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Multi Elemental Analysis of Medicinal Plant Flower Hygenya Abyssinia "Locally Called Chima" Used in Rural Part of Ethiopia (Gurage Zone, Shamene) as a Medicine to Cure from Tapeworm

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Abstract: *In the under-developed countries, the people of far-flung rural areas still depend upon herbal medicines and minerals. The foundation of usage of herbal and mineral medicine is the experience of thousands of years. The present paper deals with the characterization of the flower of a flowering plant Hygenya Abyssinia \ locally called Chima" for essential and toxic elements. The sample of the flower were collected from three different small forests, \Kotergedra, Yame and Genemar", found in the study area. The plant is rarely grown by people in the surrounding. The three forests are the main source of the plant's flower almost all the people using it. One sample is prepared for irradiation from the flower collected from each forest, so that the numbers of samples were three. The standardization of neutron activation analysis called instrumental neutron activation analysis (INAA) was employed for the qualitative and quantitative description of elements. The analysis methodologies were validated by analyzing the IAEA 1515 (apple leaves). More than twenty elements including K, Mg, Al, Cl, Fe, Ca, Mn, Na, V, Br, La, Sm, Sc, Cr, Co, Zn, Rb, Ba, Nd, Eu, Tb, Yb, and Th, were determined in all samples. The elements considered were those elements their concentration in the analysis of the IAEA standard agrees with the values given in the certificate of the standard in addition to their percentage standard deviation (SD) is 10 and below 10.*

Keywords: *INAA, Chima, Herbal medicine.*

1. Introduction

Herbal medicines, also known as medicinal plants are known for their activeness and are widely practiced in almost every part of the world. They are easily available in remote parts of Ethiopia at relatively lower cost. World Health Organization (WHO) supports the use and application of herbal medicines by its agency called International Regulatory Cooperation for Herbal Medicines. It was established in 2006 under the WHO and is a global network of regulatory authorities responsible for regulation of herbal medicines [1]. These medicines are used for curing various diseases like kidney disorder, cancer, diabetes, cardiovascular problems, urinary tract diseases, parasites. Herbal medicine could be obtained by either one of or concoction of flowers, fruits, barks, roots, seeds, and leaves from one or more plants. The major component of these medicines contains organic compounds some of which have biological activity. These medicinal plants have been little explored on their elemental contents. Four non-metallic elements (H,O, C and N) account for 99 percent of all elements in all biological systems. Seven elements Na, K, Ca, Mg, P, S and Cl provide another 0.9 percent of the total content and trace elements share the remaining 0.1 percent [1].

There are reports suggesting that trace elements deficiencies can lead towards the impaired growth during infancy and childhood. In biological system, some trace elements have been classified as essential, the others as toxic and the rest of undecided role. Trace elements play crucial role in various biochemical functions because some metals form integral part of enzymes. In addition to the essential elements present in herbs, they might contain toxic elements from the environment. Researchers have measured As, Cd, Cu, Hg and Pb in various food articles. It is therefore, very important to analyze all kind of food articles including herbal medicines for their mineral contents. Herbal medicines belonging to different countries have been explored for their elemental composition, because of the presence of research facilities like nuclear reactor. The objective of the present study is to report elemental contents and concentration of the flower of flowering plant Hygenya Abyssinia \Chima" which is Ethiopia origin by instrumental neutron activation analysis (INAA). The presentation focuses mainly on the experimental methodologies used and discusses the results obtained. Brie y the plant Hygenya Abyssinia \locally called Chima" grows in Ethiopia only. The plant is flowering plant and people in the area of this research use the powdered form of the dried flowers as an anti tapeworm medicine, it works effectively. The powder is taken after it is mixed thoroughly with a glass of water. There is no constant measurement regarding the amount of the powder for adults and children. The plant gives flower in the months November to December, people in the surrounding collect these flowers and keep dry for use in the other months of the year.

2. Theory

Neutron activation analysis (NAA) is a very precise technique mainly used to determine trace concentrations of elements in samples or to acquire information on the spatial distribution of a neutron field via neutron activation detectors [2]. In INAA a sample is first irradiated with neutrons of speed 2200m/s coming from e.g. a particle accelerator or an experimental reactor. Depending on the neutron flux, energy spectrum and reaction cross sections, the target nucleus undergoes a nuclear reaction and the resulting nucleus will immediately de-excite under emission of characteristic prompt gamma rays into a more stable configuration. This configuration is in general a radioactive nucleus with a certain half-life, $t_{1/2,}$ which will further decay under emission of characteristic delayed gamma rays into a stable product nucleus.

A sample is irradiated together with a calibrator containing a known amount of the element of interest. The calibrator is measured under the same conditions as the sample (sample-to-detector distance, equivalent sample size and equivalent matrix). From comparison of the net peak areas in the two measured spectra the mass of the element of interest can be calculated using the equation (1) [3]:

$$(M_x)_{unk} = M_{std} \frac{\left(n_\gamma/\{t_c - e^{-\lambda t_d}(1-e^{-\lambda t_c})\}\right)_{unk}}{\left(n_\gamma/\{t_c - e^{-\lambda t_d}(1-e^{-\lambda t_c})\}\right)_{std}} \qquad (1)$$

Where: $(M_x)_{unk}$ is the mass of the element of interest x in the sample, and $(M_x)_{std}$ is the mass of the element in the standard or comparator both in gram. In order to determine the count "n_Y", the irradiated sample is measured by a detector for some time (t_c) starting at (t_d), the time since the end of irradiation (t_i). The number of radio isotopes decaying in that time interval is "the radio isotopes present at t-t_c is subtracted from the number at t-t_d" In this procedure many of the experimental parameters - such as neutron fluency rate, cross section and photo peak efficiency cancel out at the calculation of the mass and the remaining parameters are all known.

In full multi-element INAA the calibrator should be multi element calibrator. It takes considerable export to prepare multi-element calibrators for all 70 elements measurable via NAA with adequate degree of accuracy in a volume closely matching the size and the shape of the samples [3].

3. Method

3.1. Sample Collection

Samples were collected directly from the flowering plant in "Kotergedra", "Genemar" and \Yame" forests. Different samples from the same forest were dried exposing to dry air at room temperature then powdered using a mortar and homogenized together to get one sample for irradiation. By the same procedure we have prepared three different samples of the flower. The three samples were packed in polythene pocket and taken to the minister of agriculture of Ethiopia to obtain permission in order to transport them abroad to where a reactor and facilities were available. After obtaining the permission by a letter reference number 334807, the samples were transported to Nigeria, Ahmadu Bello University (ABU), and Center for Research and Training (CERT) to irradiate them and count the resulting activities.

3.2. Sample Preparation

The powder form of the samples brought from Ethiopia were dried in an oven at a temperature of $45C^0$till constant mass then prepared for irradiation in the NAA sample preparation laboratory, Center for Energy Research and Training (CERT), Ahmadu Bello University, Zaria Nigeria. The samples as well a Standard Reference Material (SRM) were weighed between 150mg - 200mg using a Mettler Toledo balance, model AE 24. The reference material used was Lichen NIST- 1515 (apple leaves) supplied by International Atomic Energy Agency (IAEA), Vienna, Austria, for verification and quality control purpose. A total of eight samples were prepared, four for short irradiation and four for long irradiation. The four short irradiation samples were obtained by taking one from each of the three "Chima" samples transported from Ethiopia and the other sample is prepared from the SRM. The same procedure is applied to prepare long irradiated four samples. All the samples were coded, a code "KGR" was used for the name of the sample collected from "Kotergedra", a code name "GNMR" was used for the sample collected from "Genemar" and "YAME" was the code name of the sample collected from "Yame" and "NIST 1515" was used for the apple leaves, packed in a plastic and put in pre – cleaned air tight polyethylene vials. The samples in the vial were kept in "Desketo" an air tight container which contains silicates in order to keep them away from moisture due to humidity of the atmospheric air till the date of irradiation.

3.3. Irradiation and Counting

The irradiation facility used at Ahmadu Bello University, CERT is the Nigeria Research Reactor-1 (NIRR-1) which is a Chinese Miniature Neutron Source Reactor (MNSR) with a nominal thermal power of 31.1 kW pool type, with a maximum thermal neutron flux of 10^{12} neutrons $m^{-2}s^{-1}$. During this work the reactor was made to run at half of its maximum power. So that the flux in the inner sites of irradiation was $2.5x10^{11}$ $m^{-2}s^{-1}$ and in the outer sites was $1.25x10^{11}$ $m^{-2}s^{-1}$ as noted from the control consul.

There is an automated system which works with air pressure, to transfer samples pneumatically in to irradiation cite of the reactor and retrieve them from the reactor through a rabbit transfer tube to the counting laboratory. There were six irradiation sites of the reactor which were situated around its core, one outer channel (B4) , one cadmium fixed channel for Epithermal irradiation (A2),four inner channels (A1;B1;B2;B3).
The analysis of the plant flower in this work can be divided into two different techniques on the basis of the half-life of the radionuclide. The first technique is to use a short irradiation (5 min).In this process samples were irradiated for five minutes in the outer irradiation site, B4, with a flux of $1{:}25\times10^{11}$ $m^{-2}s^{-1}$. The second technique used was long irradiation, in this case the samples were irradiated in the one of inner channels (A1) for six hours, the ux in this cite of irradiation was 5×10^{11} $m^{-2}s^{-1}$ .Even though the number of elements determined by this technique is smaller than the number determined by the shorter irradiation, the elements to be determined in this technique were important and are affected by short life nuclide interferences. Two multi-elemental standards \lichen and tomato leaves" were used, since one multi-elemental standard would produce too much activity on irradiation to count successfully.
For calibration purposes, an energy calibration is performed with each batch of samples using two standards; Cobalt ^{60}Co, and cesium ^{137}Cs. These standards provide known energy for the energy calibration of the detector.
After irradiation the samples were left to cool until the activity of the samples become approximately 30 micro Seviert, and taken to the counting facility furnished with high purity germanium detector connected with computers supported by analysis software, MAESTRO and WINSPAN, to measure gamma-ray spectra of the samples and standards. The detector has a 30 percent relative efficiency with energy resolution of 1:95keV for ^{60}Co photo peak at 1332keV. It is connected to an ORTEC 570 Spectroscopy amplifier and ORTEC made TRUMP-PCI 8 k card ADC and multichannel analyzer. In all counting, dead time was less than 10% and pile-up corrections were applied by the software allowing the count for real time count. This system of computer and detector gives an output spectrum with energy peaks. The software calculates the concentration of each element in the spectrum which is connected with the area of the energy peaks.

4. Result and Discussion
From the experimental spectrum of irradiated samples, the spectrum shows various gamma ray energy peaks. The decay of each of gamma ray was followed and half lives of radio nuclide were used by the software to confirm the element present in the sample. Information on data about the half life and principal gamma energy of the isotopes were taken from [5]. Elemental concentrations in the three samples of “Chima” were calculated using equation 1, by a computer supported by software MAESTRO and WINSPAN.
The Standard reference material apple leaves NIST-1515 was also analyzed for the purpose of quality control and comparison in this study. The quality control of INAA system is based on the results from the analyses of these standard samples, analyzed under the same conditions as the real samples. Careful consideration of these results yields information on accuracy and precision of the analyses. The result obtained from the analysis of standard reference material in the present study is displayed in table 1. The table presents the elemental concentrations in the reference material as analyzed by this work compared to the certified and non certified values given in the certificate of the standard.
From results obtained, it is observed that most of the elemental concentrations are within 10% of certified values. Since the concentrations of most of the elements are similar to their respective certified values except for those that are below detection limit, almost all of the elements listed in the certificate of the standard were considered. Those elements which are below the detection limit during the analysis of the standard were not analyzed in the “Chima" samples too because of the uncertainty in the quality of the result.

Element	Present work (ppm)*	Certified Values (ppm)*	Non certified values (ppm)*
K	16100 161	16100	
Mg	2710 165	2710	
Al	286 5	286	
Cl	570 20	579	
Ca	15260 443	15260	
V	0.26 0.08	0.26	
Mn	54 1	54	
Na	24.4 0.5	24.4	
Br	1.8 0.3		1.8
La	20 0.08		20
Sm	3 0.01		3
Sc	0.03 0.007		0.03
Cr	0.3 0.01		0.3
Fe	83 8	83	
Co	0.09 0.01		0.09
Zn	12.5 2.7	12.5	
Rb	10.2 0.3	10.2	
Ba	49 11	49 ± 2	
Nd	17 1		17

Eu	0.2 0.05		0.2
Tb	0.4 0.05		0.4
Yb	0.3 0.06		0.3
Th	0.03 0.1		0.03

Table 1: Nist1515, certified and non certified values compared with the present work. The certified and non certified values were taken from its certificate [7]
**ppm = parts per million*

The resulting elements in the "Chima" samples in this work are listed in table 2, the table includes the elemental concentrations and their standard deviations. It is observed that SD values in most cases were <10% suggesting high order of accuracy and precision of our data. Therefore, it is assumed that elemental concentrations in the sample should be accurate within 10%. The codes "KGR" is given to sample collected from Kotergedra, "GNMR" is for the sample from Genemar and "YAME" is for a sample from Yame.

Element	E_γ (Kev)	$T_{1/2}$(second)	KGR (ppm)	GNMR (ppm)	YAME (ppm)
K	1524.6	$4.464*10^4$	24630	24090	25010
Mg	1014.4	567.6	3523	3700	3415
Al	1779	134.4	2948	2979	2750
Cl	2167.7	2232	5458	5568	5572
Ca	3084.5	523.2	8060	7898	7772
V	1434.1	289.02	4.82	6.31	4.4
Mn	2113.1	$9.288*10^3$	11.3	107.1	102.1
Na	2754.0	$5.4*10^4$	516	481	449
Br	776.5	$1.271*10^5$	32.21	31.6	32.5
La	1596.2	$1.451*10^5$	6.07	6.25	6.2
Sm	103.2	$1.667*10^5$	0.94	0.96	0.92
Sc	889.3	$7.197*10^6$	0.56	0.58	0.55
Cr	320.1	$2.3933*10^6$	0.47	0.8	0.69
Fe	1291.6	$3.845*10^6$	1379	1392	1363
Co	1332.5	$1.663*10^8$	0.23	0.29	0.2
Zn	1115.6	$2.9722*10^7$	41	28	34
Rb	1076.6	$1.6157*10^6$	34	32	38
Ba	496.3	$1.0195*10^6$	64	BDL	BDL
Nd	91.1	$9.504*10^5$	BDL	2.78	BDL
Eu	1408	$4.1972*10^8$	0.11	0.12	0.16
Tb	879.4	$6.2467*10^6$	0.17	BDL	0.18
Yb	396.3	$3.6202*10^5$	0.57	0.5	0.55
Th	312	$2.333*10^6$	0.08	0.09	0.08
LU	208.4	$5.797*10^5$	$5.1*10^{-6}$ 0	$7.1*10^{-6}$ 0	$3.41*10^{-6}$ 0

Table 2: Experimentally obtained concentration of elements in the sample Chima.
**ppm = parts per million.*

As can be seen from table 2, Magnesium (Mg), Aluminum (Al), Chlorine (Cl), Iron (Fe) and Calcium (Ca) found in a relatively higher concentration in all of the three samples. The elements, Manganese (Mn) and sodium (Na) are relatively in a moderate concentration. The other elements vanadium(V), Bromine(Br), Lanthanum(La), samarium (Sm), scandium(Sc),chromium(Cr), Cobalt(Co), Zinc(Zn), Rubidium(Rb), Barium(Ba), Neodymium(Nd), Europium (Eu), Terbium(Tb), Ytterbium(Yb),and Thorium(Th) are presented only in trace quantities.

The data showed that amounts of elements in the three different samples of "Chima" vary to certain extents; it is probably due to many factors which directly linked to the elemental Composition of soil where the plants grown and the pollution of the flower on its tree due to atmospheric pollutants. The differences seen in the concentration of some of the elements is given in the bar chart below.

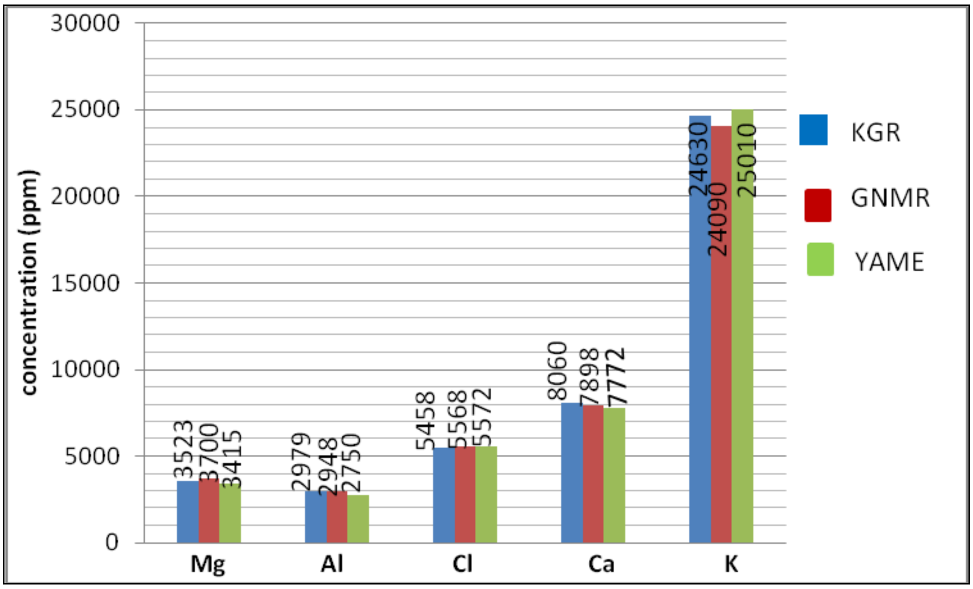

Figure 1: A column chart showing the differences in the concentration of major elements found in the "Chima" sample

The trace elements play a vital role in the medical value of plants as curative and preventive agents in combating disease, nutritive and catalytic disorders. The concentration of trace elements in plants is so meager that their importance was ignored for a long time. There is a vast scope to explore the preventive medicinal aspects of various trace elements. Health treatment is based on medicinal plants recommended as nutritional supplements for the treatment of everyday problems. There is resurgence of interest in herbal medicine for the treatment of main ailments. The main advantage of herbal medicines is that naturally occurring products without any side effects. Trace elements play a major role in health and diseases. Whereas, excessive intake of some of these essential elements may adversely act the human metabolic function [[14],[15]]. At high concentrations these essential elements can lead to poisoning [16].

The elemental concentrations were determined to verify the biological role of trace elements in anti parasitic medicinal plant flower are K, Mg, Al, Cl, Fe, Ca, Mn, Na, V, Br, La, Sm, Sc, Cr, Co, Zn, Rb, Ba, Nd, Eu, Tb, Yb, Th. In different literatures, it is explained that the elements Fe, K, Mg, Na, Ca, Co, Mn, Zn and Cu have been classified as essential elements, Ni, Cr are possibly essential while Cd, Pb and Li are non essential elements for the human body. Among the various elements detected in different medicinal plants used in the treatment of different diseases, Calcium and potassium are found in major concentrations in these plants, this work also justifies a higher concentration of these elements relative to others [17].

It is known that potassium is necessary for muscle contraction (especially cardiac fiber), for the synthesis of some proteins and as an enzymatic cofactor. The action of potassium depends on the presence of sodium [18].

Calcium is needed in the development of bone and teeth and it regulate heart rhythm, helps in normal blood clothing, maintain proper nerve and muscle functions. Magnesium (Mg) plays an important role in regulating muscular activity of heart rhythm and also Magnesium is important cofactor of convert blood glucose into energy [13]. Chloride works with sodium and potassium carry an electrical charge when dissolved body fluids and to regulate the pH in the body. Chloride is also important to digest the food properly and absorb many elements that what we need to survive. Toxic elements such as Cd, Hg and Sb were not detected in the sample. It is not mean that these elements are totally absent from the samples, but the concentrations of these elements present in the sample analyzed are found to be very low below the detection limit to cause any kind of effect.

In this study, Bromine was obtained in the three samples and its concentration in the sample "KGR" is 32.21 0.2, the concentration in "GNMR" sample is 31.6 0.2 and it was 32.5 0.2pm in the "YAME" sample. The concentration of bromine in the "KGR" and a "YAME" samples were higher than its concentration in the "GNMR" sample. Bromine is corrosive to human tissue in a liquid state and its vapors irritate eyes and throat. Bromine vapors are very toxic with inhalation. Humans can absorb organic bromines through the skin, with food and during breathing. Organic bromines are widely used as sprays to kill insects and other unwanted pests. This poisonous nature of the element may act to kill the parasite tapeworm inside human intestine after people of this research area take 'Chima\ as a medicine. High doses of bromine can cause problems in a human being too. The most known health effects that can be caused by bromine-containing organic contaminants are malfunctioning of the nervous system and disturbances in genetic materials, damage to organs such as liver, kidneys and lungs and they can cause stomach and gastrointestinal malfunctioning. Some forms of organic bromines, such as ethylene bromine, can even cause cancer, damage the nervous system and the thyroid gland [13].

Zinc was found in the all of the three samples. Its concentration variation shows that the sample "KGR" contains highest "41 3" compared to the other two, as can be seen from table 2. Zinc is one of the essential mineral elements that is found in almost every cell, where it stimulates the activity of over 100 enzymes needed for various biochemical reactions [18, 20]. In fact, it has been established that the six categories of the international nomenclature are zinc metalloenzymes [19]. Important enzymes stimulated by zinc, support metabolic processes such as the immune system, wound healing, organoleptic abilities of taste and smell, brain development, synthesis of DNA and RNA, normal growth and development during pregnancy, cell division, sexual maturation, storage and release of insulin among others [18]. Because of its roles in the biochemical processes of growth and development, zinc is considered as one of the most essential mineral elements in fetal, infant and early childhood development [12].

Na and K act as electrolytes in the human body. Na is the principal cat ion in the extracellular fluids and modulates the maintenance of the intracellular and interstitial volumes. Sodium is essential for regulation of osmotic pressure of the body and helps to maintain acid base and water balance of the body. Its deficiency causes loss of body weight and nerves disorder [18]. Sodium was present at major levels with its highest concentration found in "KGR” at 516 5ppm and the least concentration was 481 3ppm in "GNMR" sample. Potassium concentration was found to be the highest of all elements in all of the three samples. The largest concentration was found to be 250 10 ppm in “YAME" sample. Its concentration was 246 30 ppm in “KGR" and 240 90 ppm in “GNMR" samples.

Barium (Ba) and Neodymium (Nd) are the elements which were not found in all the three samples. Barium was found in the “KGR” sample with a concentration of 64 1.5 ppm but its concentration in the other two "GNMR\ and "YAME\ samples was below detection limit(BDL). In other case Neodymium was detected in GNMR sample with a concentration of 2.78 0.85 in parts per million but its concentration was below detection limit in the other two samples "KGR” and "YAME\. Below detection limit is not mean that there is no such element in the sample, but the concentration is too low to be detected, the concentration in the other two samples "KGR” and "YAME\was According to different literatures out of the known natural elements that play key role in the evolution of life, there are three groups of elements, the first group are macro elements which are found in all of green plants in high concentrations “Main organic elements", the second group called “secondary organic elements” and the third are “Trace elements”. Main organic elements: H, O, C, N, S, P Secondary organic elements: Fe, Mg, Ca, Na, K Organic trace elements: Li, B, J, Cl, Br, Al, Si, Ti, Mn, Co, Cu, Zn, Se, Mo, Bi, V, Rb.

In this work a focus was only on the investigation of the two groups, Secondary organic elements and organic trace elements. We hope that our results will very important information about the presence of various useful major and minor elements which help in the cure the parasite as mentioned in the beginning.

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