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# Elemental Analysis of Some Libyan Medicinal Plants Using Inductively Coupled Plasma Atomic Emission Spectroscopy

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## التحليل العنصري لبعض النباتات الطبية الليبية باستخدام مطيافية البلازما المقترنة بالحث

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**Abstract**
The elemental compositions of four medicinal plants grown in Libyan wilderness, particularly Juniperus Communis, Globularia alypum, Origanun majorana and Ajuga Iva, have been analyzed using four different approaches. All plant samples were analyzed for 18 elements (Ca, Mg, K, Na, P, Fe, Si, Mn, Zn, Cu, Co, Cr, Ni, Sn, Al, Hg, Pb and Cd) by ICP-MS technique .The macronutrients, calcium, magnesium, potassium, sodium, phosphorus and iron are found to be the dominate species in the selected samples ranging from calcium which has the highest concentration to iron with the lowest content. The data also demonstrated that the highest micronutrient concentrations were observed for silicon, manganese, and zinc, respectively. The concentration level of aluminum is considered to be relatively high compared with other toxic elements which could be due to the high soil and environmental content of this element or may be due to the much higher selectivity of these plants towards aluminum.
A variety of statistical methods such as Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), Hierarchical Cluster Analysis (HCA), and boxplot graphical tool were applied to examine the significant differences between the selected medical plants regarding their elemental composition, as well as to identify variations arising from different sample preparation methods. In these all, the statistical analysis revealed significant differences in yield between the water extraction procedure and other extraction methods, a discrepancy that can be explained by the limited solubility of the corresponding water-soluble salts. Meanwhile there is a slight difference between ashing method and other two methods (HNO3/H2O2 and HNO3/HClO4 methods). In general, the concentration ranges obtained using (HNO3/HClO4) acid mixture is a bit higher compared to (HNO3/H2O2) and dry ashing methods.

**Keywords**: medicinal plants, water solubility, ICP-AES, statistical analysis**.**

**الملخص**
اختص هدا البحث بدراسة التحليل العنصري لأربعة نباتات طبية نمت في البرية الليبية، وهي Jumiperus communis و Globularia alypum و Origanum majoranaو Ajuga Iva. وذلك باستخدام أربع طرق هضم مختلفة لتحضير العينات.

 

شمل التحليل قياس 18 عنصرًا وهي (Ca, Mg, K, Na, P, Fe, Si, Mn, Zn, Cu, Co, Cr, Ni, Sn, Al, Hg, Pb, Cd) باستخدام تقنية مطيافية البلازما المقترنة بالحث.(ICP-ES)
أظهرت النتائج أن العناصر الغذائية الكبرى، وهي: الكالسيوم، والمغنيسيوم، والبوتاسيوم، والصوديوم، والفوسفور، والحديد، تمثل العناصر السائدة في العينات النباتية المدروسة، إذ تراوحت تراكيزها من الكالسيوم الذي سجل أعلى نسبة تركيز إلى الحديد الذي أظهر أدنى محتوى.
كما بينت البيانات أن أعلى تراكيز للعناصر الصغرى كانت لكل من السيليكون والمنغنيز والزنك على التوالي.
لوحظ أيضا أن تركيز الألمنيوم مرتفع نسبيًا مقارنة ببقية العناصر السامة، وقد يُعزى ذلك إلى ارتفاع محتواه الطبيعي في التربة والبيئة المحيطة، أو إلى نسبة الامتصاصية العالية لهذه النباتات لعنصر الألمنيوم.
تم تطبيق بعض الأساليب الإحصائية والتي شملت تحليل المكوّنات الرئيسية(PCA) ، وتحليل التمييز بالمربعات الصغرى الجزئية(PLS-DA) ، والتحليل العنقودي الهرمي(HCA) ، بالإضافة إلى أداة المخطط الصندوقي(Boxplot) ، وذلك لدراسة الفروق المعنوية بين النباتات الطبية المختارة من حيث تركيبها العنصري، وكذلك لتحديد التباينات الناتجة عن اختلاف طرائق تحضير العينات.
بصورة عامة، أظهرت النتائج الإحصائية وجود فروق معنوية واضحة في النتائج بين طريقة الاستخلاص بالماء وبقية طرائق الاستخلاص الأخرى، ويمكن تفسير هذا الاختلاف بـ محدودية ذوبانية الأملاح القابلة للذوبان في الماء فقط.
وفي المقابل، لوحظ وجود فرق طفيف بين طريقة (Dry Ashing) وكل من طريقتي ($HNO_3/H_2O_2$)و($HNO_3/HClO_4$) .

**الكلمات المفتاحية:** نباتات طبية، قابلية الذوبان في الماء، ICP-AES، التحليل الإحصائي.

## Introduction

Medicinal herbs have long been used by humans in traditional medicine. Although their use declined over the last century with the emergence of synthetic drugs, the utilization of easily accessible and low-cost medicinal plants has persisted, continuing to coexist with modern medical practices [1]. There has been a revival of social interest in the use of herbal products because of their observed and proven efficacy and being free from some toxic effects associated with synthetic drugs. Nowadays, plant materials are widely used throughout developed and developing countries as home remedies, nutritional supplements [2] or as raw materials for the pharmaceutical industry, representing a substantial proportion of the global drug market [1].

In addition, health treatments based on medicinal plants are being prescribed by doctors in the forms of plant extracts, infusions or by direct ingestion of very fine powder plant. Likewise, they are recommended as a nutritional supplement for the treatment of everyday problems such as stress and insomnia. From a medical point of view, the most important constituents of plants are pharmacologically active compounds such as flavonoids, alkaloids, glycosides and many other organic substances. Beside, medicinal plants contain essential and trace elements, which can be available to the human body from any kind of consumption of herbs and their extracts.

For many chemical elements, there is a narrow range between deficiency and toxicity for the human body. Therefore, the adequate establishment of health guidelines for food is often difficult. The contents of major and trace elements in plants are governed both by the geochemical features of the soil where the plant grows and by the ability of plants to accumulate elements selectively. Moreover, plants can accumulate elements from the surrounding aerial or aquatic environment, enabling some plants to be used as biomonitors [3-6] for environmental pollution.

An element is considered to be essential if it satisfies some observed requirements [7, 8] including a deficiency causes functional disorder or some other form of reduced function, a supplementation with the element is of important to development and deficiency symptoms associated with decreased concentration of the element in the blood or in other body fluids.

With these considerations, the following elements H, C, O, N, P, S, F, Na, Hg , Ca, Cl, Si, V, Cr, Mn, Fe, Co, Cu, Zn, Se, Mo, Sn, I and possibly Ni have been worldwide approved to be essential for human [7], . These elements are divided into three groups depending on their concentration in the body : I- Macronutrients: This group are included the elements of which our daily requirements is more than 100 mg such as Ca, Fe, P, Na, K, Cl, Mg and S [7]. II- Micronutrients: The daily requirements of group minerals (Cu, Zn, Mn, I, Mo, Se, F, Br, Cr, Sn, Co, V and Si) ranging from less than 1 mg and up to 100 mg[7].

Based on the arbitrary division, [9] we will consider the element as major elements if it presents in the concentration range from 1% to 100 % of the investigated sample. If the concentration range is from 0.01 % to 1.0 % then the element is considered as a minor element constituent of the sample. Meanwhile, the element with concentration ranging from $10^{-7}$ % to 0.01 % is classified as Trace Element constituent. Trace Elements have been classified into three groups based on their importance for human health [7]. First, essential trace elements (micronutrients), including mostly transition metals such as Zn, Cu, Mn, Cr, Co, Mo, Si, Sn, F and I. Second, probably essential elements such as Ni. The third group consists of non-essential toxic trace elements such as Hg,

 

Pb, As, Cd which are potentially toxic even at relatively low concentrations and can cause serious damage to the human body [7].

Due to the lack of documented data concerning the levels of minerals in all of the Libyan wild medicinal herbs, this study was undertaken with two main objectives in mind: First, the establishment of a reliable data base concerning a large number of major, minor and trace elements in a number of Libyan wild medicinal herbs, which could be responsible for the alleged therapeutic values of these plants. Secondly, the development of a reliable mineralization procedure which could be applied efficiently to extract most of the minerals from the complex matrix of these wild herbs.

Several different digestion procedures were applied for destruction of sample matrix and therefore extraction of the different elements of interest before spectroscopic measurements. Since these digestion procedures were not originally specifically developed for trace elements in wild medicinal plants, a comparative study was carried out to determine the concentration of these elements and evaluate the efficiency of different digestion procedures to extract the elements of interest.

## Material and methods

### Chemicals

High purity water (deionized water) used throughout this work was produced by both distillation and ion exchange resins treatment. The analytical grade chemicals are: Nitric acid ($HNO_3$, 65w/w %, pure) was supplied by Farmitalia Cario Erba S.P.A and Perchloric acid ($HClO_4$ concentrated, pure) was obtained from Fluka chemika and Hydrogen peroxide ($H_2O_2$ 30 w/w %) was supplied by Merck (Darmstadt, Germany) and Hydrochloric acid (HCl, 37w/w %,) was obtained from Carlo Erba). Sulphuric acid ($H_2SO_4$) was obtained from BDH. Calcium chloride ($CaCl_2$, pure) and ammonium iron (II) sulfate hexa hydrate [$(NH_4)_2Fe\ (SO_4).6H_2O$] were supplied by Merck (Darmstadt, Germany). Cobalt nitrate [$CO(NO_3)_2.6H_2O$, 98w/w %] and chromium nitrate [$Cr(NO_3)_3.9H_2O$, 99 w/w %] and copper nitrate [$Cu\ (NO_3)_3.3H_2O$, 99w/w %] were purchased from Riedel-de haenag seize-Hannover. Lead nitrate [$Pb(NO_3)_2.4H_2O$, 99.5w/w %] were obtained from analyticals Carlo erba. Zinc oxide (ZnO, 99.9 w/w %), tin metal, sodium chloride (NaCl), sodium metasilicate ($Na_2SiO_3.5H_2O$), potassium chloride (KCl), nickel nitrate [$Ni(NO_3)_2.6H_2O$], manganese dichloride ($MnCl_2.4H_2O$), magnesium metal, cobalt (II) chloride ($CoCl_2.6H_2O$), aluminum metal and potassium dihydrogen orthophosphate ($KH_2PO_4$) were supplied from Ventron Gmbh.

### Collection and pretreatment of plant samples

The medicinal plants Juniperus Communis, Globularia alypum, Origanun majorana and Ajuga Iva were hand harvested from Libyan wilderness at the end of spring. The collected plant materials were washed thoroughly with double distilled and deionized water then air- dried for two to three weeks. The plant samples were then chopped up and subjected to an additional cleaning step using deionized water in an ultrasonic bath, followed by oven-drying at approximately 90 °C for at least 24 hours. Finally, the plant samples were chopped and grounded in a ceramic mortar and pestle to obtain semi powder samples and stored in tightly closed glass jar away from direct sunlight and heat.

### Methods of digestion

In this study four methods were used to prepare the samples for the analysis using Inductively Coupled Plasma Emission Spectroscopy (ICP-ES) technique as follow:

### Wet digestion procedure 1

The plant samples were dried for 1 hr at 90°C, and permitted to return to room temperature in a desiccator. Individual samples containing 2.000 grams of plant were weighed into 80-mL beakers and covered with watch glasses then carefully introduced 20.00 mL con. $HNO_3$ to each beaker and swirled periodically to remove the $NO_2$ bubble, and never allowed the foam to reach the upper edge of the beakers.

The mixtures were refluxed gently in a hood for 6 hours (until the volumes became less). The contents were allowed to cool for 5 minutes at room temperature. After that 5 mL of $HClO_4$ were added and boiled gently (around 2 hrs) to almost dryness then the residues were allowed to cool to almost room temperature.

The residues were dissolved in 0.1N $HNO_3$, heated to boiling point, then cold to room temperature and filtered through a glass filter into 50-mL volumetric flasks and diluted to the marks with 0.1N $HNO_3$. The concentration of the resulting solution is either directly measured or further diluted before measurement, as it is appropriate.

 

**Wet digestion Procedure 2**
The procedure of wet digestion method similar to the pervious method. The only difference is the using of $H_2O_2$ instead of $HClO_4$ for the treatment of the sample mixture .

**Dry ashing Procedure**
The plant samples were placed in dishes in an oven at 90°C for a minimum of 24 hrs and then allowed to cool in a desiccator. The silica crucibles were cleaned by placing in (1:1) nitric acid and heating to boiling point. Then the crucibles were transferred to a muffle furnace and fired at 500°C. After cooling, individual samples containing 2.000g of plant were weighed into the crucibles, then placed in a muffle furnace and the temperature was increased slowly to 500°C and maintained for 8hrs. The crucibles were removed and allowed to cool. The ash was wetted by a few drops of water, and added 1.00 mL of (1:1) nitric acid (suprapur; 65%) dropwise.
The crucibles were transferred to electrical hot plates. After the contents had dried, the crucibles were placed in a muffle furnace for a further 2 hrs at 500°C then allowed to cool to room temperature.
Sample solutions were prepared by dissolving the contents of each of the crucibles in 4.00 mL of (1:1) nitric acid (suprapure), then filtered through a glass filter into 50-mL volumetric flasks and carefully brought to volume with deionized water.

**Water infusion procedure**
Individual samples containing 2.000 grams of plant were weighed into 80-mL beakers and covered with watch glasses. 60.0 mL of water were added to the beakers and refluxed gently for 2 hrs, then filtered through into 50-mL volumetric flasks and diluted to the marks with 0.05N $HNO_3$.

**Samples Analysis**
The selected samples were analyzed for, Ca, Mg, K, Na, P, Fe, Si, Mn, Zn, Cu, Co, Cr, Ni, Sn, Al, Hg, Pb and Cd by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-ES), Model S, Industrial research center, Tripoli-Libya.
The ICP-MS was optimized and calibrated with internal standards before measuring the plant samples. A high level of helium gas was used to prevent from the interferences. The instrumental parameters and operating conditions used for ICP-ES are shown in the following table:

**Table**. ICP-AS spectrometer operating parameters

| ICP-MS operation parameters | Description/ value |
|---|---|
| Plasma gas | Ar X50S 5.0 |
| Makeup gas | 0.9 L/min |
| Carrier gas | 1.1 L/min |
| Plasma gas flow (Ar) | 15 L/min |
| Radio frequency power | 1550 W |
| Injector | 2.0 mm |
| Nebulizer pump | 0.1 rps |
| Sample intake | 0.5 mL/min |

**Results and discussion**
**Comparison of the levels of the studied elements within each individual studied plant**
We attempt in this part to investigate and interpret the concentration trends and profiles of each studied element in the selected Libyan wild medicinal plants. The results were obtained for Ca, Mg, K, Na, P, Fe, Si, Mn, Zn, Cu, Co, Cr, Ni, Sn, Al, Hg, Pb and Cd based on several methods of sample preparation with ICP–ES analysis.

**The macronutrients elements**
The mean concentration of macronutrients elements based on different digestion methods with a mixture of ($HNO_3$/$HClO_4$), and with a mixture of ($HNO_3$/$H_2O_2$) acids, dry ashing and water infusion procedures for the selected plants are shown in Figure 1 and Table1:

 

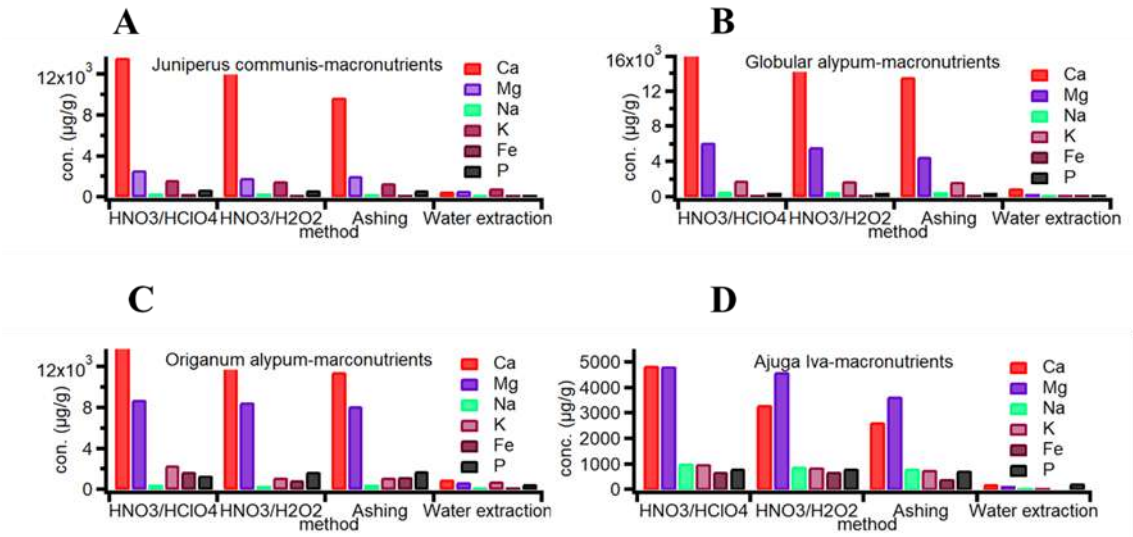


**Figure1**: The concentration of macronutrients elements in the selected Libyan herbal plants with four different procedures.

**Table 1**: The mean concentration (µg/g) of macronutrients in medical plants*

| plant | method | Ca | Mg | Na | K | Fe | P |
|---|---|---|---|---|---|---|---|
| J. Communis | $HNO_3/HClO_4$ | 15632 | 2668 | 416 | 1718 | 325 | 744 |
| | $HNO_3/H_2O_2$ | 16339 | 1883 | 379 | 1578 | 277 | 728 |
| | ashing | 11765 | 2115 | 329 | 1386 | 205 | 708 |
| | water infusion | 664 | 580 | 176 | 925 | 17 | 190 |
| G. alypum | $HNO_3/HClO_4$ | 20991 | 6194 | 641 | 1899 | 339 | 515 |
| | $HNO_3/H_2O_2$ | 22390 | 5675 | 617 | 1874 | 317 | 543 |
| | ashing | 19637 | 4607 | 573 | 1777 | 276 | 491 |
| | water infusion | 1024 | 351 | 45.51 | 131 | 11.75 | 26.34 |
| O. majorana | $HNO_3/HClO_4$ | 16217 | 8831 | 547 | 2385 | 1803 | 1423 |
| | $HNO_3/H_2O_2$ | 11486 | 8541 | 416 | 1198 | 985 | 1801 |
| | ashing | 15579 | 8168 | 510 | 1205 | 1246 | 1855 |
| | water infusion | 1023 | 793 | 111 | 832 | 11.27 | 594 |
| A. iva | $HNO_3/HClO_4$ | 4877 | 4854 | 1036 | 1011 | 723 | 842 |
| | $HNO_3/H_2O_2$ | 3342 | 4615 | 832 | 890 | 710 | 847 |
| | ashing | 2655 | 3666 | 839 | 900 | 445 | 762 |
| | water infusion | 235 | 180 | 21.94 | 100 | 1.82 | 276 |

*Average of triplicate determinations, %R.S.D.= 0.03-0.22.

According to Figure.1 and Table 1, the macronutrients: calcium, magnesium, potassium, sodium, iron and phosphorus are found to be the dominate species ranging from calcium which has the highest concentration to iron which has the lowest concentration in most plant samples. This found to be acceptable regardless of the method of sample preparation used to bring the concerned elements into the solution for ICP-MS measurement. In most samples, the concentration superiority of the alkaline earth metal calcium over that of magnesium and the alkaline metal potassium over sodium is very obvious using any method of sample preparation. However, the other important and usually less abundant macronutrient phosphorus and iron were also found in significant amounts in these claimed Libyan wild medicinal plants which may make it a good source for these important macronutrients.

In general, the concentration range obtained using ($HNO_3/HClO_4$) acid mixture is a bit higher compared to ($HNO_3/H_2O_2$) and dry ashing methods. This could be related to the oxidizing power of acids, which results in the complete destruction of the organic matter and therefore bringing into the solution most of the metal content of the sample or maybe due to the volatile of these elements during ashing process.

It is noteworthy that the mean concentration of calcium, magnesium, potassium, and sodium in water extract is significantly lower than the concentration in the solution of the acid digested samples. This most likely attributed to the lower solubility of organic and the inorganic compounds of these elements in water

.

 

From the given data table, we can also infer that the significant amounts of the macronutrients: calcium, magnesium, sodium, potassium, iron and phosphorus are indeed existed in the aqueous extract of this claimed wild medicinal plants. Therefore, this water extract could significantly contribute to satisfy the daily allowance recommended by the world health organization (WHO), which might explain the claimed healing effects of the plant when used as a remedy.

Based on the natural abundance of these elements, the observed concentrations of calcium, magnesium, sodium, iron, potassium and phosphorus were found to be in the expected order [10]. In addition, their amount in this studied wild medicinal plant were found within the concentration ranges of the other medicinal plants in literature survey data [13-19].

**The micronutrients elements**

The average data of digesting methods with acids and dry ashing is presented in Figure 2 and Table 2.

The results showed that silicon had the highest concentration among the trace elements, followed by manganese, and zinc respectively. These three micronutrients were significantly more abundant than the other measured elements: copper, cobalt, nickel, chromium, and tin.

The concentrations pattern was found for the analyzed samples as follow:

**Si** >> **Mn** > **Zn** > **Cu** > **Co** > **Ni** > **Cr** > **Sn**

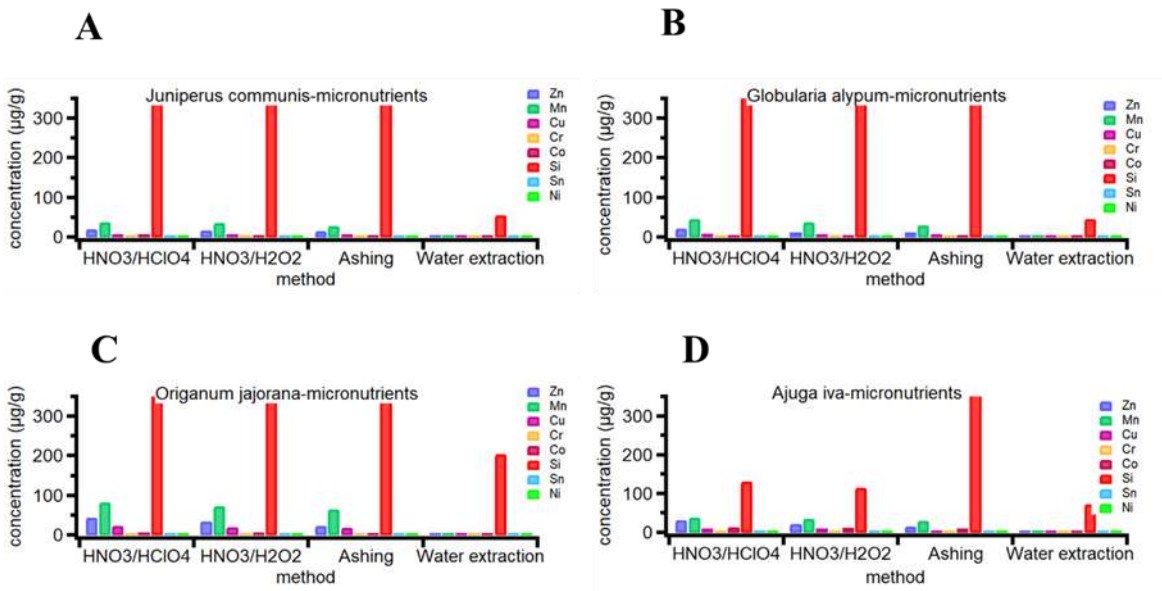


**Figure 2**: The concentration of micronutrients elements in selected Libyan herbal plants with different procedures.

**Table 2:** The mean concentration (µg/g) of micronutrients in medical plants*

| plant | method | Zn | Mn | Cu | Cr | Co | Si | Sn | Ni |
|---|---|---|---|---|---|---|---|---|---|
| J. Communis | $HNO_3/HClO_4$ | 20.88 | 38.75 | 8.6 | 3.67 | 7.57 | 1220 | 2.51 | 5.03 |
| | $HNO_3/H_2O_2$ | 17.65 | 36.84 | 7.56 | 3.59 | 6.86 | 1134 | 2.19 | 4.61 |
| | ashing | 15.52 | 29.33 | 7.25 | 3.67 | 4.19 | 1587 | 2.09 | 4.04 |
| | water infusion | 1.33 | 0.98 | 0.93 | 0.37 | 0.77 | 55.99 | 0.0 | 0.62 |
| G. alypum | $HNO_3/HClO_4$ | 22.24 | 45.99 | 10.01 | 3.65 | 5.15 | 424 | 2.11 | 5.05 |
| | $HNO_3/H_2O_2$ | 12.73 | 38.16 | 7.92 | 3.65 | 4.5 | 346 | 2.16 | 4.64 |
| | ashing | 12.56 | 29.85 | 8.24 | 3.66 | 4.01 | 479 | 2.14 | 4.48 |
| | water infusion | 2.82 | 1.25 | 0.80 | 0.38 | 0.38 | 46.52 | 0.0 | 0.49 |
| O. majorana | $HNO_3/HClO_4$ | 44.3 | 84.07 | 24.86 | 3.03 | 8.5 | 1203 | 7.11 | 7.2 |
| | $HNO_3/H_2O_2$ | 35.59 | 73.67 | 20.4 | 2.18 | 8.01 | 840 | 6.82 | 5.47 |
| | ashing | 23.41 | 66.11 | 18.93 | 3.65 | 6.87 | 1275 | 6.27 | 4.72 |
| | water infusion | 3.63 | 2.56 | 1.43 | 0.33 | 0.56 | 206 | 0.0 | 0.49 |
| A. iva | $HNO_3/HClO_4$ | 32.14 | 38.51 | 12.25 | 3.64 | 15.46 | 133 | 6.42 | 4.38 |
| | $HNO_3/H_2O_2$ | 23.45 | 35.63 | 11.08 | 3.62 | 13.16 | 116 | 6.37 | 3.77 |
| | ashing | 16.89 | 30.63 | 6.94 | 3.63 | 12.05 | 384 | 6.03 | 3.18 |
| | water infusion | 1.02 | 1.72 | 0.75 | 0.35 | 1.49 | 73.45 | 0.0 | 0.61 |

*Average of triplicate determinations, %R.S.D.= 0.04-0.23.

 

The relative concentration of these micro elements were found to be in agreement with the findings of many other researchers working on medicinal plants in different parts of the world. Based on the natural abundance of these elements, the concentration of silicon, manganese, zinc, copper, nickel and cobalt were found to be in the expected order [13, 15, 16, 19-21].

**The toxic elements**

According to Table 3 and Figure 3, the concentration of aluminum is considered to be relatively high compared with other toxic elements which could be due to high soil and environmental content of this element or may be due to the much higher selectivity of these plants towards aluminum. The concentration of toxic trace elements in these Libyan wild medicinal plants were found within the concentration ranges of the other medicinal plants in Africa**,** Europe and Asia [13, 18].

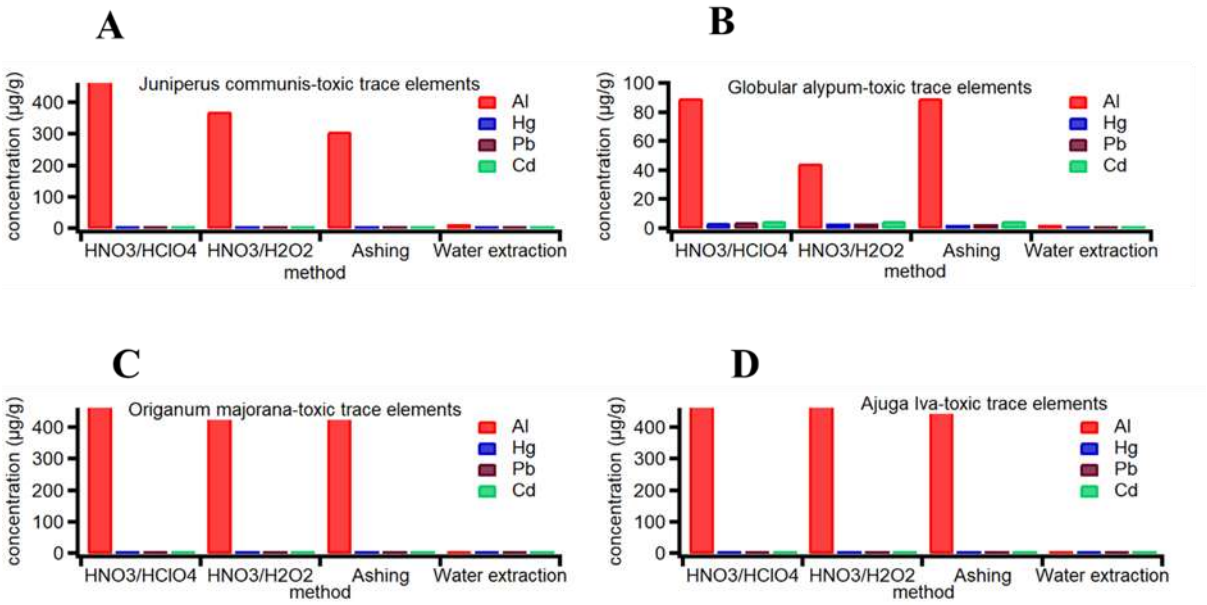


**Figure 3**: The concentration of toxic elements in selected Libyan herbal plants with different procedures.

**Table 3**: The mean concentration of toxic elements in medical plants*

| plant | method | Al | Hg | Pb | Cd |
|---|---|---|---|---|---|
| J. Communis | $HNO_3/HClO_4$ | 484 | 2.94 | 5.22 | 5.65 |
| | $HNO_3/H_2O_2$ | 373 | 2.52 | 4.37 | 5.63 |
| | ashing | 309 | 2.34 | 4.35 | 5.65 |
| | water infusion | 14.6 | 0.0 | 0.6 | 0.02 |
| G. alypum | $HNO_3/HClO_4$ | 339 | 4.14 | 4.46 | 5.65 |
| | $HNO_3/H_2O_2$ | 284 | 3.65 | 3.76 | 5.66 |
| | ashing | 386 | 2.54 | 3.37 | 5.65 |
| | water infusion | 2.54 | 0.0 | 0.42 | 0.56 |
| O. majorana | $HNO_3/HClO_4$ | 1472 | 4.31 | 6.53 | 5.66 |
| | $HNO_3/H_2O_2$ | 1489 | 2.83 | 6.27 | 5.65 |
| | ashing | 1401 | 2.83 | 5.78 | 5.65 |
| | water infusion | 7.36 | 0.0 | 1.11 | 0.94 |
| A. iva | $HNO_3/HClO_4$ | 955 | 7.14 | 5.1 | 5.62 |
| | $HNO_3/H_2O_2$ | 749 | 6.46 | 4.59 | 5.64 |
| | ashing | 602 | 3.81 | 3.8 | 5.64 |
| | water infusion | 0.75 | 0.0 | 0.28 | 0.55 |

*Average of triplicate determinations, %R.S.D.= 0.05-0.27.

**Statistical Analysis results**

The statistical techniques including Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), Hierarchical Cluster Analysis (HCA) and boxplot tool were performed to evaluate the significant differences between selected medical plants regarding their

 

elemental composition, as well as to identify variations arising from different sample preparation methods

The Principal Component Analysis (PCA) results demonstrated a strong separation between each plant from the others. For example, the PCA result showed that there is a significant difference between G. alypum and J. Communis in terms of digestion methods, with the first two principal components explaining 94% of the total variance, as depicted in Figure 4**.** Also, the PCA result exhibit that there is significant difference between water extracting method and other methods (wet and dry methods). The difference could be explained by the elemental composition that extracted by water only represent the soluble salts only. There is a slight difference between $HNO_3/HClO_4$ method and other two methods ($HNO_3/H_2O_2$ and ashing method), as seen in Figure 4.

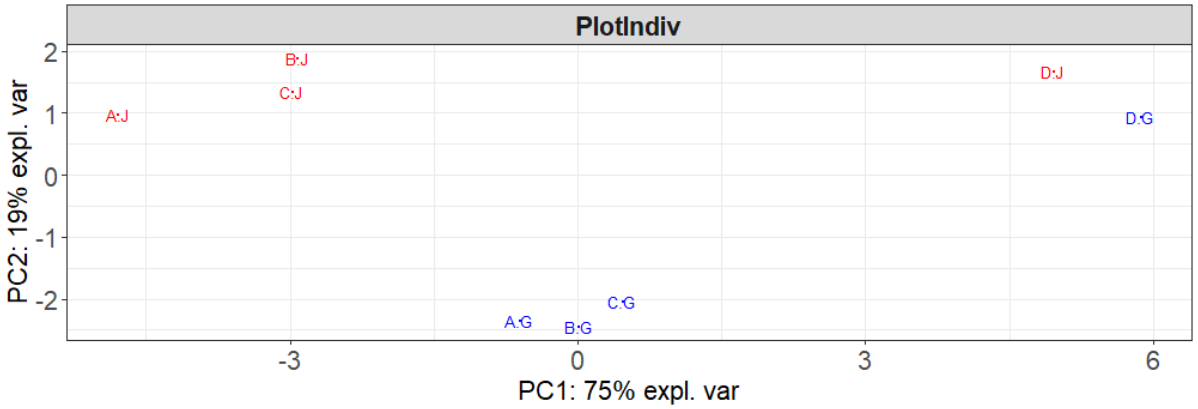


**Figure 4**: PCA Analysis of elemental composition of G. alypum and J. Communis; AG ($HNO_3/HClO_4$ for G. alypum), BG ($HNO_3/H_2O_2$ for G. alypum), CG (dry ashing for G. alypum), DG (water extraction for G. alypum), AJ ($HNO_3/HClO_4$ for J. Communis), BJ ($HNO_3/H_2O_2$ for J. Communis), CJ (dry ashing for J. Communis), DJ (water extraction for J. Communis).

The results of the Partial Least Squares Discriminant Analysis (PLS-DA) analysis (Figure 5) demonstrate a clear distinction between G. alypum and J. Communis in terms of digestion methods. The analysis also reveals distinct clustering for certain groups, water extraction method and other methods. However, there is some overlap observed between the water extraction method for both plants, suggesting partial similarity in the ability of extract the elements from the plants.

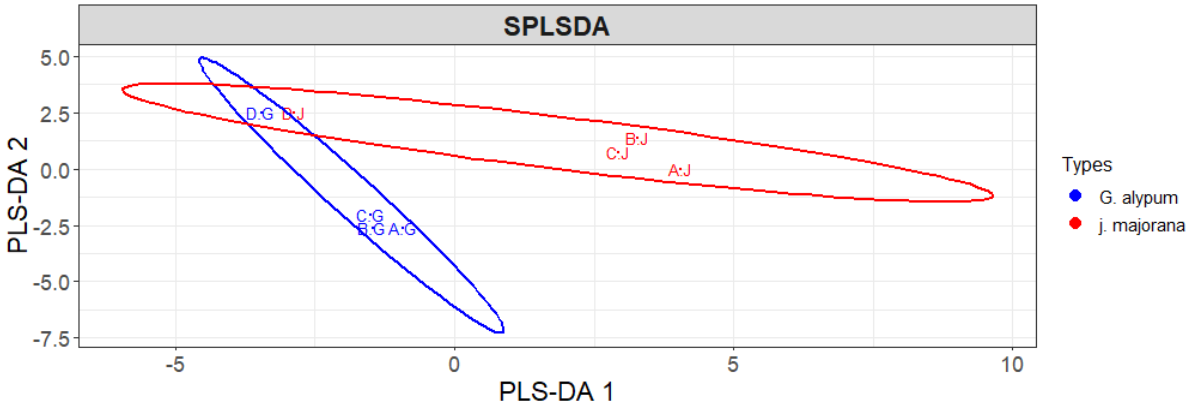


**Figure 5**: PLS-DA Analysis of digestion methods that used to extract elements from selected plants; AG ($HNO_3/HClO_4$ for G. alypum), BG ($HNO_3/H_2O_2$ for G. alypum), CG (dry ashing for G. alypum), DG (water extraction for G. alypum), AJ ($HNO_3/HClO_4$ for J. Communis), BJ ($HNO_3/H_2O_2$ for J. Communis), CJ (dry ashing for J. Communis), DJ (water extraction for J. Communis).

The Hierarchical Cluster Analysis (HCA) dendrogram plots, reveal a clear separation between water extraction method and other digestion methods as shown in Figure 6. The analysis groups similar methods together, indicating that these methods have a similar ability to extract the elements from the plants.

 

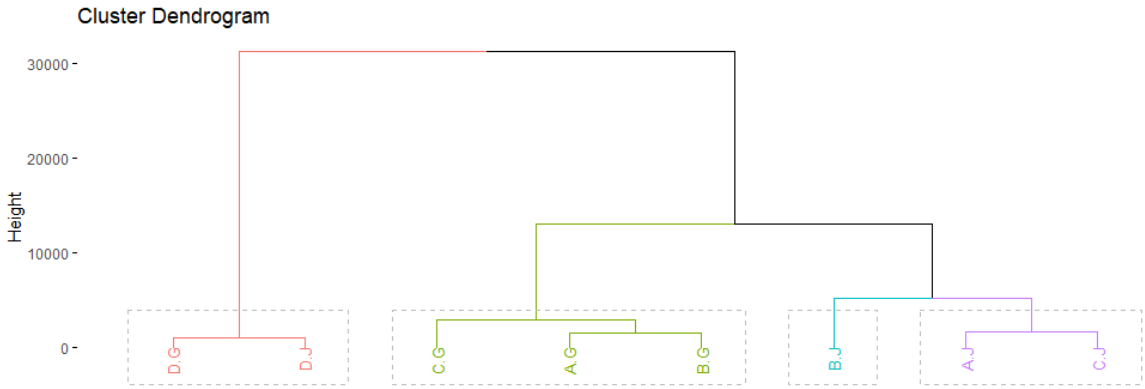


**Figure 6:** Dendrogram plots showing separation of digestion methods that used to extract elements from selected plants, AG ($HNO_3/HClO_4$ for G. alypum), BG ($HNO_3/H_2O_2$ for G. alypum), CG (dry ashing for G. alypum), DG (water extraction for G. alypum), AJ ($HNO_3/HClO_4$ for J. Communis), BJ ($HNO_3/H_2O_2$ for J. Communis), CJ (dry ashing for J. Communis), DJ (water extraction for J. Communis).

The statistical boxplot graph also shows there is significant differences in the selected medical plants in term of elemental concentration. For instance, the plants have a significant difference in concentration of silicon, cobalt and zinc. This may relate to the different ability of the plants to absorb these metals. While there are no significant differences between these plants according to the concentration of chrome and tin, as seen in the following example (Figure7).

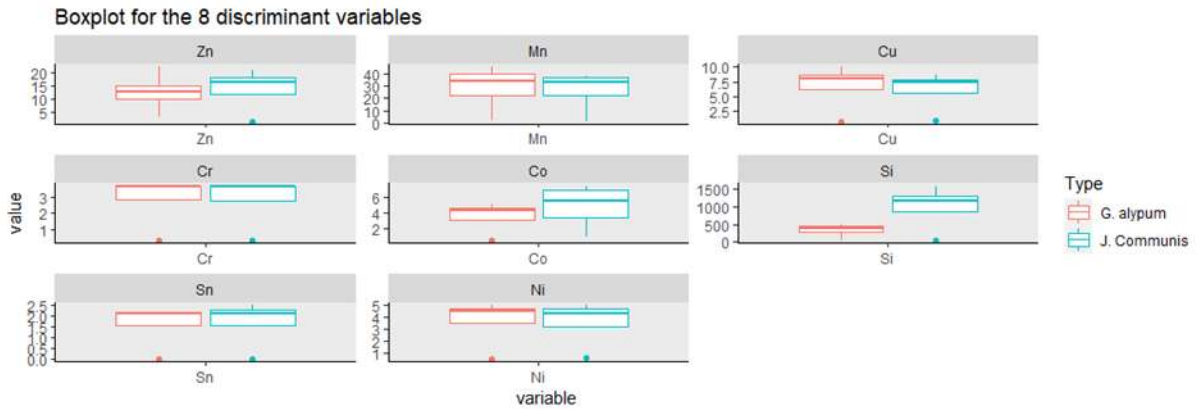


**Figure 7:** Grouped boxplots showing the possible elements that considered as marker to distinguish between selected plant

**Conclusion**

The concentrations of Macro, Micro and some of toxic elements were estimated in four of Libyan wild medicinal plants. The studied medicinal plants were: Juniperus Communis, Globularia alypum, Origanun majorana and Ajuga Iva tissues.

We conclude from this study that, the concentration levels for different measured elements in these plants changed in the following order: Ca >> Mg > K > Si > P > Al > Na > Fe > Mn > Zn > Cu > Co > Cd > Pb > Ni > Cr > Sn > Hg. As it can be a notable, the macronutrient calcium was found to present in major level, while the macronutrients magnesium, potassium, sodium, phosphorus, iron and micronutrient silicon and toxic trace element aluminum were found to be in minor level. The micronutrients zinc, manganese, copper, chromium, tin, nickel and cobalt as well as the toxic element lead were found to be in trace level.

The results of the statistical PCA, PLS-DA and HCA analysis confirmed the clear superiority of ($HNO_3/HClO_4$) as wet digestion mixture over the other discussed procedures in destroying the complex organic matrix of the studied plant tissues extracting most of the elements. In addition, boxplot exhibit a significant variation was observed in the elemental concentrations among the selected medicinal plants. Specifically, the selected plants have a clear difference of silicon, cobalt and zinc concentrations. In contrast, no significant differences were detected among the plants with respect to chromium and tin concentrations.

**Compliance with ethical standards**

*Disclosure of conflict of interest*

The authors declare that they have no conflict of interest.

 

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