

Comparison of Wet Ashing Methods for Medicinal Plants, *Celosia argentea* and *Cubeba officinalis* by Atomic Absorption Spectrophotometer

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Summary: The role of elements particularly trace elements in health and disease is now well established. In this paper we investigate the presence of various elements in two herbs *Celosia argentea* and *Cubeba officinalis*. The both herbs are used for liver ailments and other diseases.

Two wet acid digestion methods were used for the determination of total elements such as Zn, Cr, K, Mg, Ca, Na, Cu, Fe, Pb, Al, Ba, Mn, Co, Ni, and Cd in both plants. Sample digested with Nitric acid and 30% Hydrogen peroxide, is found to be more efficient to decompose the biological material as compared to the sample digested with sulphuric acid and Nitric acid mixture. It has been observed that, the level of essential elements was found to be high as compared to the trace and toxic elements. The considerable amount of amount of Zn, Fe, Co was found in these plants. The considerable amount of water extractable elements was also present in both plants.

Introduction

From prehistoric times a variety of medicinal plants are being used as potential drugs [1-4]. Herbs had been used by all culture through out history. It was an integral part of the development of modern civilization. Many patients have been turning to alternate medicine in search of the relief of which routine present day medical care has not been able to give them. The medicinal plants is also the important source of new medicine for the treatment of diseases like cancer, malaria, disorders of cardiovascular, central nervous system and many more.

Liver disease are major health problem in developing and under developing countries. Thus liver disease has become a major cause of mortality in the world. The cure of these liver disorders remain elusive in the allopathic system of medicine. While traditional medicines claim that they have effective modalities for the treatment of liver diseases.

The safety of Unani hepato protective herbal preparations *jigrine*, prepared from fourteen medicinal herbs. The other unani herbal preparation liv-52 contains *Capparis spinosa*, *Cichorium intybus*, *Solanum nigrum*, *Cassia occidentalis*, *Terminalia arjuna*, *Achillea millefolium* and *Tamarix gallica*. These formulations are effective in reduction of serum

bilirubin. Both these herbal preparation give relieve symptoms and signs of viral hepatitis [5].

A survey by some researcher that 160 phyto-constituents from 101 plants belonging to 52 plant families have hepatoprotectant report by Central Drug Research Institute, Lucknow (India). *Phyllanthus amarus* was another most important plant selected for clinical trials [6-8]. All these medicinal plants contain a variety of chemical constituents like phenols, essential oils, glycosides, flavonoids, alkaloids and xanthines.

The powder of *Eclipta alba* is believed to be the best drug for treating liver cirrhosis and infectious hepatitis. *Eclipta alba* and *Wedelia calendulacea* are widely used in India for jaundice and other liver and gall bladder ailments. One recent study showed that a liquid extract from fresh *Eclipta* leaves was effective in vivo in preventing acute carbon tetrachloride-induced liver damage in guinea pigs [9].

It is now well established that elements, particularly trace elements, play a vital role to assess medicinal value of a plant and therapy in health and disease. The traditional medicine practitioners (Hakim, Herbalists) are using zinc compound for

curing different disease. The treatment of liver and cardiac disorders using Unani herbo-metallic preparations e.g., Kushta Jast [10].

Zinc supplementation to the patients of liver disease may prove helpful in the fast recovery of many of these diseases. Present study has been undertaken in our Laboratories to see the commonly occurring elements among two medicinal plants used for curing the liver disease. The efficiency of medicinal plants described here for the treatment of liver disorders may at least in part be due to the presence of high zinc level and other beneficial metals in both medicinal plants.

Results and Discussion

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.

In wet ashing method using nitric acid and sulphuric acid was efficient but the extreme caution was taken during the digestion to prevent too vigorous acid reaction. It was observed that digestion of samples with sulphuric acid: nitric acid mixture is found to be unsatisfactory because the digestion with sulphuric acid; nitric acid were carried to completion as evidenced by the complete removal of residual sulphuric acid from the digestion flask. The sulphuric acid is very corrosive and duration of digestion and removal of extra acid is longer as compared to method 2.

Nitric acid with hydrogen peroxide was found to be superior to other acid and acid combination digestions. Although other acid combination proved satisfactory, but they were not as desirable due to they're inherent dangers and increased potential for contamination. The percentage recovery of all elements is better than other digestion methods i.e. 98 - 99%. Mean values for all elements are differed less than 1% from the certified values [11]. The

coefficient of variation was less than 2 % for different elements. The color of digesting mixture was light yellow having very low viscosity. The variation in results of Zn and Mn was differed from 5 to 8% respectively as compared to method 1 (Table 1) The variation in results of copper, iron and chromium was also observed. The sample solutions obtained by method I have yellow color and low viscosity. The percentage recovery was 96-97%.

Table-1: Comparison of wet ashing methods for the determination for total elements in two medicinal plants, (*Celosia argentea* Linn. and *Cubeba officinalis* Miq.) by using atomic absorption spectrophotometer

Elements	<i>Celosia argentea</i>		<i>Cubeba officinalis</i> (Fruits)
	(Leaves)	(Seeds)	
Sodium	1345.2 - 2114.7 (1339.5-2095.5)	1272.7 - 1857.6 (1265.5 - 1751.4)	2276.4 - 2831.6 (2267.6 - 2721.7)
Potassium	1122.1 - 2126.2 (1119.6 - 2234.9)	1175.7 - 1217.8 (114.5 - 1210.9)	4226.8 - 4349.1 (4218.7 - 4338.4)
Calcium	1513.2 - 2241.4 (1510.5 - 2131.9)	1126.5 - 1482.4 (1118.5 - 1472.8)	1317.2 - 1691.2 (1310.9 - 1684.8)
Magnesium	1744.4 - 1922.2 (1732.7 - 1911.6)	1378.4 - 1598.7 (1369.5 - 1589.6)	1244.5 - 1583.4 (1236.7 - 1578.8)
Iron	5.36-9.45 (4.44-8.55)	8.66 - 10.98 (8.54 - 10.19)	5.26 - 8.75 (4.88 - 7.95)
Zinc	9.84 - 12.45 (5.71 - 11.90)	15.62 - 18.22 (14.81 - 17.24)	17.26 - 19.84 (16.39 - 18.95)
Manganese	0.425 - 0.578 (0.315 - 0.561)	2.18 - 3.33 (1.85 - 2.94)	0.244 - 0.399 (0.232 - 0.386)
Cobalt	0.585 - 0.677 (0.515 - 0.656)	0.677 - 0.957 (0.596 - 0.921)	0.844 - 0.949 (0.789 - 0.879)
Chromium	0.281 - 0.522 (0.271 - 0.512)	0.246 - 0.352 (0.239 - 0.341)	0.181 - 0.266 (0.172 - 0.257)
Copper	0.351 - 0.665 (0.321 - 0.615)	0.347 - 0.575 (0.337 - 0.565)	0.383 - 0.537 (0.365 - 0.517)
Nickel	0.351 - 0.595 (0.341 - 0.587)	0.276 - 0.382 (0.266 - 0.371)	0.388 - 0.538 (0.378 - 0.526)
Lead	0.416 - 0.891 (0.398 - 0.871)	0.364 - 0.472 (0.355 - 0.462)	0.443 - 0.538 (0.433 - 0.524)
Cadmium	0.316 - 0.796 (0.310 - 0.786)	0.024 - 0.039 (0.021 - 0.037)	0.344 - 0.528 (0.338 - 0.518)
Barium	0.843 - 1.34 (0.823 - 1.23)	2.42 - 3.26 (1.75 - 2.86)	2.45 - 3.84 (2.25 - 3.42)
Aluminum	12.84 - 19.58 (11.89 - 18.95)	3.71 - 4.51 (3.12 - 3.98)	9.45 - 14.66 (8.96 - 14.05)

Liver disease is major health problems in developing countries. Fatty liver, alcoholic hepatitis and especially the cirrhosis are the main disorders of the liver [12]. The cure of these liver disorders remains elusive in the allopathic system of medicine. While traditional medicines claim that they have effective modalities for the treatment of liver disease, the unani (Greco-Arab) system of medicine really seems to have broken through.

The traditional medical practitioners used different metal compounds for curing different diseases [13]. The effectiveness of medicinal herbs is due to large number of constituents. The presence of high level of essential and trace elements in the herbs

may contribute toward their effectiveness the traditional system of many of them have been proved to be safe and effective.

It was observed that considerable amount of essential elements are present in total as well as water extractable ions which are more effective in liver ailments such as Ca, Mg, Fe, Zn, Co and Mn (Table 1 and 2).

Table 2: Determination of metals in decoction of medicinal plants, *Celosia argentea* Linn (surwali), and *Cubeba officinalis* Miq. (Kabab- Chini) by atomic absorption spectrophotometer (mg/100g on dried basis)

Elements	<i>Celosia argentea</i>		<i>Cubeba officinalis</i>
	(Leaves)	(Seeds)	(Fruits)
Sodium	519.4 - 712.5	424.3 - 678.2	727.3 - 871.1
Potassium	521.2 - 711.9	663.4 - 872.5	739.1 - 937.3
Calcium	418.3 - 713.9	248.5 - 322.8	789.9 - 829.8
Magnesium	469.7 - 851.6	444.5 - 589.5	712.3 - 788.2
Iron	1.24 - 3.85	1.16 - 1.22	2.64 - 3.74
Zinc	3.50 - 4.82	3.75 - 4.83	3.75 - 4.84
Manganese	0.237 - 0.241	1.55 - 1.74	0.166 - 0.186
Cobalt	0.171 - 0.341	0.257 - 0.474	0.166 - 0.199
Chromium	0.139 - 0.278	0.174 - 0.228	0.172 - 0.184
Copper	0.153 - 0.178	0.144 - 0.273	0.122 - 0.252
Nickel	0.082 - 0.177	0.116 - 0.178	0.125 - 0.156
Lead	0.141 - 0.157	0.174 - 0.182	0.099 - 0.134
Cadmium	0.141 - 0.195	0.018 - 0.027	0.089 - 0.128
Barium	0.123 - 0.364	1.24 - 1.36	1.12 - 1.26
Aluminum	4.38 - 6.55	1.12 - 1.18	1.46 - 2.64

The presence of total Fe, Co and Zn is effective in curing the liver problems. Impaired zinc metabolism is well documented in both acute and chronic liver disorders [14].

Patients with liver diseases can develop Zn depletion through inadequate dietary intake, impaired absorption or increased zinc excretion [15]. Zinc rich herbs appears to be helpful in protein synthesis, repair and regeneration of hepatic cells and has a protective and therapeutic potential in liver disease, it also induce metallothionine to afford a protection against liver damage [16]. It is also reported that Zn supplementation to the patients of liver disease may prove helpful in the fast recovery of many of these diseases.

Experimental

Material

Plant samples

Five samples of each plant was collected from different areas of Hyderabad city, Sindh univer-sity

jamshoro campus, Tandojam nd from Authentic drug dealers, Karachi, Peshawar. Reference samples was identified by coworker of Botany department of Sindh University Jamshoro, Pakistan.

Cubeba officinalis Miq., belong to family Piperaceae, called in english (*Cubeb*s, *Tail pepper*) and locally (*Kabab-Chini*). This plant is useful in liver disease and have many other pharmacological actions such as demulcent, deterrent, deobstruent, carminative, diuretic, emmenagogue, antiseptic, expectorant and stimulant [17,18].

Celosia argentea Linn. Belong to family Amaranthaceae, called in english (*Quail grass*) and locally (*Surwali*). It is useful for many liver ailments and also having many other beneficial effects. The leaves are antipyretic, aphrodisiac, and vulnerary. The seeds are useful in, anthelmintic, inflammation, skin eruption, swellings and boils, flowers are used for amenorrhoea, dysentery, haemoptysis, haemorrhoids and leucorrhoea [19].

Reagents and calibration

The supra pure nitric acid (65% w/v) and hydrogen peroxide (35% w/v) reagents (Merck), high-purity water (electrical resistivity $> 10\text{m}\Omega\text{ cm}$) was produced with a Milli-Q system Millipore, MA, USA).

Calibration was obtained with external standards. The standards solutions were prepared by diluting a 1000mg/l multi element solution (ICP Multi element standard iv, Merck, Darmstadt, FRG) with the same acid mixture used for sample dissolution. Glass ware were cleaned by soaking with the contact over night in a 10% (w/v) nitric acid solution and then rinsed with deionized water.

Instrumentation

Atomic absorption spectrophotometer, Hitachi Model 180-50, S.N. 5721-2, (Hitachi Ltd. Tokyo, Japan) was used for the determination of mineral elements. The spectrophotometer out put was connected to a Hitachi recorder 056 and back ground 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 recorded by the manufacturers.

Methodology

1. Sample digested with sulphuric acid:
Nitric acid (1:1)

2. Sample digested with Nitric acid : 30%
Hydrogen peroxide (2:1)

Digestion method No. (2) was preferred because this method is rapid and percentage recovery of all elements is better than other digestion methods i.e. 98 - 99% [20].

Sample preparation

The both plant samples (*Cubeba officinalis* and *Celosia argentea*) were washed with deionized water to remove dust particles and dried at 110°C for two hours. The dried plant samples were ground to fine powder and used for wet acid digestion methods.

Decomposition of plant samples

The duplicate two sets of each part of plant samples and reference samples were weighed into separate digestion flasks and treated with 5 ml nitric acid and 5 ml sulfuric acid. In other set of samples treated with nitric acid and hydrogen peroxide (2:1), side by side 5 ml of each mineral acid set was added in empty flasks, which served as blanks for both procedures [21-23]. The flasks were covered with watch glasses and heated to reflux on an electric hot plate at 80° - 100°C. After heating for five minutes, the contents of flasks were treated with additional 5ml of mineral acid mixtures 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 2 N Nitric acid and filtered through Whatman filter paper # 42 into 25 ml volumetric flasks, marked as stock sample solutions.

Preparation of decoction

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

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

Elemental analysis

All standards and samples readings were conducted with the Atomic absorption Spectrophotometer Hitachi model 180-50, in the absorbance mode using different flames i.e., air "acetylene and acetylene nitrous oxide gasses in different compositions. Concentration values for different elements were calculated from the absorbance values by using linear regression equation. Each regression expression was obtained by employing at least five standards made in deionized water. A blank reading was also taken and necessary correction was made during the calculation of percentage concentration of various elements.

Percentage recovery test

The efficiency of extraction method was checked by standard addition method. The duplicate samples of each plants were spiked with known amount of metal standards prior to digestion as described above. Each resultant value is the mean of at least 3 independent batches prepared in duplicate, and each sample analysed at least twice for each element. The matrix of standards and sample solutions was same by using 2 N HNO₃. The percentage recovery test for different elements by two digestion methods are 97.0 - 99 % in range

Conclusions

The wet acid digestion method with mineral acid mixture (nitric acid and hydrogen peroxide) is efficient, rapid and recovery is better as compared with other acid mixture. The efficiency of medicinal plants described here for the treatment of liver disorders may at least in part be due to the presence of high zinc level and other essential elements in the medicinal plants.

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