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ORIGINAL PAPER

ANALYSES OF THE NUTRITIONAL COMPOSITION AND MINERAL ELEMENT CONTENT OF *LONICERA FULVOTOMENTOSA* HSU ET S.C. CHENG GROWN IN DIFFERENT SOILS*

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Abstract

Lonicera fulvotomentosa Hsu et S.C. Cheng is widely distributed in southwest China. Dried flowers of this plant are used in the treatment of fever, pain, and infectious diseases, such as SARS and avian influenza. The present study aimed to evaluate the chemical composition and bioactive ingredients of dried flowers from *Lonicera fulvotomentosa* Hsu et S.C. Cheng plants grown in yellow loam and karst soil in Guizhou, China. All samples from the two different sources exhibited different ranges of changes in the content of moisture, lipids, fiber, ash, crude protein, and carbohydrates, varying from 3.25 to 3.63%, 7.76 to 9.93%, 6.93 to 7.34%, 12.32 to 12.76%, 7.85 to 8.53%, and 56.21 to 59.77%, respectively. The ICP-AAS analysis indicated that the predominant mineral elements in the dried flower were Ca, K, Fe, and Mg. The content of these elements was: 297.34-351.26 mg kg^{-1}, 132.56-140.37 mg kg^{-1}, 37.77-41.25 mg kg^{-1}, 9.47-11.36 mg kg^{-1} for Ca, K, Fe and Mg, respectively. The three phenolic acids (gallic, caffeic acid, and chlorogenic acid) and five flavonoids (rutin, luteolin, quercetin, apigenin, and kaempferol) were found with the HPLC method. We concluded that the chemical composition of *Lonicera fulvotomentosa* Hsu et S.C. Cheng is similar to *Lonicera japonica*, but the nutritional composition and mineral element content are different, and the content of chlorogenic acids and caffeic acid from the dried flowers obtained from different soils were extremely high. *Lonicera fulvotomentosa* Hsu et S.C. Cheng, grown in different soils, can be used as a substitute for *L. japonica* in traditional Chinese medicine. Due to the specific soil

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parameters which can influence the content of bioactive ingredients, plants from different sources may cause different medicinal effects.

Keywords: karst soil, yellow loam, nutritional composition, mineral elements.

INTRODUCTION

Chinese traditional medicine has been used in treating diseases for thousands of years. *Lonicera japonica* belongs to *Lonicera* sp., *L. japonica* is one of the most important medicinal plants widely used in Chinese medicine. Dried flowers of *L. japonica* are widely used with other traditional Chinese medicines to treat epidemic fever and infectious diseases, such as SARS and bird flu (Qian et al. 2017). *Lonicera fulvotomentosa* Hsu et S.C. Cheng is one of the most important species in *Lonicera* family, which has a chemical composition and pharmacological effects similar to *L. japonica and* is commonly used as a substitute for *L. japonica* (Chun et al. 2015). The phytochemical composition and nutrient composition of a medicinal plant are closely related to that of its therapeutic effect. Previous research indicated that the flower buds of *Lonicera* species contain a variety of compounds, such as phenolic acids, flavonoids, triterpenoid saponins, and iridoids (Shang et al. 2011). Medicinal plants contain a variety of trace elements. In recent years, the relationship between trace elements and human health has been gaining more attention, for instance, magnesium, iron, selenium, and zinc play an important role in metabolism (Pelus et al. 1994, Shazia et al. 2012). The content and composition of trace elements in medicinal plants are closely related to soil types (Chen et al. 2011). The bioactive ingredients of medicinal plants play a decisive role in the prevention or control of human diseases. Caffeoylquinic acid derivatives are the major active components of the Chinese herb *L. japonica*, which have a significant antioxidant effect. Furthermore, they demonstrate significant antitumor activity (Mishima et al. 2005) and potential activity against anti-human immunodeficiency virus (HIV) (Yang et al. 2005). The difference in the position of the caffeoyl ester groups on the cyclohexane ring in di-O-caffeoylquinic acids (diCQAs) will result in different anti-bacterial activities in *L. japonica* (Han et al. 2014). The development and evaluation of nutritional composition and mineral elements from medicinal plant materials is attracting more and more attention.

The southwest karst region of China, in Guizhou Province, is the largest continuous karst region in the world, characterized by fragile ecological environments and strong soil erosion (Sun et al. 2020). Features which are exclusive to karst include high concentrations of Ca, Mg and K, the lack of surface water and very slow rate of soil formation, and therefore they pose several challenges for colonizing plants (Nadia et al. 2019), all these leading to the development of specialized flora with specific growth characteristics and nutritional composition. *Lonicera fulvotomentosa* Hsu et S.C. Cheng can

grow in karst soil and tolerates high calcium, alkaline, and barren soil condition, which is beneficial to the restoration of the karst geomorphic environment. There were nine compounds from the acid hydrolysate of the flower buds of *Lonicera fulvotomentosa* Hsu et S.C. Cheng, and only ethylcaffeate, caffeic acid, and isovanillin exhibited inhibitory effects against human immune deficiency virus (HIV) protease (Wang et al. 2019). However, detailed composition of the flowers of *Lonicera fulvotomentosa* Hsu et S.C. Cheng flowers has not been reported yet. Importantly, the nutritional composition and mineral element content in a plant depend on several factors, of which the most important is the geographical environment, for example where the plant is cultivated, the type of soil, climate and water availability.

This study aimed to compare the chemical composition and nutritional status of *Lonicera fulvotomentosa* Hsu et S.C. Cheng grown in the yellow loam and Karst landform soil.

MATERIAL AND METHODS

Reagents and standards

The methanol (MeOH) and acetonitrile (ACN) used in this study were of HPLC grade and were purchased from ThermoFisher Scientific, (China), Company Limited. Gallic and chlorogenic acids, caffeic acid, rutin, luteolin, quercetin, apigenin, and kaempferol were purchased from Sigma--Aldrich (Shanghai) Trading Company Limited (China), other chemicals used in this study were of analytical grade. Water (ultrapure) was taken from an in-house Milli-Q plus purification system (Millipore, Midford, MA USA). The multielement standard mixture solutions contained Fe, P, K, Ca, Mg, Mn, Zn, B, Mo, and Cu. The digestion and decomposition system employing 70% (w/w) HNO_3 was purchased from Aladdin Reagent (Shanghai) Company Limited.

Preparation of samples

Lonicera fulvotomentosa Hsu et S.C. Cheng flowers, which came from Congjiang, Guizhou and Xinyi Guizhou (located on 26°37′24″ and 107°20′36″, 25°08′12″ and 104°09′26″) respectively, were collected from cultivars growing in yellow loam and karst landform soil. Yellow loam is a mixture of sand, clay and a small amount of calcite, light yellow, which can be easily pulverized by hand. Karst landform soil is mainly black lime soil, with poor soil particle viscosity, poor soil fertility, and weak alkaline reaction. Mineral elements were determined in an Agilent 5100 ICP-OES inductively coupled plasma emission spectrometer. The content of mineral elements in the soils is shown in Table 1. The plants were identified and authenticated by Dr. Xujun Wang, Hunan Academy of Forestry, China. The flowers were

Table 1

Mineral element type and content in karst soil and yellow loam

Soil type	Element type and content										
	total N (g kg^{-1})	total P (g kg^{-1})	total K (g kg^{-1})	total Ca (g kg^{-1})	total Mg (g kg^{-1})	total Fe (mg kg^{-1})	total Mn (mg kg^{-1})	total Zn (mg kg^{-1})	total B (mg kg^{-1})	total Mo (mg kg^{-1})	total Cu (mg kg^{-1})
Karst soil	3.63	0.35	4.52	3.22	2.64	35.58	0.53	62.49	0.12	0.76	1.13
Yellow loam	4.43	0.47	2. 55	2.36	1.53	62.73	0.81	75.26	0.19	0.61	0.89

cleaned and processed according to the method of Tan et al. (2016) with modifications. A set of six samples from each geographical origin was randomly collected. All materials were air-dried, powdered and protected from light in an acclimatization room for further analysis.

Chemical analysis

The samples were dried in a forced air oven at 60°C to constant weight for moisture determination. The crude protein content and crude lipid content were determined using standard procedures of AOAC (USA, 2000). Namely, 1.000 g of a dried sample was taken and ashed at 600°C. The crude fiber content was determined according to the method of Churkova et al. (2013). Total carbohydrates were determined with the phenol sulphuric acid method (Dubois et al. 1956). According to the method of Monica et al. (2015), the energy values were obtained from the values of carbohydrates, lipids, and proteins being multiplied by 4, 9, and 4, respectively.

Extraction of phenolic compounds

The extracts were prepared from 30 g of dry flowers and 300 mL of 80% methanolic aqueous solution. The mixtures were sonicated for 30 min, shaken 8 h, 25°C, filtered through no. 1 filter paper (Whatman Inc.). The final filtrate was evaporated in a vacuum pump heated at 45°C and the residues were lyophilized and together with the powdered extract obtained were kept in a refrigerator at 4°C for further use. All of the extractions were performed in three separate replications.

ICP-MS analysis

Multielemental analysis was performed with an optimized ICP-QMS method in a Thermo Scientific X Series 2 ICP-MS. First, the samples were homogenized and weighed in an acid-washed polytetrafluoroethylene (PTFE) digestion tube, then per 0.5 g sample was dissolved in 5 ml of HNO_3 (70% w/v). The tube was heated in a microwave oven following the procedure below: 50% and 150°C for 10 min, 70% and 220°C for 20 min, and 10% and 100°C for 15 min. At last, the digest was transferred to a 50 mL acid-washed volumetric flask, adjust volume to 50 mL with deionized water. A blank (only

deionized water) was also prepared following each batch of samples. According to Marin 's method, the total nitrogen was determined using a modified Kjeldahl procedure (Mariadas et al. 2005) .

HPLC-DAD analysis

The standards and samples were dissolved in the mobile phase, the concentration of each was 25 μg mL^{-1}, treated for 30 minutes using 30 kHz ultrasonic, filtered through the 0.45 μm filter, the solutions were separated on a C18 Reversed-phase chromatography column. The standards and samples were analysed using an HPLC Agilent 1200 Series. The HPLC was operated at 25°C, and it was equipped with a degasser, a binary pump, an autosampler, a diode array detector, and a C18 reversed-phase analytical column (150 × 4.6 mm id, 5 μm particle size). The mobile phase was a binary gradient elution of methanol added to a buffer solution, and the buffer contained TCA/water 0.1% solution with pH=2.4. The injection volume was 20 μL. The linear gradient elution began with 20% to 50% methanol over the first 30 min, followed 70% methanol over the next 30 min. The flow rate was 0.6 mL min^{-1}. The data collected at 268 and 354 nm were processed by chemometric methods. All analyses were carried out in three separate replications.

Statistical analysis

Descriptive analyses were performed and were reported using the means and standard deviations (SD) by the Holm-Sidak test following a one-way ANOVA on IBM SPSS Statistics 25.0. The differences were considered as statistically significant at $p<0.05$.

RESULTS

Chemical analysis

Phenolic acids and flavonoids as major bioactive ingredients in *Lonicera fulvotomentosa* Hsu et S.C. Cheng flowers play an important role in a number of aspects in human medicine and health. Assessment of the nutritional composition of *Lonicera fulvotomentosa* Hsu et S.C. Cheng is essential for evaluating its medicinal value. The content different of phenolic acids and flavonoids in two samples that came from different soils are presented in Table 2, and the differences between them were not significant. Other determinations show that the moisture content ranged from 3.25 to 3.63% in all samples, the lipid content was found to vary from 7.76 to 9.93%, the fiber content was in the range of 6.93 to 7.34%, the content of ashes was from 12.32 to 12.76%, crude protein – from 7.85 to 8.53%, and carbohydrates – from 56.21 to 59.77%. The principal sources of energy in *Lonicera fulvotomentosa* Hsu et S. C. Cheng are carbohydrates, lipids, and proteins, and

Table 2

Nutritional composition of dry weight (DW) of *Lonicera fulvotomentosa* Hsu et S.C. Cheng flowers collected from karst soil and yellow loam

Composition	Karst soil	Yellow loam
Moisture(%)	3.25±0.36	3.63±0.45
Lipid(%)	9.93±1.04	9.76±0.82
Fiber(%)	7.34±0.66	6.93±0.57
Ash(%)	12.76±0.84	12.32±0.45
Protein(%)	7.85±0.93	8.53±1.12
Carbohydrates(%)	59.77±3.81	56.21±3.21

Values are mean ± SD, analysed individually in triplicate

their content was converted into the energy value according to Monica's method (Monica et al. 2015), The value of energy varied from 326.21 to 362.57 kcal 100 g^{-1}. The results provide reference data for evaluating the medicinal value of *Lonicera fulvotomentosa* Hsu et S.C. Cheng.

Elemental analysis

The mineral elements taken from soil play an important role in the formation of active components in medicinal plants. The role of mineral elements in the efficacy of Chinese herbal medicine and their effect on human health have attracted great attention. The type, quantity, and bioavailability of mineral elements are closely related to soil properties and climate parameters. Eleven elements were determined by ICP-MS in *all samples* collected from karst soil and yellow loam. N, P, K, Ca, Mg were present at levels of g Kg^{-1} for dry flowers, whereas Mn, Fe, Zn, Cu, B, and Mo were present at mg Kg^{-1} levels (Table 3). The elemental analysis of our samples revealed high contents of Ca, Mg, and Mn in karst soil, which was significantly higher than that from yellow loam. While N, P, Fe, and Zn in yellow loam were significantly higher than that from karst soil. The content differences of Cu, B, and Mo were nonsignificant between two groups of samples came from yellow loam and karst soil.

Quantification of phenolic acids and flavonoids

The main phenolic acids and flavonoids compounds presented in the methanolic extracts were investigated by HPLC method. Serial dilutions of the standard compounds were performed to make the standard curves using linear regression. The compounds quantifications (μg g^{-1} dry flower) were made by processing the retention times and chromatographic peak areas of all the components in flower samples with relative retention value and relative peak area methodologies. Figure l shows a chromatogram

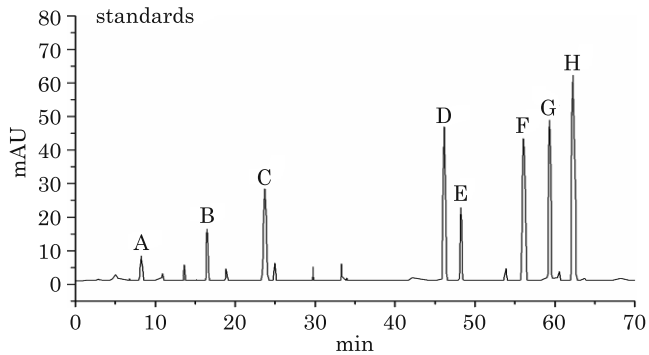

Fig.1. Chromatogram of reference substance of phenolic acids and flavonoids.
A – gallic acid, B – chlorogenic acid, C – caffeic acid, D – luteolin, E – rutin, F – quercetin, G – kaempferol, H – apigenin.

Table 3

Trace element concentration of flowers in *Lonicera fulvotomentosa* Hsu et S.C. Cheng

Elements (mg Kg^{-1})	Karst soil	Yellow loam
N	19842.25±965.36	27721.63±1348.45 **
P	1487.33±75.65	2654.43±135.76**
K	8257.34±231.65	14574.53±0.57**
Ca	20166.26±564.85*	17262.92±465.36
Mg	3827.85±125.54**	3228.53±178.34
Mn	162.19±3.81*	116.21±7.65
Fe	347.43±3.81	404.21±12.48*
Zn	74.43±3.67	115.51±5.54*
Cu	19.64±1.65	14.26±1.49
B	5.08±0.91	7.25±1.23
Mo	0.77±0.11	1.27±0.24

Values are mean ± SD, analysed individually in triplicate. Statistically significant differences between the means of both soils ** $p<0.001$; * $p<0.05$.

of a standard mixture under optimal conditions, the standard compounds were effectively separated, eight peaks were obtained within 65 min. Because the wavelengths range of phenolic classes are wide, two wavelengths detection was accomplished at 268 nm for phenolic acids and 354 nm for flavonoids. A good linearity was reached over all the phenolic compounds. The extracts from flower of *Lonicera fulvotomentosa* Hsu et S.C. Cheng grown in karst soil (Figure 2) and yellow loam (Figure 3) were detected, results showed that their chromatographic separations were similar.

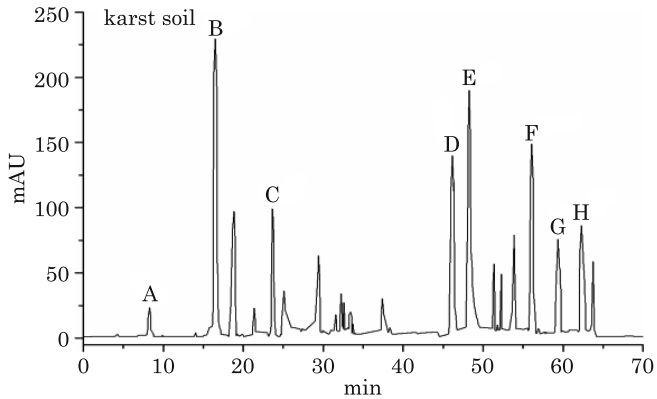

Fig. 2. HPLC analyses of *Lonicera fulvotomentosa* Hsu et S. C. Cheng sample came from karst soil. Designations see Figure 1

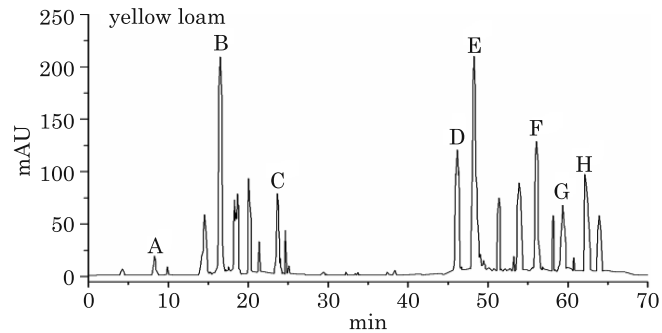

Fig. 3. HPLC analyses of *Lonicera fulvotomentosa* Hsu et S. C. Cheng sample came from yellow loam. Designations see Figure 1

The chromatographic behaviors were closely correlated with the concentration of phenolic compounds, the HPLC peak areas which determines the concentration of each compound were different. Eight phenolic compounds were identified: gallic, caffeic acid, chlorogenic acids, luteolin, rutin, quercetin, kaempferol, and apigenin.

Chlorogenic acid, an ester of caffeic acid and quinic acid, was the most abundant phenolic compound in theses samples, followed by caffeic acid. Rutin, a type of bioactive flavonoid, was the most abundant in flavonoids (Table 4). The plant cultivated in karst soil contained significantly higher luteolin and kaempferol levels than that in yellow loam. Besides, the two samples were significantly different in rutin and apigenin content, in yellow loam they were larger than in karst soil. The content of chlorogenic acid and caffeic acid are the main indicators to evaluate the quality of *L. japonica* (Cui et al. 2014), they didn't show a significant difference between two samples came from karst soil and yellow loam.

Table 4

The quantitative determination of polyphenolic compounds

Compound (μg g^{-1})	Karst soil	Yellow loam
Gallic acid	43.54±3.46	41.87±3.17
Chlorogencic acid	2149.54±104.35	1938.57±124.43
Caffeic acid	1536.22±65.43	1418.36±54.62
Luteolin	343.62±16.26*	283.48±23.92
Rutin	523.35±21.87	608.38±31.47*
Queretin	168.49±6.77	147.63±5.49
Kaempferol	98.36±4.21*	47.45±3.66
Apigenin	39.25±3.77	82.15±2.79*

Values are mean ± SD, analysed individually in triplicate. Statistically significant differences between the means of both soils ** $p<0.001$; * $p<0.05$.

DISCUSSION

Composition differences

Lonicera fulvotomentosa Hsu et S.C. Cheng is rich in minerals and unique phytochemicals, including a mixture of carbohydrates, lipids, proteins, and mineral nutrients, these ingredients are required for human health and well-being. Our results suggested moisture contents, crude protein, ash, lipid, carbohydrates, and crude fiber contents exhibited no obvious changes between two samples came from different soils, there was no statistical difference ($p>0.05$). Besides, the ash content in these samples is more than 12%, so these flowers can provide mineral resources, which are essential for maintaining human health. The distribution and bioavailability of micronutrients in the soil can affect the absorption and transport of mineral elements in plants, and the amount of different minerals present in plants seriously affect the quality and high yield of the plants.

Relationship between nutritional composition and mineral elements content

The most abundant mineral elements were observed in the samples that came from karst soil were Ca, Mg, and Mn. In contrast, the Zn and Fe content was higher in the samples that came from yellow loam than in the plants from karst soil. In the multiple linear regression analysis, the absorption of Zn and Fe had higher correlation coefficients (R=0.8742). It has been well documented that the N nutritional status of the plant is a critical tool for agronomic biofortification of wheat with Zn and Fe (Kutman et al.

2011), Zn, Fe, and Ca concentrations initially increase as applied N dose increase. Gawalko et al. (2001) found that soil factors appear to be the major controlling influences on the trace elements. The same cultivar may absorb different amounts of minerals in different soils (Perilli et al. 2010). Yellow loam helps to hold water and bind mineral nutrients, whereas in yellow loam plant roots cannot penetrate far into hard clay soils, so the concentration of dark-colored organic materials is low in yellow soils, and they are often colored yellow because of relatively more iron, aluminum, and manganese. Rainfall washes mineral nutrients out from karst soil, so karst soil has a low mineral nutrient content. The content of N, P, and K in samples from karst soil also was lower than in those that came from yellow loam, which may be related to the lower content of Zn and Fe in samples that came from yellow loam. Better knowledge of metal homeostasis is a basic step to sufficiently understand plant mineral acquisition and storage, which can help us to efficiently raise medicinal plants yield and improve medicinal herb quality.

Abundance and diversity of flavonoids

The determinations of polyphenolics have shown that the *Lonicera fulvotomentosa* Hsu et S.C. Cheng samples that came from karst soil contained a higher amount of chlorogenic acid (2149.54±104.35 μg g^{-1}), luteolin (343.62±16.26 μg g^{-1}) and kaempferol (98.36±4.21 μg g^{-1}), whereas samples from yellow loam had higher amounts of rutin (608.38±31.47 μg g^{-1}) and apigenin (82.15±2.79 μg g^{-1}). It was suggested that the abundance and chemodiversity of flavonoids in *Lonicera fulvotomentosa* Hsu et S.C. Cheng is the main factor explaining their therapeutic effectiveness against diverse diseases (Wang et al. 2019). The methanolic extract of *Lonicera species* flowers has been proven to have high antioxidant activity (Ting et al. 2016), and polyphenolics are responsible for most of the antioxidant activities of the *Lonicera* species.

Chlorogenic acid and its isomers isochlorogenic acid and neochlorogenic acid, which occur in flower, fruit and leaves of dicotyledonous plants, can promote various pharmacological activities that mainly act against obesity, reduce liver lipids, and heal acute lung illnesses (Zhang et al. 2010). On the other hand, substantial amounts of rutin are present in *Lonicera fulvotomentosa* Hsu et S.C. Cheng flowers. Rutin, one of the most abundant flavonoids in nature, has been shown to exert intestinal anti-inflammatory effects in experimental models of colitis (Suzuki et al. 2005, Mascaraque et al. 2014). It is hoped that specific anti-inflammatory drugs could be developed from *Lonicera fulvotomentosa* Hsu et S.C. Cheng. However, in order to better understand the association of flavonoids and suppression of the growth of bacteria, classification of flavonoids and clinical studies are required.

CONCLUSIONS

The phenolic acids and flavonoids as major bioactive ingredient in *Lonicera fulvotomentosa* Hsu et S.C. Cheng play an important role in a number of aspects in human medicine and health. Assessment of the nutritional composition of *Lonicera fulvotomentosa* Hsu et S.C. Cheng is essential for evaluating its medicinal value. Eleven elements were determined in *all samples* collected from karst soil and yellow loam, the content of Ca, Mg, and Mn in karst soil was higher than that in yellow loam, but the content of N, P, K, Fe, and Zn was lower. The type and quantity of mineral elements in *Lonicera fulvotomentosa* Hsu et S.C. Cheng are closely related to soil properties and nutrient availability. The content of phenolic acids and flavonoids of two samples which came from different soils were compared, and the differences were not significant. Eight phenolic compounds were identified in the flowers of *Lonicera fulvotomentosa* Hsu et S.C. Cheng: gallic, caffeic acid, chlorogenic acids, luteolin, rutin, quercetin, kaempferol, and apigenin. The plant cultivated in karst soil contained significantly higher luteolin and kaempferol levels than that in yellow loam. The content of rutin and apigenin in yellow loam was higher than in karst soil. The content of chlorogenic acid and caffeic acid, which are the main indicators to evaluate the quality of *L. japonica*, did not show a significant difference between two samples. *Lonicera fulvotomentosa* Hsu et S.C. Cheng grown in different soil can be used as a substitute for *L. japonica*. *Lonicera fulvotomentosa* Hsu et S.C. Cheng from different sources may cause different medicinal effects.

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DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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