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Determination and Evaluation of Mineral Constituents of Medicinal Plants used for the Treatment of Asthma and other Ailments by Atomic Absorption Spectrophotometry

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Abstract

Mineral contents have been determined for thirty samples from three medicinal plants (*Hibiscus rosa-sinensis, Salvadora oleoides* and *Euphoria hirta)* . Fifteen essential trace and toxic elements were determined, using atomic absorption spectrophotometer. Ten samples from each plant were collected from the vicinity of Jamshoro and Tandojam Agricultural University and drug stores. The edible parts of all three plants were digested with two known wet asking methods. It was observed that the levels of essential micronutrient Ca, Mg, Fe, Mn and Zn, in all there medicinal plants are found to be (3.491.3-3928.2), (2269.3-3617.3), (3.23-5.42), (6.13-7.33) and (4.22-6.94) mg/100g respectively on dried basis. The efficiency of digesting mineral acid mixtures was checked by certified reference sample of Spinach NBS-1570.

***Keywords*:** Asthma; medicinal plants; Digestion methods; essential metals; decoction.

Introduction

Allergies and asthma are the diseases which are result of an imbalance in the immune system. Vitamins such as A, C and E, minerals like as Zn and Se are antioxidants and are essential to immune system for the health [1]. The extract of medicinal plants raises the body's immune system and lowers the allergies and asthma. Selenium deficiency may causes asthma and its existence helps to utilize vitamin E. Besides that, many substances derived from plant kingdom are still used for commercial medication that may be used for heart disease, all type of pain, asthma, high blood pressure and other diseases [2]. One of the important herbs *Ephedra* was used by Chinese as a traditional medicine for the treatment of as thma and other respiratory diseases for more than two thousand years. The bark extraction of the white oak tree *Qquercus alba*, Quercetin is used for the treatment to all types of sinus reactions, asthma, allergies and hay fever. This extraction of the mentioned bark can also stabilize those cell membranes, which produce histamine that is important for allergic reactions. When we look to the medicines of the developed countries we find that about 25% of all prescribed medicines are derived from medicinal plants [3-6]. Large number of medicinal plants belong to different genera are cultivated in different areas of Pakistan, among which few plants have been studied for minerals contents and large number of plants still remain for the analysis of minerals. However, all essential and trace elements play vital role in medicine and

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therapy in health and disease, therefore, it is essential to analyze all parts of plants for determination of the excellent power of healing for human being in numerous ailments and disorders [7-9].

The intracellular levels of calcium ions are responsible to control muscles contraction smoothly as well as it has important function for asthma. Energy needed for muscles contraction is produced in the presence of calcium, so that ATPase activated to hydrolyze ATP, which provide energy for muscles functioning. Magnesium has also same role to produce energy but this energy is required for the muscles of chest wall and diaphragm for breathing. It also helps to functioning lungs healthy by acting as a bronchodilator and stops bronchial routes from going in to tremor [10-12].

The comparative study of iron was carried out by taking the biological samples such as serum, urine, serum thyroid hormones, thyroid stimulating hormone, free triiodothyroxine, and free thyroxin of goitrous female patients to find out the deficiency of iron in women [13, 14]. Microchip was used for the purification of proteins and magnetic resonance imaging while as metal ions was examined by means of chemical and biological separations [15]. Selenium level from different medicinal plants was found by using the method of Microwave-assisted extraction at different concentrations of nitric acid and hydrochloric acid solutions. The concentration of selenium present in medicinal plants was also examined on highly sensitive equipment electrothermal atomic absorption spectrometry [16]. The concentration of heavy metals in scalp hair of hypertensive patients were observed through wet digestion method by using flame and graphite furnace atomic absorption spectrometry [17].

Fluorescence spectrofluorimetry was used for the determination of aluminum after separating it through solid phase extraction [18]. The analytical study of copper was carried out [19-22] by various researchers. Alarming levels of trace and toxic elements were observed in water and soil of that area where solid waste are dumped [23].

The purpose of this work is to find out the variable concentration of minerals accumulated in various parts of medicinal plants and to compare this concentration by using two different methods i.e. wet ashing and decoction methods.

Materials and Methods

Materials and Methodology

Nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) of analytical grade (Merck, UK) were used for acid digestion of plants samples. Certified 1000 ppm standards reference solution of each metal (Fluka chamicals, UK) was used and aliquots of these were diluted with a solution of 2 N Nitric acid to yield working standards. Milli – Q water was used throughout this experimental work. Certified standard reference (Spinach – NBS 1570) was purchased from the U.S. Dept. of Commerce, National Bureau of standards.

Instrumentation

Measurement of fifteen elements was made by Atomic Absorption Spectrophotometer Hitachi model 180-50, S.N. 5721-2, (Hitachi Ltd. Tokyo, Japan) in linear (least square) mode, equipped, with a model 056 Hitachi recorder.

Sampling

Ten samples from each plant were collected from different locations in Hyderabad including University of Sindh, Jamshoro campus, Sindh Agriculture University, Tandojam campus and from drug stores in Karachi and Hyderabad. All plants were taxonomically characterized including vernacular names, plant habits and their medicinal importance as per literature [24-27], which are frequently used in Asia and other parts of world. Reference samples of *Hibiscus rosa-sinensis* (China Rose), *Salvadora oleoides* (Tooth Brush Tree) and *Euphorbia hirta* (Small Euphorbia or Australian asthma herb) was also identified.

Determination of total elements in medicinal plants

Preparation of plant samples for determination of essential, trace and toxic elements

Triplicate samples of each medicinal plant were washed with distilled water to remove foreign particles dried at 75-80 ^{0}C in oven until achieving the constant weight. All samples were separately ground to powder form and wet digested to destroy organic matrix to leave only minerals for analysis, as Atomic Absorption Spectrophotometry technique required transparent solution, which is free from organic matter [28-30].

Wet ashing methods

Variable ratio of acids were used for digestion of medicinal plants, both are known wet ashing methods as follows:

1. Sulphuric acid, nitric acid and perchloric acid were in ratio (1:1:1)
2. Nitric acid and 30% Hydrogen peroxides were in ratio (2:1)

Three replicate samples of each medicinal plant, reference sample as well as certified samples were weighed in to separate 100 ml conical flasks and treated with 5 ml of nitric acid. Similarly, same amount of identical acid was transferred to an empty flask to use as a blank [31].

All flasks were covered with watch glasses; heated at controlled temperature on a hot plate at 80-100 ^{0}C approximately 50-65 minute until semi dried content was obtained. The flasks were removed from hot plates and cooled down for 15-20 minutes. In addition, further 5 ml of nitric acid was added to each flask and again heated similarly as describe above. Finally, 2-3 ml of 30% hydrogen peroxide was added to flasks and heating was continued for further 50-65 minutes until semidried content obtained. The watch glasses were removed from all flasks and 50 ml of deionized water was added and heated similarly until water reduced to half. All flasks were cooled down and filtered through Whatman # 42 paper to volumetric flasks up to mark (25ml), with adding few drops of 2N HNO_3 as preservative. This stock sample solution was used to measure total elements.

Determination of Elements in Medicinal Plants by Decoction Method

Three replicates of three dried plant samples and reference samples were boiled with deionized water on an electric hot plate for 30-40 minutes. The content was cool down to room temperature and filtered through Whatman # 42 filter paper. This was stock sample solution used as water extract elements.

Calibration procedure

Standard solutions (1000 ppm) from stock solution of fifteen elements i.e. potassium, sodium, magnesium, calcium, manganese, iron, zinc, copper, cobalt, chromium, cadmium, nickel, aluminum, barium, and lead were prepared in 2N nitric acid. The calibration curve of each element was drawn by using Atomic absorption spectrophotometer. Calibration curve was obtained and plot of concentration versus absorbance was constructed using MS Excel software. The calibration summary results of these elements are presented in (Table 1 and 2).

Employing the above-mentioned experimental conditions, the NBS- 1570 Spinach was analyzed to check the accuracy of the procedures. The result is given in Table 3, which are fairly in good agreement with the reference values.

Results and Discussion

Comparison of wet ashing methods

Atomic absorption has been extensively used to analyze trace elements in plant samples. Mineral acids are widely used for sample digestion and preservation. The sample preparation by digestion with different mineral acid in different combinations used to get rapid and better recovery percentage. The decomposition of organic matter must be completed to avoid interference by organic residue and must be soluble in very small volumes of dilute acid. The extraction of plant samples with acid mixture 1 was found to be unsatisfactory. The variation in results of

Zn and Mn was differed from 5 to 8 % respectively as compared to method 2 (Table 1). The variation in results of copper iron and chromium was also observed. The digested samples by method 2 have dark brown color and higher viscosity than samples prepared by method 1. Digestion method No. 1 was rapid and versatile and the percentage recovery of all elements is better than digestion method 2 i.e. 98 - 99%. The color of digesting mixture was light yellow having very low viscosity.

Table 1. **Comparison of wet ashing methods for determination of total metals in Standard Reference Materials NBS- 1570 (Spinach) mg/100g**

Elements	Reference values	Method 2	Method 1
Sodium	----	608 ±2.8	605±3.6
Potassium	3560 ± 5	3568 ± 12	3566±7
Calcium	-----	678± 5.6	675±6
Magnesium	------	145.0 ± 1.8	143±5
Iron	55 ± 2	53 ± 3.5	51±4
Zinc	5.0 ± 0.2	4.8 ± 0.28	4.6±0.45
Cobalt	16.5± 0.6	16.6 ± 0.7	16.5±0.8
Manganese	0.15	0.47 ± 0.05	0.13±0.006
Chromium	0.46	0.44 ± 0.05	0.43±0.007
Nickel	0.6	0.62 ± 0.04	0.61±0.03
Copper	1.2 ± 0.2	1.22 ± 0.3	1.16±0.5
Lead	0.12± 0.02	0.12 ± 0.025	0.117±0.024
Cadmium	0.15	0.148 ± 0.004	0.142±0.005
Barium	87.0±2	86.5±3.5	87±4.0
Aluminum	----	2.56 ±0.56	2.5±0.6

Evaluation of mineral constituents of medicinal plants

The use of alternative therapies is increasing particularly in patients with lung disease. Asthma patients may resort to dietary supplements of herbal remedies because they want to avoid conventional drug therapy or because such treatment does not control their symptoms. The role of zinc is also important in asthma. It is found that zinc level in patients with asthma were significantly lower than were the level in healthy persons [32-35]. The magnesium depletion leads to respiratory fatigue. Magnesium promotes healthy lung function by acting as a bronchodilator, preventing the bronchial passages from going into spasm.

The essential and trace elements found in three medicinal plants are given in (Table 2 and Table 3). The high levels of Na found in whole plant of *Euphorbia hirta* folloed by *salvadora oleoides* (leaves and fruits) and *Hibiscus rosa-sinensis* leaves. Almost same trend was observed for K, Ca and Mg where as an inverse fashion was seen for Fe, Zn, Co, Mn, Cr, Ni, Cu, Pb, Cd, Ba and Al respectively (Table 2). The level of K is present in considerable amount in both forms i.e; total and water extractable form. The total K was found in all medicinal plants on dry basis. The minimum level was found in *Salvadora oleoides* and high amount of this important micronutrient was observed in *Eupharbia hirta*. The levels of Ca and Mg were found elevated in all plants. Maximum level of Ca was found in *Eupharbia hirta* where as minimum in *Salvadora oleoides*. The minimum level of Mg was observed in *Hibiscus rosa-sinensis* Linn and maximum amount found in *Euphorbia hirta*. The Zinc level was high in *Hibiscus rosa-Sinensis* where as low values for Zn were found in *Euphorbia hirta*. The different ranges of iron and manganese were determined in these three plants.

Considerable amounts of other essential trace elements namely copper, iron, zinc and manganese were also present in all medicinal plants which are very important in view point of human health and fitness.

Table 2. **Determination of total metals in medicinal plants, *Hibiscus rosa- sinensis* Linn (Jasun), *Salvadora oleoides* Decne (Jhal khabar) and *Euphorbia hirta* Linn. (Tanbeshari) by two digestion methods**

Elements	*Hibiscus rosa- sinensis* (Leaves)	*Salvadora oleoides*		*Euphorbia hirta* (whole plant)
		(Leaves)	(Fruits)	
Sodium	1121.7 - 1624.3* (1112.4 - 1614.5)	1431.7 - 1682.3 (1421.5 - 1672.4)	1486.3 -1752.2 (1477.5 - 1745.3)	1668.8 - 1926. 9 (1659.3 - 1916.4)
Potassium	1265. 2 - 1621.3 (1255.3 - 1619.2)	2453.8 - 3142.2 (2446.5 -3133.4)	1218.3 -1624.8 (1215.4 - 1621.2)	2939. 2 - 3326.6 (2935.4 - 3322.3)
Calcium	2219. 8 - 2956.6 (2212.4 - 2947.3)	1583.4 - 2135.2 (1589.2 - 2129. 3)	3491.3 - 3928.2 (3487.5 - 3921.4)	2464.5 - 2824.9 (2459.2 – 2818.4)
Magnesium	1228.6 - 1955.3 (1223.7 - 1949.3)	1242. 8 - 1569.4 (1236.4 - 1563.2)	2269.2 - 3617.6 (2259.9 - 3612.3)	2243.3 - 2849.3 (2243.4 – 2844.2)
Iron	4.44 - 6.15 (3.46 - 5.55)	4.26 - 6.83 (3.23 - 5.87)	3.23 - 5.42 (2.27 - 4.38)	4.19 - 6.32 (3.26 - 5.66)
Zinc	3.97 - 6.63 (3.32 - 6.36)	3.23 - 4.26 (3.12 - 4.22)	6.13 - 7.33 (5.98 - 7.28)	2.63 - 3.12 (2.19 - 2.99)
Cobalt	1.94 - 2.48 (1.55 - 2.38)	3.25 – 4.50 (3.12 - 4.47)	1.15 - 2.29 (1.12 - 2.22)	1.85 - 2.19 (1.77 - 2.11)
Manganese	4.36 - 6.87 (4.29 - 6.77)	2.25 - 3.68 (2.19 - 3.61)	4.22 - 6.94 (4.19 - 6.88)	2.52 - 9.28 (2.47 - 9.23)
Chromium	0.364 - 0.568 (0.355 - 0.556)	0.464 - 0.646 (0.455 - 0.641)	0.345 - 0.477 (0.336 - 0.466)	0.334 - 0.483 (0.329 - 0.479)
Nickel	1.54 - 1.73 (1.44 - 1.66)	0.619 - 0.725 (0.612 - 0.719)	0.533 - 0.655 (0.522 - 0.649)	0.519 - 0.612 (0.512 - 0.611)
Copper	0.323 - 0.479 (0.319 - 0.477)	0.212 - 0.267 (0.199 - 0.259)	0.473 - 0.566 (0.467 - 0.555)	0.545 - 0.733 (0.535 - 0.728)
Lead	0.240 - 0.493 (0.236 - 0.488)	0.157 - 0.340 (0.147 - 0.333)	0.348 - 0.715 (0.341 - 0.712)	0.424 - 0.605 (0.416 - 0.699)
Cadmium	0.146 - 0.202 (0.138 - 0.298)	0.061 - 0.075 (0.0542 - 0.068)	0.126 - 0.315 (0.119 - 0.312)	0.329 - 0. 548 (0.324 - 0.539)
Barium	2.36 - 5. 85 (2.25 - 4.65)	2.24 - 2.97 (1.98 - 2.77)	1.12 - 1.28 (1.11 - 1.22)	1.16 - 2.68 (1.12 - 2. 51)
Aluminum	1.46 - 1.75 (1.39 - 1.66)	1.25 - 1.87 (1.15 - 1.66)	3.25 - 4.29 (3.13 - 4.22)	2.15 - 3.66 (2.11 - 3.55)

*Sample digested with method 2
( ) Method 1

Table 3. **Determination of metals in decoction *of Hibiscus rosa- sinensis* Linn. (Jasun), *Salvadora oleoides* Decne. (Jhal khabar) and *Euphorbia hirta* Linn. (Tanbeshari) by atomic absorption spectrophotometer.**

Elements	*Hibiscus rosa- sinensis* (Leaves)	*Salvadora oleoides*		*Euphorbia hirta* (whole plant)
		(Leaves)	(Fruits)	
Sodium	129.6 - 344.2	124.6 - 321.2	861.3 - 945.4	824.2 - 942.7
Potassium	612.9 - 787.2	936.3 -1192.5	829.4 - 997.3	1047.7 - 1214.5
Calcium	748.3 - 907.5	612.4 - 818. 2	989.4 - 1038.3	955.5 – 1064.8
Magnesium	574.8 - 877.9	629.5 - 763.8	992.9 - 1135.5	966.4 – 1217.5
Iron	1.16 - 2.53	1.14 - 1.47	1.16 - 2.19	1.24 - 1.32
Zinc	1.32 - 1.92	1.12 - 1.22	2.63 - 3.28	1.19 - 2.42
Cobalt	1.25 - 1.68	1.62 -2.36	0.023 - 0.029	0.86 - 1.24
Manganese	0.147 - 0.188	0.131- 0.186	1.13 - 2.27	1.16 - 2.25
Chromium	0.142 - 0.164	0.148 - 0.208	0.059 - 0.079	0.028 - 0.122
Nickel	0.244 - 0.496	0.486 - 0.562	0.122 - 0.239	0.137 - 0.239
Copper	0.107 - 0.129	0.062 - 0.084	0.121 - 0.215	0.135 - 0.294
Lead	0.087 - 0.122	0.063 - 0.087	0.108 - 0.159	0.144 - 0.178
Cadmium	0.058 - 0.064	0.024 - 0.035	0.064 - 0.083	0.045 - 0.038
Barium	1.67 - 2.45	0.893 - 1.12	0.539 - 0.925	0.362 - 0. 512
Aluminum	0.299 - 0.472	0.264 - 0.333	1.13 - 1.22	0.226 - 0.382

Conclusion

Three medicinal plants; *Hibiscus rosa-sinensis* Linn. (China Rose), *Salvadora oleoides* Decne (Tooth Brush Tree) and *Euphorbia hirta* Linn (Small Euphorbia or Australian asthma herb) were examined to determine the minerals and essential elements. The studies showed that the level of calcium and magnesium was found to be high in all plants. The considerable amounts of water extractable Ca and Mg were also observed. The level of potassium was also present in considerable amount in both forms i.e. total and water extractable forms. It was reported that when asthmatics patients are put on intravenous fluid repletion in an emergency room situation, potassium is the first component. Zinc, iron and manganese are also present in all three medicinal plants, which support in asthma and many physiological problems.

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