Determination of Mineral Constituents in Medicinally Important Plants *Nigella sativa, Myristica fragrans* Houtt *and Allium sativum* Linn. Using Atomic Absorption Spectrophotometry

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Summary: Medicinal properties have been attributed to a large variety of plants cultivated in different parts of Pakistan. Only few of these plants have been analyzed for their mineral content and large number remain unanalyzed. It is imperative to analyse the plants for their trace element content, which have healing power for mankind in numerous ailment and disorders.Present study has been undertaken in our laboratories to see the commonly occurring elements among some medicinal plants and in its decoction such as *Nigella sativa, Allium sativum, Myristica fragrans houtt,* for fifteen elements has been carried out using atomic absorption spectrophotometer. Two procedures were employed for decomposition of organic matter present in the plant samples. The result obtained from both the procedures was compared with each other. For all three plant samples relatively higher results were obtained when samples were decomposed with Nitric acid and Hydrogen per oxide mixture. The level of essential elements was to be found high as compared to concentration of toxic elements. The level of important elements such as Zinc, Iron was present in considerable amount.

Introduction

Trace elements are crucial to virtually all biochemical and physiolcgical processes in plant, animal and human beings. Of these iron, zinc, cobalt, manganese, nickel, copper, chromium and molybdenum are now thought to be essential for animal life [1,2,3].

Medicinal properties has been attributed to a large variety of plants [4,5] cultivated or naturally grown in different parts of Asian countries. For example, garlic has been advised for various diseases, worms, cancer, infections and in the fields of cardiovascular disease [6]. In Pakistan and several other developing countries garlic has been used as an effective herbal medicine product. In Germany, garlic products are the highest selling over the counter drugs.

A balanced diet provides sufficient intake of mineral salts. Those supplied by the medicinal plants are associated with the other active principals and the medicinal action of such mineral constituents have supplementary importance [7,8].

In order to understand the use of medicinal plants and their modes of action, it is necessary to know some thing about their active constituents [9,10].

Pharmacoloical Importance

Allium sativum commonly called Garlic, belong to family Lilaceae [11,12] have many pharmacological actions such as *disinfectant* and diuretic. Garlic is anticholesterolemic agent [13,14], Blood purifier [15], and alternative tonic for the gastro-intestinal tract and for the diseases of lungs. Garlic contains thiosulfinates (allicin) essential to most of the microbial and hypocholesterolemia effects of garlic, and antioxidant effects. Fruit of *Allium sativum* is used for analysis [16,17,18,19].

Myristica fragrans commonly called Jaiphal belong to family Myristicaceae have many Pharmacological actions such as aromatic, carminative, mildly irritant, stimulant, astringent, aphrodisiac [20]. The oil of Jaiphal with sesame oil useful as massage against disease of cold origin e.g. Paralysis, facial paralysis, rheumatism. Seed kernel of *Myristica fragrans* is used for analysis.

Nigella sativa commonly called Kolonji belongs to family Ranunculaceae. Seeds of kalonji were used in the indigenous [21] system of medicine. It has many pharmacological actions such as a antiphlegmatic, stimulant, carminative, [22] detergent, diuretic, emmenagogue, stimulant expectorant, anticancer, antifertility stomach and liver tonic. According to an Arab Proverb it is said

Table 1: Determination of Total Metals in *Nigella sative, Myristica fragrans* and *Allium sativum* and in Their Reference Samples by Atomic Absorption Spectrophotometer Hitachi Model 180-50

Elements	Nigella (Seed)	Jaiphal (fruit)	Garlic
Sodium	120.6 - 250.5	195.7- 236.3	107.8 -162.3
	(159.69±3.4)*	(215.67± 2.4)	(98.04 ± 1.6)
Calcium	370.06 - 781.25	431.74 - 503.70	635.6 -720.8
	(665.5± 3.4)	(414.4±3.02)	(682.21±7.92)
Potassium	93.70- 113.3	99.61-129.13	138.98 -190.16
	(120.5±2.8)	(119.61± 3.43)	(178.98± 2.6)
Magnesium	(130.1- 160.4)	110.3 - 151.70	156.6 – 240.5
	(153.67± 2.84)	(140.45 ±3.6)	(224.01± 2.4)
Zinc	2.4 – 5.26	2.01 - 2.56	6.25 - 10.15
	(3.83 ±0.2)	(2.82 ± 0.32)	(9.36 ± 1.20)
Iron	7.95- 8.58	3.27- 3.90	180.6 - 220.8
	(9.24 ±1.24)	(4.0 ±0.64)	(245.5 ± 4.67))
Manganese	2.72 - 3.97	4.75 - 6.56	2.42- 3.52
	(4.58 ± 0.5)	(7.12 ± 1.26)	(2.57 ±0.084)
Nickel	0.34 - 0.37	0.47 - 0.55	0.410 - 0.490
	(0.42± 0.028)	(0.529± 0.11)	(0.487 ±0.037)
Cobalt	0.259 - 0.343	0.187- 0.274	0.347- 0.485
	(0.30 ± 0.04)	(0.32 ±0.064)	(0.42 ± 0.062)
Chromium	0.144 – 0.176	0.135 - 0.164	0.204 - 0.584
	(0.21 ±0.044)	(0.174 ±0.06)	(0.632 ±0.046)
Lead	0.143 - 0.180	0.148 - 0.197	0.256- 0.368
	(0.226 ±0.038)	(0.165± 0.051)	(0.326 ± 0.063)
Copper	1.14- 2.46	0.538- 0.64	0.278 - 0.38
	(2.87± 0.23)	(0.724 ± 0.14)	(0.416 ±0.041)
Cadmium	0.107 – 0.242	0.081 - 0.17	0.0352 - 0.444
	(0.26 ± 0.02)	(0.14 ±0.035)	(0.386 ± 0.015)
Aluminum	1.56 - 3.12	3.12-14.06	8.5 - 8.05
	(3.25± 0.45)	(12.87 ± 0.65)	(9.67 ± 0.74)

* Values in parentheses are due to reference sample

that in the black seed is the medicine for every disease except death, and it is used for analysis.

Results and Discussion

Decomposition Procedures

Identical reference samples of each plant were analysed after decomposition by two wet digestion method. Although a number of methods have been recommended for destroying organic matter to produce soluble inorganic salts which is preliminary requirement of atomic absorption technique. The digestion of plant materials by both methods was completed in 1-2 hours. As revealed by the table 3 showing the results, found in case of method 1 indicate the low value of lead, iron, aluminum, chromium and nickel as compared to values obtained by method 2.

Elemental Concept

In developing nations, alternative medicine could help to solve many of the common as well as peculiar medicinal problems facing the people. The role of elements in health and disease is now an established fact. Our goal is to confirm whether the elements occurring in a plant are responsible the therapeutic action or it is due to some organic matter and if established that it is due to partially or fully due to elements, then we can prevent diseases by giving those elements in other form also.

The distribution of the elements in the various genera species of plants will highlight the knowledge of the distribution of certain useful trace elements and their availability from medicinal plants. In all three samples the level of essential elements such as Na, K, Mg, Ca is high except level of Na is low in garlic. The garlic is beneficial in lowering cholesterol and hypotensive, so the nature help to mankind the level of sodium is low in consumable part of garlic. Level of zinc and iron is also high in all three plants especially in garlic, which are beneficial in many diseases such as blood anemia, diabetic and hypercholeterolemia. The level of other essential trace elements is present in considerable amount.

Unani crude drugs are mostly used as 'Joshanda' Persian word prepared by boiling, which is aqueous extract containing some water soluble organic principles such as glycoside, saponin and

Table 2: Determination of Metals in Decoction of *Nigella sative, Myristica fragrans* and *Allium sativum* by Atomic Absorption Spectrophotometer Hitachi Model 180-50

Elements	Nigella (Seed)	Jaiphal (fruit)	Garlic (fruit)
Sodium	92.3 - 114.9	83.35-98.67	77.04 - 82.30
	(120.5 ± 2.5)[&]	(92.6 ± 3.45)	(75.8 ±2.6)
Calcium	105.06-167.92	114.04-123.02	142.21-167.92
	(147.6 ± 3.56)	(119.65 ± 4.6)	(158.6 ±5.78)
Potassium	47.9 - 61.68	58.36 - 67.21	77.7- 87.9
	(76.5 ± 3.7)	(65.8 ± 3.2)	(79.85 ± 5.3)
Magnesium	54.56 - 128.63	71.45 - 73.36	23.01- 32.14
	(114.65 ± 3.8)	(72.75 ± 1.8)	(28.75 ±1.56)
Zinc	2.03 - 3.00	1.65 - 2.32	1.06 - 1.28
	(2.76 ± 0.45)	(1.95 ± 0.5)	(1.34 ± 0.23)
Iron	4.36 - 5.24	1.57 - 2.38	2.80- 3.74
	(4.84 ±0.78)	(1.74 ±0.42)	(2.98 ± 0.34)
Manganese	1.58 - 2.15	1.96-2.66	0.925-0.1.52
	2.34 ±0.35)	(2.18 ±0.3)	(0.1.71 ±0.21)
Nickel	0.096- 0.118	0.129- 0.118	0.21- 0.287
	(0.23 ± 0.05	(0.25 ±0.063)	(0.223 ±0.08)
Cobalt	0.159 - 0.243	0.064- 0.105	0.248 - 0.347
	(0.28 ± 0.025)	(0.12 ±0.024)	(0.264 ± 0.032)
Chromium	0.044- 0.094	0.081- 0.16	0.079 - 0.124
	(0.12 ±0.032)	(0.19 ±0.02)	(0.106 ±0.018)
Lead	0.049- 0.075	0.0265- 0.051	0.059- 0.063
	(0.084 ±0.012)	(0.065 ±0.01)	(0.078 ±0.012)
Copper	0.538- 0.642	0.12 - 0.245	0.14- 0.162
	(0.716 ±0.034)	(0.18± 0.02)	(0.154 ±0.02)
Cadmium	0.0246- 0.0302	0.046- 0.056	0.034- 0.0415
	(0.04 ±0.006)	(0.058 ±0.006)	(0.043 ±0.005)
Aluminum	1.25 – 1.65	1.85 – 2.565	0.684 – 0.824
	(1.52 ±0.15)	(2.625 ±0.13)	(0.768 ±0.041)

& Values in parentheses are due to reference sample

Table 3:Comparative data of total elements of Reference samples of Nigella sativa, Myristica fragrans, and Allium sativum using two method of digestion by Atomic Absorption Spectrophotometer

Elements	Nigella (Seed)	Jaiphal (fruit)	Garlic
Sodium	156.5± 3.8)	206.38±3.6	96.3 ± 1.45
	(159.69±3.4)*	(215.67± 2.4)	(97.04 ± 1.6)
Calcium	658.25±2.8	413.7±3.7	735.6 ±5.8
	(665.5± 3.4)	(414.4±3.02)	(742.21±7.92)
Potassium	121.7 ±1.8	120.6± 4.48	176.8 ±3.76
	(120.5±2.8)	(119.61± 3.43)	(178.98± 2.6)
Magnesium	(163.6 ±3.4)	160.3 ±5.4	240.45 ±3.2
	(173.67± 2.84)	(160.45 ±3.6)	(243.01± 2.4)
Zinc	2.68 ± 0.5	2.75 ± 0.28	10.45 ± 1.34
	(2.83 ±0.2)	(2.82 ± 0.32)	(11.36 ± 1.20)
Iron	8.87 ± 0.45	3.30 ±0.48	227.8 ± 3.4
	(9.24 ±1.24)	(4.0 ±0.64)	(245.5 ±4.67)
Manganese	4.17 ± 0.64	6.85 ±1.34	0.546 ± 0.1
	(4.58 ± 0.5)	(7.12 ± 1.26)	(0.571 ±0.084)
Nickel	0.36 ±0.03	0.45 ±0.14	0.426 ±0.05
	(0.42 ±0.028)	(0.529± 0.11)	(0.487 ±0.07)
Cobalt	0.34± 0.06	0.286 ± 0.05	0.46 ±0.084
	(0.39 ± 0.04)	(0.32 ±0.064)	(0.52 ± 0.092)
Chromium	0.184 ±0.06	0.148 ± 0.075	0.576 ± 0.038
	(0.21 ±0.044)	(0.174 ±0.06)	(0.632 ±0.046)
Lead	0.164 ± 0.03	0.178 ± 0.025)	0.32 ± 0.045
	(0.226 ±0.038)	(0.265 ± 0.051)	(0.426 ± 0.063)
Copper	2.37 ±0.34	0.684 ±0.09	0.376 ±0.05
	(2.87± 0.23)	(0.724 ± 0.14)	(0.416 ±0.041)
Cadmium	0.178 ± 0.03	0.127 ± 0.02	0.294 ±0.02
	(0.26 ± 0.02)	(0.14 ±0.035)	(0.346 ± 0.015)
Aluminum	3.82 ±0.56	12.06 ±1.0	8.95 ± 0.85
	(4.25± 0.45)	(12.87 ± 0.65)	(9.67 ± 0.74)

* Values in parentheses obtained via digestion method 2

sugars and mostly inorganic compounds. It is unlikely that glycosides or saponin should have an antiviral activity. The decoction of three medicinal important plants have considerable amount of essential elements, which are beneficial in many ailments of human beings.

Experimental

Plant Samples

Five to ten samples of all plants i.e. bulb of *Allium sativum*, seed kernel of *Myristica fragrans*, and seeds of *Nigella sativum*, were collected from different areas of Hyderabad city, Sindh University Jamshoro campus and drug dealers. Reference samples was identified by co-worker of Botany Department of Sindh University Jamshoro, (Pakistan). Reference sample of *Myristica fragrans*, seeds of *Nigella sativum* was purchased from Hamdard drug store.

Equipment and Chemical Reagents

All the reagents and chemical used were of AR grade. All the solution of standards and samples were prepared in deionized water.

Mineral elements analysis of three plant samples and reference materials was carried out by atomic absorption spectrophotometer Hitachi model 180-50 equipped with standard hollow cathode lamps and Zeeman background correction. The flame absorption mode used with air-acetylene and nitrous oxide-acetylene flame.

Two methods were adopted for the digestion of plant samples namely:

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

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

Digestion method No. (2) 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%.

Decomposition of Plant Samples

All plants were washed with distilled water and dried at 120°C in electric oven to a constant weight. The dried plant material was then ground to powder. In next step the two set of each part of sample plants and reference sample was weighed into separate digestion flasks and treated with 5ml nitric acid and 5 ml sulphuric acid, side by side 5 ml nitric acid and 5 ml sulphuric acid was also added in empty flasks which served as blanks for both procedures [23,24]. The flasks were covered with watch glasses and heated to reflux 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 and sulphuric acid separately, followed by 2ml of 30% hydrogen peroxide in each flasks, and the heating at gentle reflux was continued until a clear and transparent solution was obtained. The volume of contents was reduced to semi dried mass, again added 5ml 2N HNO_3 and 2N H_2SO_4, heated for 2 minute and the contents of the flasks were cooled, diluted appropriately with 2N HNO_3 and 2N H_2SO_4 filtered through Whatman # 42 paper into volumetric flasks marked as stock sample solutions.

Preparation of Decoction of Plant Materials

Triplicate 1 g a dried sample of each plant material was heated with 50 ml deionized water in separate flasks on electric hot plate for one hour. The content of flasks were cooled and filtered through Whatman filter paper No. 42. Flittered material poured into volumetric flasks and marked as stock sample solutions.

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 Mineral Elements

Appropriate 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. Fifteen elements were determined in medicinal plants using air-acetylene and Nitrous oxide- acetylene flame mode.

A blank readings was also taken and necessary correction was made during the calculation of percentage concentration of various elements.

Percentage Recovery Test

Precision of both procedures used for the decomposition of plant materials was checked by decomposing and analysing five identical samples of each plant, and calculating the relative standard deviation in both cases.

The efficiency of extraction methods was checked by standard addition method. The matrix of standards and sample solutions was same in both cases. The percentage recovery test for different elements by digestion methods No. 1 and 2 was 97.0 – 98.5 and 99.0 to 99.5 % in range, respectively.

Conclusions

It was concluded that the level of toxic elements was very low in medicinal plants and in its decoction as compared to essential elements. Mineral elements: Na, K, Mg, Mn, Zn, Co, Cr, and Fe are present in all medicinal plants and may be directly or indirectly helpful in the management of many diseases.

The level of zinc was observed high in all three samples of plants. However, it is very effective in killing virus. The inorganic trace elements are active in very low concentration, so in therapeutic activity of medicinal plants the trace elements play major role.

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