Note

Determination of Mineral Elements in Nanyang Mugwort (*Artemisia argyi*) Leaves Harvested from Different Crops by Inductively Coupled Plasma Mass Spectrometry and Inductively Coupled Plasma Atomic Emission Spectrometry

Chao Li,^a Zhan-hu Cui,^b Zhe Li,^c Li Gao,^a Chao-yun Zhang,^a Dan-xia Li,^a Zhong-ming Zhang,^a and Xian-zhang Huang^{*,a}

^aHenan Province Key Laboratory of Zhang Zhongjing Formulae and Herbs for Immunoregulation, Nanyang Institute of Technology; Nanyang 473004, China; ^bCollege of Agriculture, Fujian Agriculture and Forestry University; Fuzhou 350002, China; and ^cTang-ai Ecological Agriculture Development Limited Liability Company; Nanyang 473400, China. Received October 29, 2020; accepted January 16, 2021; advance publication released online January 30, 2021

Due to high need for medical purposes, multiple harvests of mugwort (*Artemisia argyi*) have been extensively applied in China for the increase of mugwort yield recently. However, the investigation on the mineral elements in different crops, which are significantly related to mugwort growth and the clinical efficacy of this medicinal herb, has not been conducted. This study provided an analytical method and quality evaluation for mineral elements in Nanyang mugwort leaves harvested from three different crops. The contents of 35 mineral elements were determined by inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectrometry (ICP-AES). ANOVA, principal component analysis and factor analysis were applied to evaluate the results. Four principal components were identified and their comprehensive evaluation function was as follows: $F = 0.7008F_1 + 0.1236F_2 + 0.0936F_3 + 0.0321F_4$. The comprehensive scores of the mugwort leaves from different crops were ranked as follows: 3rd crop > 2nd crop $\approx$ 1st crop. These findings can provide a reference for the quality control and clinical use of mugwort leaves, and a guidance of differential nourishing strategies for different crops.

Key words *Artemisia argyi*; mineral element; quality evaluation

Introduction

Traditional mugwort leaves are dried leaves of *Artemisia argyi* Levl. et Vant., an annual plant of the *Artemisia* genus of the *Asteraceae* family endemic to northern temperate regions. They are widely used as dietary functional material, food flavoring and coloring agent in China, Japan, Korea and other East Asian countries.^{1–3)} They can also be used both orally and externally for medical purposes, such as channeled to the liver, spleen and kidney.⁴⁾ They are proved to have various clinical effects, e.g., meridian warming, bleeding stopping, cold dispersing, pain relieving, and miscarriage preventing.^{5–8)} Therefore, the mugwort leaves market grew at a rate of 30% during the last 10 years. Due to the high need, multiple crops for mugwort leaves within a year is developed in Nanyang, the largest mugwort leaves base in China. Based on the long-term cultivation practices of our research group, Nanyang mugwort is generally harvested at a height of 90 cm and grown in three crops per year. However, the quality of mugwort leaves from each crop for the consumption and clinical application has not been analyzed.

Previous studies of annual mugwort leaves have focused on the chemical components and their pharmacological effects.^{9–13)} This study provided the contents of 35 mineral elements in mugwort leaves from three crops have been determined by inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectrometry (ICP-AES). The results can provide a rational basis and useful supplement for the quality control and evaluation of mugwort leaves.

Results and Discussion

The validation data for the analytical procedure was summarized in Table S1. Each element showed a good linear relationship, low detection limit, and reasonable linear range, indicating that the experimental method meet the analytical requirements. In addition, the relative standard deviation (RSD) values were below 3.06%, and their recoveries were 98.66–102.17%.

The contents of 35 kinds of mineral elements in mugwort leaves from the 1st, 2nd and 3rd crops were determined and ANOVA and multiple comparisons (Table 1). The overall quality score of each element showed the following order: $K > Ca > Mg > Al > Fe > Mn > Na > Cu > Sr > Zn > Ba > Ti > Cr > Ni > V > Mo > La > Pb > Zr > As > Y > Co > Cd > Sc > Sn > Ga > Nb > Tl > Be > Sb > Bi > Ge > Ag > Hg > Se$. Duncan analysis showed that the contents of Mo, La and Tl in leaves were significantly different between the 1st crop and the 2nd crop ($p < 0.05$), while the contents of the remaining 32 elements were about same ($p > 0.05$). The contents of Ca, Al, Fe, Sr, Ba, Ti, Cr, V, La, Pb, Zr, As, Y, Co, Cd, Sc, Sn, Ga, Tl, Bi, Ge, Ag, Hg and Se dramatically differed between the 1st crop and the 3rd crop; and the contents of Ca, Al, Fe, Cu, Sr, Zn, Ba, Ti, V, La, Pb, Zr, As, Y, Co, Sc, Sn, Ga, Be, Bi, Sb, Ge, Ag, Hg and Se largely change from the 2nd crop to the 3rd crop. In terms of accumulation pattern, mineral contents in leaves were similar from the 1st crop to the 2nd crop, but largely different to those from the 3rd crop.

K, Ca, Mg, Fe, Mn, Cu, Ni and Mo, which are essential mineral elements for plant growth,^{14,15)} showed low variation

* To whom correspondence should be addressed. e-mail: nylgxyhxz@126.com

Table 1. Analytical Results and Differential Analysis

Element	Unit	Mineral element content (Mean $\pm$ S.D., $n = 6$)		
		First crop	Second crop	Third crop
K	g/kg	34.591 $\pm$ 0.642a	29.816 $\pm$ 3.907a	37.674 $\pm$ 8.286a
Ca		13.526 $\pm$ 0.576b	12.528 $\pm$ 1.270b	19.340 $\pm$ 4.588a
Mg		3.824 $\pm$ 0.210a	3.487 $\pm$ 0.325a	3.892 $\pm$ 0.853a
Al		1.724 $\pm$ 0.033b	1.385 $\pm$ 0.280b	5.264 $\pm$ 0.301a
Fe		0.618 $\pm$ 0.002b	0.530 $\pm$ 0.062b	1.527 $\pm$ 0.169a
Mn		0.138 $\pm$ 0.005a	1.570 $\pm$ 0.030a	1.522 $\pm$ 0.029a
Na		73.546 $\pm$ 8.881a	82.638 $\pm$ 9.607a	79.916 $\pm$ 23.904a
Cu		41.145 $\pm$ 2.146ab	45.770 $\pm$ 5.708a	33.998 $\pm$ 6.127b
Sr		36.648 $\pm$ 1.548b	38.858 $\pm$ 3.351b	56.977 $\pm$ 14.445a
Zn		35.433 $\pm$ 1.737ab	31.246 $\pm$ 2.248b	45.333 $\pm$ 9.711a
Ba	mg/kg	32.473 $\pm$ 0.900b	28.551 $\pm$ 2.092b	57.799 $\pm$ 12.547a
Ti		29.585 $\pm$ 3.112b	22.241 $\pm$ 2.089b	59.947 $\pm$ 14.078a
Ni		3.062 $\pm$ 1.672a	2.491 $\pm$ 1.233a	1.619 $\pm$ 0.317a
Cr		4.104 $\pm$ 0.656b	4.414 $\pm$ 0.919ab	5.691 $\pm$ 0.574a
V		2.272 $\pm$ 0.189b	1.677 $\pm$ 0.226b	5.893 $\pm$ 0.845a
Mo		1.138 $\pm$ 0.159a	0.851 $\pm$ 0.090b	0.941 $\pm$ 0.081ab
La		1.174 $\pm$ 0.031b	0.786 $\pm$ 0.099c	2.042 $\pm$ 0.225a
Pb		0.939 $\pm$ 0.122b	1.296 $\pm$ 0.090b	2.187 $\pm$ 0.533a
Zr		0.888 $\pm$ 0.099b	0.675 $\pm$ 0.048b	1.818 $\pm$ 0.292a
As		0.552 $\pm$ 0.011b	0.451 $\pm$ 0.012b	1.148 $\pm$ 0.168a
Y	μ g/kg	0.502 $\pm$ 0.038b	0.383 $\pm$ 0.545b	1.100 $\pm$ 0.157a
Co		0.316 $\pm$ 0.067b	0.198 $\pm$ 0.028b	0.502 $\pm$ 0.081a
Sc		0.156 $\pm$ 0.025b	0.099 $\pm$ 0.003b	0.352 $\pm$ 0.090a
Cd		0.180 $\pm$ 0.003b	0.209 $\pm$ 0.008ab	0.296 $\pm$ 0.079a
Sn		0.132 $\pm$ 0.013b	0.100 $\pm$ 0.014b	0.220 $\pm$ 0.061a
Ga		0.131 $\pm$ 0.007b	0.097 $\pm$ 0.014b	0.302 $\pm$ 0.032a
Nb		0.127 $\pm$ 0.019a	0.392 $\pm$ 0.052a	0.227 $\pm$ 0.054a
Tl		36.506 $\pm$ 10.255b	73.991 $\pm$ 8.332a	57.479 $\pm$ 12.439a
Be		27.771 $\pm$ 6.944ab	17.623 $\pm$ 4.969b	34.353 $\pm$ 10.375a
Sb		31.183 $\pm$ 3.534ab	28.323 $\pm$ 0.377b	33.460 $\pm$ 1.792a
Bi	μ g/kg	32.670 $\pm$ 2.137b	31.282 $\pm$ 1.120b	61.480 $\pm$ 9.099a
Ge		14.766 $\pm$ 0.669b	11.222 $\pm$ 1.666b	29.536 $\pm$ 3.582a
Ag		13.010 $\pm$ 1.661b	11.833 $\pm$ 1.841b	56.369 $\pm$ 16.137a
Hg		11.188 $\pm$ 0.872b	9.994 $\pm$ 0.629b	19.515 $\pm$ 2.425a
Se		3.305 $\pm$ 0.712b	2.687 $\pm$ 0.651b	6.863 $\pm$ 0.143a

Note: Different lowercase letters indicate that the difference is statistically significant ($p < 0.05$). S.D.: standard deviation.

coefficients, suggesting that they accumulate in the leaves of different crops most likely, these elements behave as the most important limiting factors for the growth of mugwort leaves. Regarding to the heavy metals, the contents of Pb, Cd, Hg and As were below the specified limits according to Chinese pharmacopoeia regulations (the content of Pb, Cd, As, Hg and Cu should not be higher than 5.0, 1.0, 2.0, 0.2 and 20.0 mg/kg, respectively).⁴⁾ While, the content of Cu exceeded the limit by 2- to 3-fold. This may be explained by the Cu-enriching capability of leaves, or the high Cu content of the soil or environment.

Principal component analysis was further performed to explore the relationships between the mineral elements in the mugwort leaves from different crops. As shown in Table S2, four principal components were extracted with a cumulative variance contribution of 94.991%, indicating that the four principal components comprehensively reflect the overall information of the mineral elements. The variance contributions of four principal components were as follows: $F = 0.7008F_1 + 0.1236F_2 + 0.0936F_3 + 0.0321F_4$. The values of F_s from the 3rd crop were generally highest, while those from the 1st or

2nd crop were lower and overlapped (as shown in Table S3). The order of the weighted scores of the comprehensive evaluation function was as follows: the 3rd crop (2.504) > the 2nd crop (−1.217) $\approx$ the 1st crop (−1.286). The observed results indicate that the accumulation and distribution of mineral elements in the mugwort leaves from the 3rd crop are much higher than those from the 1st or 2nd crop. They may be closely correlated to the growth period of each crop (around 50, 60 and 90 d to the 1st, 2nd and 3rd crop, respectively).

Conclusion

The current study revealed that the mineral elements in the mugwort leaves from 3rd crop are highly enriched compared to the 1st or 2nd crop, indicating that the longer growth period enables greater accumulation of mineral elements in the leaves. The results provide supportive information for the quality control and safety evaluation of mugwort leaves. Further comprehensive evaluation of mugwort leaves by combining the contents of mineral elements and active ingredients should be further considered.

Experimental

Plant Material Nanyang mugwort plant was cultivated in the mugwort production base of the Tang'ai Ecological Agriculture Development Corporation and harvested when reaching a plant height of approximately 90 cm. The harvested time of the 1st, 2nd and 3rd crop was in mid-June, mid-August and mid-November of 2019. After collection, the leaves were dried and thoroughly grinded.

Sample Digestion Approximately 0.4 g of mugwort leaf sample was weighed and placed in a digestion tank. The sample was treated with 5 mL of HNO₃ (guaranteed-reagent pure, Merck, Germany) overnight, incubated at 160 °C for 6 h. After cooling down, the acid was evaporated, the digestion was then transferred into a 25 mL volumetric flask. A controlled sample was set up by the same procedure. The controlled and digested samples were injected into the instrument for analysis.

Measurement Parameters The working parameters of ICP-MS (Model Thermo X Series II, Thermo Fisher Scientific, U.S.A.) were as follows: radio frequency (RF) power: 1200 W; atomizer pressure: 1.0 bar; auxiliary gas flow rate: 0.7 L/min; plasma gas flow rate: 13.0 L/min; peristaltic pump speed: 30 r/min for analysis and 70 r/min for flushing. The working parameters of ICP-AES (Model ICA P-6300, Thermo Electron, Beverly, MA, U.S.A.) were as follows: RF power: 1150 W; peristaltic pump speed: 50 r/min; auxiliary gas flow rate: 0.5 L/min; atomizer gas flow rate: 0.7 L/min.

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Conflict of Interest The authors declare no conflict of

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