by Atomic Absorption spectrophotometer. *(mg/100 g on dried basis)*

Elements	Leaves	Fruit	Stem
Sodium	1355.9- 1596.6 (1343.4 – 1576.9)*	1415.7-1826.2 (1411.6 – 1821.2)	1545.4- 1772.9 (1535.2 – 1761.6)
Potassium	1415.35-1925.9 (1411.5- 1918.6)	1232.2-1429.1 (1221.4 – 1419.6)	1661.1- 1935.4 (1651.7 – 1924.8)
Calcium	1393.6-2454.0 (1387.8 – 2444.7)	2396.5-2568.9 (2381.6 – 2551.5)	2154.6 - 2511.4 (2144.5 – 2510.6)
Magnesium	1827.8-1963.8 (1819.5– 1951.7)	1905.7- 2234.8 (1902.8 – 2225.4)	1181.9-1415.9 (1179.6 – 1408.6)
Iron	4.25- 6.86 (3.23 – 5.76)	7.45 - 15.44 (6.34 -13.33)	3.95- 5.68 (2.88 – 4.85)
Zinc	4.56 - 6.36 (4.16- 6.18)	5.59 - 6.33 (5.12- 5. 49)	7.45- 8.57 (7.13-7.94)
Manganese	2.83- 4.16 (2.73 – 3.92)	1.46 - 1.78 (1.43 – 1.67)	1.35 -1.85 (1.33 – 1.76)
Cobalt	0.427- 0. 569 (0.412 - 0.526)	0.844- 0. 968 (0.7 94- 0.93)	0.433- 0. 786 (0.133- 0.261)
Chromium	0.198- 0. 277 (0.145 - 0.249)	0.152 - 0.232 (0.138 – 0.195)	0.198- 0. 277 (0.175- 0.218)
Nickel	0.295- 0.435 (0.243- 0.419)	0.184- 0. 212 (0.164 - 0.194)	0.128- 0. 295 (0.106 - 0.239)
Copper	0.329- 0.476 (0.312 - 0.423)	0.569- 0. 958 (0.497- 0.896)	0.336- 0.483 (0.31- 0.413)
Lead	0.127- 0. 249 (0.098- 0.215)	0.188- 0. 249 (0.166 - 0.219)	0.183- 0. 218 (0.155 - 0.185)
Cadmium	0.061- 0.139 (0.058 - 0.129)	0.089 - 0.139 (0.076 – 0. 126)	0.049- 0.132 (0.043 - 0.126)
Barium	2.26- 3.48 (2.12-3.26)	3.25 - 4.38 (2.92- 4.15)	2.66 - 3.48 (2.36 - 2.96
Aluminum	55.26- 88.87 (49.48- 83.16)	66.51- 99.29 (62.74 - 93.54)	48.35- 58.72 (44.48 - 54.46)

* Samples digested with method II

Table 2. Determination of metals in decoction of *Azadirachta indica* (neem) by Atomic Absorption Spectrophotometer (mg/100 g on dried basis)

Elements	Leaves	Fruit	Stem
Sodium	128.8 - 229.8	113.5-241.4	266.7- 468.7
Potassium	384.8 - 542.3	213.8 - 577.6	384.8 - 512.7
Calcium	411.3- 573.2	(884.2- 956.3	256.8 - 362.6
Magnesium	268.9 - 575.7	801.3 - 995.7	310.6 - 653. 9
Iron	2.64- 3.73	3.26- 4. 87	1.92- 2. 85
Zinc	1.46- 2.18	1.28- 2. 49	1.33-1.54
Manganese	1.39- 1.43	0.765 - 0.889	0.856 - 0.998
Cobalt	0.247- 0.386	0.194- 0.233	0.133- 0.261
Chromium	0.014 - 0.049	0.068 - 0.095	0.075- 0.098
Nickel	0.129- 0.169	0.084 - 0.174	0.096 - 0.139
Copper	0.165 - 0.293	0.297- 0.486	0.169- 0.273
Lead	0.074- 0.137	0.136 - 0.159	0.075 - 0.125
Cadmium	0.032 - 0.099	0.036 - 0.046	0.043 - 0.036
Barium	1.22 - 1 .56	1.32 - 1.45	1.36 - 1.49
Aluminum	9.44- 12.16	10.74 - 13.54	8.48 - 11.96

The digested samples by method (I) were clear with a light yellow tint and have low viscosity, indicating complete sample digestion as compared to sample obtained by method (II), have brownish colour and high viscosity.

Numerous enzymes activities exhibited by the skin is a reflection of the metabolic role of that organ. These enzymes depending turn on the presence of the special metal ion. The unique processes of Keratinization and melanine formation are enzyme dependant and therefore could be influence by trace metal deficiency or excess.

The level of toxic elements found to be very low in all three parts of this plant as compared to the essential elements such as Na, K, Mg, Mn, Zn, Co, Cr, and Fe. These essential elements may be directly or indirectly helpful for management of many diseases.

Copper, aluminum and zinc were present in both form, i.e. total as well as water extractable form and they have important role in curing the skin problems. These important elements and their salts are used in skin infections as disinfectant and cleansing agent. The Zn is cofactor for many enzyme required for the healing damaged skin [11]. The level of aluminum was found to be high in all three parts of plant especially in leaves. When paste of leaves is applied on infected skin these elements may play roles such as antiseptic, soothing and cooling effects.

Experimental

Plant samples

Ten samples of *Azadirachta indica* (L) were collected from different areas of Hyderabad city and campus area. However, the reference samples were identified with help of Botanists and vouchers specimens were grown on the plot of Center of Excellence Analytical Chemistry, University of Sindh, Jamshoro.

Chemicals and Glass Wares

Nitric acid and hydrogen peroxide of suprapure analytical grade (Merck) were used for acid digestion of plant samples. Certified 1000 ppm standard reference solution of each metal of Fluka Kamica was used and aliquots of these were diluted with a solution of 2N Nitric acid to yield working standards. Milli-Q water was prepared using a Milli- Q deionization unit, which was used through out the experiments. All glass wares was

Table 3. Analytical data for standards of elements

Elements	Conc. range ppm (x)	Absorption range (Y)	Statistical calculation y = mx +c		
			m	c	r
Sodium	0. 0 – 1. 0	0.0 – 0.313	0.3079	0.0032	0.999
Potassium	0. 0 –1. 0	0.0 – 0.565	0.5654	0.0026	0.999
Calcium	0. 0 -1. 0	0.0– 0.0305	0.0304	0.0002	0.999
Magnesium	0. 0 –1. 0	0.0 – 0.883	0.886	0.0133	0.996
Iron	0. 0 – 4.0	0.0 – 0.255	0.0641	0.001	0.999
Zinc	0. 0 – 1.0	0.0 – 0.286	0.2861	0.0006	0.999
Manganese	0. 0 –1. 0	0.0 – 0.09	0.0886	0.0018	0.998
Chromium	0 .0 – 0.5	0.0 – 30*	60*	0.35	0.999
Cobalt	0 .0 –1. 0	0.0 – 72*	72*	0.4	0.999
Copper	0. 0 –1. 0	0.0 – 0.097	0.0966	0.001	0.999
Aluminum	0. 0 – 10	0.0– 0.031	0.0032	0.001	0.996
Nickel	0. 0 – 0.5	0.0 – 45*	89.6	0.4	0.999
Lead	0. 0 – 0.5	0.0 – 41*	82	0.175	0.999
Cadmium	0. 0 – 0.5	0.0 – 88*	176.8	0.35	0.999

• Chart divisions

washed and rinsed with distilled water followed by deionized water. Washed glass ware was placed in 20% Ultrex nitric acid for 48 hours and then rinsed extensively with distilled and deionized water.

Instrumentation

Mineral elemental analysis of plant samples and reference materials were assessed by Atomic Absorption Spectrophotometer Model Hitachi 180-50, S.N. 5721-2, (Hitachi Ltd. Tokyo, Japan) with linear (least square) mode and equipped with a recorder model 056 Hitachi along with Zeeman background correction. The flame absorption mode was used with air acetylene and nitrous oxide-acetylene flame. Other operational parameters including lamp current and wave lengths were those recommended by the manufacturers.

All standards and samples readings were carried out using Atomic Absorption Spectrophotometer in the absorbance mode applying different flames i.e. air acetylene: air and acetylene; nitrous oxide gasses in different compositions. Concentration values for different elements were calculated from the absorbance value by use of linear regression equation. Each regression expression was obtained by employing at least 5 standards concentration made in deionizd water.

Methodology

In case of digestion of plant samples two methods were adopted in present study namely;

Method I: Sample digested with Nitric acid 30% hydrogen peroxide (2:1)

Method II: Sample digested with sulfuric acid 30% hydrogen peroxide (1:1)

Digestion method (I) was preferred and used, because this method is rapid and percentage recovery of all elements is better than method (II).

Decomposition of plant samples

According to the part used, plant source can be divided in to stem, leaves, fruit. All parts of the plants were washed with distilled water and dried at 120 ^{0}C in an electric oven to a constant weight. The dried plant material was then ground to powder. In the next step sample plant and reference sample was weighed into separate digestion flasks and treated with 5ml nitric acid and 5ml sulfuric acid, 5ml nitric acid and was also added in empty flasks which served as blanks for both procedures [20-22]. The flasks were covered with watch glasses and heated to reflux on an electric hot plate at 80° to 100°C. After heating for five minutes, the contents of flasks were treated with additional 5ml of nitric acid and sulfuric acid separately, followed by 2ml of 30% hydrogen peroxide in each flask and the heating at gentle reflux was continued until clear and transparent solution was obtained . The contents of the flasks were cooled and diluted with 2N Nitric acid and filtered through Whatman # 42 paper into 25 ml volumetric flasks, marked as stock sample solutions..

Preparation of Decoction

Each dried part of plant was boiled with deionized water for one hour on electric hot plate. After cooling, it was filtered through Whatman # 42 and kept it as stock sample solution.

The aqueous extract gave +ve test for the presence of glycosides, saponin and water soluble sugars.

Percentage recovery test

The efficiency of extraction method was checked by standard addition method. The duplicate sample of each part of the plant spiked with known amount of metal standard (Fluka Kamica) prior to digestion as described above. Sample blanks were also prepared in both cases. Each result values is mean of at least 3 independent batches prepared in duplicate and each sample analysed at least twice for each elements. The matrix of standards and sample solutions was same by using 2N Nitric acid. The percentage recovery test for different elements by both methods are 97.0 -99 % in range.

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