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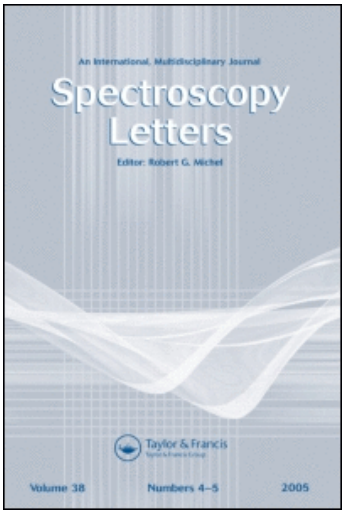

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The Mineral Content of *Rhizona cyperi* after Microwave-Assisted Digestion and Determination by AAS

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The Mineral Content of *Rhizona cyperi* after Microwave-Assisted Digestion and Determination by AAS

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ABSTRACT *Rhizoma cyperi* (tuberal part of *Cyperus rotundus* Linn) obtained from 15 different zones of China was studied to determine the contents of 16 trace elements such as 4 minor (Ca, K, Mg, and Na), 9 trace (Co, Cr, Cu, Fe, Mn, Mo, Ni, V, and Zn), and 3 toxic (Ag, Cd, and Pb) elements. The concentration determination of 16 elements was performed by atomic absorption spectrophotometry (AAS) after microwave-assisted digestion. A microwave-assisted digestion procedure based on the mixture nitric acid–hydrogen peroxide was evaluated. The method was successfully validated with the good recoveries (97–105%) against CRM GBW07603 (bush twigs and leaves). The calibration curve furnished good linear correlation coefficients ($r = 0.9956$–0.9999), excellent recoveries (99.35–103.7%), and limits of detection (LOD = 1–50 $ng \cdot mL^{-1}$) suitable to determine in *Rhizoma cyperi*. The results showed that K, Ca, Mg, and Na were the most abundant of the major elements in *Rhizoma cyperi* with average concentrations of K, 26,221 $\mu g \cdot g^{-1}$; Ca, 1097 $\mu g \cdot g^{-1}$; Mg, 714 $\mu g \cdot g^{-1}$, and Na, 293 $\mu g \cdot g^{-1}$, respectively. K element was determined for the first time in this plant.

KEYWORDS atomic absorption spectrophotometry, microwave-assisted digestion, *Rhizoma cyperi*, trace elements

INTRODUCTION

Cyperus rotundus Linn. (coco-grass, purple nut sedge, red nut sedge) is a cosmopolitan sedge (*Cyperaceae*) native to Africa, southern and central Europe, and Asia. This plant grows naturally in tropical, subtropical, and temperate regions.[1] It is a traditional medicinal plant appearing among Chinese, Japanese, Indian, and Mediterranean basin areas and is a natural drugs used against spasms and stomach disorders.[2] *Rhizoma cyperi*, the tuberal part of *C. rotundus*, known in Chinese as *xiangfu* or *xiangfuzi*, is one of the oldest known medicinal plants used widely as an analgesic, sedative, antispasmodic, antimalarial, for stomach disorders, and to relieve diarrhea.[3] It stimulates the thyroid, increases metabolism, and acts as a carminative.[4] Infusion of this herb has been used in pain, fever, diarrhea, dysentery, as an emmenagogue, and for other intestinal problems.[5–10]

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The essential oil of *Cyperus* also shows many bioactivities. It exhibits antibacterial and antimutagenic, anti-*Candida*, antioxidant, cytotoxic, and apoptotic properties.[11–14]

Cyperus rotundus has been reported to contain oils, mono- and sesquiterpenes, alkaloids, steroid glycosides, saponins, flavonoids, tannins, starch, and carbohydrates.[15–19] Only scant references exist on the elemental contents of *Cyperus*. Jin et al.,[20] using inductively coupled plasma–optical emission spectroscopy (ICP–OES), compared the trace element contents in *Cyperus* before and after processing.

In the present study, we have analyzed 15 samples of *C. rotundus* collected from 15 different zones of China for four minor (Ca, K, Mg, and Na), nine trace (Co, Cr, Cu, Fe, Mn, Mo, Ni, V, and Zn) and three toxic (Ag, Cd, and Pb) elements using flame and graphite furnace atomic absorption spectrophotometer (AAS) after microwave-assisted digestion, respectively. Certified reference material (CRM) obtained from the National Research Center for Certified Reference Materials of China (Beijing) were also analyzed for quality assurance and data validation.

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MATERIALS AND METHODS

Samples

Fifteen samples of *Rhizoma cyperi* (RC) from 15 different zones were collected from the local drug market within a time interval of 6 months during summer of 2007. Surface contamination was wiped with tissue paper and left to air dry and then in an oven at ~80°C. The samples were powdered in agate mortar and passed through 100-mesh sieve. Certified reference material such as bush twigs and leaves GBW07603 was also dried per recommended procedures before use.[21–24]

Apparatus

An Analytik Jena AG AAS ZEEnit 700 AAS (Jena, Germany) equipped with a transverse-heated graphite furnace and with deuterium and a Zeeman background corrector was used in the experiments. WinAAS Version 3.16.0 software was used. For flame measurements, a 10-cm-long slot-burner head, a hollow cathode lamp, and an air–acetylene flame were used. For graphite furnace measurements, argon was used as inert gas. Na, Ca, K, and Mg were determined in flame AAS (FAAS). The other elements were determined in a graphite furnace AAS (GFAAS). The operating parameters for working elements are given in Table 1. Each sample was read five times to get the average value and concentration was corrected for the volume of acid used in sample preparation.

TABLE 1 Operating Parameters for Working Elements

Ion	Wavelength (nm)	Lamp intensity (mA)	Split (nm)
Ag	328.1	4	0.8
Cd	228.8	3	0.2
Co	240.7	7	0.2
Cr	357.9	4	0.8
Cu	324.7	2	0.8
Fe	248.3	6	0.5
Mn	279.5	5	0.5
Mo	313.3	7	0.8
Ni	232.0	5	0.2
Pb	283.3	4.0	0.8
V	318.4	6.0	0.8
Ca	422.7	4.0	1.2
K	404.4	5.0	0.5
Mg	285.2	2.0	0.7
Na	330.3	3.0	1.2
Zn	213.8	3.0	0.8

A Sineo MDS-6 (Shanghai, China) closed vessel microwave digestion system (maximum pressure 750 psi, maximum temperature 250°C) was used. Teflon vessels were used all the digestion procedures. The operating parameters for working elements were set as recommended by the manufacturer. The reaction vessels were cleaned using 5 mL of concentrated nitric acid before each digestion.

Reagents

All reagents were of analytical reagent grade unless otherwise stated. Double deionized water (Milli-Q Millipore 18.2 resistivity (Massachusetts, USA)) was used for all dilutions. Ultrapure grade HNO_3 was used throughout the work. All plastic and glassware were cleaned by soaking in dilute HNO_3 (1:7) for 2 days and were rinsed with distilled water prior to use. The element standard solutions used for calibration were prepared by diluting stock solutions of 1000 $mg \cdot L^{-1}$ of each element supplied from the National Research Center for Certified Reference Material (CRM) of China (Beijing) and were stored at 4°C in the dark. Working solutions in the $\mu g \cdot L^{-1}$ or $ng \cdot L^{-1}$ range were obtained daily

by stepwise dilution. Bush twigs and leaves GBW07603 (CRM) were obtained from the National Research Center for CRMs (NRCCRM).

Procedures

In order to achieve a shorter digestion time, a microwave-assisted digestion procedure was applied for all RC samples and CRM. The samples were washed with deionized water, dried, and triturated, resulting in a homogeneous mass. Five replicates samples of CRM and RC (0.3 g) were directly weighed into Teflon vessels. Two milliliters of a fresh mixture of concentrated HNO_3–H_2O_2 (2:1, v/v) was added to each vessel and was kept at room temperature for 10 min. The vessels were placed in a polytetrafluoroethylene (PTFE) container, closed, and heated following a one-stage digestion programmed at 80% of total power (1000 W). The heating program for the digestion procedure[25,26] is listed in Table 2. After cooling, the contents of each vessel were evaporated to a semidried mass and then diluted with deionized water. The final solutions were transferred to 10-mL calibrated flasks and their volumes completed with deionized water. The microwave vessels were cleaned with hot nitric acid solution and subsequently washed with deionized water. Each sample preparation step was performed in a laminar flow fume cupboard to avoid external contamination.

Calibration Curves

The calibration curves were constructed by the plots of the absorbance intensities of the analyte of the concentrations of the working solutions and one blank solution. A linear least-squares regression analysis was performed for the analyte. The concentrations of the analyte in unknown samples were determined by interpolation from the calibration curve. The analytical characteristics of the AAS method are shown in Table 3.

TABLE 2 Heating Program for the Digestion Procedure

Step	Time (min)	Power (W)
1	2	250
2	2	0
3	6	250
4	5	400
5	8	550
Ventilation	5	0

TABLE 3 Analytical Characteristics of the AAS Method

Analyte	Linear range (ng/mL)	LOD[a] (ng/mL)	LOQ[b] (ng/mL)	Correlation coefficients (r)
Ag	30–3000	30	90	0.9991
Cd	1–50	1	3	0.9986
Co	1–100	2	6	0.9956
Cr	100–5000	30	90	0.9999
Cu	1–50	1	3	0.9996
Fe	30–5000	30	90	0.9998
Mn	10–3000	10	30	0.9998
Mo	1–100	2	6	0.9996
Ni	30–8000	10	30	0.9997
Pb	10–200	10	30	0.9965
V	50–1000	4	12	0.9992
Ca	100–6000	20	60	0.9999
K	50–4000	30	90	0.9999
Mg	10–600	2	6	0.9999
Na	50–2000	10	30	0.9999
Zn	50–1000	50	150	0.9999

[a]LOD: limit of detection.
[b]LOQ: limit of quantification.

RESULTS AND DISCUSSION

Various sources contribute to the metal composition of the RC. The sample pretreatment procedure must take into account the analyte of interest, the matrix characteristics, and the minimal required time period of the analytical technique considered. The recovery values were nearly quantitative (≥95%) for the microwave-assisted digestion method. The relative standard deviations were less than 10% for all investigated elements. A t-test was used to determine significant differences between mean values ($p < 0.05$). The accuracy of the method was evaluated by means of trace element determination in CRM. The results are given in Table 4. The achieved results were in good agreement with certified values.

In this work, Zn, Na, Ca, K, and Mg were analyzed by FAAS. Cu, Cd, Pb, Ni, Cr, Mn, Fe, Co, Mo, Ag, and V were analyzed by GFAAS. The results of the analyses are summarized in Table 5. In the analysis of trace element contents, 16 trace elements were quantified: cobalt, argentums, molybdenum, cadmium, vanadium, chromium, lead, nickel, copper, manganese, zinc, iron, natrium, magnesium, calcium, and potassium.

The contents of cobalt, argentums, molybdenum, cadmium, vanadium, chromium, lead, and nickel in RC from different zones in China ranged between

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TABLE 4 Trace Metal Concentrations in Certified Reference Material (Bush Twigs and Leaves GBW07603)[a,b]

Element	Certified value ($\mu g \cdot g^{-1}$)	Our value ($\mu g \cdot g^{-1}$)	Recovery (%)
Ag	0.049	0.045 ± 0.004	99
Ca	1.68	1.67 ± 0.11	105
Cd	(0.38)[c]	0.37 ± 0.09	97
Co	0.41	0.39 ± 0.05	99
Cr	2.6	2.6 ± 0.2	98
Cu	6.6	6.7 ± 0.5	103
Fe	1070	1073 ± 43	98
K	0.92	0.91 ± 0.09	102
Mg	0.48	0.46 ± 0.05	97
Mn	61	63 ± 3	99
Mo	0.28	0.31 ± 0.075	98
Na	1.96	1.98 ± 0.24	98
Ni	1.7	1.6 ± 0.4	99
Pb	47	48 ± 4	98
V	2.4	2.5 ± 0.5	99
Zn	20.6	20.3 ± 1.7	103

[a]n = 5.
[b]Means expressed as 95% tolerance limit.
[c]Values in the parentheses are not certified.

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0.09 and 0.39, 0.08 and 0.72, 0.10 and 1.58, 0.34 and 2.09, 0.46 and 7.16, 0.27 and 6.26, 1.44 and 4.89, and 1.06 and 8.96 $\mu g \cdot g^{-1}$, respectively. Copper, manganese, zinc, iron, natrium, magnesium, calcium, and potassium in RC from different zones in China ranged between 3.51 and 18.7, 6.13 and 54.8, 13.8 and 64.7, 84.5 and 156, 166 and 459, 409 and 830, 475 and 1908, and 17,794 and 42,997 $\mu g \cdot g^{-1}$, respectively. The recommended dietary allowance (RDA) for copper is 900 μg a day for both men and women. The RDA for molybdenum is 45 μg per day for both men and women. The RDA for zinc was set at 11 mg per day for men and 8 mg per day for women. Adequate intake for chromium of 35 μg for men and 25 μg for women is recommended. Adequate intake level for manganese is set at 2.3 mg per day for men and 1.8 mg per day for women.[27]

A comparison of trace elemental contents of RC from different zones (Table 5) shows that RC-2 shows the highest Mg ($830 \pm 76.5 \mu g \cdot g^{-1}$) concentrations. RC-4 shows the highest Ag ($0.72 \pm 0.04 \mu g \cdot g^{-1}$), Cd ($2.09 \pm 0.21 \mu g \cdot g^{-1}$), V ($7.16 \pm 0.60 \mu g \cdot g^{-1}$), Cu ($18.7 \pm 1.5 \mu g \cdot g^{-1}$), and Fe ($156 \pm 14.2 \mu g \cdot g^{-1}$) concentrations. RC-5 shows the highest Co ($0.39 \pm 0.01 \mu g \cdot g^{-1}$), Cr ($6.26 \pm 0.57 \mu g \cdot g^{-1}$), Ni ($8.96 \pm 0.76 \mu g \cdot g^{-1}$), and Mn ($54.8 \pm 4.9 \mu g \cdot g^{-1}$) concentrations. RC-8 shows the highest Na ($459 \pm 42.3 \mu g \cdot g^{-1}$)

TABLE 5 Mineral Contents in Microwave-Digested *Rhizoma cyperi* Samples ($\mu g \cdot g^{-1}$)[a]

Sample	Co	Ag	Mo	Cd	V	Cr	Pb	Ni	Cu	Mn	Zn	Fe	Na	Mg	Ca	K
RC-1	0.18 ± 0.01	0.48 ± 0.02	0.10 ± 0.01	0.52 ± 0.02	0.65 ± 0.02	0.70 ± 0.02	3.42 ± 0.32	4.70 ± 0.45	4.02 ± 0.39	22.4 ± 2.2	25.8 ± 2.8	96.9 ± 9.8	166 ± 14.3	700 ± 56.9	787 ± 80.1	25355 ± 2141
RC-2	0.13 ± 0.01	0.64 ± 0.03	0.43 ± 0.02	0.34 ± 0.01	2.21 ± 0.19	0.34 ± 0.01	3.29 ± 0.31	4.49 ± 0.43	4.19 ± 0.41	14.4 ± 1.3	18.1 ± 2.1	88.3 ± 9.1	261 ± 25.4	830 ± 76.5	532 ± 51.2	20960 ± 1542
RC-3	0.18 ± 0.01	0.21 ± 0.01	0.43 ± 0.02	0.41 ± 0.01	1.03 ± 0.10	0.45 ± 0.02	3.01 ± 0.29	8.77 ± 0.85	6.42 ± 0.63	15.4 ± 1.5	32.6 ± 3.1	113 ± 10.1	326 ± 28.7	617 ± 51.3	1284 ± 102	18827 ± 1754
RC-4	0.19 ± 0.01	0.72 ± 0.04	1.07 ± 0.10	2.09 ± 0.21	7.16 ± 0.60	1.43 ± 0.12	4.54 ± 0.43	1.06 ± 0.10	18.7 ± 1.5	18.6 ± 1.9	63.2 ± 5.9	156 ± 14.2	433 ± 35.4	699 ± 54.1	877 ± 85.6	25863 ± 2348
RC-5	0.39 ± 0.01	0.24 ± 0.01	0.54 ± 0.02	0.35 ± 0.01	3.36 ± 0.29	6.26 ± 0.57	4.31 ± 0.41	8.96 ± 0.76	6.31 ± 0.58	54.8 ± 4.9	13.8 ± 1.4	132 ± 12.8	186 ± 17.6	745 ± 71.2	1365 ± 121	31890 ± 2954
RC-6	0.09 ± 0.01	0.25 ± 0.01	0.31 ± 0.01	0.37 ± 0.01	1.20 ± 0.11	3.14 ± 0.31	2.71 ± 0.26	2.64 ± 0.31	5.07 ± 0.49	12.9 ± 1.1	27.1 ± 2.8	112 ± 10.9	192 ± 18.5	825 ± 78.3	547 ± 55.1	19584 ± 1851
RC-7	0.21 ± 0.01	0.33 ± 0.11	0.88 ± 0.04	0.35 ± 0.02	3.12 ± 0.31	5.76 ± 0.48	1.44 ± 0.12	1.09 ± 0.11	17.3 ± 1.7	18.8 ± 1.7	36.6 ± 3.7	134 ± 13.2	243 ± 21.6	709 ± 54.1	1637 ± 153	30352 ± 2992
RC-8	0.18 ± 0.01	0.31 ± 0.01	0.16 ± 0.01	0.39 ± 0.02	1.36 ± 0.12	0.27 ± 0.01	3.44 ± 0.31	4.56 ± 0.43	3.51 ± 0.31	18.9 ± 1.6	36.1 ± 3.5	101 ± 9. 8	459 ± 42.3	712 ± 55.3	925 ± 90.1	22390 ± 2157
RC-9	0.26 ± 0.01	0.08 ± 0.01	0.84 ± 0.04	0.52 ± 0.02	2.84 ± 0.24	5.88 ± 0.51	2.73 ± 0.28	4.13 ± 0.39	12.3 ± 1.2	31.1 ± 3.0	33.2 ± 3.1	139 ± 12.7	453 ± 41.5	707 ± 57.1	1591 ± 161	20148 ± 2014
RC-10	0.10 ± 0.01	0.30 ± 0.02	0.24 ± 0.01	0.44 ± 0.02	1.54 ± 0.13	3.12 ± 0.31	4.89 ± 0.45	2.72 ± 0.26	5.98 ± 0.61	16.6 ± 1.5	39.1 ± 4.1	103 ± 11.2	224 ± 21.8	409 ± 39.5	1188 ± 107	35255 ± 3621
RC-11	0.19 ± 0.01	0.12 ± 0.01	0.18 ± 0.01	0.57 ± 0.01	0.46 ± 0.02	0.48 ± 0.02	2.58 ± 0.23	4.66 ± 0.50	4.36 ± 0.41	28.8 ± 2.9	21.9 ± 2.0	133 ± 12.9	304 ± 29.7	699 ± 55.7	1389 ± 131	17794 ± 1524
RC-12	0.13 ± 0.01	0.62 ± 0.02	0.52 ± 0.02	0.42 ± 0.02	1.29 ± 0.11	5.86 ± 0.57	3.59 ± 0.37	3.22 ± 0.31	4.81 ± 0.47	22.6 ± 2.1	27.4 ± 2.9	134 ± 13.8	250 ± 26.8	735 ± 59.4	1395 ± 140	29110 ± 2841
RC-13	0.14 ± 0.01	0.28 ± 0.02	1.58 ± 0.21	0.52 ± 0.02	3.68 ± 0.27	2.26 ± 0.26	3.49 ± 0.36	3.83 ± 0.37	4.33 ± 0.42	35.3 ± 3.4	64.7 ± 5.9	127 ± 12.5	313 ± 30.5	719 ± 58.3	1908 ± 184	32024 ± 3124
RC-14	0.10 ± 0.01	0.19 ± 0.01	0.13 ± 0.01	0.41 ± 0.01	1.43 ± 0.12	0.39 ± 0.02	3.36 ± 0.32	2.79 ± 0.30	11.6 ± 1.1	11.6 ± 1.0	16.3 ± 1.4	91.6 ± 9.2	273 ± 28.9	791 ± 70.5	562 ± 51.2	20765 ± 1987
RC-15	0.11 ± 0.01	0.22 ± 0.01	0.30 ± 0.01	0.46 ± 0.01	1.29 ± 0.11	1.08 ± 0.09	3.41 ± 0.33	4.33 ± 0.45	11.2 ± 1.0	6.13 ± 0.58	28.0 ± 3.1	84.5 ± 8.0	307 ± 30.1	810 ± 68.4	475 ± 50.1	42997 ± 3587
Mean	0.17	0.33	0.52	0.54	2.17	2.49	3.35	4.13	7.99	21.89	32.25	116	293	714	1097	26221

[a]n = 5.

TABLE 6 Recovery of Mineral Elements from *Rhizoma cyperi* after Microwave-Assisted Digestion[a]

Analyte	Ag	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	V	Ca	K	Mg	Na	Zn
Added amount (ng/mL)	100	30	50	350	30	1000	500	30	90	50	100	200	2000	250	500	100
Found (ng/mL)	101.20	29.80	50.30	351.6	29.87	1012.3	498.7	31.12	89.86	49.64	102.3	198.7	2012.2	252.3	508.2	99.47
% Recovery	101.2	99.30	100.6	100.5	99.57	101.23	99.74	103.7	99.84	99.28	102.3	99.35	100.6	100.9	101.6	99.47

[a]n = 5.

TABLE 7 Comparison of Our Data with Other Similar Medicinal Plants

Sample	Cu	Mn	Zn	Fe	Mg	Ca	K	Ref.
Rhizoma cyperi[a]	7.99	21.89	32.25	116	714	1097	26,221	Our data
Bupleurum scorzonerifolium Willd.	74.8	—	44.3	869.3	1790.5	5588.9	—	[24]
Gentiana macrophlla	50.7	154.3	33.3	196.1	1028.9	3665.1	—	[25]
Curcuma wenyujin Y.H. Chen et C. Ling	15.7	102.3	7.9	600.2	1688	1160	53,102	[26]

[a]Mean values; —: means not reported.

concentrations. RC-10 shows the highest Pb ($4.89 \pm 0.45\,\mu g \cdot g^{-1}$) concentrations. RC-13 shows the highest Mo ($1.58 \pm 0.21\,\mu g \cdot g^{-1}$), Zn ($64.7 \pm 5.9\,\mu g \cdot g^{-1}$), and Ca ($1908 \pm 184\,\mu g \cdot g^{-1}$) concentrations. RC-15 shows the highest K ($42{,}997 \pm 3587\,\mu g \cdot g^{-1}$) concentrations.

It is observed that RC are enriched in K ($>17\,mg \cdot g^{-1}$), Ca, Mg, and Na ($>0.1\,mg \cdot g^{-1}$) as minor constituents with trace amounts of Fe, Mn, and Zn ($>20\,\mu g \cdot g^{-1}$). However, V and Cr, whose compounds are considered insulin-like, only are found at 0.54 and $2.17\,\mu g \cdot g^{-1}$, respectively.

The recoveries of the mineral elements from the RC sample after microwave-assisted digestion were studied. Satisfactory results obtained for the elements examined are given in Table 6. These results confirm the validity of the method for the determination of the investigated mineral elements. The recoveries of the mineral elements were in the order of 99.28–103.7%.

A comparison of our data with other similar medicinal plants (including *Bupleurum scorzonerifolium* Willd., *Gentiana macrophlla*, and *Curcuma wenyujin* Y. H. Chen et C. Ling) are given in Table 7. Our values for Zn are in excellent agreement with *Bupleurum scorzonerifolium* Willd. and *Gentiana macrophlla*,[28,29] whereas Ca matches with *Curcuma wenyujin* Y. H. Chen et C. Ling.[30] Concentrations of Cu, Mn, Fe, Mg, and K are substantially higher value than our data.

Trace elements play important roles in human life.[31–34] Elemental uptake by a plant is its characteristic property and may depend on the use of fertilizers, irrigation water, and different climatological/geo-environmental factors. It seems that bioavailability rather than the total amount of an element is important.

CONCLUSIONS

In this article, 15 RC samples from different zones of China were analyzed for 16 elements using flame and graphite furnace atomic absorption spectrophotometry and a microwave-assisted digestion method. The microwave digestion system in RC samples provides a simple, effective, and accurate method of sample digestion. The validity of the microwave digestion procedure was confirmed by the good recoveries (97–105%) obtained for the mineral elements for the certified reference material. The calibration curve furnished good linear correlation coefficients (r = 0.9956–0.9999), excellent recoveries (99.35–103.7%), and limits of detection (LOD = $1–50\,ng \cdot mL^{-1}$) suitable to determine in RC samples.

This study is expected to provide important insight into the disparity of the mineral element concentrations in medicinal plants obtained from different zones. The results show that the concentration of mineral element varies over a wide range, attributed to varying climatological and geo-environmental

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conditions, local soil characteristics, season of the year, and rainfall from one zone to another.

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