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Short Communication

Qualitative and Quantitative Content Determination of Macro-Minor Elements in *Bryonia Alba* L. Roots using Flame Atomic Absorption Spectroscopy Technique

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Abstract

***Purpose*:** To determine the elements in *Bryonia alba* L. roots, collected from the Crimean Peninsula region in Ukraine.
***Methods*:** Dry ashing was used as a flexible method and all elements were determined using atomic absorption spectrometry (AAS) equipped with flame and graphite furnace.
***Results*:** The average concentrations of the determined elements, expressed as mg/100 g dry weight of the sample, were as follow: 13.000 for Fe, 78.000 for Si, 88.000 for P, 7.800 for Al, 0.130 for Mn, 105.000 for Mg, 0.030 for Pb, 0.052 for Ni, 0.030 for Mo, 210.000 for Ca, 0.130 for Cu, 5.200 for Zn, 13.000 for Na, 1170.000 for K, 0.780 for Sr, 0.030 for Co, 0.010 for Cd, 0.010 for As, and 0.010 for Hg. Toxic elements such as Cd and Pb were also found but at very low concentration. Among the analyzed elements, K was the most abundant followed by Ca, Mg, P, Si, Fe, Na, and Zn, whereas Hg, As, Cd, Co, Mo, and Pb were found in low concentration.
***Conclusion*:** The results suggest that the roots of *Bryonia alba* L. plant has potential medicinal property through their high element contents present. Moreover, it showed that the AAS method is a simple, fast, and reliable for the determination of elements in plant materials. The obtained results of the current study provide justification for the usage of such fruit in daily diet for nutrition and for medicinal usage in the treatment of various diseases.

Introduction

Bryonia alba L., is one of the smallest genus in the family cucurbitaceae, consists of 12 species distributed throughout the Europe and West Asia.[1,2] It has been traditionally used in the treatment of different diseases such as cough, frontal pain, inflammation of serous tissues, peritonitis, pneumonia, jaundice, typhoid, rheumatism, brain disorders with serous exudation and as a heart tonic.[2]

Nowadays, the interest in chemical composition analysis of medicinal herb products is growing owing to the continuing developments in nutrition and in biochemical surveying and mineral prospecting.[3,4] Additionally, the studies related to therapeutic plants not only aim to characterize the active components found in plants, but also for scientific support of its therapeutic properties.[5]

Macro, micro and trace elements are known to play a vital role in biological functions in plants and in human metabolic reactions. Moreover, trace elements play an important role in the formation of bioactive chemical constituents in medicinal herb plants and thus are responsible for their medicinal and toxic properties accordingly.[6,7]

Several number of techniques such as voltammetery, atomic absorption spectrometry (AAS),[8] inductively coupled plasma atomic optical emission spectrometry (ICP-OES),[9] X- ray fluorescence (XRF),[10] differential pulse cathode stripping voltamperometry (DPCSV)[11] and instrumental neutron activation analysis (INAA)[12] are normally used for the determination of trace elements in medicinal herbal plants. Because of its specificity, sensitivity, high precision, simplicity, rapid analysis, low cost, low detection limit, and wide linear range, AAS is the most widely recommended instrument used in analytical procedures for the trace metal analysis found in complex biological samples.[3] AAS methods are considered as direct aspiration determinations where they are accomplished as single element analysis and are relatively free of interelement spectral interferences. In

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other words, the use of special light sources produced by the cathode lamp is emitted from excited atoms of the same element of interest and specific spectrum selection allows the quantitative determination of individual components of a multielement mixture.[13] AAS as an accurate and rapid technique was chosen for the present work. The most commonly used methods for the sample preparation of plant species are dry ashing, wet ashing and microwave assisted treatment.[14]

In the present work, nineteen minor and trace nutrient elements (Fe, Si, P, Al, Mn, Mg, Pb, Ni, Mo, Ca, Cu, Zn, Na, K, Sr, Co, Cd, As, and Hg) in *Bryonia alba* L. roots growing in Ukraine were determined by AAS coupled with dry ashing method.

Material and Methods

Reagents and solutions

Standard stock solutions with a concentration (1000 mg L^{-1}) of the individual metal element were used to prepare the requested concentrations by dilution using a 1% (v/v) nitric acid solution. The diluted standard solutions were used to build the calibration curves. Metal element standards were purchased from Sigma-Aldrich (St Louis, MO, USA). An analytical reagents grade of concentrated nitric acid (70%) and hydrogen peroxide (30%) were purchased from Sigma-Aldrich (St Louis, MO, USA). In all experiments carried out, the glass /plastic containers were cleaned by soaking in10%, v/v HNO_3 for at least 24 h and rinsing with distilled water prior to use. All other chemicals used were of analytical grade. The ultrapure deionized water obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used for preparing the solutions and for all dilutions.

Instrumentation

An AAS instrument (Perkin Elmer AAnalyst 700 model AAS) with deuterium background corrector was used for the determination of Fe, Si, P, Al, Mn, Mg, Pb, Ni, Mo, Ca, Cu, Zn, Na, K, Sr, Co, Cd, As, and Hg. Pb, Cd, and Ni were determined by HGA graphite furnace using high purity argon while other measurements were carried out in an air/acetylene flame. The operating parameters for working elements were set according to the recommendations of the manufacturer.

Plant materials

Fresh roots of *Bryonia alba* L. were collected from the Crimean Peninsula region, which is located south of the Ukrainian region of Kherson and west of the Russian region of Kuban. The collection has been done in autumn during 2014 – 2015. The temperature recorded at harvesting time ranged between 15 – 20°C. Botanical identification/authentication was performed at Bogomolets National Medical University, Kiev, Ukraine.

Macroscopic techniques for identification/authentication of Bryonia alba L.

It is well known that authentication/identification of raw material is the essential starting point in developing a botanical product. However, inherent chemical variability will certainly be observed with any botanical. Additionally, each step of harvest, storage, processing, and formulation may significantly change the quality and uniformity of the final product, whether by preserving the desired marker components or by eliminating unwanted contaminants.[15] Therefore, methods to assure quality control in manufacturing and storage are required tools not only to ensure optimal efficacy but also safety of these products. Additionally, such controls are important for the evaluation of toxicological, pharmacological, or clinical studies involving botanical dietary supplements.[16]

Macroscopic study

The macroscopic study of the morphological description of the plant parts was conducted by our naked eye and with the aid of magnifying lens.

Sample preparation

The collected roots were washed thoroughly with tap water followed by deionized water and then allowed to dry in an oven at 50 – 60 °C. The sample was dried to constant weight (0.0005 g). One gram sample was accurately weighed and ground with the aid of a food processor Power Plus 1300 (Braun, Germany) for 3 min (instead of using mortar and pestle for better homogeneity as it allows for faster decomposition, thus more precise results). Then it was sieved through a 0.5 mm diameter sieve. The obtained powder of the plant material was stored in a dry and dark place at room temperature in the polyethylene bags till used.

Dry ashing procedure

One gram of sample was transferred into a porcelain crucible. The muffle furnace temperature was gradually increased from room temperature to 450˚C in 1 h. The sample was redried for 1h in the oven, cooled, and reweighed. The steps were repeated at 1h drying intervals until the differences in the variations in the released water were less than 0.05%. The obtained sample was ashed for about 8 h until a gray or white ash residue was obtained. The residue was dissolved in 5 mL of HNO_3 (25%, v/v) and, if necessary, the mixture was heated slowly to dissolve the residue. Then the digestion solution was heated up using an electric hot plate at 150°C until evaporated to near dryness. The residue was filtered through Whatman filter paper and transferred into a volumetric flask and made up to 25 mL with 3% HNO_3. The blank digestion experiment was also prepared in the same way. This procedure was adopted from the work of Soylak *et al*.[17]

Analytical procedure

AAS is a widely used technique for determining a large number of metals. In AAS, an aqueous sample containing the metal analyte of interest is aspirated into an air-acetylene flame, causing evaporation of the solvent as well as vaporization of the free metal atoms (atomization). Fe, Si, P, Al, Mn, Mg, Pb, Ni, Mo, Ca, Cu, Zn, Na, K, Sr, Co, Cd, As, and Hg in *Bryonia alba* L. root sample was analyzed using AAS equipped with flame and graphite furnace. Graphite furnace was used for the determination of trace and ultra-trace concentrations (Pb, Ni, Mo, Co, Cd, As, Hg). The following solutions (La(III) ions when determining Mg or Ca, and CsCl solutions were used as an ionization buffer in measurements of K, Mg and Na and were added to both sample and standard solutions in order to overcome chemical interferences in the flame upon determination.[18] The operation conditions used to operate AAS instrument were as recommended by the manufacturer. Data were rounded off properly based on the value of standard deviation from measurement conducted in triplicate.

Results and Discussion

Some of the trace elements known to be essential to our body such as As, Co, Cu, Fe, Mn, Ni, Si, Zn and the other essential major elements are Ca, K, Na, and Mg. So, different trace elements in the different medicinal plants will have their definite role in the functioning of our body. The roles of the detected elements are given below.

In our preliminary trials we examined the accuracy of the current work by analyzing the spiked samples at three level concentrations (low, medium and high), which were taken from stock solution of each metal and spiked in a 100 mL Erlenmeyer flask containing 1g sample. The recoveries of all metals in the spiked samples were ranged from 90 to 101 %.

The average results of elemental analysis obtained by the AAS technique for the analysis of *Bryonia alba* L. roots are shown in Table 1 in mg /100 g dry weight of the sample. It is to be noted that each result is an average of three independent triplicate measurements. As can be seen in this table, K, Ca, Mg, P, and Si are the most abundant elements in roots presenting concentrations ranging from 78 to 1170 mg/100 g dry weight of the sample levels.

Table 1. The content of macro-minor elements of *Bryonia alba* L. roots using flame atomic absorption spectrophotometry (n = 3).

Elements found in B. alba L.	Content, mg/ 100 g dry weight of the sample (Mean ± SD)
Fe	13 ± 0.12
Si	78 ± 1.21
P	88 ± 2.31
Al	7.8 ± 0.91
Mn	0.13 ± 0.020
Mg	105 ± 2.31
Pb	<0.03 ± 0.011
Ni	0.052 ± 0.0033
Mo	<0.03 ± 0.0023
Ca	210 ± 2.52
Cu	0.13 ± 0.02
Zn	5.2 ± 0.34
Na	13 ± 0.21
K	1170 ± 4.20
Sr	0.78 ± 0.07
Co	<0.03 ± 0.097
Cd	<0.01 ± 0.015
As	<0.01 ± 0.092
Hg	<0.01 ± 0.015

The concentrations of trace elements Fe, Al, Zn, and Na were found in the medium range of 5.2 to 13 mg/100 g dry weight of the sample. Ni, Cd and Pb are toxic elements which occur naturally in plants as a result of uptake, generally in places with high concentration caused by atmospheric and industrial fallout.[3] These are found in <0.01 to 0.0052 mg /100 g dry weight of the sample. Other elements such as Mo, Co, As, Hg, Mn, Cu, and Sr were found in the range 0.01 to 0.13 mg/100 g dry weight of the sample.

Iron (Fe)

Iron (Fe) is considered an essential element necessary for human body. It is the main component of myoglobin, hemoglobin, and a number of enzymes that play an important role in the oxygenation of red blood cells. It is needed for a healthy immune system as well as for energy production. It has been reported that iron severe deficiency results in anemia, which ranges from a fall in plasma ferritin to the strict iron deficiency that characterized by small red blood cells and low hemoglobin concentration level.[3,19] The daily requirement of Fe for a child is 10 mg/day, whereas for an adult is 20 mg/day. The Fe concentration in *Bryonia alba* L. roots sample analyzed was found 13 mg /100 g dry weight of the sample.

Manganese (Mn)

As known pyruvate carboxylase and superoxide dismutase are enzymes which contain manganese. [19] The manganese content found in *Bryonia alba* L. roots sample was 0.13 mg /100 g dry weight of the sample.

Magnesium (Mg)

It has been reported that magnesium (Mg) is considered the most imperative mineral for stress relief,[3] and as a non-essential element for living organisms.[20] *Bryonia alba* L. roots sample was found to contain 105 mg /100 g dry weight of the sample. The concentrations of this element upon analysis the roots of four medicinal samples by Dafalla, 2014 ranged from 1.42 to 42 ppm.[20]

Lead (Pb), Cadmium (Cd), and Nickel (Ni)

Bryonia alba L. roots sample contains Pb <0.03, Cd <0.01, and Ni 0.052 mg /100 g dry weight of the sample. These elements are considered to be toxic in nature, and thus their presence at trace level in various medicinal plant samples analyzed may be due to the pollution occurring from industrial activities and automobile.[19] Cadmium is considered a very hazardous to human health. Additionally, it causes high blood pressure, damages kidneys and liver.[21] The permissible limits set by WHO[22] are 0.2 to 0.81 ppm and from 0.1 to 10 ppm for cadmium and lead, respectively. Thus the concentrations of cadmium and lead found in *Bryonia alba* L. roots were within the limits. In the work of Dafalla, 2014,[20] who studied the roots of four medicinal plants, Cd concentrations varied from 1.8 to 14.4 ppm compared to the obtained results.

Calcium (Ca)

Calcium (Ca) plays a vital role in the absorption of dietary vitamin B, also for the activation of enzymes like the pancreatic lypase, and for the synthesis of the neurotransmitter acetylcholine. The recommended daily allowance for taking Ca is 800 mg for adults and for children 500 - 1000 mg. Therefore, in order to attain a Ca level of practically one percent of the total diet would be difficult.[3] The concentration of Ca found in *Bryonia alba* L. roots sample was 210 mg /100 g dry weight of the sample.

Sodium (Na) and Potassium (K)

The concentration of sodium (Na) and potassium (K) in *Bryonia alba* L. roots sample was found to be 13, and 1170 mg /100 g dry weight of the sample, respectively. Potassium is considered the richest element among all elements found. Usually, plants absorb potassium and sodium from the soil in the form of K and Na ions. Moreover, it is very important for enzyme activation, photosynthesis, water use efficiency, starch formation and protein synthesis.[21] The obtained data in our study indicate that the *Bryonia alba* L. roots sample is not deficient in potassium. Therefore, it is useful to be used as a food source, rich in K, for humans as it might help in the case of potassium deficiency.[3] The high concentration of K was found as well in the roots of the four medicinal plants studied by Dafalla, 2014 where the concentrations ranged from 400 - 842 ppm.[21]

Zinc (Zn)

Zinc is considered as one of the main components of over 200 enzymes having both catalytic and structural roles including the alcohol dehydrogenase, ribonucleic polymerases, alkaline phosphatase, and carbonic anhydrase.[23] Scientific studies conducted on animals have shown that zinc deficiency occurred during pregnancy may cause developmental disorders in the offspring.[24] Low intake of zinc may cause coronary artery disease. The concentration of zinc in *Bryonia alba* L. roots sample was 5.2 mg/ 100 g dry weight of the sample.

Copper (Cu)

Following zinc and iron, copper is considered the third most abundant trace element in the body. It has also been reported that it is an important catalyst for iron absorption. Its deficiency may be a risk factor for cardiovascular disease. When obvious copper deficiency occurs, symptoms such as neuropenia, cardiac disorders, osteoporosis and anemia may occur.[25]

In *Bryonia alba* L. roots sample, copper was found to be 0.13 mg/ 100 g dry weight of the sample. Excess copper is toxic. Normal daily intake of copper is 2-5 mg per day. In edible plants permissible limit set by FAO/WHO[26] in 1984 was 3.00 ppm. Thus, in *Bryonia alba* L. roots copper is less than the permissible limit.[21]

Cobalt (Co)

Cobalt is one of the most important essential components for the B12 vitamin and thyroid metabolism.[3] It is necessary in very small amounts in all mammals. It is used to treat different types of cancer in humans anemia treatment. The intake of high amount can cause heart diseases.[13] Cobalt is present in *Bryonia alba* L. roots sample at very low concentration of about <0.03 mg/ 100 g dry weight of the sample.

Silicon (Si)

Silicon (Si) is one of the most abundant elements found in the earth's crust and is commonly distributed in nature in different forms. It is well known that Si is associated to bone structure and its strength (Osteoporosis), reducing the risk of developing Alzheimer's disease, and preventing collagen metabolism abnormalities.[27] The concentration of silicon in *Bryonia alba* L. roots sample was 78 mg/ 100 g dry weight of the sample.

Phosphorus (P)

Phosphorus (P) is the constituent of more than 240 enzymes. Its deficiency in the organism is accompanied by multisystem dysfunction. Moreover, P is responsible for fetus development, sperm manufacture, and suitable function of the immune response.[20,28] The concentration of P in *Bryonia alba* L. roots sample was 88 mg/ 100 g dry weight of the sample. According to Dafalla work,[20] P concentrations were from 2.61 to 14.24 ppm.

Aluminum (Al)

Aluminum (Al) ions are considered toxic to most plants. It has negative results in being suppressed root growth and causing a series of abnormal metabolic effects.[29] Being an important herbal plant in the world, *Bryonia alba* L. roots also contain a concentration of Al of about 7.8 mg/ 100 g dry weight of the sample, and can be considered an important source of dietary Al.

Molybdenum (Mo)

Molybdenum (Mo) concentration found in *Bryonia alba* L. roots sample was <0.03 mg/ 100 g dry weight of the sample. Although Mo is required in trace amounts for the body as it is mostly present in the pancreas and plays a significant role in the production of insulin. Deficiency of Mo results in the disorder of the liver, and the daily intake should not exceed 1.0 mg. Beyond this level is toxic.[30] When analyzed in the roots of four medicinal plants namely; Ocimum basilicum, Poupulus nigra, Taraxacum officinale, and Convallaria maialis, its concentration varied from 0.82 to 9.2 ppm as most samples usually have concentrations between 0.51 and 0.72 ppm are considered the highest Mo concentration.[20]

Strontium (Sr)

Strontium (Sr) is an element which can be found in the environment in a large concentration ranges. The transfer of such a contaminant from the environment to plants will lead to contamination of the food pathway and finally into the human body. Then it deposits in bones and teeth and can cause bone and renal diseases accordingly.[31] It has been reported by Brambilla *et al*.[32] that plants can be contaminated by three main pathways namely; leaf uptake, root uptake, and deposition of contaminant on plant aerial parts.[33] Additionally, it is also affected by the properties of soil such as organic matter content, ionic composition, and pH.[34] The concentration of strontium in *Bryonia alba* L. roots sample was 0.78 mg/ 100 g dry weight of the sample.

Arsenic (As)

Arsenic (As) is a toxic pollutant in the environment, which results from anthropogenic and natural sources. It has been reported that plants growing in As contaminated soils accumulate As in their biomass.[35] Additionally, As may accumulate in soils and sediments owing to the use of arsenical pesticides, fertilizers, irrigation, and oxidation of volatile arsine in air disposal of industrial, municipal and animal waste.[36] Once a plant is contaminated with arsenic, it causes toxicity such as leaf chlorosis and necrosis, and also reduces growth. Different As species in plants show different toxicity, so it is important to quantify As in plants to better understand their metabolism. The concentration of arsenic in *Bryonia alba* L. roots sample was <0.01 mg/ 100 g dry weight of the sample.

Mercury (Hg)

Mercury (Hg) is extremely toxic trace metal pollutant. Bio-accumulation of Hg in plants and then its entry into the food chain results in long term health hazards.[37] The toxicity of Hg depends on its chemical state. Some forms of Hg are relatively non-toxic and have been used as medicines, e.g., for the treatment of syphilis. Speciation of mercury at trace and ultra-trace levels is a matter of current interest. The concentration of Hg in *Bryonia alba* L. roots sample was <0.01 mg/ 100 g dry weight of the sample.

In this study, the concentration of K, Ca, Mg, P, and Si were found to be the highest followed by the remaining elements in trace levels. Referring to the elemental concentration in Table 1, we can use the particular medicinal plant as requested by knowing the functional values of each element. Extra attention must be paid not to take those medicinal plants containing a large concentration of the above elements for a long time daily. For toxicity, the presence of arsenic in *Bryonia alba* L. roots sample will not lead to any undesirable effect because its concentration is very low as indicated by the World Health Organization Maximum Tolerable Daily Intake (WHO-MTDI, 2008)[38] value (2μg/day/ kg body weight). The low level observed for Pb and Cd leads to the conclusion that the plant is grown in the pollution free soil as elemental uptake by the plant mainly depends on regional soil as well as climate conditions and thus could be used as a medicinal plant.

Conclusion

The different concentrations of elements present in *Bryonia alba* L. roots leads to the conclusion that these roots will have different specific roles in the treatment of different diseases. The results obtained from the present study provide vital data on the availability of diverse essential elements, which can be useful to provide dietary information for designing value –added foods and for food bio fortification. This study provides a comprehensive investigation of the contents of 19 trace elements in *Bryonia alba* L. roots sample growing in Ukraine. The dry ashing method coupled with atomic absorption spectrometry was used for the determination of trace elements in *Bryonia alba* L. roots. The results indicated that *Bryonia alba* L. roots had a high content of K, Ca, Mg, P, and Si. The contents of the toxic heavy trace elements Cd and Pb were very low (<0.03 mg/ 100 g dry weight of the sample) and could not cause any threat to the consuming population. This technique is considered reliable for routine analysis elements determination in a wide range of botanicals and dietary supplements.

Ethical Issues

Not applicable.

Conflict of Interest

The authors declare no conflict of interests.

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