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TRACE ELEMENTS MAY BE RESPONSIBLE FOR MEDICINAL EFFECTS OF *SAUSSUREA LANICEPS*, *SAUSSUREA INVOLUCRATE*, *LYCIUM BARBARUM* AND *LYCIUM RUTHENICUM*

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Abstract

Background: The pharmacodynamics of *Saussurea laniceps*, *Saussurea involucrata*, *Lycium barbarum* and *Lycium ruthenicum* have been researched, and trace elements have been considered as the essential elements, but little attention has been paid to the trace elements of the herbal medicine. We would like to report on the content of copper (Cu), manganese (Mn), iron (Fe), zinc (Zn) and selenium (Se) levels in the four herbal medicines.

Materials and Methods: A total of 20 whole plant materials were collected of each species in China. The content of Cu, Mn, Fe and Zn in the dried aerial parts was estimated by the standard atomic absorption spectrophotometry. The level of Se was detected using hydride generation atomic fluorescence spectrometry.

Results: The mean concentrations of Cu, Mn, Fe, Zn and Se in *S. laniceps* were 7.758±0.924μg/g, 201.3±16.24μg/g, 222.7±35.10μg/g, 18.48±2.913μg/g and 1.42±0.16μg/g, respectively; *S. involucrata* were 19.56±2.20μg/g, 88.75±8.53μg/g, 812.7±126.9μg/g, 34.85±3.81μg/g and 1.04±0.05μg/g, respectively; *L. barbarum* were 10.83±0.26μg/g, 9.598±0.32μg/g, 55.65±3.83μg/g, 11.92±0.27μg/g and 11.84±0.59μg/kg, respectively; *L. ruthenicum* were 12.67±0.39μg/g, 13.78±1.13μg/g, 98.04±5.03μg/g, 14.46±1.27μg/g and 35.12±2.34μg/kg, respectively.

Conclusion: This study provided the trace elements content of Cu, Mn, Fe, Zn and Se in the four herbal medicines. The trace elements are maybe other functional compounds for medicinal effects. Deep relationship between pharmacological and trace elements contents, especially its mechanism of action should be future research.

Key words: *Saussurea laniceps*, *Saussurea involucrata*, *Lycium barbarum*, *Lycium ruthenicum*, trace elements content, traditional Chinese Medicine

Introduction

Related medicinal plants from the same family or genus have been and are being used for similar therapeutic purposes in various Chinese Medicines (Yi et al., 2010). “Snow lotus” flowers are famous Chinese Herbal Medicine (CHM) for the treatment of rheumatoid arthritis, stomachache and dysmenorrhea in traditional Chinese Medicine (TCM), Tibetan medicine, Uyghur medicine, Mongolian medicine and Kazakhstan medicine (Chinese Pharmacopoeia

Commission, 2010; Yi et al., 2010, Chik et al., 2015; Chen et al., 2016). *Saussurea involucrata* and *Saussurea laniceps* are two species of “snow lotus”. *S. involucrata* is Composite family and grows in the mountains at heights of 4000–5000m in Xinjiang areas in China. *S. laniceps* mainly distributed in the Qinghai-Tibet plateau at heights of 3500–5300m. The two snow lotuses have different macroscopic and microscopic features (Chen et al., 2014). The main compositions of *S. involucrata* were phenolic acids, flavonoids, and lignanoids; but the main compositions of *S. laniceps* were phenylpropanoids, lignans, flavonoids, coumarins, sesquiterpenes, steroids, ceramides, and polysaccharides (Yi et al., 2009a and b; Wang et al., 2010, Chik et al., 2015; Chen et al., 2016). Although S. laniceps are primarily harvested as medicinal plants, they also have become popular souvenir items with tourists because they are strange-looking, rare, and grow in exotic locations (Yang et al., 2003). *Lycium barbarum*, known as goji berry, belonging to the *Solanaceae* family, has been used as a TCM to nourish the liver and kidney, to protective against chronic diseases, and to improve vision (Bo et al., 2016). It has long been favorited in Southeast Asia and China, and is increasingly becoming popular as a so-called ‘superfruit’ in western diets due to its potential health-promoting constituents (Hempel et al., 2017). *Lycium ruthenicum* is also *Solanaceae* family and widely distributes in salinized desert of Qinghai-Tibet plateau, which is a unique nutritional food, and has widely been used for treatment of abnormal menstruation, heart disease and menopause (Zheng et al., 2011). Active constituents of *L. ruthenicum* are reported to be having a variety of biological activities, including anti-aging, immunoregulation, lowering blood-fat and blood-sugar levels and anti-fatigue. Because of overexploitation and deterioration of its habitat, *L. ruthenicum* is now considered to be threatened in China (Liu et al., 2012).

Whereas the studies of pharmacological properties and chemical composition have been mainly researched, little attention has been paid to the inorganic components of the rare medicine. Unlike synthetic drugs, herbal medicine is a synergistic system comprising multiple and complicated components (Yi et al., 2014 and 2016). Trace metal levels are important for human health. Trace elements play an important role in the plant metabolism and biosynthesis as cofactors for enzymes, and also play an important role in the formation of the active chemical constituents present in medicinal plants (Tokalıoğlu, 2012). Copper (Cu), manganese (Mn), iron (Fe), zinc (Zn) and selenium (Se) are essential for correct growth and development of all animals and can be considered as trace minerals with a central role in many metabolic processes throughout the body (Rahman et al., 2006; Stef and Gergen, 2012). Cu is required for iron metabolism, enzyme systems, immune systems, connective tissue metabolism and mobilization (Wang et al., 2014b). Mn participates in a wide range of adsorptive and redox reactions (Tebo *et al*., 2004). Fe is needed for normal growth of animals, and synthesis of Fe-requiring enzymes (Hostetler et al., 2003). Zn deficiency causes increased susceptibility to infection and impaired immune function (Hotz and Brown, 2001). Selenoenzymes have a major role as antioxidant that inhibit proinflammatory cell metabolisms and protect cell components (Rayman, 2000). Trace elements have been added to the list of elements considered as essential components of herbal medicine (Rajasekaran et al., 2005). In order to better clarify the pharmacodynamics, the objective of the present study was to estimate the trace elements content in *S. laniceps, S. involucrate, L. barbarum* and *L. ruthenicum*.

Materials and Methods

Sampling

S. laniceps, *S. involucrate*, *L. barbarum and L. ruthenicum* (Figure 1) were collected in August, 2016 in Shannan district (east longitude 92°14′–93°22′, north latitude 28°08′–29°07′, altitude 3800–5200m) of Tibet Autonomous Region, Tianshan Mountains (east longitude 88°05'–88°54', north latitude 42°05'–43°00', altitude 4000–4500m) of Xinjiang Autonomous Region, Zhongning county (east longitude 105°30'–105°44', north latitude 37°15'–37°28', altitude 1200–1500m) of Ningxia Autonomous Region, and Yumen district (east longitude 96°20'–96°36', north latitude 39°54'–40°05', altitude 1400–1700m) of Gansu province, People's Republic of China, respectively. A total of 20 samples were collected for each species. The four herbal medicines were identified by professor Chaoying Luo, Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS. Voucher specimens with accession numbers 20160805, 20160814, 20160816 and 20150827 were submitted to the Herbarium of the Traditional Chinese Veterinary Medicine (Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS).

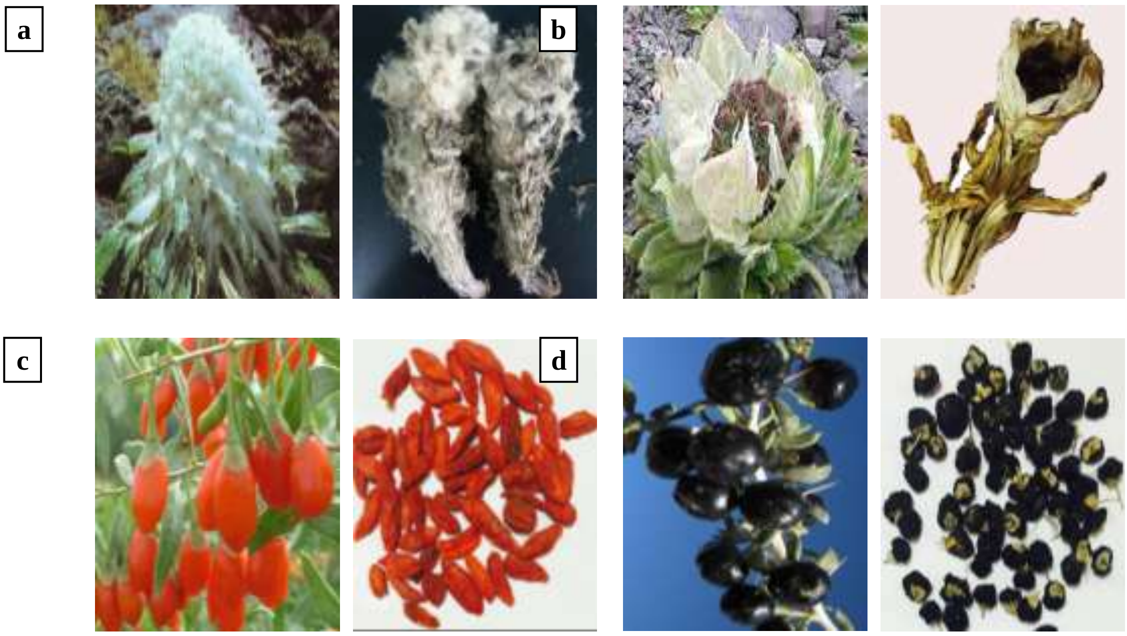

Figure 1 Photos of *S. laniceps* (a), *S. involucrata* (b), *L. barbarum* (c) and *L. ruthenicum* (d) plants and their medicinal materials.

Processing and digestion of samples

All chemical reagents were guaranteed reagent grade. Standard stock solutions were prepared from Chinese Certified Referenced Material (100μg/mL) and were diluted to the corresponding metal solution. The working solution was freshly prepared by diluting an appropriate aliquot of the stock solutions. Ultra pure water was used for all dilutions.

The four herbal medicines samples were dried in an oven at 70°C for 48 h, crushed, and sieved through 0.25mm sieve. The powder (1.0g) were placed in PTFE digestion tubes and 10mL diacid mixture (HNO_3 : H_2O_2=8 : 2) was added to each digestion tubes for overnight. After that the samples were digested using microwave digestion system (MARS5, CEM Company, America). The optimal digestion procedure was described in our previous publication (Wang et al., 2014a). The digested samples were cooled to room temperature, transferred to glass tube, and removed the diacid mixture until 1mL was left using intelligent temperature controller in 130°C. The digested solution was transferred to volumetric flask, and volume made up to 50mL with ultra pure water. A blank digest was carried out in the same way.

Four elements (Cu, Mn, Fe, Zn) were measured using flame atomic absorption spectrometry (FAAS) (ZEEnit 700, Analytik Jena, Germany) with a deuterium background corrector. The hydride generation atomic fluorescence spectrometry was used for Se determination (Lu et al., 2004). The optimal operating condition and procedure was developed as described in our previous publication (Wang et al., 2014a) with slightly updated for higher sensitivity and lower detection limits in Table 1.

Table 1: Working conditions of the atomic absorption spectrometer and atomic fluorescence spectrometry

Element	Absorption line (nm)	Slit (nm)	Lamp current (mA)	Gas consump-tion (L h^{-1})	Burner height (mm)	Flame type	Input dosage (mL min^{-1})	Linear equations
Cu	324.8	1.2	3.0	50	6	C_2H_2-air	5.0	$A = 0.1325C - 0.0023$ $r^2 = 0.9999$
Mn	279.5	0.8	6.5	60	6	C_2H_2-air	5.0	$A = 0.1212C + 0.0021$

								$r^2 = 0.9998$
Fe	248.3	0.2	6.0	65	6	C_2H_2-air	5.0	$A = 0.0209C + 0.0011$ $r^2 = 0.9999$
Zn	213.9	0.5	4.0	60	6	C_2H_2-air	5.0	$A = 0.1542C + 0.0009$ $r^2 = 0.9990$
Se	217.6	-	60.0	24	-	Ar	0.5	$A = 0.1059C - 0.0014$ $r^2 = 0.9989$

Where ***A*** denotes absorbance value, ***C*** denotes concentration (μg/g or μg/kg)

Results and Discussion

Medicinal herbs have been particularly recognized as being readily available, valuable and affordable resource for health care, and as an integral part of traditional medical practices for thousands of years (Tripathy et al., 2015). Potential healthcare applications of herbal medicines are very much related to their chemical composition and bioactive constituents, such as total phenolics, flavonoids, polysaccharides, anthocyanins, proanthocyanidins, alkaloids, as well as minerals (macro-, micro- and trace elements) (Szentmihalyi et al., 2006; Zengin et al., 2008). Trace elements are essential elements required to all cells for a number of biochemical functions, acting as structural components of tissues, constituents of the body fluids, and enzymes in major metabolic pathways (Lozak et al., 2002; Wang et al., 2016). Determination of the element composition of herbal medicines is essential for understanding their health effects and nutritive.

As show in Figure 2, the mean concentrations of Cu, Mn, Fe, Zn and Se in *S. laniceps* were 7.758±0.924μg/g, 201.3±16.24μg/g, 222.7±35.10μg/g, 18.48±2.913μg/g and 1.42±0.16μg/g, respectively; in *S. involucrata* were 19.56±2.20μg/g, 88.75±8.53μg/g, 812.7±126.9μg/g, 34.85±3.81μg/g and 1.04±0.05μg/g, respectively. And the trace elements content has significant differences between *S. laniceps* and *S. involucrate* (p<0.01 or p<0.05). The mean value of the total trace elements contents in “snow lotus” followed a descending order as: Fe > Mn > Zn > Cu > Se. In our study, we found that the “snow lotus” is rich in Mn, Fe and Zn, especially abundant in Mn compared with other plants (Niu et al., 2009; Zhao et al., 2009). Mn participates in a wide range of redox and adsorptive reactions, and plays a significant role in the bioavailability and geochemical cycling of many essential or toxic elements (Tebo et al., 2004). The therapeutic usage of herbal medicine in curing of various diseases due to presence of trace elements is already a widespread approach in medicinal purposes (Rajan et al., 2014). Most of the herbal medicines are rich in trace elements, thereby providing a possible link to the therapeutic action of the medicine (Kolasani et al., 2011). The trace elements present in “snow lotus” may play a direct or indirect role in the formation of the active chemical constituents and pharmacological nature especially Mn. Therefore, trace elements can be responsible for medicinal properties. The further research in the relationship of pharmacodynamics and trace elements should be needed.

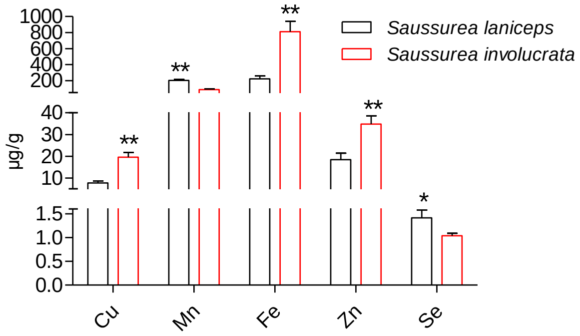

Figure 2: Mean concentrations of the trace elements analyzed in *S. laniceps* and *S. involucrate*. ** means $p<0.01$, * means $p<0.05$.

The TCM plants *L. barbarum* and *L. ruthenicum* are valued for the abundance of bioactive carotenoids and anthocyanins in their fruits, respectively (Liu et al., 2014). However, the trace elements contents remain poorly understood. As show in Figure 3, the mean concentrations of Cu, Mn, Fe, Zn and Se in *L. barbarum* were 10.83±0.26μg/g, 9.598±0.32μg/g, 55.65±3.83μg/g, 11.92±0.27μg/g and 11.84±0.59μg/kg, respectively. But the contents of Cu, Mn, Fe, Zn and Se in *L. ruthenicum* were 12.67±0.39μg/g, 13.78±1.13μg/g, 98.04±5.03μg/g, 14.46±1.27μg/g and 35.12±2.34μg/kg, respectively; which were significantly higher than *L. barbarum* ($p<0.01$). *L. barbarum* is one kind of the famous food plants and traditional medicine (Gong et al., 2016), have been gradually regarded as a health functional food in Asia, Europe and United States based on it is beneficial in immunity improvement, antioxidation, enhancing hemopoiesis, antiradiation, anti-age and anti-cancer (Dong et al., 2009). *L. ruthenicum* processed abundant anthocyanins, and with wide range of physiological and biological activities such as antitumor effect and antioxidant activity. Adding certain amount of *L. ruthenicum* to daily diet will be beneficial for people's health (Liu et al., 2012). The biochemical mechanisms of the medicinal effects are primarily attributed to the anthocyanins, flavonoids, polysaccharides, essential oils and carotenoids (Yao et al., 2011). Researchers reported that for enhancing the immune system and curing of skin diseases, body required small quantity of trace elements, which recover quickly from serious infection and defend from pathogen (Rajan et al., 2014). From our research the contents and composition of trace elements in *L. barbarum* and *L. ruthenicum* is maybe another functional compound. And *L. ruthenicum* can be regarded as an additional supplementary source of many important trace elements in human daily diet and nutrition.

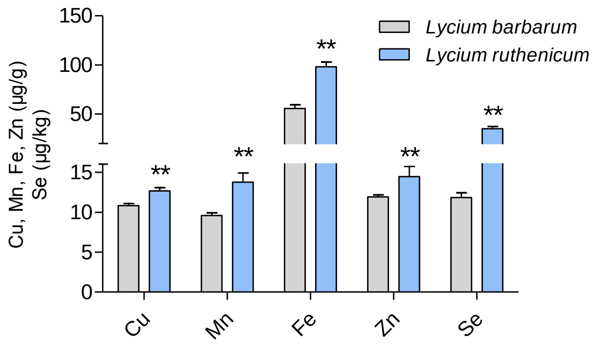

Figure 3: Mean concentrations of the trace elements analyzed in *L. barbarum* and *L. ruthenicum*. ** means $p<0.01$, * means $p<0.05$.

Conclusion

S. laniceps, *S. involucrata*, *L. barbarum* and *L. ruthenicum* are worth additional attention because of wide uses, extensive biological activities, and reliable clinical efficacy in various ethno-medical systems. This study provided the trace elements content of Cu, Mn, Fe, Zn and Se in the four herbal medicines. The results suggest that the four herbs may provide a beneficial source of some elemental micronutrients. The trace elements are maybe other functional compounds for medicinal effects. The therapeutic activity of the four herbs is maybe associated with the content of trace elements to some extent. Deep relationship between pharmacological and trace elements contents, especially its mechanism of action should be future research.

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Conflict of Interest: Authors declare that there is no conflict of interest

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