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Statistical Approach and Neutron Activation Analysis for Determining Essential and Toxic Elements in Two Kinds of Algerian *Artemisia* Plant



Samir Begaa¹ · Mohammed Messaoudi¹ · Adel Benarfa^{2,3}

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Abstract

In this study, two kinds of Artemisia plant, *Artemisia campestris* L. and *Artemisia herba-alba* Asso., collected from different locations in Djelfa province, Algeria, were subjected to an instrumental neutron activation analysis (INAA) in order to determine their essential and toxic elements for the first time. The obtained results for both types revealed the existence of twenty-one elements, namely, As, Ba, Br, Ca, Ce, Co, Cr, Cs, Eu, Fe, Hf, K, La, Na, Rb, Sb, Sc, Sm, Sr, Yb, and Zn, where, the elements K, Ca, Fe, and Na respectively showed a significant concentration. On the other hand, the tolerable daily intake (TDI) of the studied plants for an adult person per day was within the tolerance limits imposed by the World Health Organization (WHO). Hence, these findings might therefore be used to offer scientific basis for an optimum usage of the studied plants and so enriches the database of medicinal herbs.

Keywords Artemisia campestris L. · Artemisia herba-alba Asso. · Essential elements · Toxic elements · Instrumental neutron activation analysis

Introduction

In recent years, despite globalization and scientific progress, the system of alternative medicine and/or traditional medicine was considered as a sensory system of primary health care in many countries like China, India, and Pakistan, as well as regarded as an important constituent in the indigenous medical systems [1, 2]. Indeed, these days, aromatic plants are playing a strategic role after using it at various traditional medicines and physiotherapy to maintain good health and relieve ailments and diseases. Hence, aromatic plants have gained a great attention from researchers and scientific community in all across the world [3–6]. The World Health Organization

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(WHO) indicated that between 60 and 80% of the populations of countries throughout the world, particularly those in Asia, Africa, Latin America, and the Middle East, are currently using traditional medicine, including herbal medicines, as remedies [7]. Among the famous herbal used worldwide is the genus Artemisia (family Asteraceae); this genus is widespread over the world and growing wildly and comprises over 400 species. Recently, some reports suggested that a domain of non-pharmaceutical products produced from Artemisia plant material-like herbal teas-might be effective in treating or preventing malaria. This information makes us say that Artemisia plant material could be a potential solution or have a curative effect on COVID-19. Indeed, the recent work of Faiz Ul Haq et al. [8] reported that Artemisia annua ethanolic extract showed a remarkable inhibition against SARS-CoV, with 50% effective concentration (EC₅₀) value of $34.5 \pm 2.6 \,\mu$ g/mL and 50% cytotoxic concentration (CC₅₀) of $1053 \pm 92.8 \ \mu g/mL$.

Eleven species of *Artemisia* genus could be found in the Algerian flora including, *A. campestris* L. and *A. herba-alba* Asso. These two plants are in fact widely used by the Algerian population and considered as a therapeutic herb to treat several stomach aches and disorders. The consumption way of these plants for almost all the Algerian people is by preparing them as remedies after soaking them in hot water for at least half an

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hour [6, 9, 10]. A. campestris L., known in Algeria as "Dgouff" and/or "Armoise rouge," grows wildly in steppe and desert zones; this plant was used for a long time ago in the Algerian folk medicine in both rural and urban territories, and it is well-known for its valuable effects in the treatment of gastrointestinal complaints, including the most common stomach ache "peptic ulcer." Moreover, this plant is renowned as a versatile source of components with bioactive properties [6, 9]. On the other side, unlike A. campestris L., A. herba-alba Asso., known as "desert wormwood" and called in the Algerian vernacular language as "shih" and/or "Armoise blanche", grows spontaneously in arid and semi-arid areas of the Mediterranean basin, and spreads even far to the northwestern Himalayas; this plant has been used in traditional medicine by many cultures since ancient times as hemostatic agents, analgesic, antibacterial, and antispasmodic [10].

For the determination of chemical elements of a biological sample, one can select a technique from a variety of reliable and routinely used methods; the technique based on the principles of neutron activation like instrumental neutron activation analysis has been most extensively used in many areas of life, such as nutrition, environment fields, and biology, due to its remarkable high sensitivity, accuracy, versatility, and multi-elemental character [11–13]. Accordingly, this study was meant, for the first time, to employ a sensitive nuclear analytical approach (Instrumental Neutron Activation Analysis (INAA)), to investigate and assess both essential and toxic elements amounts presented in *A. campestris* L. and *A. herba-alba* Asso. plants. Hence, provides reliable data to the scientific literature for an optimum usage of the studied plants and so enrich the database of the inorganic medicinal herbs.

Materials and Methods

Sampling and Sample Preparation

Two types of *Artemisia* plant, *A. campestris* L. and *A. herbaalba* Asso. (Fig. 1), collected in June 2013 from different locations in Djelfa province, Algeria, were oriented to an elemental analysis; firstly, the samples (three samples for each type) were



Fig. 1 Photographs of (a) A. campestris L. and (b) A. herba-alba Asso

washed several times with deionized water in order to remove soil particles and dust, then dried in shade for 3 weeks at room temperature. Next, the samples were grounded using an agate mortar and pestle, then sieved to get identical fine powder. After that, the resulted powder of each sample was stored in precleaned polyethylene-capped bottles until use [14, 15]. In this work, the standard reference material GBW 07605 was used to determine the elemental concentration, whereas the analytical results obtained for two standard reference materials NIST-SRM 1573a (tomato leaves) obtained from the National Institute of Standard and Technology (NIST) and Chinese tea leaves (GBW 07605) procured from the National Research Center of CRM, Langfang, China, were subjected to internal quality control procedure. Finally, both samples and standards were placed in aluminum irradiation capsule.

Irradiation and Counting

The prepared aluminum capsule was placed in the appropriate irradiation site for 6 h at a thermal neutron flux of 4.5×10^{13} cm⁻² s⁻¹ in a vertical experimental channel of the Algerian Es-Salam research reactor. After an appropriate cooling time, the irradiated samples together with the standard were measured using a coaxial HPGe detector (Canberra) with 1.8-keV resolution at 1332 keV of ⁶⁰Co with 40% relative efficiency with GENIE-2k software. Cooling time was in the range of 2–5 days and the counting time was about 10,800 s. The second measurements were executed after 16–20 days.

Quality Control and Quality Assurance

Quality assurance is a proactive operation to minimize the probability of errors in an analytical procedure. It also includes quality control, the operation by which the occurrence of errors is inspected once the analysis has been completed. Quality assurance procedures are described for individual steps in neutron activation analysis, namely, preparation of the test portion, selection of analytical protocol, calibration, instrument performance checks, irradiation, decay, measurement, spectrum analysis and interpretation, and internal and external quality control, as well as for ensuring the technical competence of the personnel involved. For INAA determinations, two CRMs like NIST1573a (tomato leaves) and GSV4 (tea leaves) were used for quality control purposes (Tables 1 and 2).

In order to evaluate the laboratory performance, we determined the *U*-score, *Z*-score, and relative bias (RB). These parameters were calculated according to the following equations (1-3):

$$U_{\text{score}} = \frac{|X_{\text{Lab}} - X_{\text{Ref}}|}{\sqrt{\mu_{\text{Lab}}^2 + \sigma_{\text{Ref}}^2}} \tag{1}$$

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Table 1 Comparison of measured values with certified values in the certified reference materials of standard reference materials NIST-SRM 1573a. Values represent mean \pm standard deviation (*n* = 3)

Element	NIST-SRM 1573a (tomato leaves)											
	Certified value	This work measured value	U-score	Z-score	Bias							
Ba (µg/g)	(63)	66.3 ± 9.0	0.26	-	5.24							
Br (µg/g)	(1300)	1268 ± 133	0.13	-	-2.46							
Ca (µg/g)	$50,500\pm900$	$51,194 \pm 5541$	0.12	0.77	1.37							
Ce (µg/g)	(2)	2.04 ± 0.24	0.09	-	2.00							
Co (µg/g)	0.57 ± 0.02	0.53 ± 0.05	0.69	2.00	- 7.02							
Fe (µg/g)	368 ± 7	360.6 ± 27.6	0.26	1.06	-2.01							
K (%)	2.70 ± 0.05	2.752 ± 0.103	0.13	1.04	1.93							
La (µg/g)	(2.30)	2.34 ± 0.18	0.10	-	1.74							
Na (µg/g)	136 ± 4	146.5 ± 17.1	0.60	2.63	7.72							
Rb (µg/g)	14.89 ± 0.27	14.8 ± 1.3	0.07	0.33	- 0.60							
Sc (µg/g)	(0.1)	0.101 ± 0.062	0.02	-	1.00							
Sm (µg/g)	(0.19)	0.205 ± 0.029	0.36	-	7.89							
Th ($\mu g/g$)	(0.12)	0.111 ± 0.015	0.39	-	- 7.50							
Zn (µg/g)	30.9 ± 0.7	29.4 ± 1.4	0.92	2.14	-4.85							

Values between parentheses are non-certified

$$Z_{\text{score}} = \frac{|X_{\text{Lab}} - X_{\text{Ref}}|}{\mu_{\text{Lab}}} \tag{2}$$

Relative bias (RB) =
$$\frac{X_{\text{Lab}} - X_{\text{Ref}}}{X_{\text{Ref}}} \times 100$$
 (3)

where X_{Lab} , μ_{Lab} , X_{Ref} , and σ_{Ref} are the laboratory results, standard deviation, recommended uncertainty, and standard uncertainty respectively.

The laboratory performance is evaluated as **follows**: satisfactory if *U*-score ≤ 1 , and satisfactory if *Z*-score ≤ 2 , questionable for 2 < Z-score < 3, and unsatisfactory for *Z*-score ≥ 3 .

Results and Discussions

Concentrations of Trace Elements

Elemental concentrations of the studied plants *A. campestris* L. and *A. herba-alba* Asso. were calculated using INAA technique. The illustrated results in Table 1 and Table 2 show that all the element concentrations are in good arrangement with the certified values after using two CRMs: NIST-SRM 1573a and GBW 07605. This calculation exposed a great quality results found in this examination which could be observed

Table 2 Comparison of measured
values with certified values in the
certified reference materials of
GBW 07605 (tea leaves). Values
represent mean \pm standard
deviation $(n = 3)$

Elements	GBW 07605 (tea leaves)										
	Certified value	This work measured value	U-score	Z-score	Bias						
Ba (µg/g)	58 ± 3	55.09 ± 9.43	0.29	0.97	- 5.02						
Br (µg/g)	3.4 ± 0.4	3.62 ± 0.55	0.32	0.55	6.47						
Ca (µg/g)	4300 ± 200	4180 ± 620	0.18	0.60	-2.79						
Ce (µg/g)	1.0 ± 0.1	1.01 ± 0.30	0.03	0.10	1.00						
Co (µg/g)	0.18 ± 0.02	0.190 ± 0.026	0.30	0.50	5.56						
Fe (µg/g)	264 ± 10	272 ± 29	0.26	0.80	3.03						
K (%)	1.66 ± 0.06	1.60 ± 0.03	0.88	1.00	- 3.61						
La (µg/g)	0.6 ± 0.03	0.59 ± 0.09	0.11	0.33	- 1.67						
Na (µg/g)	44 ± 4	46.6 ± 5.4	0.39	0.65	5.91						
Rb (µg/g)	74 ± 4	71.2 ± 5.9	0.40	0.70	-3.78						
Sc (µg/g)	0.085 ± 0.009	0.084 ± 0.013	0.06	0.11	-1.18						
Sm (µg/g)	0.085 ± 0.017	0.091 ± 0.015	0.26	0.35	7.06						
Th ($\mu g/g$)	0.061 ± 0.06	0.065 ± 0.015	0.25	0.67	6.56						
Zn (µg/g)	26.3 ± 0.9	27.1 ± 2.1	0.33	0.89	3.04						

through the statistical assessment and evaluation where, the statistical parameters "Relative bias (RB)", Z-score, and U-score calculated for all elements are acceptable. The amounts of both essential and non-essential chemical elements detected and calculated in both studied plants are illustrated in Table 3; in this work, CRM of GBW 07605 was employed to determine the chemical elemental concentrations for both studied samples of Artemisia. It is obvious that the analyzed samples are characterized by a large variety within investigated groups.

The determined concentrations for the studied plants (*Artemisia campestris* L. and *Artemisia herba-alba* Asso.) are shown in Table 3. From the illustrated results in Table 3, twenty-one chemical elements were identified after using instrumental neutron activation analysis (INAA) technique, these elements could be divided into three main groups: essential chemical element group includes K, Ca, Fe, Na, Co, and Cr; non-essential chemical element group includes La, Sm, Ba, Ce, Cs, Eu, Hf, Rb, Sc, Sr, and Yb; and, last, the potential toxic elements

Table 3 Mean values of chemical component mass fractions ($\mu g/g$ on dry mass basis) determined in two types of *Artemisia (A. campestris L.* and *A. herba-alba* Asso.) by INAA technique. SD represents standard deviation (n = 3)

Elements (µg/g)	A. campe	estris I	L.	A. herba-alba Asso.								
	Mean	Mean		Mean		SD						
Essential chemical elements												
Ca	8700	±	500	11,500	±	600						
K	10,000	±	300	17,000	±	122						
Na	248	±	14	645	±	37						
Fe	631	±	23	617	±	21						
Zn	13.15	±	0.52	18.2	±	0.71						
Со	0.30	±	0.04	0.27	±	0.03						
Cr	1.50	±	0.05	0.74	\pm	0.02						
Potential toxic che	emical elem	ents										
As	0.46	±	0.05	0.88	±	0.10						
Br	36.02	±	4.26	63.32	±	7.48						
Sb	0.03	±	0.01	0.040	±	0.01						
Other chemical ele	ements											
Ba	16.58	±	1.50	13.16	±	1.26						
Ce	1.58	±	0.17	1.62	\pm	0.17						
Cs	0.09	±	0.012	0.08	\pm	0.010						
Eu	0.040	±	0.01	0.030	±	0.001						
Hf	0.08	±	0.01	0.09	±	0.01						
La	0.74	±	0.04	0.62	±	0.04						
Rb	2.76	±	0.19	3.61	±	0.24						
Sc	0.015	±	0.02	0.15	±	0.02						
Sm	0.11	±	0.04	0.10	±	0.04						
Sr	129.9	±	15.2	121.6	±	14.2						
Yb	0.07	±	0.02	0.04	±	0.01						

group contained Br > As > Sb which presented in a descending content pattern.

Human health needs several elements which considered essential for its growth; these elements are in fact necessary components and play a significant physiological role, where calcium is an essential mineral for human health, contributing in bones and teeth healthy, and plays an important role as a cofactor in enzyme reactions. The element potassium is the most common intracellular ion in both human and animal cells and is also abundant in dietary matter [16, 17]; the concentration of calcium for both studied plants ranged from 8700 to 11,500 μ g/g and the concentration of potassium started from 10,000 up to 17,000 μ g/g, noticing maximum values in A. herba-alba Asso. samples; for sodium element, we noticed quite small values comparing with calcium and potassium, ranging between 248 and 645 μ g/g. The essential element iron, which plays an important role in the transportation of oxygen through hemoglobin [18], showed closed concentration values for both studied plants (617 μ g/g and 631 μ g/g). The essential element zinc plays an important role in the body immunity, by keeping it vital, healthy, and free from disease and common illnesses [19]; zinc element showed an acceptable and almost similar concentration for both plants (13 to 18 μ g/g). Cobalt is closely associated with the physiological role of vitamin B_{12} in the production and maintenance of red blood cells. The concentration of cobalt for both samples was found almost comparable (0.27 to 0.30 μ g/g) [20]; this component is important and essential and considered as a cofactor for insulin and a constituent of the glucose tolerance factor (GTF) [20, 21]. The concentration of chromium was found ranging between 0.74 and 1.50 µg/g, whereas, A. campestris L. samples contained much chromium amounts than A. herbaalba Asso. samples. The toxic element Br is comparable in both Artemisia kinds. The concentration of elements such as Ba, Cs, Eu, Hf, La, Sc, Sm, Sr, and Yb in A. herba-alba Asso. represented lower values than that in A. campestris L., whereas the concentrations of elements Ce, Hf, and Rb in A. herbaalba Asso. are higher than that those in A. campestris L. These differences could probably be due to the plant's nutrition, climate, water, and soil condition [22].

Statistical Analysis and Inter-elemental Correlation

Concentration data obtained for both kinds of *Artemisia* samples were subjected to statistical analysis, with one-way ANOVA followed by Tukey's multiple comparisons. One-factor analysis of variance (ANOVA) showed statistically significant differences in mean concentrations of (Ba, Yb), (Br, Zn), (Ca, Eu), (Ce, Rb), (Co, Fe), (Sb,Zn), and (Eu, K). The level of significance was set at P < 0.05.

The Pearson correlation coefficients (r) of chemical elements in *A. herba-alba* Asso. are given in Table 4. A high positive relationship was detected between some essential and toxic

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Table 4	Pearson's correlation	coefficient (r) of c	hemical elements	in A. herba-alba	a Asso. Th	is coefficient	was applied to	evaluate the	degree	of the
relationshi	ip between chemical e	elements in A. herbe	<i>a-alba</i> Asso. samp	oles						

	Ba	Br	Ca	Ce	Co	Cr	Cs	Eu	Fe	Κ	Na	Rb	Sb	Yb	Zn
Ba	1														
Br	0.725	1													
Ca	-0.345	-0.434	1												
Ce	0.035	0.707	-0.145	1											
Со	0.457	0.944	-0.383	0.897	1										
Cr	-0.270	-0.019	-0.811*	0.110	0.093	1									
Cs	0.350	0.574	0.487	0.590	0.584	-0.730	1								
Eu	-0.262	-0.345	0.995**	-0.096	-0.308	-0.858	0.567	1							
Fe	0.471	0.934	-0.218	0.896	0.983*	-0.086	0.722	-0.135	1						
Κ	-0.084	-0.136	0.949	0.033	-0.123	-0.925	0.726	0.976*	0.058	1					
Na	0.516	0.769	-0.904	0.492	0.737	0.599	-0.071	-0.862	0.613	-0.736	1				
Rb	0.083	0.677	0.128	0.951*	0.839	-0.200	0.802	0.189	0.894	0.330	0.276	1			
Sb	0.268	0.854	-0.438	0.943	0.973*	0.266	0.458	-0.379	0.932	-0.226	0.751	0.836	1		
Yb	0.961*	0.509	-0.296	-0.242	0.196	-0.290	0.175	-0.229	0.210	-0.092	0.367	-0.182	0.000	1	
Zn	0.644	0.991**	- 0.493	0.764	0.970*	0.092	0.517	-0.411	0.944	-0.212	0.817	0.701	0.910	0.414	1

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

elements, e.g., Co–Fe (r = 0.983, P < 0.01), Co–Zn (r = 0.97, P < 0.05), Br–Zn (r = 0.991, P < 0.01), Ce–Rb (r = 0.951, P < 0.05), Co–Sb (r = 0.973, P < 0.05), and Eu–K (r = 0.976, P < 0.05). However, negative correlations were also found, e.g., between Ca–Cr (r = -0.811, P < 0.05). These differences in elemental relationships may be attributed to the plant physiological antagonism between some essential and toxic elements.

A high level of correlations was found between some essential and toxic elements in the analyzed sample. Table 5 presents the value of the correlation coefficients between the different elements in *A. campestris* L. samples. Ba element showed positive correlation with Hf (r = 0.964, P < 0.05), as well as Br element showed also high positive correlation with Zn (r = 0.956, P < 0.05). Element pairs Br–Co, Co–Sc, and

 Table 5
 Pearson correlation coefficient (r) of chemical elements in A. campestris L.

	Ba	Br	Ca	Ce	Со	Cs	Fe	Hf	К	Sb	Sc	Sm	Sr	Zn
Ва	1													
Br	0.554	1												
Ca	-0.563	-0.761	1											
Ce	0.860	0.702	-0.901	1										
Со	0.196	0.899	-0.470	0.319	1									
Cs	0.969*	0.742	-0.671	0.895	0.426	1								
Fe	0.240	0.941	-0.650	0.462	0.972*	0.471	1							
Hf	-0.964*	-0.355	0.523	-0.838	0.053	-0.880	-0.020	1						
Κ	0.393	-0.237	0.536	-0.130	-0.260	0.255	-0.426	-0.383	1					
Sb	0.521	0.381	-0.883	0.844	0.000	0.522	0.221	-0.608	-0.482	1				
Sc	0.169	0.912	-0.671	0.442	0.95*	0.405	0.994**	0.038	-0.524	0.258	1			
Sm	-0.847	-0.825	0.906	-0.981*	-0.494	-0.922	-0.613	0.773	0.135	-0.757	-0.586	1		
Sr	-0.498	-0.224	0.788	-0.786	0.173	-0.457	-0.047	0.626	0.410	-0.985*	-0.086	0.669	1	
Zn	0.744	0.956*	-0.859	0.877	0.732	0.880	0.810	-0.599	-0.167	0.578	0.778	-0.954*	-0.451	1

Inter-elemental correlations of Artemisia campestris L. samples were evaluated for all combinations

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

Elements are expressed in terms of the correlation coefficient, r

	Essential	chemical ele	ements RDA	A/AI	Potential toxic chemical elements TDI (adult with 70 kg (BW))				
Elements	Ca (mg/day)	Fe (mg/day)	K ^a (mg/day)	Na ^a (mg/day)	Zn (mg/day)	As µg/day/person	Br mg/day/person	Sb µg/day/person	
Artemisia campestris	87	6.31	100	2.48	0.13	4.6	0.36	0.3	
Artemisia herba-alba	115	6.17	170	6.45	0.18	8.8	0.63	0.4	
Males 19-50 years	1000	8	4700	1500	11	150	70	420	
Females 19-50 years	1000	18	4700	1500	8	150	70	420	
Pregnancy 19-50 years	1000	27	4700	1500	11	150	70	420	
Lactation 19-50 years	1000	9	5100	1500	12	150	70	420	

 Table 6
 Intake values (in mg/day, person) of some essential elements and tolerable daily intake of adult (TDI/70 kg) of some toxic elements (WHO/FAO [23])

RDA, recommended daily allowance/^aAI, adequate intake, expressed in mg/day/person for adult men and women.

Ce–Zn showed positive correlation with correlation coefficient values 0.90, 0.95, and 0.877 respectively. Fe showed strong positive correlation with Sc (r = 0.994, P < 0.005). Br was negatively correlated with Ca, Hf, K, Sm, and Sr. Element pairs Ba–Hf, Ce–Sm, Sb-Sr, and Sm-Zn showed high negative correlation values: -0.964, -0.956, 0.985, and -0.954 respectively.

Estimation of the Dietary Intake of Trace Elements

Medicinal and/or aromatic plants might be a worthy source of indispensable elements for human body. However, medicinal herbs in general are not completely inoffensive; sometimes allergic and toxic reactions happened as well as drug interactions. Hence, the consumption rate of herbs must be monitored and strictly control by an herb expert or a nutritionist. The present study attempts to provide an estimation of the dietary consumption of essential and toxic elements contained in both Artemisia plants (A. campestris L. and A. herba-alba Asso.). As well as delivers reliable information and facts to the scientific literature, the results' values of the estimation of micronutrients and theoretically toxic elements in the studied samples are given in Table 6 with the suggested daily tolerance limits. The assessed data of the studied plants were compared with those provided by the World Health Organization (WHO)/FAO. The typical consumption values per day and per person for essential and toxic elements contained in those plants were determined assuming an intake of 10 g (dry weight) of the studied plant ration per person [24]. The probable intake estimation of the plants indicated that the contents of toxic elements (As, Br, Sb) are well below toxicological reference values provided by the WHO/FAO and were found within nutritional recommendation values; thus, the studied plant toxic effects are considered negligible.

Conclusion

The present investigation tried to determine and evaluate the chemical elements toxicity contained in two types of Artemisia plant, A. campestris L. and A. herba-alba Asso., which are used widely by the Algerian people, after using instrumental neutron activation analysis (INAA) method for the first time on those plants. Twenty-one chemical elements were determined, including seven micro-nutritional elements such as K, Ca, Fe, Na, Co, Cr, and Zn. Three possible toxic elements which were: As, Br and Sb, and finally, fourteen non-essential chemical elements. An inter-elemental correlation was assessed which represented a high correlation level exists between some element pairs rather than others. On the other side, it can be reasoned that the essential and toxic element concentrations in both plants were considerably below the estimated human consumption tolerable limits set by the WHO/FAO. Finally, the outcomes of the present investigation might be used for rich medicinal herb databases.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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