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A RESEARCH ARTICLE

Quantitative Determination of Mineral Composition in Thymus Capitatus and Rosmarinus Officinalis

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Abstract

The mineral compositions for two herbals plants grown in Libyan wilderness, namely Thymus Capitates and Rosmarinus Officinalis have been detected using four different digestion methods. plant samples were measured for calcium, magnesium, potassium, sodium, phosphorus, iron, manganese, zinc, copper, cobalt, chromium, nickel, lead and cadmium by flame atomic absorption spectrometry (AAS) technique. The macrominerals calcium, magnesium potassium and sodium were found to dominate species in the selected plants ranging from calcium which has the highest level to sodium with the lowest concentration. Iron, zinc, manganese and nickel concentrations were detected in minor levels while copper, lithium and lead were found in trace levels. Calcium, magnesium, potassium and sodium were detected in water extraction of these plants at significant amount while other minerals were not detected in water extraction. Besides, chromium, cobalt and cadmium were not detected by any digestion methods.The findings from the study revealed that the water extract contains respectively the amount of Ca, Mg, K and Na that is equivalent to the Recommended Daily Allowance (RDA) of these macronutrients which are important for human health. However, the water extract of Fe, Zn, Mn, Ni, Cu and Li is not enough to supple the RDA of these metals. Consuming enough amount to ground particles of these herbals may supply the RDA of these micronutrients. The very low level of Pb make these plants safe for disease treatment.
The statistical analysis exhibited significant variance in yield between the water extraction method and other extraction procedures. The discrepancy that can be explained by the limited solubility of the corresponding water-soluble salts. On the other hand, there is a slight variance between ashing procedure and other two procedures (HNO_3/H_2O_2 and $HNO_3/HClO_4$). In general, the concentration ranges obtained using (HNO_3/HClO4) acid mixture is a bit higher compared to (HNO_3/H_2O_2) and dry ashing procedures

1. Introduction

Industrialization of last decades has led to newly discovers in medical sciences and a better understanding infectious diseases as well as metabolic disorders. Modern drugs which manufactured by pharmaceutical companies are still unable to meet all needs of majority of the people in poor countries [1, 2]. Recent study by the World Health Organization (WHO) has proven that about 80% of the people in the world still relies on the traditional treatment for many diseases [3]. Through it, Medicinal plants play an important role in traditional treatment for many diseases and are utilized as home remedies. Thus, it can be used a traditional therapy includes the utilize of plant extracts [4-6].

More than 40 elements have been considered to be essential for life systems in both mammals and plants. An element is considered essential when it satisfies the body requirements. Supplementation with the element is therefore necessary to treat deficiencies associated with its low concentration in body fluids [7-10].

Minerals (inorganic elements) necessary for normal body functions can be classified into two categories relaying on the quantity required for the body or the amount found in the plants: macrominerals and microminerals. Macrominerals such as calcium, potassium and magnesium found to be relatively in a high level while microminerals such as copper, nickel and manganese found to be

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in relatively low level [11,12]. Although minerals are often essential for many living organisms, they become toxic if found at high levels. In addition some elements are consider to toxic even present at trace concentrations [13,14].

The significance of the selected elements in this study will be briefly discussed: calcium is the fifth most abundant element in the earth [7]. Adult male body contains approximately 1200 g of calcium. About 99% of these amount present in the skeleton [15]. Calcium helps functions such as muscle growth and impulses in the brain [16]. The deficiency of calcium could result in osteoporosis and rickets. In addition, the excess of calcium could deposit in gall bladder and kidney [15]. Magnesium represents the tenth abundant metals in the earth [7]. Magnesium is an important in the regulation of cellular sodium, potassium and calcium [17]. The deficiency of magnesium in human body could cause convulsions and behavioural disturbances [18]. The relative high level of magnesium could cause symptoms such as depressed deep tendon reflexes and respiration [19]. Sodium is an important element for blood plasm, maintenance of the osmotic pressure of the body fluid and nervous system [20]. The deficiency of sodium leads to dehydration, weakness, and mental confusion [21]. Sodium toxicity could lead to hypertension [22]. Potassium is essential cation of the intracellular fluid and extracellular fluid and also influences muscle activity [23]. Potassium deficiency could produce kidney [24]. Potassium toxicity could lead to renal failure, advanced dehydration and Addison's disease [25].

Iron is required for metalloenzymes such as nitrogenase, various oxidase, reductases, dehydrogenase and haemoglobin [26]. Iron deficiency could lead to anaemia [27]. Excess levels of iron in the tissues could cause hemosiderosis [28]. Zinc is essential for many enzymes for their activity. It is important for RNA and DNA. It is also required for healthy immune system [29]. Zinc deficiency results in under- performing immune system, loss of smell, skin problems, falling hair, etc. [30]. The high levels of zinc could result in toxicity symptoms such as drowsiness, diarrhoea and hallucinations [31]. Manganese is required for immune system, involved in production of energy, regulates blood sugar levels and cell reproduction [32]. Some research indicates the manganese deficiency could lead to impairs glucose metabolism and reduce insulin production [33]. High levels of manganese in air may lead to mental and emotional disturbances [34]. Copper is required for several proteins and metalloenzymes including B-hydroxylase, dopamine and cytochrome oxidase [35]. Copper deficiency could lead to thyroid gland, heart disease and nervous system problem [36]. The excess levels of copper will cause gastric pain, coma, learning disabilities and liver damage [37]. Chromium (III) is an essential nutrient that helps the body to utilize sugar and may also may involve in protein and fat metabolisms [38]. Chromium deficiency could lead to decrease insulin effectiveness [39]. Ingesting and breathing large amounts of excess of chromium (VI) may cause runny nose, nosebleeds and ulcers [40]. Cobalt is necessary for normal red blood cell formation [41]. The effect of cobalt deficiency is degeneration of nervous system [42]. Co (II) compound could lead to DNA strand breaks, sister-chromatid exchanges and aneuploidy [43]. Lithium is used to reduce the developing dementia risk and increase bone density [44]. Lithium deficiency could be one of the factor that cause Alzheimer's disease [45]. The toxicity side of lithium could cause commonly affect renal function [46]. Many scientists though that nickel maybe necessary for human body [47]. Lower levels of nickel were observed in blood of anaemic children compared to healthy children [48]. High levels of nickel could cause altered hormone and enzyme activities and can impact glucose tolerance, blood pressure and immune function [49]. Lead and cadmium are considered to be toxic even excess in trace levels [50]. The ingestion of these metals in food or in drink could cause several symptoms such as nausea, vomiting abdominal cramp and headache within minutes of ingestion [51].

Therefore, the present work aims to investigate the mineral composition of Thymus capitatus and Rosmarinus officinalis through the quantitative determination 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 to estimate the Recommended Daily Amounts (RDA) of these minerals which are necessary for human health. Moreover, to make sure these plants are safe in aspect of toxic metals levels.

Several statistical methods were employed to evaluate significant differences in mineral composition among the herbal plants, as well as to assess variations arising from the sample preparation methods [52-54].

2. Materials And Methods

2.1. Chemicals

Deionized water was produced through distillation and ion exchange resins process and was supplied by West Tripoli Power Station. The chemicals that were utilized in this research were analytical grade and supplied by Ventron GmbH, Farmitalia Carlo Erba S.P.A., Fluka Chemika, and Merck (Darmstadt, Germany).

2.2. Sample preparation

Medicinal plants were harvested in the spring season and washed with deionized water. The plants were air dried for around three weeks, then the herb species were finely chopped and placed in an oven at 90C° for about 24 hours. After that, the plant samples were grounded in a ceramic mortar and stored in dry glass jar. In this study, three digestion methods and water extraction were applied to extract the minerals from plant matrix.

2.3. Wet digestion Procedure 1

Twenty grams of each herbal sample were transferred into 80 ml beakers then, 20 ml of con. HNO3 were added to each beaker and covered with watch glasses after NO2 bubble was released. In the next step, the samples mixtures were refluxed gently until their volume became less, then the contents were allowed to cool for approximately 5 minutes at room temperature. 5 ml of HClO4 were added and boiled to almost dryness then the contents were allowed to cool to room temperature. The content of the beaker was dissolved in 0.1N HNO_3, boiled, then cold to room temperature then filtered by a glass filter into 50-ml volumetric flasks and diluted with 0.1N HNO_3 to marks.

2.4. Wet digestion Procedure 2

The wet digestion procedure 2 followed the same procedure as the pervious method except of that, H2O2 was supplied instead of $HClO_4$ for the treatment of the herbal mixture.

2.5. Dry ashing Procedure

Two grams of each individual plant sample were introduced into crucibles and then transferred into a muffle furnace, where they were heated at 500°C for 8 hrs. The crucibles were transferred from the furnace and allowed to cool at room temperature. The ash 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 HNO_3.

2.6. Water infusion procedure

Two grams of each herbal sample were transferred into 80-mL beakers and covered with watch glasses. A sufficient water was added 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 traditional procedure to extract some of pharmacologically active organic compounds and elements.

2.7. 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: potassium, sodium, and lithium were measured by using flame photometer. All peaks were background subtracted to remove interferences from the media such as deionized water and oxidizing agents.

3. Results And Discussion

Comparison of the concentrations of the studied minerals within each individual medicinal plant

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

Table1. The mean concentration (µg/g) of macrominerals and microminerals in T. capitates and R. officinalis with different digestion methods. Recommended daily amounts (RDA) *.

element	plant	digestion with HNO_3 / $HClO_4$	digestion with HNO_3 / H_2O_2	ashing	water infusion	RDA
Ca	T. capitates	26274	22279	17303	2044	1.0g/day
	R. officinalis	15292	12458	10159	1196	
Mg	T. capitates	9046	9210	7080	2524	340 mg
	R. officinalis	10587	10612	8633	2751	
Na	T. capitates	2409	2481	1958	1226	1.5 g/day
	R. officinalis	5120	4811	4708	2134	
K	T. capitates	14699	14490	1958	1226	4 mg/day
	R. officinalis	13173	15117	13606	8387	
Fe	T. capitates	575	605	390	ND	18 mg/day
	R. officinalis	786	758	572	ND	
Zn	T. capitates	40.10	45.79	25.75	ND	8 mg/day
	R. officinalis	37.32	36.21	26.22	ND	
Mn	T. capitates	40.47	34.84	23.15	ND	5 mg/day
	R. officinalis	30.11	32.13	22.22	ND	
Ni	T. capitates	91.14	90.23	8.75	ND	160 µg/day
	R. officinalis	45.67	43.23	7.28	ND	
Cu	T. capitates	16.46	12.25	23.52	ND	1.7 mg/day
	R. officinalis	9.85	12.50	25.56	ND	
Li	T. capitates	7.30	5.2	ND	ND	1mg/day
	R. officinalis	7.95	8.2	ND	ND	
Pb	T. capitates	9.14	9.23	ND	ND	-
	R. officinalis	7.20	6.02	ND	ND	

**Average of triplicate determinations, %R.S.D.= 0.03-0.10; *ND = not detected * Cr, Co and Cd not detected *RDA = The recommended daily amounts [55-60].*

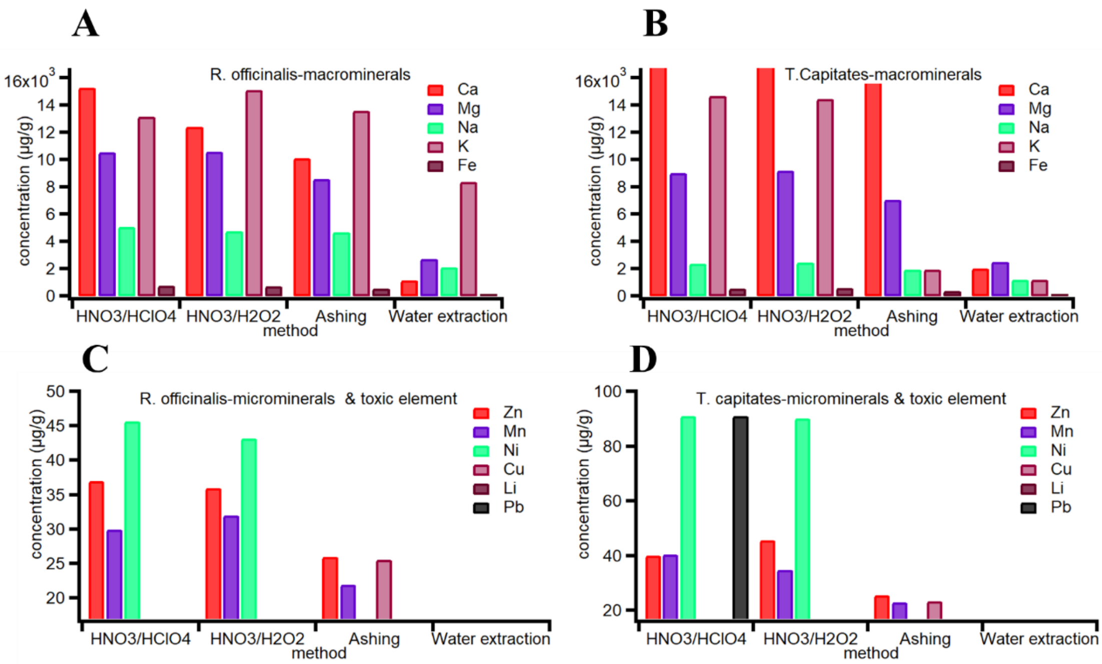

Figure 1. The concentration of microminerals, microminerals and toxic elements in the selected Libyan medicinal plants with four different procedures; *A (macrominerals in R. officinalis), B (macrominerals in T. capitates), C (microminerals and toxic element in R. officinalis), D (microminerals and toxic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

3.1. The macronutrients elements

The dependence of macrominerals concentrations on digestion methods with ($HNO_3/HClO_4$), (HNO_3/H_2O_2), dry ashing and water infusion procedures for chosen plants are shown in Table1 and Figure 1(A&B). The data revealed that the macrominerals: calcium, potassium, magnesium, sodium and iron are existed in highly predominate species in the samples of these herbal plants regardless of the method digestion. Among these, calcium exhibited the highest concentration, whereas iron was present at the lowest level in these samples. The concentration superiority of calcium over that of magnesium and potassium of sodium is very noticeable using sample preparation method. These observations are strongly supported by the previously published literature data [61]. However, the important of iron usually less abundant and found in a significant amount in these herbals which could be a good source for iron deficiency in human body. Iron concentration in these plants was found in a good agreement in some previous studies in some other countries [62-64].

Dry ashing procedure to digest the samples resulted in concentrations with considered to be inferior compared to the concentrations obtained using $HNO_3/HClO_4$ and HNO_3/H_2O_2 procedures. The order of relative concentrations of the detected macrominerals based on digestion procedures is:

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

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. With simple calculation we can conclude that about 976 mL, 420 mL, 2058 mL and 1220 mL of water extract obtained using 20 g sample of this medicinal plant are expected to contain respectively the amounts of calcium, magnesium, Potassium and sodium that are equivalent to the Recommended Daily Allowance (RDA) of the these mineral elements proposed by different institutions and authors, we can realize that this plant can be an important source of certain mineral elements namely calcium, magnesium, potassium and sodium as macronutrients. The content of iron in studied plant samples was not detected by refluxing 2.0g of plant tissues. However, since iron is indeed proven to exist in the acid mixture extract and in dry ashed samples, thus the disappearance of iron in water extract could be due to the lower solubility of its compounds in pure water. This problem could to certain extent be solved by increasing the sample size of this plant for water extract or by consuming as ground particles in capsules to benefit from the iron,

3.2. The microminerals and toxic elements

The concentration of microminerals and toxic elements with different digesting methods are presented in Table 1 and Figure 1(C&D). The results indicated that nickel had the highest concentration in both plants followed by zinc and manganese which found in the same level in both plants. In addition, copper was presented at moderate levels, whereas lithium was detected at the lowest concentration among the micronutrients in both plant samples. It is also noteworthy that, the concentration of nickel in T. capitatus was approximately twice that observed in R. officinalis. This may reflect a higher selectivity of T. capitatus for this element.

The microminerals Ni, Zn, Mn and Cu have been recognized as essential for plant and human. These elements have enormous impact on human health. Insufficient dietary intakes of these minerals impair several functions of the reproductive system, central nervous system, energy metabolism and enzyme activities and thus could cause serious illnesses [65]. The presence of the trace metals zinc, manganese, copper and nickel not detected in the water extract under our experimental conditions. Thus, the consume of water extraction of 2.0 grams of plant samples are not equivalent to the Recommended Daily Allowance (RDA) of these elements. The consuming sufficient amount of ground particles of these plants could supply the RDA of these minerals.

The toxic element lead found in lower levels compared to other macrominerals. Furthermore, the microminerals chrome, cobalt and toxic elements, Cd, were not detected. The lowest level of Pb and non-detected data of Cr, Co and Cd make these plants safe, for consuming these plants to supply the Recommended Daily Allowance (RDA) of these elements, in the point of view of toxic levels.

The concentrations pattern of microminerals and toxic elements were found for the analyzed samples as follow:

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

The mean concentration of these microminerals and toxic elements, Pb, were found to be with the range of many studies around the world [6, 9, 66-69].

3.3. Statistical analysis

The Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), Hierarchical Cluster Analysis (HCA) and boxplot were utilized to assess the significant differences between herbal plants toward their mineral composition, as well as to estimate variations arising from the methods that used to prepare the samples. PCA results exhibits variation between the selected plants. The first two principal components explaining 84% of the total variance, as seen in Figure 4. Moreover, PCA data reveal that there is significant variation between water extracting method and other methods (wet and dry methods). This could be related to the mineral composition which extracted by water infusion only represent the soluble salts minerals only. In addition, there is slight variance between ashing method and wet methods (HNO_3/H_2O_2 and $HNO_3/HClO_4$ methods), as seen in Figure 4.

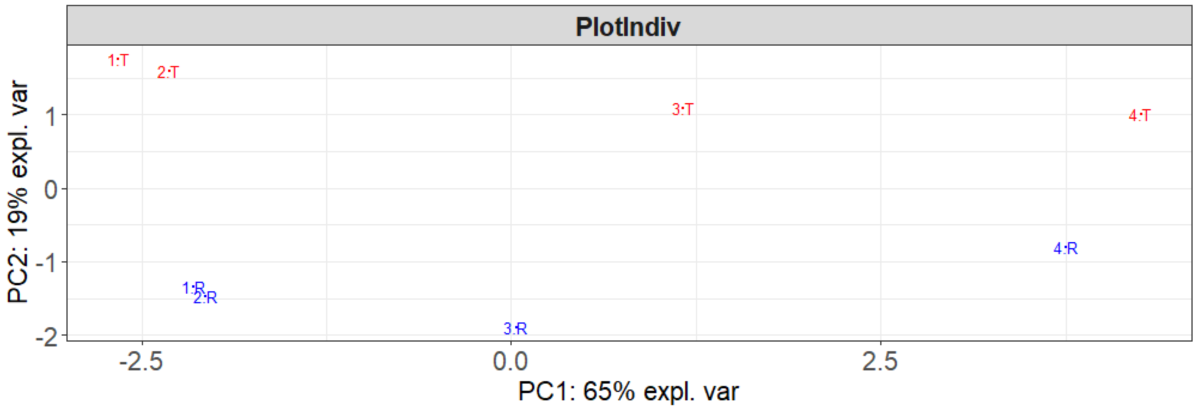

Figure 4. PCA analysis of elemental composition of R. officinalis and T. capitates; *1.S ($HNO_3/HClO_4$ for R. officinalis), 2.S (HNO_3/H_2O_2 for R. officinalis), 3.S (dry ashing for S. Officinalis), 4.S (water extraction for R. officinalis, 1.T ($HNO_3/HClO_4$ for T. capitates), 2.T (HNO_3/H_2O_2 for T. capitates), 3.T (dry ashing for T. capitates), 4.T (water extraction for T. capitates).*

PLS-DA result indicates that a clear variation between T. capitates and R. officinalis in terms of digestion methods. The result also exhibits distinct clustering for certain groups, water infusion method and other digestion methods. In addition, there are slight differences between ashing method and wet methods (HNO_3/H_2O_2 and $HNO_3/HClO_4$ methods), as seen in Figure 5.

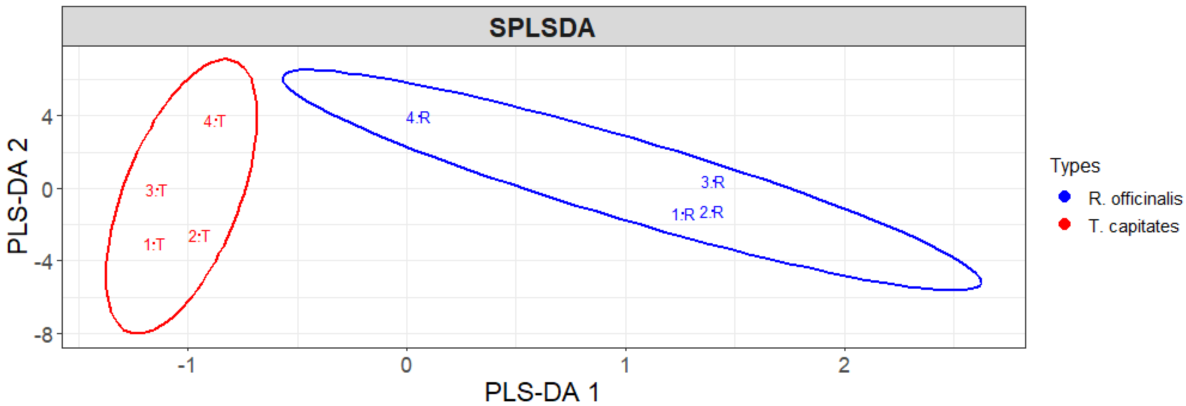

Figure 5. PLS-DA analysis of elemental composition of R. officinalis and T. capitates; *1.S ($HNO_3/HClO_4$ for R. officinalis), 2.S (HNO_3/H_2O_2 for R. officinalis), 3.S (dry ashing for S. officinalis), 4.S (water extraction for R. officinalis, 1.T ($HNO_3/HClO_4$ for T. capitates), 2.T (HNO_3/H_2O_2 for T. capitates), 3.T (dry ashing for T. capitates), 4.T (water extraction for T. capitates).*

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

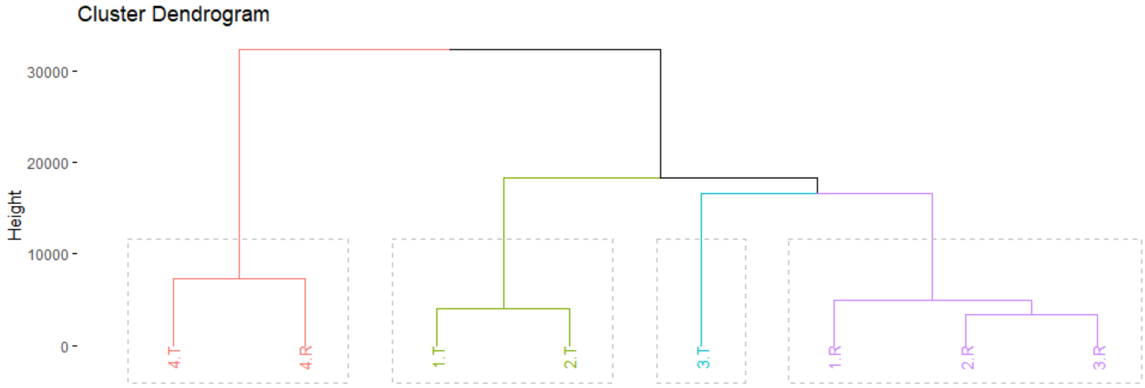

Figure 6. Dendrogram Plots showing separation of digestion methods which used to extract elements from R. officinalis and T. capitates; *1.S ($HNO_3/HClO_4$ for R. officinalis), 2.S (HNO_3/H_2O_2 for R. officinalis), 3.S (dry ashing for S. officinalis), 4.S (water extraction for R. officinalis, 1.T ($HNO_3/HClO_4$ for T. capitates), 2.T (HNO_3/H_2O_2 for T. capitates), 3.T (dry ashing for T. capitates), 4.T (water extraction for T. capitates).*

Boxplot graph indicates there is significant variation in these plants in terms of mineral concentration. For instance, these plants have a significant variation in concentration of calcium, potassium, sodium, magnesium, nickel. While there are small significant differences between these herbals according to zinc and lead, as seen this example, Figure 7.

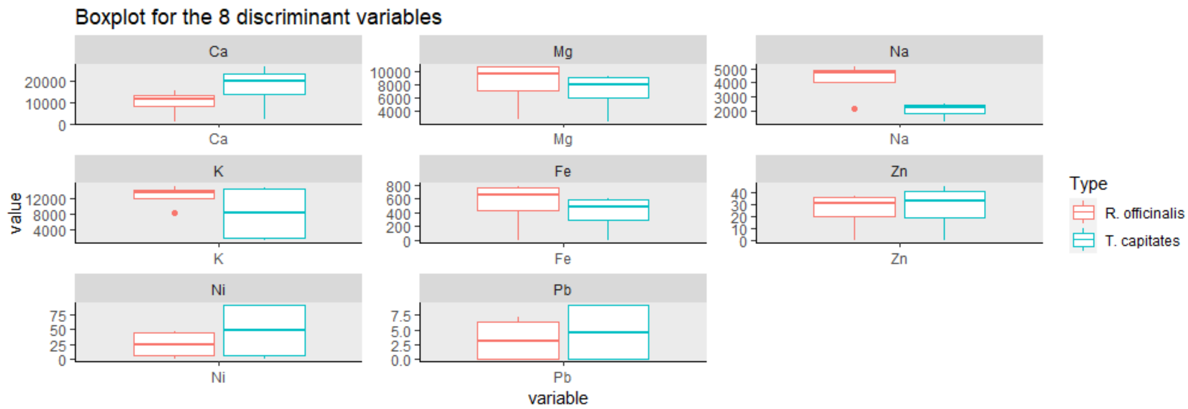

Figure 7. Grouped Boxplots showing the possible minerals that considered as marker to differentiate between R. officinalis and T. capitates.

4. Conclusion

The concentration of macrominerals, microminerals and some of toxic elements were determined in R. officinalis and T. capitates. From the data, the concentration levels of these minerals in the selected plants were found to be in this order: Ca > K > Mg > Na > Fe > Ni > Zn ≥ Mn > Cu > Li > Pb. The macrominerals calcium and sodium were found to present in major levels. It is noticeable that, the concentration of Ca in T. capitates higher than in R. officinalis (two folded), while the concentration of Mg and Na found to be higher in R. officinalis compared to the level of these minerals in T. capitates. The concentration of Fe, Zn, Mn and Ni were found in minor levels while Cu, Li and Pb were detected in trace levels. The concentration of Ca, Mg, K and Na were detected in water extraction at significant amount, while other minerals not detected in water extraction. This could be related to the solubility of these elements' salts in water. Cr, Co and Cd not detected by any digestion methods.

The statistical analysis revealed that clear superiority of ($HNO_3/HClO_4$) as wet digestion method followed by (HNO_3/H_2O_2) method to prepare the samples for measurements. While the water extract represents the lowest procedure to extract the minerals. The statistical analysis also demonstrated that there are significant differences in the selected medical plants in term of mineral concentrations. For instance, the herbals have a significant difference in concentration of Cu, Na and Ni. This may relate to the different ability of the plants to absorb these metals. While there is no significant variance between these herbals according to the concentration of Mg and Zn.

Conflict of Interest

The authors confirm that there are no financial or personal conflicts of interest that could affect the work reported in this paper.

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الإمكانات العلاجية وتقييم السلامة لنباتي الزعتر الليبي وإكليل الجبل: دراسة كمية للعناصر المعدنية وربطها بالإرشادات الصحية للإنسان

الملخص

تم تحديد التركيب المعدني لنباتين عشبيين ينموان في البرية الليبية، وهما Thymus capitatus (الزعتر البري) و Rosmarinus officinalis (إكليل الجبل/الروزماري)، باستخدام أربع طرق مختلفة للهضم الكيميائي. وقد تم قياس تراكيز عناصر الكالسيوم، والمغنيسيوم، والبوتاسيوم، والصوديوم، والفوسفور، والحديد، والمنغنيز، والزنك، والنحاس، والكوبالت، والكروم، والنيكل، والرصاص، والكادميوم باستخدام تقنية مطيافية الامتصاص الذري(AAS).

أظهرت النتائج أن العناصر الكبرى(Macrominerals) ، وهي الكالسيوم والمغنيسيوم والبوتاسيوم والصوديوم، كانت العناصر السائدة في النباتات المدروسة، حيث تراوحت تراكيزها من الكالسيوم الذي سجل أعلى مستوى إلى الصوديوم الذي سجل أقل تركيز. كما تم الكشف عن الحديد والزنك والمنغنيز والنيكل بتراكيز منخفضة نسبيًا، في حين وُجد النحاس والليثيوم والرصاص بكميات ضئيلة جدًا . وتم الكشف عن الكالسيوم والمغنيسيوم والبوتاسيوم والصوديوم في المستخلص المائي لهذه النباتات بكميات ملحوظة، بينما لم يتم الكشف عن العناصر الأخرى في المستخلص المائي. إضافة إلى ذلك، لم يتم الكشف عن الكروم والكوبالت والكادميوم باستخدام أي من طرق الهضم المستخدمة.

أوضحت نتائج الدراسة أن المستخلص المائي يحتوي على كميات من الكالسيوم والمغنيسيوم والبوتاسيوم والصوديوم تعادل أو تساهم بشكل ملحوظ في تلبية الاحتياج اليومي الموصى به (RDA) لهذه العناصر الكبرى، والتي تُعد ضرورية لصحة الإنسان. ومع ذلك، فإن كميات الحديد والزنك والمنغنيز والنيكل والنحاس والليثيوم الموجودة في المستخلص المائي غير كافية لتلبية الاحتياج اليومي الموصي به من هذه العناصر الضئيلة. ومن ناحية أخرى، فإن استهلاك كميات مناسبة من المسحوق النباتي الكامل لهذين النباتين العشبيين قد يساهم في توفير الاحتياج اليومي الموصي به من هذه العناصر الضئيلة. كما أن الانخفاض الشديد في مستوى الرصاص يدل على أن هذه النباتات آمنة للاستخدام في الأغراض العلاجية.

أظهر التحليل الإحصائي وجود فروق واضحة في كفاءة الاستخلاص بين طريقة الاستخلاص بالماء وطرق الاستخلاص الأخرى. ويمكن تفسير هذا الاختلاف بمحدودية ذوبانية الأملاح القابلة للذوبان في الماء. في المقابل، لوحظت فروق طفيفة فقط بين طريقة الرماد الجاف Dry Ashing)والطريقتين الأخريين المعتمدتين على الهضم الحمضي باستخدام خليط حمض النيتريك/فوق أكسيد الهيدروجين (HNO_3/H_2O_2) وخليط حمض النيتريك/حمض البيركلوريك .($HNO_3/HClO_4$) وبشكل عام، كانت تراكيز العناصر المقاسة باستخدام خليط $HNO_3/HClO_4$ أعلى قليلًا مقارنةً بتلك الناتجة عن طريقتي HNO_3/H_2O_2 والرماد الجاف.

الكلمات المفتاحية :البرية الليبية، الذوبانية في الماء، مطيافية الامتصاص الذري(AAS) ، التحليل الإحصائي