Comparison of Sample Preparation Methods for the Determination of Essential and Toxic Elements in Important Indigenous Medicinal Plant *Aloe barbadensis*

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Summary: The role of elements particularly trace elements in health and disease is well established. In this paper the authors investigate the presence of various elements in very important herb *Aloe barbadensis (Aloe vera)*, it is commonly used in different ailments especially of elementary tract and skin problems.

Four acid digestion methods for the determination of total elements in *Aloe barbadensis* were made. The samples of the plant were collected from surrounding of Hyderabad city, Sindh University, Jamshoro and vouchers specimens were prepared following the standard herbarium techniques.

In total 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 the plant, and in its decoction, using Atomic absorption spectrophotometer Hitachi Model 180-50. The procedure found to be more efficient than other methods of decomposes the biological material in nitric acid and 30% hydrogen peroxide. The validation of the methods was checked with the NBS-1570 (Spanish) as standard reference material. The yielded element values were in close agreement with certified values. The percentage recovery of different elements was found to be better i.e. (98-99%) as compared to other method of digestion. It was noted that level of essential elements was high as compared to the level of toxic elements

Introduction

Medicinal plants play the most important role in traditional medicine, and this system of medicine use elements for curing many diseases [1-4]. The study of the role of elements in increasing the body's resistance to environmental stresses, reducing the risk of diseases is very important topic in last few decades.

Many scientists claim that the regular intake of protective elements supplements in appropriate doses and correct proportions may one day be recognized as an important measure in the maintenance of health and prevention of disease [5, 6].

As human beings in the twenty first century, our body 's metabolic and detoxification systems are under ever increasing stress from foreign chemicals, nutrient depleted foods, and immune damaging infectious agents. In order to control and prevent the inevitable progression of the immune system destruction that these stresses cause, therapy must be multifactorial involving all levels of health, diet and life style. The whole leave of *Aloe vera* will be effective on all levels of this therapeutic program [7, 8].

Elements in biological sources are more efficient than pure elemental status, because of presence of elements as well as presence of vitamins and other physiological active compounds. The whole leaves of *Aloe vera* will be effective on all levels of this therapeutic program.

Pharmaceutical importance

Aloe barbadensis (*Aloe vera*) belongs to family Liliaceae. It is used in long term symptomatic treatment of rheumatoid arthritis, insect bite, stomach disorder , constipation, hemorrhoids, headaches,

mouth and gum diseases, kidney ailment and diabetes [9, 10]. Mucopolysacharides (MPC) [11-13] are found in large amounts in fresh *Aloe vera* which enhance the functioning of the entire immune, repair, detoxification, digestive and elimination systems of the body. The aloe polysaccharides also protect the bone marrow from damage by toxic chemicals and drugs.

The juice of leave contain phenolic compounds such as Aloin, these compounds protect the skin from radiation, burns. Aloe gel is a mild anesthetic, relieving itching, swelling and pain. It also have antibacterial and antifungal activity, and increase the blood flow to wounded areas and stimulate fibroblasts, the skin cells responsible for wound healing [14-16].

Results and Discussion

We live in a world where-in medicines are constantly prescribed for the slightest ailments and new strains of virus and bacteria are increasingly becoming immune to our battle against them. This is partly due to an over medicated, synthetic drug dependent population. It is therefore, little wonder that people are seeking natural means, as well as traditional medicine for the management and healing of illness and pain.

Medicinal plants have for many years provided an important source of natural products, many of which have formed the basis for the development of medicinally important drugs [17]. Even today almost 25 % of all the prescribed medicines in the developed world contain ingredients derived from medicinal plants. The trace elements play a vital role in the medicinal value of a plant and therapy in health and disease. It is imperative to analyze the plants for their trace element contents, which have healing power for mankind in numerous ailments and disorders. Elemental losses are not so common as errors due to contamination, but they can be equally severe, the danger of losses is especially high during the step of drying and ashing.

Methodology

Atomic absorption spectrophotometry has been extensively used to analyse trace and toxic elements in biological samples. A suitable dissolution method for the biological samples to yield homogenous solution is a crucial first step in many spectrochemical analysis.

The main object for the sample preparation by digestion with different mineral acid in different combinations was to get:

1. The decomposition of organic matter must be completed to avoid interference by organic residue.
2. The decomposition product must be soluble in very small volumes of dilute acid
3. The decomposition method should be simple, rapid and economical.

Decomposition procedures

It was observed that digestion of plant samples with Sulphuric acid : Nitric acid : Perchloric acid mixture is found to be unsatisfactory, because digestion with Sulphuric acid and Perchloric acid were carried to completion as evidenced by the complete removal of residual Sulphuric acid and Perchloric acid from the digestion flasks. The Sulphuric acid and Perchloric acid are very corrosive and duration of digestion and removal of extra acid is longer as compared to method 4 as seen in (table 1). The variation in results of Zn and Mn was differed from 5 to 8 % respectively as compared to method 4. The variation in results of copper iron and chromium was also observed (table 1). The sample solution has yellow colour and low viscosity. The percentage recovery was 95- 96%.

In wet ashing method using Nitric acid is efficient but the value of iron, , zinc, manganese and copper (4.45 -6.0, 6.73 -7.98, 2.08-3. 15, 0.621-0.873) is lower as compared to the values observed by method 4 as (5.15 –7.24, 7.34- 8.78, 2.78 – 3.67, 0.686-0.953). A yellow opacity was a consistent physical characteristic of these samples and they have low viscosity

The digestion with sulphuric acid and 30% hydrogen peroxide provided the values of many elements are deviated from value obtained by other methods (table 1). The sample has dark brown colour and higher viscosity than samples of other methods.

Nitric acid with hydrogen peroxide was found to be superior to other acid and acid combination

Table 1: Determination of metals in *Aloe barbadensis (Aloe vera)* by different method of Digestion using Atomic Absorption Spectrophotometer Hitachi Model 180-50 (mg/100g Dried basis)

Elements	Method 1	Method 2	Method 3	Method 4
SODIUM	2573.8-2605.4	2543.5-2580.6	2567.8-2643.5	2608.5-2698.6
CALCIUM	3405.1-4841.9	3385.1-4671.5	3425.1-4895.6	3515.1-4984.9
POTASSIUM	280x.4-3081.6	2696.4-2988.5	2906.4-3141.4	2916.4-3386.6
MAGNESIUM	1416.3-1437.6	1398.5-1419.8	1429.3-1427.5	1489.3-1547.0
MANGANESE	2.08-3. 15	2.26- 3.23	2.58 – 3.34	2.78 - 3.67
ZINC	6.73 -7.98	6.25- 7.85	6.5 – 8.34	7.34- 8.78
IRON	4.45 -6.0	4.40 -6.18	4.78 -6.84	5.15 –7.24
NICKEL	0.241-0.442	0.26 – 0.475	0.25 - 0.457	0.31 – 0.523
COBALT	1.06 -1.14	1.0 -1.054	1.12-1.34	1.24 -1.85
CHROMIUM	0.275-0.368	0.25 – 0.364	0.27 – 0.387	0.312- 0.406
LEAD	0.324-0.585	0.332-0.558	0.314-0.535	0.374-0.615
COPPER	0.621-0.873	0.60-0.793	0.612-0.763	0.686-0.953
CADMIUM	0.062-0.09	0.06-0.86	0.067-0.097	0.072-0.12
BARIUM	2.46-3.84	2.14-3.44	2.78-3.97	2.48-4.13
ALUMINIUM	2.65-3.48	2.49-3.18	2.87-3.35	2.93-3.86

digestions. Although other acid combination proved satisfactory, but they were not as desirable due to their inherent dangers and increased potential for contamination. Extreme caution was taken during the digestion to prevent too vigorous acid reaction such as initial HNO_3 and $HClO_4$ reactions with high organic materials.

Digestion method No. (4) was preferred and used for investigation of elements in samples. Because this method is rapid and versatile, the percentage recovery of all elements is better than other digestion methods i.e. 98 - 99%. Mean values for all elements is differed less than 1% from the certified values. The coefficient of variation was less than 2 % for different elements The color of digesting mixture was light yellow having very low viscosity.

The considerable amount essential and trace elements are present in decoction of the plant (table 2), these elements in medicinal plant *Aloe vera* may be directly or indirectly helpful in the management of many diseases especially skin diseases.

Essential, trace and toxic elements found in Aloe vera

The medicinally important plant *Aloe vera* leaves are filled with gel, 96% water with the other 4%, containing 75 known substances [14]. Important mineral elements such as Na, K, Mg, Mn, Zn, Co, Cr, and Fe are present in total as well as in decoction of *Aloe vera* (Table1 and 2) and these elements may be directly or indirectly helpful in the management of many diseases.

The considerable amount of potassium, calcium, and magnesium (2916.4 -4984.9, 3515.1-4984.9 and 1489.3-1547.0) respectively are present in *Aloe vera*. Potassium is important for reducing blood pressure and also increasing blood circulation, as well as preventative aid on general heart health.

Table 2: Comparative values of different elements as total and available form in decoction, of *Aloe barbadensis (Aloe vera)* using Atomic Absorption Spectrophotometer Hitachi Model 180-50 (mg/100g Dried basis)

Elements	Total elements Range	Elements in decoction Range
Sodium	2608.5-2698.6	860.0-929.5
Calcium	3515.1-4984.9	553.1-768.6
Potassium	2916.4-3386.6	699.8-724.4
Magnesium	1489.3-1547.0	160.1-178.7
Manganese	2.78 – 3.67	0.93-1.24
Zinc	9.34 - 12.78	4.40-5.54
Iron	6.15 – 9.24	2.44 -3.67
Nickel	0.31 – 0.523	0.042 - 0.074
Cobalt	1.24 -1.85	0.161- 0.232
Chromium	0.312- 0.406	0.137- 0.275
Lead	0.374 - 0.615	0.063 - 0.087
Copper	0.686 - 0.953	0.031- 0.062
Cadmium	0.072 - 0.12	0.024- 0.036
Barium	2.48 - 4.13	0.523 - 0.597
Aluminium	12.93 - 17.86	2.14 -3.65

Calcium help in the transporting of long chain fatty acid which aid in prevention of heart diseases, high blood pressure and other cardiovascular diseases. Magnesium work with Ca to help transmitting nerve impulse in the brain. It also has calming effect and works on the nervous systems of the people with depression.

Metals and their compounds have been used since ancient times for their therapeutic as well as cosmetic effects on skin. As Aloe gel applied on the wound it relieving itching, and it is also antibacterial and anti fungal. The considerable amount of zinc and aluminum (9.34- 12.78), (12.93 - 17.86) respectively are present, their salts are used in skin infections as disinfectant and cleansing agent. The lotions of zinc has a soothing and cooling effects on the skin. So the elements present in *Aloe vera* may play role for skin problems.

Experimental

Equipment

A Hitachi 180-50 Atomic Absorption Spectrophotometer equipped with standard hallow cathode lamps and air-acetylene and Nitrous oxide-acetylene flames was used for absorption measurements of fifteen elements.

Reagents:

All metals standards (1000µg/ml) were purchased from Fluka kamica. The internal standard was also prepared by dissolving pure metal of

analytical grade or metal compounds in 2N Ultrex grade of Nitric acid in deionized water. Ultrex grade of concentrated Nitric acid (16N), Sulphuric acid (18N), Hydrochloric acid (12N) and Perchloric acid (12N) and Hydrogen peroxide (30%aqueous solution) was obtained from Merck.

Material

Plant samples

Five to ten samples of *Aloe vera* , were collected in October 2000, from different area of Hyderabad city and Sindh University Jamshoro campus. Reference vouchers specimens were prepared following the standard herbarium techniques. Reference samples was identified with help of Botanists of Sindh University, Jamshoro, Pakistan. Certified sample of spinach NBS-1570 was used as reference material.

Methodology

Four methods were adopted for the digestion of triplicate of five samples collected from different areas . Each sample of *Aloe vera* was dried at 100 °C separately.

1. Sample digested with sulphuric acid; Nitric acid; perchloric acid (1:1:1)
2. Sample digested with Nitric acid
3. Sample digested with sulphuric acid: 30% hydrogen peroxide(2:1)
4. Sample digested with Nitric acid: 30% Hydrogen peroxide (2:1)

Digestion method No. (4) was preferred and used for investigation as for this method is rapid and percentage recovery of all elements is better than other digestion methods i.e. 98 - 99%.

Procedure

The plants were washed with distilled water and dried at 80-100 °C in electric oven to a constant weight. The dried plant material was then ground to powder.

In next step each part of sample plants and reference sample was weighed into separate flasks and treated with 5ml nitric acid [18-20]. The flasks were covered with watch glasses and heated to boil on an electric hot plate at 80 to 100 °C. After heating for one hour, the contents of flasks were treated with additional 5ml of nitric acid, followed by 2ml of 30% hydrogen peroxide, and the heating was continued for another hour. The watch glasses were removed from top of the flasks, and heating was continued until the volume of the contents was reduced to semi dried mass, the contents of the flasks were cooled, diluted appropriately with 2N HNO_3 and filtered through Whatman # 42 paper into volumetric flasks marked as stock sample solutions for the determination of metal ions.

In all cases two blank samples containing only the wet ashing reagents for each group were also prepared, for the assessment of possible contamination level in the procedure adopted.

Preparation of decoction

Each dried duplicate sample of each batch collected from different places were ground in small pieces and boiled with deionized water for one hour on electric hot plate. After cooling, filter through Whatman # 42 and keep it as stock sample solution.

The aqueous extract gave +ve test for the presence of glycosides, saponin and sugar only which are water soluble, other water insoluble organic compounds were absent

Determination of elements

Sample solution were aspirated into AAS and absorbance measurements were made for each elements using optimum instrumental conditions for different flame atomization modes.

Working standard solutions of Aluminum, Calcium, Cadmium, Cobalt, Chromium, Copper, Silver, Iron, Lead, Manganese, Magnesium, Nickel, Potassium, Sodium, and Zinc were prepared from stock standard solution (1000 ppm), in 2N nitric acid and calibration curves were drawn for each element using Atomic absorption spectrophotometer Hitachi model 180-50. The calibration curves obtained for concentration VS absorbance data were statistically analyzed using fitting of straight line by least square method. These fifteen elements were determined in medicinal plants. The results of these measurements are presented in table 1and 2

Reference standards from Fluka kamika were also run in parallel for inter calibration of our own prepared standards. A blank reading was also taken and necessary correction were made during the calculation of percentage concentration of various elements. Each result value is mean of at least five independent batches prepared in triplicate, and each sample analysed at least twice for each element.

Percentage recovery test

Percentage recovery test was also performed by digesting three standards of each fifteen elements spiked with samples and digested by each method described above in the same manner as the samples of alovera were digested, so it experiences the same matrix effect as the analyte.

Conclusions

The author emphasize that digestion of large test portions of plant materials using these procedures is not straight forward and requires extraordinary care and safety precautions. The main objectives for the sample preparation of biological samples by digestion with different mineral acid mixture was to get , the decomposition of organic matter must be completed to avoid interference by organic residue , and the decomposition method should be simple rapid and ecnomical.

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