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Determination of elements in Marrubium vulgare L and Brassica oleracea L

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Abstract
The concentration of minerals for two herbal Libyan plants, Marrubium vulgare L and Brassica oleracea L have been determined utilizing four digestion methods to prepare plants samples. These elements include Ca, Mg, K, Na, P, Fe, Mn, Zn, Cu, Co, Cr, Ni, Pb and Cd which are detected by flame atomic absorption spectrometry and flame photometer. Regardless of the digestion methods, Ca, K, Mg, Na, and Fe as macronutrients are found to be in the highest level in these herbals. The concentration of the micronutrient Ni has the highest concentration followed by Zn and Mn. While, Co and Li were found to be at a low concentration. Pb as a toxic element was found in a relatively moderate level. moreover, the microminerals Cr, Co and the toxic element, Cd, were under a detection limit. Statistical (PCA, PLS-DA HCA and Boxplot) results reveal the significant variation between extraction method and other digestion methods. These differences could relate to the ability of water extracting only soluble salts. In the meantime, (HNO_3/$HClO_4$) digestion method has the superiority over other digestion methods. The boxplot results also reveal the significant variance according to the mineral composition. For example, these herbals have a significant variance in the levels of Ca, K, Fe, Pb and Ni. While there are small significant differences between these herbals in terms of Zn and K.

Key words: minerals, herbs, elements, digestion, water extract

Introduction
Over the past decades, the usage of the herbal plants has increased in the developing countries [1-4]. More than 80,000 herbal species are used for medical purposes and 80% of world's population utilize herbal plants for diseases treatments [5-9]. Through it, herbals plants can play an important role used as home remedies. Thus, it can be utilized as traditional therapy which involves the use of plant extracts [7-9].
Inorganic elements (minerals) are important for human body functions and can be divided into two classes depending on the quantity that is required for the human body or the amount that exists in the plants: macrominerals and microminerals. Macrominerals which are found in relatively high concentrations such as calcium, potassium and magnesium, while microminerals they can be found in relatively low concentrations such as copper, nickel and manganese [10, 11]. Although these elements are often essential for many organisms, they become toxic if they exist at high concentrations. Also, some minerals are considered to be toxic even though they exist in trace levels [10, 11].

The aim of this research is to determine the elemental composition of Marrubium vulgare L and Brassica oleracea L through the quantitative analysis of fourteen elemental concentrations namely calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), iron (Fe), zinc (Zn), manganese (Mn), nickel (Ni), copper (Cu), lithium (Li), chrome (Cr), cobalt (Co), lead (Pb) and cadmium (Cd). In addition, statistical analysis was applied to determine if there's a significant difference between the preparation methods [2-4].

Sample preparation

The herbal plants were collected during the spring season and were washed using distilled and deionized water. The plant samples were then air dried for around two weeks, after that the plant species were chopped and placed in an oven at 90C° overnight. afterwards, the herbal samples were grounded in a ceramic mortar and stored in a dry container. In this study, three digestion methods and water extraction were applied to extract the elements from the herbal matrix

Wet digestion method 1

20 g of each medicinal sample was transferred into 80 ml beakers then, 20 ml of con. HNO_3 was added to each beaker and the beakers were then covered with watch glasses after the NO_2 bubbles were removed. After that, sample mixtures were refluxed gently until their volume became less, then the samples were allowed to cool at room temperature. 5 ml of $HClO_4$ was added and boiled to almost dryness then the samples again, were allowed to cool to room temperature. The content of the beaker was dissolved in 0.1N HNO_3, boiled, then was allowed to cool at room temperature. Ater that, the samples were filtered by a glass filter into 50-ml volumetric flasks and diluted with 0.1N HNO_3 to marks.

Wet digestion method 2

The wet digestion method 2 followed the same trend as the pervious method except that, H_2O_2 was added instead of $HClO_4$ for the preparing of the herbal samples.

Dry ashing method

2 g of each individual herbal sample were introduced into crucibles and then transferred into a muffle furnace, where they were heated at 500°C for around 6 hrs. The crucibles were transferred from the furnace and allowed to reach room temperature. The contents were 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 HNO_3.

Water infusion method

2 g of each medicinal sample were transferred into 80-mL beakers and covered with watch glasses. A sufficient water was supplied to each beaker and refluxed for 1 hour, then filtered and transferred into 50-mL volumetric flasks and diluted with 0.1N HNO_3 to the marks. This method is similar to the traditional method in the way of extracting pharmacologically active organic compounds and elements.

Instruments

plant samples were analysed 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: potassium, sodium, and lithium were measured using a flame photometer.

Results and discussion

The concentration of the minerals within each herbal plant

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

Table1: The mean concentration (µg/g) of macrominerals, microminerals and toxic elements in Marrubium vulgare L and Brassica oleracea L with the digestion methods*.

elements	plant	digestion with HNO_3 / $HClO_4$	digestion with HNO_3 / H_2O_2	ashing	water infusion
Ca	M. vulgare	20070	13573	14515	501
	B. oleracea	31771	26631	25588	1889
Mg	M. vulgare	11615	11830	11175	4127
	B. oleracea	12055	13391	11913	5367
Na	M. vulgare	1448	1169	1514	470
	B. oleracea	4671	3917	5184	2648
K	M. vulgare	14252	13878	20984	8602
	B. oleracea	12973	13313	20139	6077
Fe	M. vulgare	794	619	926	ND
	B. oleracea	191.25	134.58	149.38	ND
Zn	M. vulgare	31.56	23.54	33.37	ND
	B. oleracea	36.06	31.71	35.71	ND
Mn	M. vulgare	31.881	28.38	26.574	ND
	B. oleracea	25.35	22.45	21.73	ND
Ni	M. vulgare	11.97	7.61	6.67	ND
	B. oleracea	11.07	9.94	7.02	ND
Cu	M. vulgare	11.19	9.88	16.52	ND
	B. oleracea	ND	ND	13.65	ND
Li	M. vulgare	3.33	ND	ND	ND
	B. oleracea	7.12	4.17	ND	ND
Pb	M. vulgare	20.8	13.72	2.85	ND
	B. oleracea	30.68	13.45	6.83	ND

*Average of triplicate determinations, %R.S.D.= 0.04-0.15

*ND = not detected * Cr, Co and Cd not detected

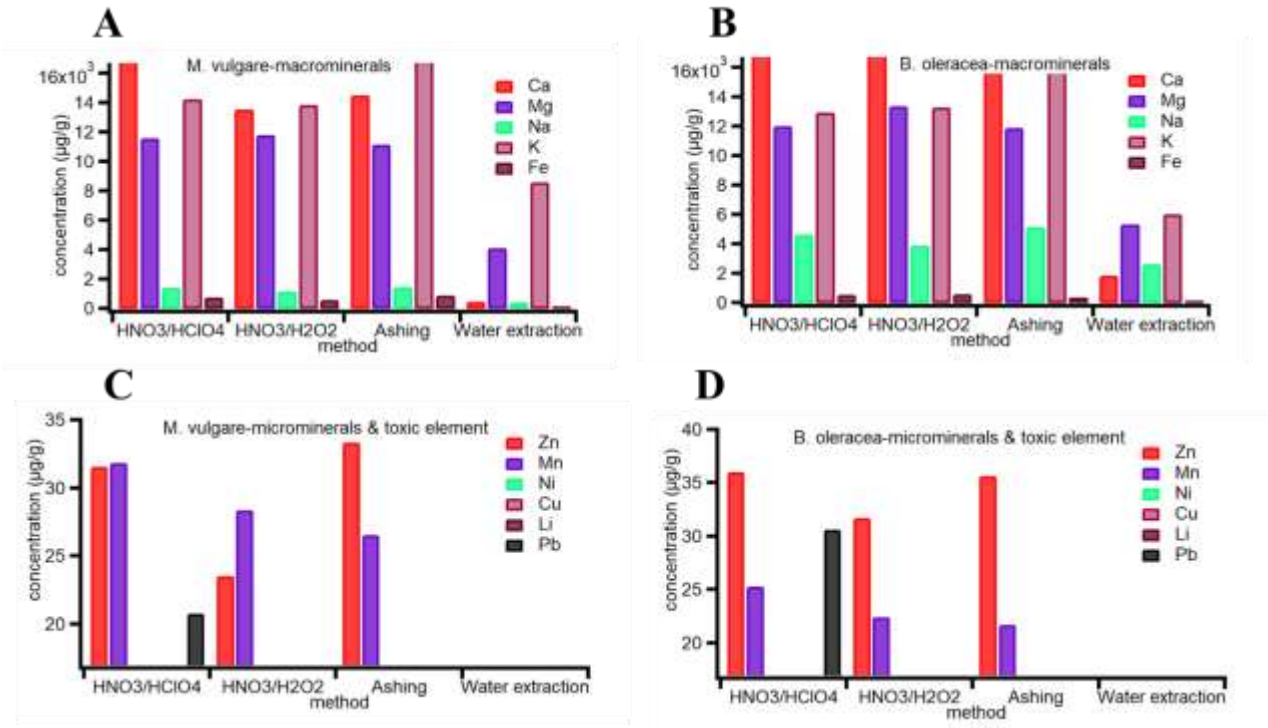

Figure.1: The concentration of microminerals, microminerals and toxic elements in the selected Libyan medicinal plants with four different procedures; A (macrominerals in M. vulgare), B (macrominerals in B. oleracea), C (microminerals and toxic element in M. vulgare), D (microminerals and toxic element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

The macronutrient elements

The concentration of macrominerals in M. vulgare and B. oleracea plants that were extracted by digestion methods with ($HNO_3/HClO_4$), (HNO_3/H_2O_2), dry ashing and water infusion procedures are shown in Table1 and Figure 1(A&B). The result exhibit that the macrominerals: calcium, potassium, magnesium, sodium and iron are found in highly predominate species in the herbal samples regardless of the digestion procedure. Among these macrominerals, calcium exists in the highest concentration, whereas iron was exhibited in the lowest concentration in these plants. The highest levels of calcium over that of other macrominerals is very noticeable using any sample preparation procedure. The previously published literature data are strongly supported by these observations [9]. However, the concentration of iron is usually less abundant but still found in a significant concentration in these medicinal plants which could be a good source for iron deficiency in the human body. Iron level in these herbal plants was found in a good agreement in some previous researches in many countries [12-14]

The order of relative levels of these macrominerals based on digestion procedures is:

$$Ca > K > Mg > Na > Fe$$

It is noteworthy that, calcium, magnesium, potassium and sodium levels in water extract is significantly lower compared to the levels of these macrominerals that were extracted using other digestion procedures. In addition, the level of iron is not detected in aqueous solution of these herbals. This may relate to the lower solubility of salts of this element in water.

The microminerals and toxic elements

The microminerals and toxic elements concentration with different digesting methods are presented in Table 1 and Figure 1(C&D). The data exhibits that nickel had the highest level in these plants followed by zinc and manganese. Moreover, copper and lithium were presented at low levels. The concentration of toxic element Pb found in a moderate level compared to the microminerals. In addition, the microminerals chrome, cobalt and toxic element, cadmium, were not detected. The order of microminerals and toxic elements were found to follow this trend:

$$Ni > Zn > Mn > Pb > Cu > Li$$

The concentration of these microminerals and toxic the element, Pb, were found to be on an agreement with many studies around the world [9, 15-19].

Statistical analysis

Statistical tools, The Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), Hierarchical Cluster Analysis (HCA) and boxplot, were used to estimate the variation between the selected plants according to their mineral composition as well as to assess the differences arising from the methods which were used to prepare the plant samples. PCA results estimate the variation between these plants. The first two principal components explaining 84% of the total differences, as shown in Figure 4. In addition, PCA result indicate that there are significate differences between the water extracting method and other digestion methods. This could be represented only by the soluble salts minerals which were extracted by water infusion. moreover, there is a slight variance between the ashing procedure and other wet digestion methods (HNO_3/H_2O_2 and $HNO_3/HClO_4$ methods), as shown in Figure 4.

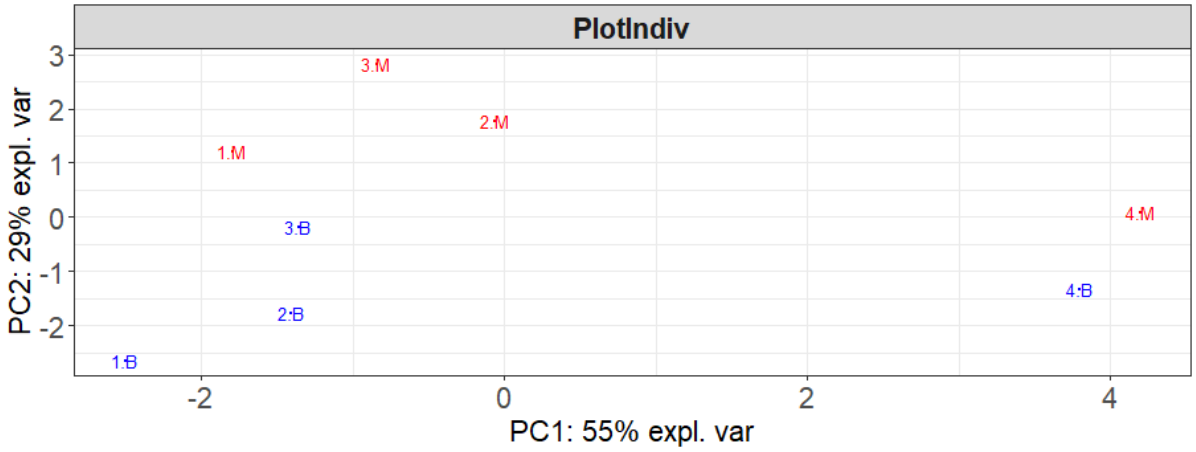

Figure 4: PCA analysis of elemental composition of M. vulgare and B. oleracea; 1.M ($HNO_3/HClO_4$ for M. vulgare), 2.M (HNO_3/H_2O_2 for M. vulgare), 3.M (dry ashing for M. vulgare), 4.M (water extraction for M. vulgare), 1.B ($HNO_3/HClO_4$ for B. oleracea), 2.B (HNO_3/H_2O_2 for B. oleracea), 3.B (dry ashing for B. oleracea), 4.B (water extraction for B. oleracea).

PLS-DA data exhibit that a clear variation between M. vulgare and B. oleracea in terms of digestion methods. The results also indicate distinct clustering for certain groups, water infusion method and other digestion methods. moreover, there are slight variances between the ashing method and the wet methods (HNO_3/H_2O_2 and $HNO_3/HClO_4$ methods), as shown in Figure 5

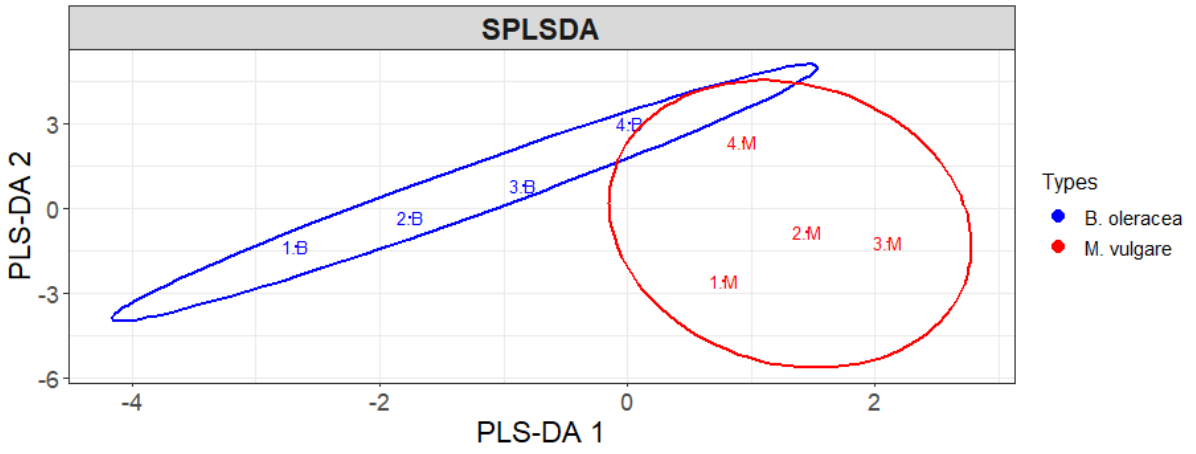

Figure 5: PLS-DA analysis of elemental composition of M. vulgare and B. oleracea; 1.M ($HNO_3/HClO_4$ for M. vulgare), 2.M (HNO_3/H_2O_2 for M. vulgare), 3.M (dry ashing for M. vulgare), 4.M (water extraction for M. vulgare), 1.B ($HNO_3/HClO_4$ for B. oleracea), 2.B (HNO_3/H_2O_2 for B. oleracea), 3.B (dry ashing for B. oleracea), 4.B (water extraction for B. oleracea).

The cluster dendrogram plot reveals clear separation between the water extraction procedure and other digestion methods. Similar groups clustered together exhibit that these procedures have similar ability to extract these minerals from the sample, as shown in Figure 6.

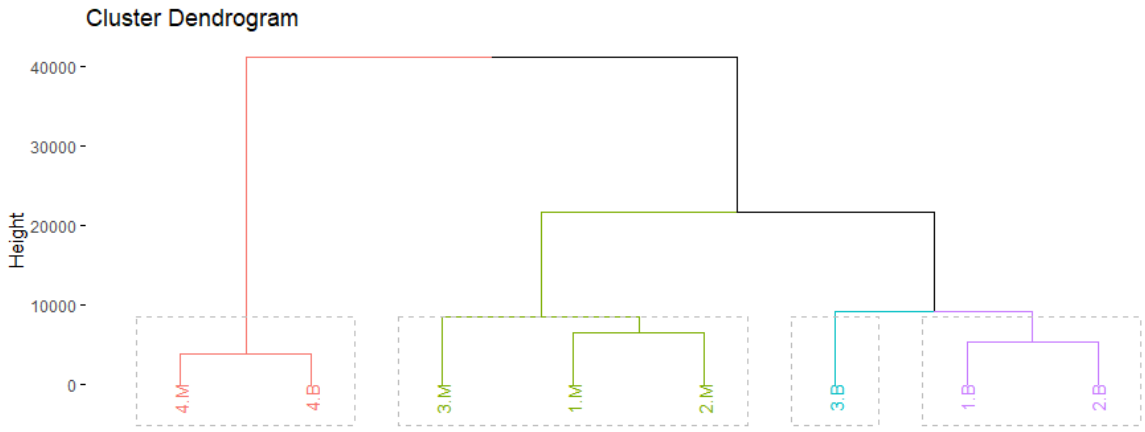

Figure 6: Dendrogram Plots showing the separation of digestion methods which are used to extract elements from M. vulgare and B. oleracea; 1.M ($HNO_3/HClO_4$ for M. vulgare), 2.M (HNO_3/H_2O_2 for M. vulgare), 3.M (dry ashing for M. vulgare), 4.M (water extraction for M. vulgare), 1.B ($HNO_3/HClO_4$ for B. oleracea), 2.B (HNO_3/H_2O_2 for B. oleracea), 3.B (dry ashing for B. oleracea), 4.B (water extraction for B. oleracea).

Boxplot graph reveals that there are significant differences in selected plants in terms of mineral composition. For instance, these plants have a significant difference in the concentration of calcium, sodium, iron, lead and nickel. While there are small significant differences between these plants according to zinc and potassium, as shown in this example, Figure 7.

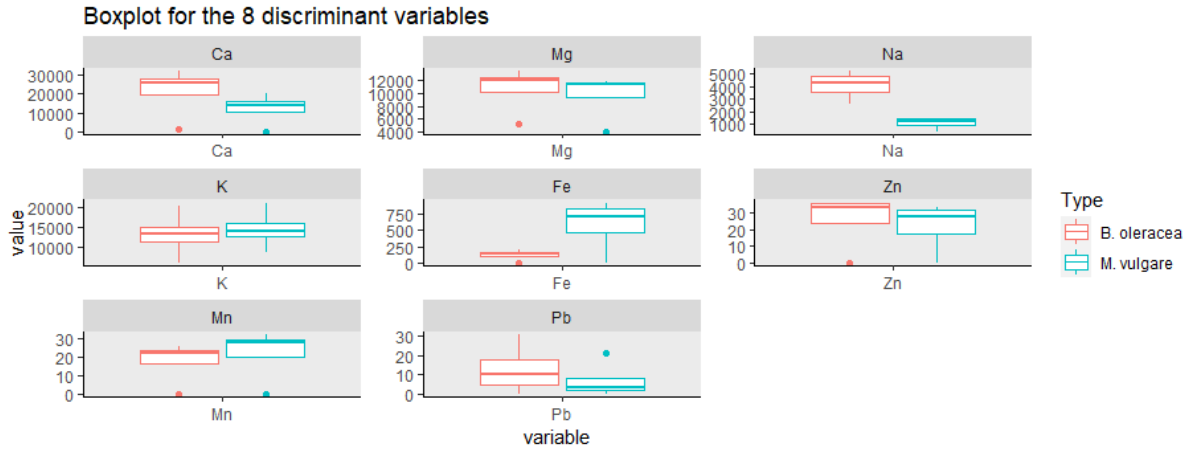

Figure 7: Grouped Boxplots showing the possible minerals that considered as marker to differentiate between M. vulgare and B. oleracea.

Conclusion

Macrominerals, microminerals and some of toxic element's concentration were detected in M. vulgare and B. oleracea. From the result, the concentration levels for these elements in the herbal plants were found to follow this order:

Ca > K > Mg > Na > Fe > Ni > Zn > Mn > Pb> Cu > Li

Calcium and sodium concentrations were existed in major levels. It is noticeable that, the concentration of nickel, zinc, manganese and lead was found in minor levels while copper and lithium detected in trace levels. The concentration of iron, manganese, nickel, copper, lithium and lead were not detected in water extract. In addition, chrome, cobalt and Cd, were not detected. The statistical data exhibits that there is a slight significant variance between the dry ashing method and other wet digestion methods ($HNO_3/HClO_4$ and HNO_3/H_2O_2). For

extracting the elements. The water extract represents the lowest method to extract these minerals. In addition, statistical analysis also exhibits that there are some significant differences in these plants according to their mineral concentrations such as calcium, sodium, iron, lead and nickel.

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