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Original Article

Multi-elemental analysis of *Ziziphora clinopodioides* from different regions, periods and parts using atomic absorption spectrometry and chemometric approaches

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ABSTRACT

In this study, ten trace elements in *Ziziphora clinopodioides* Lam., Lamiaceae, from different regions, periods and parts in Xinjiang were determined by atomic absorption spectrometry following microwave-assisted acid digestion. The decreasing sequence of elements levels was K > Ca > Mg > Fe > Cu > Zn > Na > Mn > Cd > Pb. Chemometric approaches, such as correlation analysis, principal component analysis, and hierarchical cluster analysis were applied to classify *Z. clinopodioides* according to its elements contents. Principal component analysis revealed 83.51% of the variance with the first four principal component variables. Hierarchical cluster analysis indicated five groups from the eighteen regions, and the result of classification can correspond to the geographical distribution for the most regions. Variation in the elements exhibited a decreasing trend, but of different types in the studied periods. Elemental contents distributed in leaves were higher than those in flowers and stems. Therefore, chemometric approaches could be used to analyze data to accurately classify *Z. clinopodioides* according to origins. This study provided some elemental information on chemotaxonomy, diversity, changing pattern, distribution, and metabolism of *Z. clinopodioides* at spatial and temporal levels, and could be used as a reference of planting and quality standards.

Introduction

Ziziphora clinopodioides Lam., a perennial plant belonging to the Lamiaceae, is a well known traditional Uyghur medicinal herb from Xinjiang, China. In folk society, fresh and dry *Z. clinopodioides* has been used as spice in noodle and tea. Moreover, this aromatic plant is rich in essential oil, which is applied in food, perfume, and medical treatments, thus, local people call *Z. clinopodioides* as "wild peppermint." This plant is also used to treat hypertension, fever, edema, heart disease, neurasthenia, insomnia, tracheitis, lung abscess, and hemorrhoids (Liu et al., 1999; Senejouxa et al., 2010). Other ethnobotanical uses of *Ziziphora* species as stomachic, carminative and wound healing material have been recorded in Iranian and Turkish folk medicines (Meral et al., 2002; Ozturk and Ercisli, 2007). Previous pharmacological investigations also revealed that this species exhibits antimicrobial (Ji et al., 2012), antifungal (Behravan et al., 2007), antioxidative (Tian et al., 2011), and anti-hypertensive (Guo et al., 2014) properties attributed to its essential oil. Phytochemical investigations have shown that this genus is a source of flavonoids, polyphenols, polysaccharides, fatty acids and sterols (Yu et al., 2012). The herb also biosynthesizes phenolic compounds, such as caffeic acid, rosmarinic acid, and flavonoid derivatives, including luteolin, linarin, diosmin, and thymonin (Yang et al., 2008; Tian et al., 2012). Moreover, this aromatic plant is rich in essential oil. Pulegone, *trans*-isopulegone, menthol, 1,8-cineole and limonene were found to be the major components of *Z. clinopodioides* essential oil (Ding et al., 2014a,b).

Trace elements play an important role in an organism's biological activities. In plants, trace elements are implicated in several physiological activities, including metabolism and biosynthesis, as well as enzyme cofactors (Maiga et al., 2005). Traditional medicine plays an important role in the general health status of a population, and the demand for medicinal herbs and its products have

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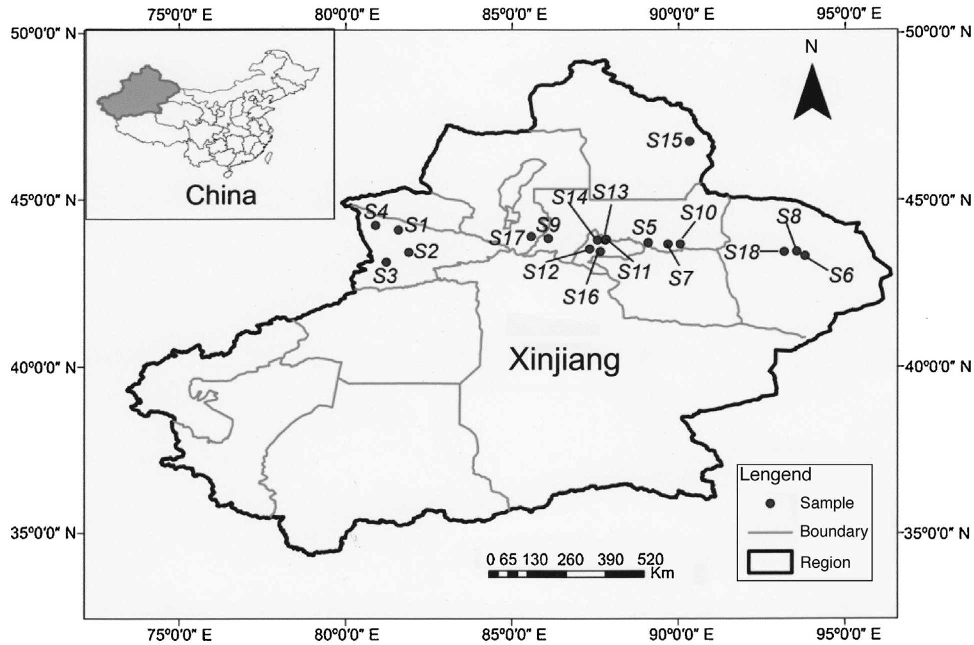

Figure 1. Geographical distribution of the collected *Z. clinopodioides* samples in Xinjiang, China.

continuously increased. However, health risk is posed by various medicinal herbs and their products because of the presence of toxic elements. The human body requires metallic and nonmetallic elements within certain permissible limits for growth and good health. As such, various trace element concentrations should be quantitatively estimated to evaluate the effectiveness of medicinal plants for the treatment of various diseases and understand pharmacological activities (Tokalıoglu, 2012). Superior quality control for medicinal herbs should be implemented to protect consumers from contamination. Various techniques, such as atomic absorption spectrometry (AAS) (Wang and Liu, 2010), inductively coupled plasma optical emission spectrometry (ICP-OES) (Liu et al., 2014), inductively coupled plasma-mass spectrometry (ICP-MS) (Bu et al., 2013), and X-ray fluorescence (XRF) (Kierdorf et al., 2014), are used to analyze trace elements in medicinal herbs. AAS as rapid and accurate technique for this purpose was chosen for the presented work.

This study aimed to determine ten trace elements (Ca, Cd, Cu, Fe, K, Mg, Mn, Na, Pb, and Zn) in *Z. clinopodioides* samples from different regions, periods and parts in Xinjiang, China. These elements were determined by flame and graphite furnace atomic absorption spectrometry, after microwave-assisted acid digestion was performed. To provide better insights into the patterns of elements, we performed common chemometric approaches, such as correlation analysis (CA), principal component analysis (PCA), and hierarchical cluster analysis (HCA), as classification techniques to evaluate data. Semi-monthly variation of elemental concentrations and the distribution of elements in leaves, flowers and stems were also investigated.

Materials and methods

Reagents and materials

All of the reagents used throughout the experiments were of analytical-reagent grade. Ultrapure grade HNO_3 (65%) and H_2O_2 (37%) were purchased from Tianjin Chemical Co., Ltd., China. Double-deionized water was produced using MilliQ water purification system (Millipore, Bedford, MA, USA). The standard solutions of Ca, Cd, Cu, Fe, K, Mg, Mn, Na, Pb, and Zn were obtained from the National Research Center for Certified Reference Material (CRM) of China (NRCCRM), and used to prepare the stock solution. Glassware and Teflon digestion vessels were cleaned by soaking overnight in diluted HNO_3 (1/9, v/v) and by subsequently rinsing with double-deionized water and drying before use.

Instruments

A Hitachi Z-2000 atomic absorption spectrometer (Hitachi Co., Ltd., Japan) equipped with a multi-elemental hollow cathode lamp and a Deuterium and Zeeman background correction system were used to determinate of trace elements. This equipment was operated using an air/acetylene flame and transverse-heated graphite furnace controlled by a personal computer. Operating parameters of the working concentrations of elements were set according to manufacturer's recommendation. Each sample was read three times to obtain a mean value. Concentration was corrected on the basis of volume of acid used in sample preparation.

A Sineo MDS-6 closed vessel microwave digestion system (Shanghai EU Analytical Instrument Co., Ltd., China) was used for extraction. Teflon reaction vessels were used for all digestion procedures. Operating parameters were set according to the manufacturer's recommendations.

Procedure

Eighteen samples of *Ziziphora clinopodioides* Lam., Lamiaceae, were collected in eighteen different regions in Xinjiang (Fig. 1) in July 2013. Nine samples were collected semi-monthly during May to August from one stationary place, one of the samples was divided into three parts (flowers, stems, and leaves) for study. All of the samples were identified by Yonghe Li, a chief apothecary of the Chinese Medicine Hospital of Xinjiang, China. These samples were washed with double-deionized water and then oven-dried at 60 °C. Dried samples were grinded and homogenized using a homogenizer. Afterward, these samples were screened with a 200 mesh sieve and stored in plastic bags.

A microwave-assisted digestion procedure was carried out to achieve a shorter digestion time. Two grams of herbal samples was accurately weighed into a Teflon digestion vessel. Afterward, 8 ml