



Rapid assessment of elements in Brassica tounefortii and Artemisia Campestris L Toraia M. Takteek^{1*}, Sanaa M. Abushhewa², Aref M. Ballut³ and Saleh A. Ouheda⁴

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Abstract

Mineral concentrations for Brassica tounefortii and Artemisia Campestris L have been detected using four digestion methods to prepare herbal samples. These mineral's include Ca, Mg, K, Na, P, Fe, Mn, Zn, Cu, Co, Cr, Ni, Pb and Cd which are measured by flame atomic absorption spectrometry and flame photometer. Regardless of the digestion procedures, Ca, K, Mg, Na, and Fe as macronutrients are found to be in the major level in these plants. Zn has the highest concentration followed by Cu and Mn. While, Ni was shown to be at a low concentration. Pb as a toxic element was existing in a relatively moderate concentration. In addition, the microminerals Cr, Co and the toxic element, Cd, were under a detection limit. Statistical (PCA, PLS-DA HCA and Boxplot) data indicates that there is a significant difference between the extraction method and other digestion methods. These differences could arise from the ability of water extracting only soluble minerals. In the meantime, the (HNO₃/HClO₄) digestion procedure has the superiority over other digestion procedures. The boxplot data also reveals the significant differences according to the mineral composition. For instance, these plants have a significant variance in the concentrations of Ca, Na, K, Fe and Mn. While there is a small significant variance between these herbals in terms of Zn and Mg.

Key words: elements. minerals, herbs, water extract and digestio

Introduction

Herbal plants play a pivotal role in traditional medicine and are used as home remedies [1-4]. Many studies were conducted by the World Health Organization (WHO) have exhibited that around 80% of the world's population still depend on traditional medicine for treatments [5-9]. Through it, medicinal plants can take apart in treatment for many metabolic disorders. Thus, it can safely be presumed that the part of traditional treatment involves the utilize of herbal extracts [7].

Minerals are necessary for many functions in the human body and can be classified into two categories depending on the quantity that is necessary for the human body or the concentration which exists in plants: macrominerals which exist in relatively high amounts such as calcium, potassium and magnesium while microminerals can exist in relatively low amounts such as copper, nickel and manganese. Although these minerals are often necessary for many organisms, they can become toxic if found at high concentrations. Also, some elements are considered to be toxic even found in trace concentrations [10, 11].

The aim of this study is to investigate the elemental composition of *Brassica tounefortii* and *Artemisia Campestris* L. Through the quantitative analysis of fourteen elemental concentrations namely calcium, magnesium, sodium, potassium, iron, zinc, manganese, nickel, copper, lithium, chrome, cobalt, lead and cadmium. In addition, statistical analysis was applied to investigate if there's a significant variance between sample preparation methods.

Sample preparation

The samples of medicinal plants were collected and washed with distilled and deionized water. The herbal samples were air dried for around two weeks, then the plant species were chopped and dried in an oven at 90C° overnight. Afterwards, the plant samples were grounded using a ceramic mortar and stored in dry containers. In this research, three digestion procedures and water extraction were used to extract the minerals from these plants.

Wet digestion method 1

20 grams of each herbal sample was transferred into 80 ml beakers and 20 ml of con. HNO₃ was added to each beaker. After the NO₂ bubbles were removed, the beakers were covered with watch glasses. The sample mixtures were refluxed gently until their volume became less, after that the samples were allowed to cool at room temperature. 5 ml of HClO₄ was added and boiled to almost dryness then the samples were allowed to cool to around room temperature. The beaker content was dissolved in 0.1N HNO₃, boiled, then was allowed to cool at room temperature. then, the samples were filtered using a glass filter into 50-ml volumetric flasks and diluted with 0.1N HNO₃ to marks.

Wet digestion method 2

In this method, the sample procedure was followed as the pervious method except that, H₂O₂ was added instead of HClO₄ for samples preparation.

Dry ashing method

2 grams of each individual plant sample were introduced into crucibles and transferred into a muffle furnace, where they were fired at 500°C for around 6 hrs. The crucibles were transferred from the furnace and allowed to cool at room temperature. The mixture was dissolved in 10 ml of nitric acid (1:1) and filtered through a glass filter. The filtrate was transferred into 50-mL volumetric flasks and diluted to the marks with 0.1N HNO₃.

Water extraction method

2 grams of each herbal sample was transferred into 80-mL beakers and covered with glasses. A sufficient water was introduced to each beaker and refluxed for 1 hour, then filtered and transferred into 50-mL volumetric flasks and diluted with 0.1N HNO₃ to the marks. This procedure is similar to the traditional method in the way of extracting pharmacologically active organic compounds and elements.

Instruments

herbal samples were measured for, Ca, Mg, K, Na, P, Fe, Mn, Zn, Cu, Co, Cr, Ni, Pb and Cd by flame atomic absorption spectrometry (AAS) using a Philips atomic spectrometer Model PU9400X England. The elements: K, Na, and Li were analysed using a flame photometer.

Results and discussion

The concentration of the minerals within each medicinal plant

The first part of the discussion explains the profiles of the mineral's concentration for the selected medicinal plants. The mean concentration of each mineral in the selected plants are presented in Table 1, and Figure 1.

Table1: The mean concentration ($\mu\text{g/g}$) of macrominerals, microminerals and toxic elements in *Brassica tounefortii* and *Artemisia Campestris* L with the digestion methods*.

elements	plant	digestion with $\text{HNO}_3 / \text{HClO}_4$	digestion with $\text{HNO}_3 / \text{H}_2\text{O}_2$	ashing	water infusion
Ca	B. Tounefortii	24902	20988	17414	2065
	A. Campestris	11969	12140	7958	1100
Mg	B. Tounefortii	10483	11660	9903	3179
	A. Campestris	4162	4495	3097	1287
Na	B. Tounefortii	5849	5116	1238	3447
	A. Campestris	3353	3171	1972	1606
K	B. Tounefortii	11659	16097	23109	7915
	A. Campestris	8426	8398	6267	5027
Fe	B. Tounefortii	162	165	128	ND
	A. Campestris	314	275	235	ND
Zn	B. Tounefortii	38.46	37.43	36.23	ND
	A. Campestris	48.92	43.42	56.94	ND
Mn	B. Tounefortii	27.22	26.33	17.43	ND
	A. Campestris	24.53	25.04	20.33	ND
Ni	B. Tounefortii	11.50	7.33	5.04	ND
	A. Campestris	7.74	5.43	ND	ND
Cu	B. Tounefortii	14.13	11.12	24.12	ND
	A. Campestris	13.24	13.42	ND	ND
Li	B. Tounefortii	7.23	4.83	ND	ND
	A. Campestris	ND	ND	ND	ND
Pb	B. Tounefortii	21.20	8.70	ND	ND
	A. Campestris	8.10	ND	ND	ND

*Average of triplicate determinations, %R.S.D.= 0.05-0.16

*ND = not detected

* Cr, Co and Cd not detected

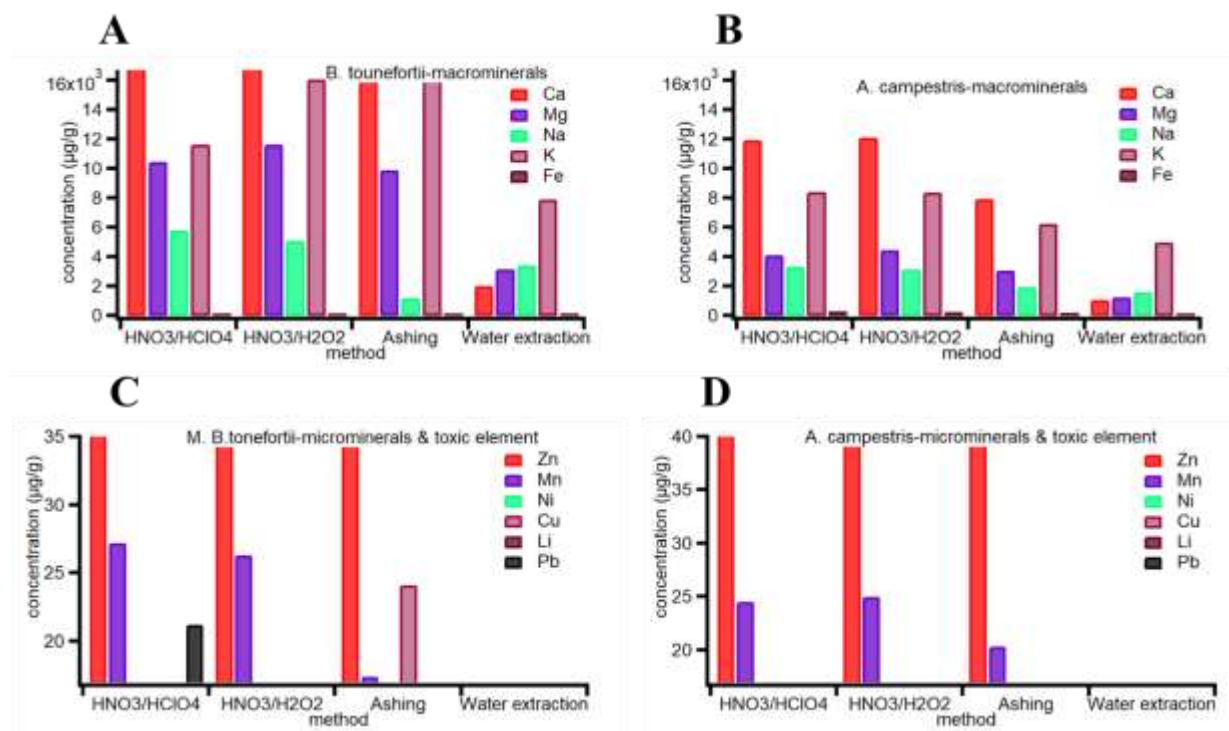


Figure.1: The concentration of microminerals, microminerals and toxic elements in the selected Libyan medicinal plants with four different procedures; A (macrominerals in *B. Tounefortii*), B (macrominerals in *A. Campestris*), C (microminerals and toxic element in *B. Tounefortii*), D (microminerals and toxic element in *A. Campestris*).

The macronutrient elements

Macrominerals concentrations in *B. Tounefortii* and *A. Campestris* plants which were extracted using four digestion methods; (HNO₃/HClO₄), (HNO₃/H₂O₂), dry ashing and water infusion procedures are shown in Table 1 and Figure 1(A&B). The result indicates that the macrominerals calcium, potassium, magnesium, sodium and iron exist in highly predominate species in plant samples regardless of the digestion method. Calcium was found with the highest concentration, whereas iron concentration represented the lowest. It is noticeable that the concentration of calcium represents the highest level compared to other macrominerals using any sample preparation procedure and several published literature data supported these observations [9, 12]. However, iron concentration is usually less abundant but still exists in a significant level in these herbals which is considered a good source for iron deficiency in the human cells. Many previous literatures were found in a good agreement with these findings [13-15]. The general order of relative concentrations of these macrominerals based on digestion procedures is:

$$\text{Ca} > \text{K} > \text{Mg} > \text{Na} > \text{Fe}$$

It is worth that the concentration of the macrominerals in water extract is significantly lower compared to the levels of these macrominerals which were extracted with other digestion methods (wet and dry ashing). Moreover, iron level is not detected in aqueous solution for these plants. These could be related to the lower solubility of iron salts in aqueous solution.

The microminerals and toxic elements

The concentration of microminerals and toxic elements that were obtained by digesting procedures are presented in Table 1 and Figure 1(C&D). the results reveal that zinc has the highest concentration in these herbals followed by copper, manganese and nickel. In addition, lithium, cobalt and the toxic element, cadmium, was not detected by any digestion methods. The order of microminerals and toxic elements were found to follow this trend:

$$\text{Zn} > \text{Cu} > \text{Mn} > \text{Ni} > \text{Pb}$$

Microminerals and toxic elements concentrations were found to be in a good agreement with several articles [9, 16-20].

Statistical analysis

The Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), Hierarchical Cluster Analysis (HCA) and boxplot, were applied to determine the variation between the selected herbals according to their mineral composition as well as to estimate the differences arising from the methods which were used to prepare the herbals samples. PCA data estimates the variation between these herbals. The first two principal components explaining 78% of the total differences, as shown in Figure 2. Moreover, PCA result indicate that there is significant variation between the water extracting procedure and other digestion procedures. This may represent only by the soluble salts which were extracted by water infusion. In addition, there is a slight difference between the ashing method and other wet digestion procedures ($\text{HNO}_3/\text{H}_2\text{O}_2$ and $\text{HNO}_3/\text{HClO}_4$ methods), as seen in Figure 2.

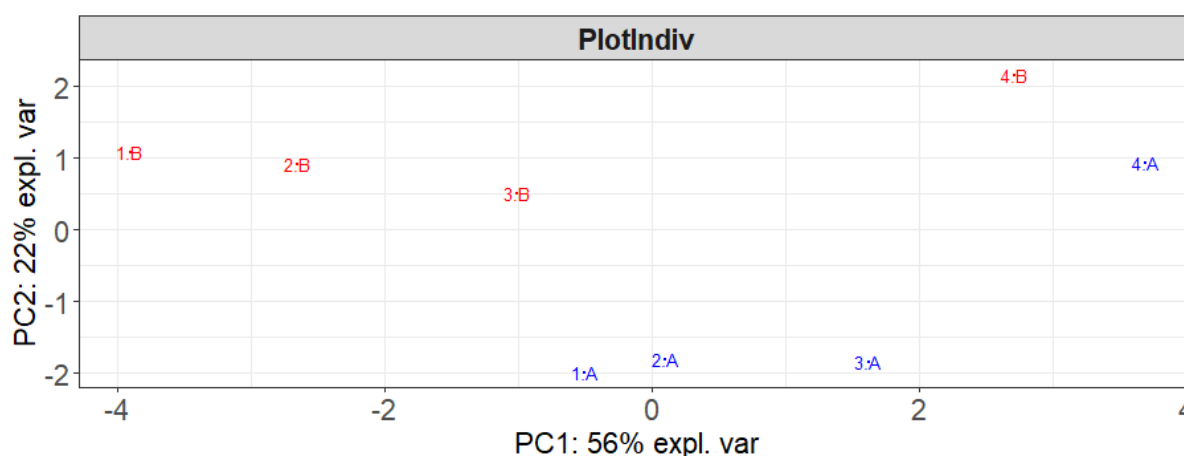


Figure 2: PCA analysis of elemental composition of *B. Tounefortii* and *A. Campestris*; 1.M ($\text{HNO}_3/\text{HClO}_4$ for *B. Tounefortii*), 2.M ($\text{HNO}_3/\text{H}_2\text{O}_2$ for *B. Tounefortii*), 3.M (dry ashing for *B. Tounefortii*), 4.M (water extraction for *B. Tounefortii*), 1.B ($\text{HNO}_3/\text{HClO}_4$ for *A. Campestris*), 2.B ($\text{HNO}_3/\text{H}_2\text{O}_2$ for *A. Campestris*), 3.B (dry ashing for *A. Campestris*), 4.B (water extraction for *A. Campestris*).

PLS-DA result reveals a clear difference between *B. Tounefortii* and *A. Campestris* in terms of digestion procedures. The results also exhibit distinct clustering for certain groups, water infusion procedure and other digestion procedures. In addition, there are slight differences between the ashing procedure and the wet procedures ($\text{HNO}_3/\text{H}_2\text{O}_2$ and $\text{HNO}_3/\text{HClO}_4$ methods), as shown in Figure 3.

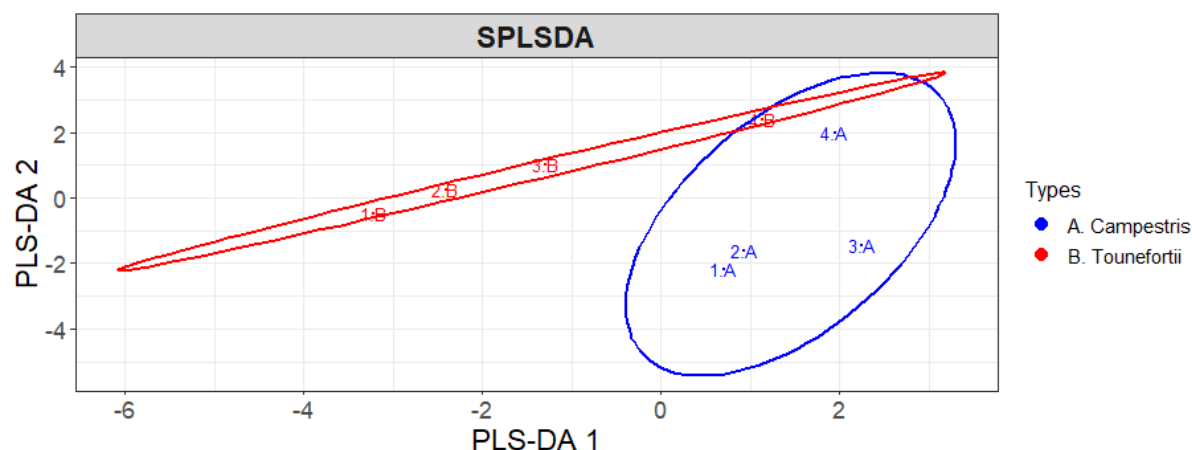


Figure 3: PLS-DA analysis of elemental composition of *B. Tounefortii* and *A. Campestris*; 1.M ($\text{HNO}_3/\text{HClO}_4$ for *B. Tounefortii*), 2.M ($\text{HNO}_3/\text{H}_2\text{O}_2$ for *B. Tounefortii*), 3.M (dry ashing for *B. Tounefortii*), 4.M (water extraction for *B. Tounefortii*), 1.B ($\text{HNO}_3/\text{HClO}_4$ for *A. Campestris*), 2.B ($\text{HNO}_3/\text{H}_2\text{O}_2$ for *A. Campestris*), 3.B (dry ashing for *A. Campestris*), 4.B (water extraction for *A. Campestris*).

The cluster dendrogram plot exhibits clear separation between the water extraction method and other digestion procedures. Similar groups clustered together reveals that these methods have a similar ability to extract these minerals from the sample, as seen in Figure 4.

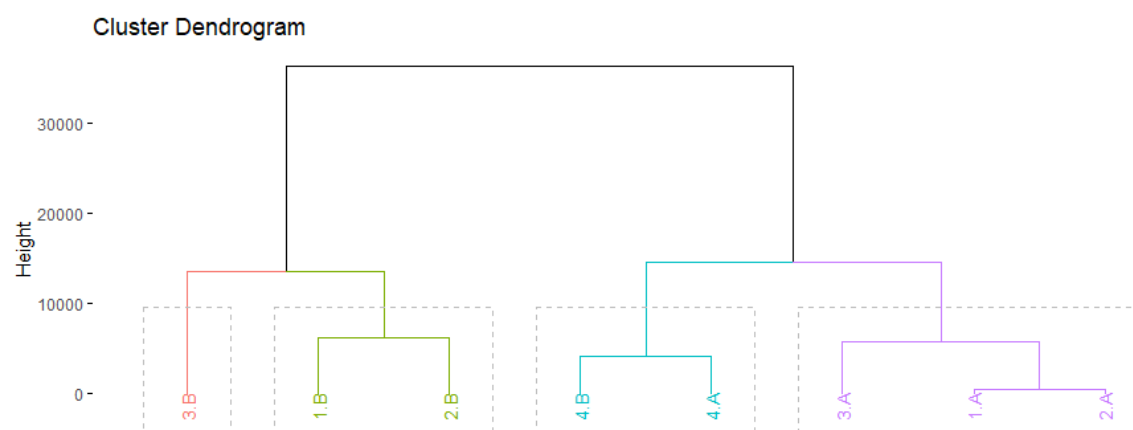


Figure 4: Dendrogram Plots showing the separation of digestion methods which are used to extract elements from *B. Tounefortii* and *A. Campestris*; 1.M ($\text{HNO}_3/\text{HClO}_4$ for *B. Tounefortii*), 2.M ($\text{HNO}_3/\text{H}_2\text{O}_2$ for *B. Tounefortii*), 3.M (dry ashing for *B. Tounefortii*), 4.M (water extraction for *B. Tounefortii*), 1.B ($\text{HNO}_3/\text{HClO}_4$ for *A. Campestris*), 2.B ($\text{HNO}_3/\text{H}_2\text{O}_2$ for *A. Campestris*), 3.B (dry ashing for *A. Campestris*), 4.B (water extraction for *A. Campestris*).

The Boxplot graph indicates that there is a significant variance in selected herbals in terms of mineral composition. For example, these herbals have a significant variance in the concentration of calcium, sodium, potassium, iron, and magnesium. While there is a small significant variance between these herbals according to zinc and manganese, as seen in this example, Figure 5.

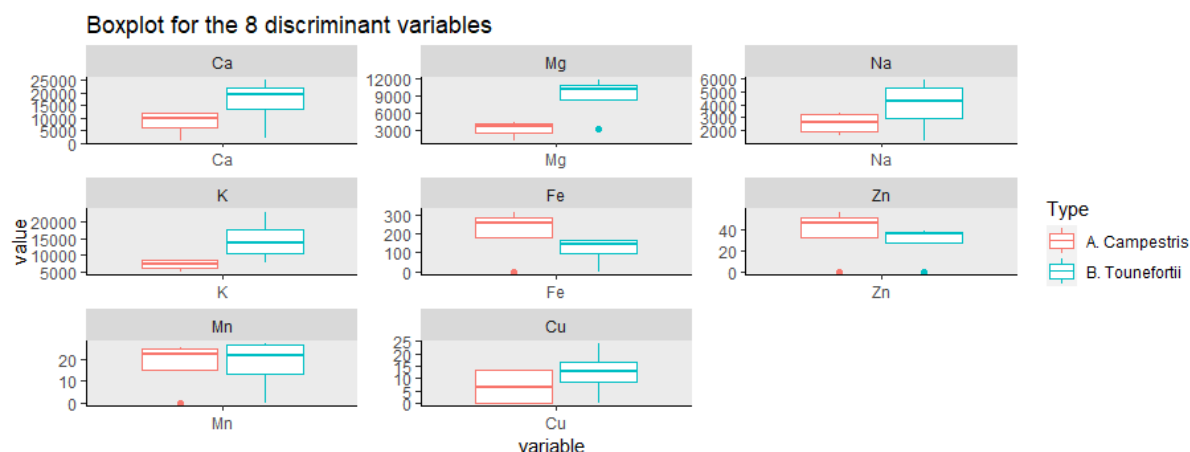


Figure 5: Grouped Boxplots showing the possible minerals that considered as marker to differentiate between *B. Tounefortii* and *A. Campestris*.

Conclusion

Macrominerals, microminerals and some of toxic element's concentrations were determined in *B. Tounefortii* and *A. Campestris*. From the data, the concentration levels for these elements in the herbal plants exist in this order:

$$\text{Ca} > \text{K} > \text{Mg} > \text{Na} > \text{Fe} > \text{Zn} > \text{Cu} > \text{Mn} > \text{Ni} > \text{Pb}$$

Calcium and sodium concentrations were found in high levels. It is worth that, the concentration of nickel, zinc, manganese and lead was found in lower levels while copper and lithium detected in trace levels. The amount of iron, manganese, nickel, copper, lithium and lead were not detected in water extract procedure. Moreover, chrome, cobalt and Cd, were not detected by any digestion method. The statistical results reveals that there is a slight significant difference between the dry ashing procedure and other wet digestion procedures ($\text{HNO}_3/\text{HClO}_4$ and $\text{HNO}_3/\text{H}_2\text{O}_2$). The water extract represents the lowest method to extract these minerals. In addition, statistical analysis also reveals that there are some significant variances in these herbals according to their mineral levels such as calcium, sodium, iron, lead and nickel.

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