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Determination of some mineral and heavy metals in Saudi Arabia popular herbal drugs using modern techniques

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The contents of 22 essential mineral elements and trace, heavy and toxic metals in 14 herbal drugs collected from the local markets of the western province of Saudi Arabia have been determined. All investigated elements were detected using inductively coupled plasma–atomic emission spectrometer (ICP-AES) after pretreatment of the tested samples with microwave digestion system. The levels of the most dangerous heavy metals Cd and Pb in the samples were below the maximum permitted levels reported by World health Organization (WHO) standards. K and Ca were present at high levels in samples 2 (Chamomile) and 11 (Becham), respectively. Ca and Mg were the most abundant mineral elements in all herbal samples. Moreover, it is observed that the concentrations of most of the tested toxic metals in the investigated herbal plants are found below the permitted levels reported by the international regulatory standards of the medicinal plants.

Key words: Herbal drugs, mineral elements, toxic metals, inductively coupled plasma–atomic emission spectrometer (ICP-AES), microwave digestion.

INTRODUCTION

Herbal plants are important and widely used in folk therapeutic treatments worldwide. It is well known that about 75% of the world's population relies on non-conventional medicines or herbal plants. These plants are sometimes contaminated with toxic heavy metals depending on their nature and origin, which impose serious health risks to consumers (Xudong et al., 2011). Herbal drugs may be contaminated easily during growing and processing. Consequently, recent developments have been reported in the environmental pollution control and treatment to reduce its undesired effects on the human health (Pesavento et al., 2009; Sitko et al., 2012).

The unexpected and undesirable effects arise from damage caused by various toxic constituents of beneficial herbs themselves, such as heavy and toxic metals. Therefore, WHO and European Pharmacopeia (*Ph. Eur.*) has developed guidelines and tolerance limits for assessing quality of herbal medicines with reference to contaminants and residues (WHO Guidelines, 2007; Gasser et al., 2009). On the other hand, the essential mineral constituents represent important elements as macronutrients or micronutrients in the herbal drugs. However, these essential elements can also have harmful effects when their in-takes exceed the recommended

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Table 1. List of the investigated herbal drugs samples.

Sample No.	Plant name (Common name)	Latin name (Genus, Species)	Family	Origin
1	Mentha (Peppermint)	*Mentha piperita*	Labiatae	Egypt
2	German Chamomile (Chamomile)	*Matricaria chamomile*	Compositae	Syria
3	Foenugreek	*Trigonella foenum graecum*	Leguminosae	Yemen
4	Juniper	*Juniperus communis*	Cupressaceae	Saudi Arabia
5	Curcuma (Turmeric)	*Curcuma domestica*	Zingiberaceae	India
6	Sweet Basil (*Ocimum*)	*Ocimum basilicum*	Labiatae	Saudi Arabia
7	Hibiscus	*Hibiscus sabdariffa*	Malvaceae	Sudan
8	Cumin	*Cuminum cyminum*	Umbelliferae	Syria
9	Thyme	*Thymus vulgaris*	Labiatae	Syria
10	Half-Bar (*Cymbopogon*)	*Cymbopogon proximus*	Graminae	Egypt
11	Becham	*Commiphora opobalsamum*	Burseraceae	Saudi Arabia
12	Santonica (*Artemisia*)	*Artemisia cina*	Compositae	Egypt
13	Fennel	*Foeniculum vulgare*	Umbelliferae	India
14	Black seed (*Nigella*)	*Nigella sativa*	Ranunculaceae	Syria

quantities significantly in herbs (Mustafa et al., 2004). It is important to have a good quality control for herbal medicines in order to protect consumers from contamination (Chwan-Bor et al., 2003). Therefore, it is a critical challenge to determine the concentration of essential elements, heavy and toxic metals in herbal plants in order to ensure that their levels meet the related standards or regulations limiting their concentration in herbal drugs. Toxic and heavy metals such as Pb, Cd, As, and Cr are widely considered as potential contaminants in our environment due to their toxicities to human. Consequently, their quantification in such popular medicinal plants is essential for herbal drugs quality control purposes.

In order to enhance the awareness about the toxicity in medicinal plants, several authors all across the world have recently reported many studies on the assessment of inorganic constituents of the herbal drugs (Sumontha et al., 2006; Remigius et al., 2003; Slavica et al., 2006; Alwakeel 2008; Lasisi et al., 2005; Galia et al., 2010; Bushra et al., 2011; Sembratowicz et al., 2009; Pharidhavi and Agrrawal, 2007; Muhammad et al., 2010a, b; Ram et al., 2010; Shazia et al., 2010; Munish and Jaspreet, 2012; Jinyu et al., 2011; Mahwash et al., 2011; Archana et al., 2011; Petenatti et al., 2011; Ragavendran et al., 2012; Amirah 2008). Prior to the metal detection, the mineralization procedure is of great interest for obtaining accurate, precise and reliable results in the metallic analysis of medicinal plants. Many procedures have been reported for the pretreatment step, namely, the wet method, the dry ashing procedure and the microwave digestion method (Sumontha et al., 2006; Remigius et al., 2003; Slavica et al., 2006; Alwakeel 2008; Lasisi et al., 2005; Galia et al., 2010; Bushra et al., 2011; Sembratowicz et al., 2009; Pharidhavi and Agrrawal 2007; Muhammad et al., 2010a, b; Ram et al., 2010; Shazia et al., 2010; Munish and Jaspreet, 2012; Jinyu et al., 2011; Mahwash et al., 2011; Archana et al., 2011; Petenatti et al., 2011). Microwave digestion method has many advantages including rapid, efficient and has reproducible results (Sumontha et al., 2006; Remigius et al., 2003; Slavica et al., 2006; Alwakeel, 2008; Lasisi et al., 2005; Galia et al., 2010). In order to assess the essential and toxic heavy metals in herbal plants, several techniques have been used after the dissolution step, such as: atomic absorption spectroscopy (Remigius et al., 2003; Slavica et al., 2006; Alwakeel, 2008; Lasisi et al., 2005; Galia et al., 2010; Bushra et al., 2011; Sembratowicz et al., 2009; Pharidhavi and Agrrawal, 2007; Muhammad et al., 2010a, b; Ram et al., 2010; Shazia et al., 2010; Mahwash et al., 2011; Archana et al., 2011; Petenatti et al., 2011), neutron activation analysis (Jinyu et al., 2011; Amirah, 2008), inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Xudong et al., 2011; Sumontha et al., 2006; Alwakeel, 2008; Galia et al., 2010; Petenatti et al., 2011), scanning electron microscopy-energy dispersive X-ray (SEM-EDX) analyzer (Xudong et al., 2011; Ragavendran et al., 2012) and inductively coupled plasma–mass spectroscopy (ICP-MS) (Qing-hua et al., 2012; Rao et al., 2007).

The purpose of the present work was to estimate the concentration of some macronutrients, micronutrients and trace toxic metals in some marketed herbal plants in the western province in Saudi Arabia using ICP-AES after pretreatment by microwave digestion system.

MATERIALS AND METHODS

Sampling

A total of 14 different herbal plant samples were collected from local markets in western province of Saudi Arabia (Table 1). The investigated herbal samples were cut into small pieces, washed 3 times with de-ionized water and dried overnight at 100±5°C. Five grams quantities of the plants were stored in clean and dry Petri-

dishes at room temperature until analysis.

Reagents

All reagents used were of analytical reagent grade unless otherwise stated. Hydrogen peroxide (35% w/v) and nitric acid (69%) were purchased from Avonchem (UK) and Sigma-Aldrich (Germany), respectively. De-ionized water with conductivity <0.2 µS/cm obtained from a Milli-Q water system (Millipore, France, Elix 10) was used to prepare standard samples and washing all glassware throughout. All plastic and glassware were cleaned by soaking in dilute HNO_3, rinsed with de-ionized water and air dried before use. Mixed working standard solutions of the investigated mineral and toxic heavy metal ions prepared by appropriate stepwise dilutions of certified stock atomic spectroscopy standards (5% HNO_3, 3 to 500 mg/kg, Perkin Elmer, USA) were used for ICP-AES validation measurements.

Apparatus

CEM (model Mars, USA) microwave digestion system (maximum power, 1600 W; maximum pressure, 800 psi; maximum temperature, 300°C) equipped with closed vessel (EasyPrep) of Teflon reaction vessels was used in all the digestion procedures of plants samples. The reaction vessels were cleaned using 5 ml of concentrated nitric acid and thoroughly rinsed with de-ionized water before each digestion.

The simultaneous determination of the investigated mineral and heavy metal were carried out using a Perkin-Elmer (Optima 2100 DV, Norwalk, CT, USA) ICP-AES instrument connected with an AS 93 Plus auto-sampler. The 40-MHz free-running generator was operated at a forward power of 1300 W; the outer, intermediate and Ar carrier gas flow rates were 15.0, 0.2 and 0.8 L/min, respectively. The pump flow rate was 1.5 ml/min. The carrier gas flow rate was optimized to obtain maximum signal-to-background ratios.

Microwave digestion pretreatment

One gram of each herbal sample was digested with 4 ml of H_2O_2 (35% w/v) and 10 ml of nitric acid (69%) in the microwave digestion system via temperature ramping (ramped to 120°C for 10 min, held for 5 min, then ramped to 200°C over 10 min, then held for 15 min). A two blank digests were carried in the same way. The resulting clear digested solutions were quantitatively diluted with de-ionized water before analysis by ICP-AES.

Assessment of mineral and heavy metals using ICP-AES

The investigated mineral and toxic heavy metal ions were analyzed using ICP-AES under optimized plasma condition. Using the auto-sampler, the measured samples were nebulized downstream to the plasma and the concentrations were automatically determined using the standard calibration graph. The ranges of standard concentrations used varied between 0.03 (e.g. Pb) and 50 (e.g. Ca) mg/L depending on the levels in the matrix of the investigated metal ions. The system was adjusted to measure the samples in triplicates and the relative standard deviation was automatically calculated. The relative standard deviation (RSD) was <2% and the correlation coefficient was >0.99998.

RESULTS AND DISCUSSION

Safety and efficacy of herbal medicines are two main issues of a drug therapy to which, the source and quality of the raw materials play an important role. For this purpose, the concentration of 22 elements of macronutrients, micronutrients and trace toxic heavy metals were determined in different 14 herbal plants using ICP-AES under the optimized conditions and after microwave digestion. Using highly sophisticated ICP-AES in combination with the microwave digestion system provides accurate, precise and reliable measurements of the investigated elements (Slavica et al., 2006). The precession is on average lower than 3% (RSD). The variation in elemental concentrations is mainly attributed to the difference in botanical structure and the plant origin as well as the preferential absorbability of the plant.

Macronutrients

Metals play a vital role as structural and functional components of proteins and enzymes in cells. Each mineral plays a number of different functions in the body. The most important pathway of metals to transport into human is from soil to plant and from plant to human. The levels of the mineral essential elements (Ca, Mg, Na, K and Fe) detected in the investigated herbal drugs are presented as mg/kg in Table 2. The data revealed that all analyzed elements were accumulated by the plants species at different concentration. Levels of the essential elements were found to be higher than those of the non-essential metals.

The results indicate that samples 1, 11, 2, and 13 contained the highest (Fe and Mg), Ca, K and Na, respectively, among the 14 herbal drugs studied. There are no international limits for the macronutrients in the herbal drugs. However, the results obtained agreed with the earlier studies of elemental distribution in herbal plants species (Sumontha et al., 2006; Lasisi et al., 2006; Shazia et al., 2010; Munish and Jaspreet, 2012). The results obtained reveal that Fe levels in herbs did not exceed the physiological limits for plants. The abundance of Ca, Mg, Na and K in the present study was also in agreements with the previous studies, which indicate that these elements were the most abundant elements in many herbal drugs.

Micronutrients

Some types of metals such as Se, Cr, Mn, Zn, Cu, and Ni are natural essential micronutrients. The levels of these metals at studied herbal drugs are presented in Table 3. The results indicated that samples 1, 3, 5, and 12 contained the highest Cu, Se, (Cr, Mn and Ni) and Zn, respectively. In all cases, the results were similar to, or lower than the case of Cr, those reported in several studies (Mustafa et al., 2004; Chwan-Bor et al., 2004; Sumontha et al., 2006; Slavica et al., 2006). The

Table 2. Macronutrients contents (µg/g) in the tested herbal species (mean), n=3.

Sample No.	Plant name	Fe	Ca	Mg	K	Na
1	Mentha	1068	15375	5725	23160	958.9
2	German Chamomile	466.5	6141	1867	25365	2485
3	Foenugreek	72.7	2043	1055	10428	373.1
4	Juniper	315.1	11164	730.9	3591	137.5
5	Curcuma	726.0	1851	2062	24120	132.7
6	Sweet Basil	225.0	14136	2697	15825	190.0
7	Hibiscus	132.0	16335	3636	18765	905.4
8	Cumin	134.1	7849	2556	12391	344.7
9	Thyme	274.1	20160	2380	14185	937.2
10	Half-Bar	937.8	3552	1113	2400	96.4
11	Becham	54.4	25364	482.2	5034	836.1
12	Santonica	863.9	13215	5265	15810	27.0
13	Fennel	104.7	13654	3037	14164	2664
14	Black seed	59.3	6486	2007	7270	37.8

Table 3. Micronutrients contents (µg/g) in the tested herbal species (mean), n=3.

Sample No.	Plant name	Se	Cr	Mn	Zn	Cu	Ni
1	Mentha	2.01	2.44	102.88	18.46	22.63	1.33
2	German Chamomile	1.74	1.32	43.06	31.41	14.26	1.24
3	Foenugreek	3.36	0.70	16.15	34.45	8.23	0.340
4	Juniper	1.26	0.72	29.67	4.33	3.48	0.310
5	Curcuma	1.60	2.68	570.6	37.33	6.01	3.45
6	Sweet Basil	0.66	0.61	20.02	21.93	7.38	0.135
7	Hibiscus	1.29	0.525	180.0	30.10	4.69	1.62
8	Cumin	3.04	0.78	17.43	27.09	7.83	0.750
9	Thyme	1.08	1.24	22.29	19.56	8.83	1.87
10	Half-Bar	0.225	2.38	44.31	14.16	3.36	1.12
11	Becham	0.255	0.240	2.55	0.495	2.17	0.045
12	Santonica	0.780	2.02	69.46	56.37	15.60	1.41
13	Fennel	0.765	0.750	69.69	29.56	13.90	1.08
14	Black seed	0.525	0.525	20.85	41.14	13.80	2.59

concentration range detected for Mn (2.55 to 570.6 mg/kg) was relatively high. However, the concentration of Mn was within the same range as previous studies (824.8 mg/kg) (Sumontha et al., 2006; Slavica et al., 2006). It was reported that a variety of medicinal plants show great Mn accumulating ability (Sembratowicz et al., 2009).

Trace toxic heavy metals

Contamination of herbal drugs with chemically toxic substances can be attributed to environmental pollution, soil composition and fertilizers. In addition, pesticides containing arsenic and mercury are widely used in some countries (WHO Guidelines, 2007). It was reported also, that the accumulation of heavy metals in herbal plants depends on climate factors, plant species, air and soil pollution (Lasisi et al., 2005). Trace toxic elements of Pb, Be, Cd, As, Ag, Sb, Al, Ba, Tl, Co, and V were determined in the investigated herbal plants using ICP-AES under the optimized conditions and after microwave digestion. The results obtained are summarized in Table 4. The results obtained indicated that Be, As, Ag and Tl were not detected in all investigated herbal drug samples (not presented).

Indeed, the analyzed herbal plants are widely used in Saudi Arabia as traditional drugs. However, the toxic metals present in high concentration in species are of particular importance in relation to WHO standards for Pb and Cd as toxic metals. The maximum permissible levels (MPL) for Pb and Cd are 10 and 0.3 mg/kg, respectively (Mustafa et al., 2004; Lasisi et al., 2005), while our values are below the WHO MPL for Pb and Cd for all investigated herbal plants. Moreover, the levels of Pb and Cd in

Table 4. Trace elements contents (µg/g) in the tested herbal species (mean), n=3.

Sample No.	Plant name	Pb	Cd	Sb	Al	Ba	Co	V
1	Mentha	0.825	0.075	1.08	685.5	26.23	0.09	1.99
2	German Chamomile	1.03	0.120	0.870	670.8	3.42	ND	0.28
3	Foenugreek	1.59	0.195	2.05	28.47	ND	ND	ND
4	Juniper	ND	ND	0.405	497.2	4.42	ND	ND
5	Curcuma	1.54	0.360	0.930	731.9	60.42	0.87	0.66
6	Sweet Basil	ND	ND	0.045	245.9	6.34	ND	ND
7	Hibiscus	1.18	0.180	0.285	227.4	39.34	ND	ND
8	Cumin	ND	0.030	0.975	181.3	8.88	ND	ND
9	Thyme	1.36	0.120	0.465	496.8	12.67	ND	0.330
10	Half-Bar	0.405	0.030	0.195	1237	6.64	0.060	2.14
11	Becham	ND	ND	ND	97.42	32.20	ND	ND
12	Santonica	0.345	0.105	0.045	695.9	192.9	ND	1.51
13	Fennel	ND	0.045	0.105	83.4	5.50	ND	ND
14	Black seed	ND	0.060	0.045	31.84	3.10	ND	ND

ND: No detection.

all investigated herbal plants come with a good agreement (or lower) with those reported in similar studies (Mustafa et al., 2004; Chwan-Bor et al., 2003; Sumontha et al., 2006; Slavica et al., 2006; Bushra et al., 2011).

The highest concentration of antimony is 2.05 mg/kg and was detected in *Foenugreek*. Although, this value is slightly higher than the antimony levels reported in previous studies in tea products and *Nigella sativa* seeds (Sumontha et al., 2006; Amirah, 2008); this value is still lower than those reported for antimony levels in earlier studies in some herbs (He et al., 2012; Müllera et al., 2009). The range of Al concentration in the investigated herbal plants varied between 28.47 mg/kg in sample 3 and 731.9 mg/kg in sample 5. Although the concentration of Al is relatively high in most of the investigated herbal plants, our values are below the averages levels reported for Al in tea products (2014 mg/kg) (Sumontha et al., 2006). Ba, Co, and V contents detected in all investigated samples (Table 4) are considerably lower and comparable with those reported in previous studies (Sumontha et al., 2006; Galia et al., 2010; Ram et al., 2010; Muhammad et al., 2010; Shazia et al., 2010).

Conclusion

Twenty-two elements of macronutrients, micronutrients and toxic heavy metals have been determined in 14 popular herbal plants which are widely used in Saudi Arabia using ICP-AES under optimized conditions and after microwave digestion. Based on the obtained results, the herbal plants tested in this work are good source of important elements, and therefore, they could serve as supplements of macro and micro nutrients elements in the body. The concentrations of the essential and nonessential elements were found safe in most of the investigated herbal plants. The results of this study may provide a useful reference for analysis of essential and toxic elements in herbal plants.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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