Inductively Coupled Plasma-Optical Emission Spectrometric Determination of Some Elements from *Anchusa azurea Mill.* Collected in Iraqi Kurdistan Region

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Abstract: The *Anchusa azurea Mill.* (Boraginaceae) is naturally grown in the Kurdistan Region of Iraq and one of those plants that are used by many Kurdish people for cooking especially in the villages. It is a perennial plant and represented in wild Iraq by 26 genera and about 93 species. The present study includes the estimation of elements in the seeds, stems, roots, and leaves of *Anchusa azurea Mill.* Firstly, the percentage of moisture and total ash were determined. After that, the concentration of twenty-one elements was determined from thirty-three elements in plant parts using inductively coupled plasma optical emission spectrometry (ICP-OES). The determined elements were: (Ca, Mg, P, K, Na, S, Al, Ba, B, Cd, Co, Cu, Cr, Fe, Mn, Ni, Sr, Ti, V, Zn, and Zr); among them nine elements were very abundant (≥ 100 ppm) and said to be macro-elements (K, Ca, P, S, Mg, Na, Fe, Al, and Ba). The potassium has recorded the highest concentration in stems (68.9 g/kg), followed Ca (32.6g/kg), P (4.33 g/kg), S (3.98g/kg) and Mg (3.73g/kg) from leaves. The concentrations of eight micro-elements (Sr, Zn, B, Mn, Cu, Ni, Ti, and Cr) were between (1-100 ppm), and the other four trace elements (Co, V, Zr, and Cd) were at (< 1 ppm). On the other hand, the nitrogen percentage (N %) and crude protein percentage (CP %) were determined from the plant parts using the micro-Kjeldahl method. The highest level of (N %) and (CP %) was from leaves part (4.09 % and 25.56 %) respectively. The occurrence of these elements especially in the leaves in addition to the other bioactive compounds makes sure to show the importance of *A. azurea* for a human body reasonable meal.

Keywords: *Anchusa azurea,* heavy metals, Microwave digestion, ICP-OES

1. Introduction

Iraq is well known for its great variation in wild plants due to the countries geographical diversity and variable climatic conditions, especially Kurdistan Region in which Erbil Province is located. Traditional medicine in Iraq can be traced back to the Sumerian period (3000–1970 B.C.), and then to the Babylonian and Assyrian periods (1970–589 B.C.) (Al-Douri, 2014). Hopper and Field (1973) also reported on the useful plants and drugs of Iran and Iraq. Erbil Province boasts a great diversity of plant species given the regions climatic variation and diverse ecological habitats, such as mountains, hills, plains, valleys, and lakes (Hooper & Field, 1937).

Based on their importance elements can be essential (like K, Mg, Ca, Mn, Fe, Co, Cu, and Zn) and they are very important for growth and health, or they may be non-essential (like Cd, Ag and Pb).

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Based on the amount needed nutritionally minerals are grouped into macro-minerals and trace. Elements, such as Mn, Cr, Fe, Co, Cu, Zn, Se, Mo, F and I are essential trace elements, while elements like Ca, Mg and K are grouped under essential macro elements (Kimura, Itokawa, & Nutr, 1990). Essential elements, including the main elements and a number of trace elements, fulfill various functions: as electrolytes, in enzymes, vitamin, hormone constituents (Belitz & Grosch, 1987; Gruchow, et al., 1988) The importance of mineral composition is due to their nutritional properties and beneficial health effects, as well as their meeting of dietary guidelines required for a healthy diet (Welna, et al., 2008). Essential trace elements or micro-nutrients such as cobalt (Co), copper (Cu), manganese (Mn), molybdenum (Mo) and selenium (Se) are elements necessary for maintaining the life processes in plants and/or animals including humans. The required amounts of trace elements are much lower than the required amounts of macronutrients such as Ca, Mg, K, N, and P. For most essential trace elements, while too low uptake/intake causes deficiency, too high causes toxicity (Johnsson, 2005). Before the detection of metals, the procedure which uses to mineralization is very important for obtaining accurate, precise and reliable results in the metallic analysis of plants. Many procedures have been reported for the pretreatment step, specifically, the methods of, wet, dry ashing, and the microwave digestion. Microwave digestion method has many advantages including rapid, efficient and has reproducible results (Maghrabi, 2014).

To estimate the metals in plants, numerous techniques have been used after the dissolution step, for instance: colorimetric, polarimetry, voltammetry, capillary electrophoresis, neutron activation analysis, scanning electron microscopy-energy dispersive X-ray analyzer, flame atomic absorption spectrometry and electrothermal atomic absorption spectrometry, inductively coupled plasma-atomic emission spectroscopy which is the same to inductively coupled plasma optical emission spectrometry, inductively coupled plasma mass spectrometry. One of the most powerful technique is inductively coupled plasma, due to their quick multi-elemental analysis capability and high sensitivity (Ibrahim, et al., 2015; Osw & Masum, 2017). The use of ICP-OES is an appropriate choice for their determination of the higher concentration of metals because no further dilution of the sample solution is needed (Rodushkini & Huhtasaari, 1999; Wu et al., 1997).

Anchusa azurea Mill. (Synonym: Anchusa italica Retz.) is a species of flowering plant known by the common name Italian bugloss and has been used by many Kurdish people for cooking. It is a perennial plant (Tutin et al., 1972) and locally it has many names like Gormza, Kawlla Shina, Chizy, Lsan Al-thor, Ward Mawe (Osw et al., 2017; Hussain et al, 2019). Anchusa azurea (Figure 1) belongs to the Boraginaceae family, which included a variety of shrubs, trees, and herbs, totaling about 2000 species in 146 genera found worldwide, whereas it is represented in wild Iraq by 26 genera and about 93 species (Almusawy, 1987). *A. italica* is native throughout whole Europe, and also found in some countries of Western Asia and Tropical Asia. The species of this family, especially Anchusa are used in folk medicine for wound healing and as a diuretic agent (Yesilada et al. 1995; Honda et al., 1996). The whole plant is antitussive, depurative, and diuretic. The dried and powdered herb is used as a poultice to treat inflammations. Use internally with caution, the plant contains the alkaloid cynoglossine which can have a paralyzing effect (Chiej, 1984) and carcinogenic (Harpestreng et al., 2004). Flowers used to like *tea, tonic to invalids and children; lowers pulsation (Chiej, 1984). The seeds of Anchusa* italica Retz. are rich sources of several saturated and unsaturated fatty acids like; palmitic acid, linoleic acid, oleic acid, γ-linolenic acid, eicosenoic acid (Peshawa et al., 2017; Lopez, et al., 2005; Guil-Guerrero et al., 2001). Furthermore, the plant containing some other compounds like; triterpene glycosides (saponins), polyphenols (flavonoids) (Kuruuzum et al., 2010) toxic and nontoxic pyrrolizidine alkaloids (ESCO, 2009) vitamin E and tannins (Khare, 2007).

Worldwide, there are many investigations have been recorded on *A. azurea* (Lopez et al., 2005; Guil et al., 2001; Kuruuzum et al., 2010; ESCO, 2009; Khare, 2007), at which all of them belong to the phytochemical screening, while no previous mineral study had been done on *A. azurea* neither in Iraq nor in the world. Therefore, the present study aimed to analyze and determine the elemental content in seeds, stems, roots and leaves of *A. azurea* which were collected from Safeen Mountain in Erbil governorate by applying ICP-OES technique after pretreatment by microwave digestion system.

2. Materials and Methods

2.1 Chemicals

All used chemicals were of analytical grade. For ICP-OES (Varian 730-ES) nitric acid (65-71%) and H_2O_2 (30%) (Sigma-Aldrich) were used. For Micro-Kjeldahl digestion method the used chemicals are included; concentrated H_2SO_4 (97%) and 10 ml of H_2O_2 (30%), 0.05 N HCl, 10 N NaOH, H_3BO_3 solution, mixed indicator (methyl red and bromo cresol green indicator) and absolute EtOH.

2.2 Instrument

An inductively coupled plasma-optical emission spectrometry (Varian 730-ES) instrument was taken at ALS Laboratory group (Czech Republic). To estimate moisture% and total ash% the oven and muffle furnace were used respectively, and for determination of N% and crude protein%, Micro-Kjeldahl apparatus was used.

2.3 Plant Material (Collection)

A. azurea was collected during May 2009 from the Garota village (Safeen Mountain) which belongs to Shaqlawa-Erbil/Kurdistan region. The collected plant materials were identified and classified from ESUH (Education Salahaddin University Herbarium). The plant raw materials were washed and air-dried under shade at room temperature. After drying, the plant parts were separately ground into fine powder using a laboratory blender, passed through a 0.71 mm mesh sieve, to provide homogeneous powder for the analysis. Powdered materials were stored in dark bottles and maintained at room temperature until required for analyzes.

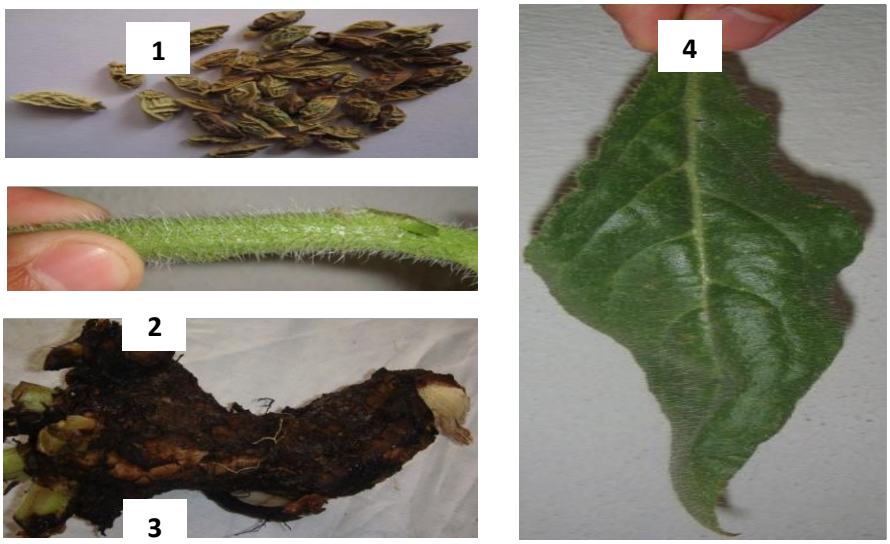

Figure 1: Anchusa azurea Mill.: 1- seeds; 2- stem; 3- root; and 4- leaf

2.4 Determination of Moisture%

Moisture content (%) and total ash content (%) of the study plant parts were determined according to the method suggested by Kaya (Kaya, 1998) and European pharmacopeia (Alaadin, 2002). One gram of each part (seeds, leaves, stems, and roots) of *A. azurea* plant was transferred to porcelain crucibles and incubated for 7 hr. in an oven at 105 °C. The specimens removed from the incubator were re-weighted at room temperature to determine the moisture content (%).

2.5 Determination of Total Ash%

After determination of the moisture, the samples were ignited in porcelain crucibles to constant mass in a muffle furnace at 550-600 °C and the specimens turned into gray color. The crucibles were allowed to cool in a desiccator. The weight of the specimens that were removed from the furnace was re-measured at room temperature to determine the total ash content (%).

2.6 Determination of Elements

Elements were determined by the method of inductively coupled plasma with optical emission spectrometry (ICP-OES). Thirty-three elements (Ca, Mg, P, K, Na, S, Al, Sb, As, Ba, Be, Bi, B, Cd, Co, Cu, Cr, Fe, Pb, Li, Mn, Mo, Ni, Se, Ag, Sr, Te, Tl, Sn, Ti, V, Zn, and Zr) were tested by this technique from four parts of *A. azurea* plant, which are seeds, stems, roots and leaves as shown in Table (2). Elements were extracted from plant samples by a mixture of acids and hydrogen peroxide using microwave assisted acid digestion. Extracts or mineral sates measured directly. Finally, quantification is performed by ICP-OES.

2.7 Sample Preparation

One gram of each sample accurately weighted, then transferred to the vessel of the microwave digestion system with 10 ml of ultra-pure concentrated (Sigma-Aldrich) nitric acid (65-71%) and 2-3 ml H_2O_2 (Sigma-Aldrich) (30% v/v) and then digested in 95 °C in Microwave digestion program. For digestion of Ti and Cr (2 ml) of HCl also was added. Then the Deionized Distilled Water (D.D.W) was added to supplement the volume to 100 ml. A blank was also prepared using all the reagents as in samples and SRM preparation procedure and concurrently analyzed (Feng et al., 1999).

2.8 Parameters for the Setting of ICP-OES Instrument Varian 730-E

RF incident power (1.2 kW), Plasma argon flow rate (15 L/ min), Auxiliary argon flow rate (1.5 L/min), Nebulizer argon flow rate (0.85 L/min), Nebulizer (Cyclonic type), Replicate read time (10 s), Number of readings per replicate (3), Instrument stabilization delay (30 s), Rinse time (60 s), Sample uptake delay (10 s), Pump rate (15 rpm), Detector (Polychromator), Detector temperature (-34.4 ^{o}C)

2.9 Micro-Kjeldahl Determination of Total Nitrogen% and Estimation of Crude Protein% (Akparov & Stepanov, 1961; AOAC, 1990)

Dried powder (1 g) of seeds, leaves, stems, and roots of *A. azurea* were digested in a 100 ml Kjeldahl digestion flask by boiling with 10 ml of concentrated H_2SO_4 (97%) and 10 ml of H_2O_2 (30%) until the solution to be clear. The digest was cooled and then introduced to 50 ml of distilled water in a beaker. The diluted digest was filtered by Whatman no. 1 filter paper and the solution made up to 100 ml with distilled water in a volumetric flask.

1. 10 N NaOH: 40 g of sodium hydroxide dissolved in D.W 100 ml.
2. H_3BO_3 solution: 2 g of dry H_3BO_3 was dissolved in 100 ml of distilled water in a volumetric flask.
3. Mixed indicator: Methyl red indicator 0.066 g and bromo cresol green indicator 0.099 g were dissolved in absolute EtOH (100 ml) and mixed well.

Ammonia in the digest was steam distilled from 10 ml of each digest (as shown above in I) to which had been added 20 ml of solution (1). The liberated ammonia was collected in a conical flask that containing 10 ml of solution (2) and a few drops of mixed indicator solution (3). The color was changed from purple to green, and the liberation of ammonia was continued until the volume was reached to 50 ml. The liberated ammonia then estimated by titrating with standard 0.05 N HCl solution. The blank determination was carried out in a similar manner. The total nitrogen and protein percentage were calculated according to equation (1) and (2) respectively.

% N = (ml acid of sample – ml acid of blank) x 0.05 x 14.01/1000 x 100/ wt. of sample x 100/1 [1]

% protein = % N x protein coefficient [2]

Where protein coefficient = 6.25 for Dicotyledonous plants.

3. Results and Discussion

3.1 Determination of Moisture% and Total Ash Content%

Moisture content % and total ash content % of the studied *A. azurea* parts were determined according to the method suggested by Kaya (1998) and European pharmacopeia (Alaadin, 2002). Moisture content measurement of the plant parts was based on the principle of "removal of plant water at a certain temperature" and "detection of moisture content according to the resulting weight loss". The highest content of moisture % was from leaves part while the lowest content was from roots part as shown in Table (1). Ash detection was based on the principle of "burning a certain amount of a specimen and converting it into ash". Also, the highest content of total ash % was from leaves part, but the lowest content was from stems part of the plant, Table (1).

Table 1: Percentage of moisture and total ash content from A. azurea parts

Plant parts	Moisture%	Total ash %
Seeds	7.43	20.33
Stems	6.88	17.52
Roots Leaves	6.83	18.31
	7.69	22.88

3.2 Determination of Elements by ICP-OES Analysis

Uptake of metals by medicinal plants is influenced by a number of factors, including metal concentrations in soils, cation exchange capacity, organic matter content, types and varieties of plants. However, the existing environmental condition and the composition of the plant is mainly dependent on the composition of the soil, which influenced primarily by the nature of the rocks from which the soil is derived. The special flow, temperature and humidity conditions of the air layer right above the

ground entail extreme fluctuations of element concentration. Furthermore, contamination problems which are not easy to assess (Bakhru, 2006).

The elements in the medicinal plants play an important role in the treatment of diseases. Some of the common elements in medicinal plants are K, Ca, Fe, Zn, Sr, etc. and the quantities of these elements in different medicinal plants or different parts from one medicinal plant are found to be varied leading to the conclusion that they are used for specific purposes. Analysis of mineral nutrients and trace elements in the *A. azurea* parts were performed by ICP-OES technique which is one of the most powerful techniques for its quick multi-elemental analysis capability and high sensitivity. Twenty-one elements were detected from the analyzed thirty-three elements. There are various methods for the classification of nutritional elements, but the most common one is that which based on their uses by the plant which are; macro, micro, and trace element.

Macro-elements are needed by the plant in relatively higher amounts than the other groups, which reaches (≥100 ppm) for the plant to grow naturally. Also, macro-elements is said to be major elements because they are present at the high level. The concentration of each macronutrient was varying from the plant parts. The First macronutrient is potassium, and its concentration is very high in stems of *A. azurea* (68.9g/kg), but its lowest concentration from seeds (24.1g/kg). The second macronutrient is calcium, its highest level from leaves part of the plant (32.6g/kg) while its lowest level from stems (9.56g/kg). Phosphorous is the third macronutrient, the concentration of P is ranged between (4.330g/kg) from leaves part and (2.0g/kg) from stems part of the plant. The highest level of Mg and S were from leaves part (3.73 and 3.98g/kg) respectively, whereas the lowest level of them was from stems part (1.03 and0. 85g/kg) respectively, Table (2).

Micro-elements are those nutritional elements needed by the plant in relatively lower amounts which are between (1 – 100 ppm). Although essential for plant growth, micronutrients can produce toxic effects if they reach a very high concentration in the plant tissues. The first micronutrient is strontium, its present in the highest level in roots (0.0778 g/kg) and the lowest level in seeds part of the plant (0.0268 g/kg). After strontium, the zinc is the second micronutrient that is present in highest amounts, the highest concentration of Zn was from leaves part (0.0467 g/kg) whereas the lowest concentration was from stems part (0.0141 g/kg), Table (2).

Trace elements are those nutritional elements needed by the plant in very trace amounts, which are (< 1 ppm), therefore they may be called minor-elements. Trace elements were divided into two groups. The first group was detected and consists of four trace elements which are (Co, V, Zr, and Cd). All of them were present from roots of the plant in a high concentration except Cd. The second group consists of twelve trace elements, it cannot be detected at that concentration, because they mainly exist in very low concentration or perhaps where there are no such elements, Table (2).

Table 2: Concentration of determined elements from *A. azurea* parts

Elements	Symbol	LOD (ppm)	Anchusa azurea elemental content in (ppm)			
			Seeds	Stems	Roots	Leaves
Aluminum	Al	2	25.9	46.4	303	234
Antimony	Sb	1	<1	<1	<1	<1
Arsenic	As	2	<2	<2	<2	<2
Barium	Ba	0.5	60.4	75.5	178	94.7
Beryllium	Be	0.05	<0.05	<0.05	<0.05	<0.05
Bismuth	Bi	2	<2	<2	<2	<2
Boron	B	2	20.8	19.1	19.2	71.7
Cadmium	Cd	0.1	<0.1	<0.1	0.11	0.12
Calcium	Ca	10	18500	9560	31500	32600
Chromium	Cr	0.2	0.7	0.4	1.8	1.4
Cobalt	Co	0.5	<0.5	<0.5	0.6	<0.5
Copper	Cu	0.2	11.1	10.5	12.6	15.6
Iron	Fe	1	65.2	72.3	426	342
Lead	Pb	1	<1	<1	<1	<1
Lithium	Li	0.5	<0.5	<0.5	<0.5	<0.5
Magnesium	Mg	3	2540	1030	1580	3730
Manganese	Mn	0.1	22.8	10.3	31.7	41.4
Molybdenum	Mo	0.5	<0.5	<0.5	<0.5	<0.5
Nickel	Ni	0.5	1.15	0.91	6.50	2.24
Phosphorus	P	2	3750	2000	3760	4330
Potassium	K	10	24100	68900	45700	51300
Selenium	Se	5	<5	<5	<5	<5
Silver	Ag	0.5	<0.5	<0.5	<0.5	<0.5
Sodium	Na	10	128	250	545	334
Strontium	Sr	0.2	26.8	39.3	77.8	55.5
Sulfur	S	15	2010	850	856	3980
Tellurium	Te	2	<2	<2	<2	<2
Thallium	Tl	1	<1	<1	<1	<1
Tin	Sn	2	<2	<2	<2	<2
Titanium	Ti	0.1	0.29	1.07	3.96	2.14
Vanadium	V	0.1	<0.1	0.12	1.07	0.58
Zinc	Zn	0.2	41.3	14.1	29.2	46.7
Zirconium	Zr	0.1	0.14	<0.1	0.18	<0.1

LOD; Limit of detection in ppm

Some of the micronutrients and trace elements are known to be essential for our body which is: Cr, As, Co, Cu, F, I, Fe, Mn, Mo, Ni, Se, Si, Sn, V, Zn and the other essential major elements are C, H, O, N, S, Ca, P, K, Na, Cl and Mg totaling twenty-six essential elements (Madan & Choudhury, 1991). Most of them are present in the *A. azurea*. So different trace elements in the different medicinal plants will have their definite role for the smooth functioning of our body.

3.3 Determination of Total Nitrogen (N %) and Crude Protein (CP %) from *A. azurea* Parts

Micro-Kjeldahl method was used to determine the percentage of total nitrogen and crude protein from four parts of *A. azurea* plant. The results obtained from this method showed that the highest N % and CP % were from leaves part (4.09 % and 25.56 %) respectively, while the lowest of them were from stems part (1.11 % and 6.93 %) respectively, as shown in Table (3).

Table 3: Percentage of N and CP from *A. azurea* parts

Plant parts	N %	Protein %
Seeds	2.31	14.43
Stems	1.11	6.93
Roots	1.57	9.81
Leaves	4.09	25.56

The Comparison of these results with those in the literature (Cover Crop Chart 2010) (Clark, 2010). Table (4) showed that the *A. azurea* (whole plant) contains a higher percentage of crude protein.

Table 4: Crude protein percentage for some plants

No.	Plant name	Protein %
1	Amaranth (Amaranthus sp.)	≈14
2	Beet (Beta vulgaris)	7-15
3	Carrot (Daucus carota var. sativus L.)	10
4	Cowpea (Vigna unguiculata L.)	19-24
5	Medic (Medicago spp.) black medic	19-21
6	Mung bean (Vigna radiataL.)	16-23
7	Spinach (Spinacia oleracea L.)	≈20
8	Teff (Eragrostis tef (Zuccagni) Trotter)	10-18
9	Turnip (Brassica rapa L. var. rapa)	12-16
10	Vetch (Viciasp.)	13-20

4. Conclusion

The present investigation includes the estimation of elements in different parts of *A. azurea*, which collected from Safeen Mountain (Erbil, Kurdistan Region-Iraq) by ICP-OES technique for the first time. The obtained results showed the presence of elements which are very important for human life such as K, Ca, P, Fe…..etc, and the concentration of some of these elements were very high like K and Ca. On the other hand, it has been found that *A. azurea* contains a high percentage of crude protein if compared with other plants.

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