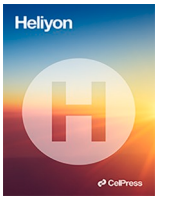
Heliyon 9 (2023) e16344

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Alkaloid variations within the genus *Stephania* (Menispermaceae) in China

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ARTICLE INFO

Keywords:
Stephania genus
Root
Alkaloids
Genetic variation
Traditional Chinese medicine

ABSTRACT

The genus *Stephania*, which is rich in alkaloids, has been used as a traditional medicine or folklore herb against numerous ailments in China. However, the understanding of the variation within the genus *Stephania* is obscure, which limits the optimal utilization of the genus. An evaluation of the variation within the genus *Stephania* would help screen the ideal *Stephania* genotypes for drug utilization. In the present study, alkaloids in the tubers of four commonly cultivated *Stephania* species in China, i.e., the genotype *Stephania kwangsiensis* Lo. (SK-guangxi) from Guangxi Province and three *Stephania yunnanensis* H.S. Lo. genotypes (SY-xueteng, SY-hongteng, and SY-lvteng) sourced from Yunnan Province, were investigated, and the genus variations were compared. The results revealed significant variations in the abundance of alkaloids in tubers within the genus *Stephania*. The *Stephania* genotypes SY-xueteng and SY-hongteng showed a relatively high abundance of total alkaloids compared with the *Stephania* genotypes SK-guangxi and SY-lvteng. Specifically, the *Stephania* genotype SY-xueteng had a relatively high abundance of palmatine in tubers, and the *Stephania* genotype SY-hongteng exhibited a high abundance of stephanine in tubers. Our study provides foundations for further utilization of ideal *Stephania* genotypes by clarifying the variations in the alkaloid contents within the genus in China.

1. Introduction

The genus *Stephania* is a medicinal plant belonging to the family Menispermaceae [1]. Thirty-nine species of the *Stephania* genus are found in China, and these are mainly distributed in the area south of the Yangtze River [2]. Most *Stephania* plants are herbaceous lianas with huge swollen tubers, which are also medicinal parts. A large tuber from an adult *Stephania* plant has a diameter of approximately 23 cm and a weight of up to 30 kg [3] and thus constitutes a large part of the whole plant. In particular, *Stephania* plants are rich in alkaloids and have been used in traditional Chinese medicine (TCM) to treat a wide range of diseases, e.g., inflammation, asthma, tuberculosis, hyperglycemia, cancer and neurodegenerative diseases [4–6]. Recent studies showed that the replication of novel coronavirus (COVID-19) can be effectively inhibited by a small amount of stephanine [7], which indicates that this compound has great potential for preventing the spread of COVID-19.

Pharmacologically, the compounds isolated from *Stephania* tubers show analgesic, anti-inflammatory and bacteriostatic activities

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https://doi.org/10.1016/j.heliyon.2023.e16344
Received 28 July 2022; Received in revised form 9 May 2023; Accepted 12 May 2023
Available online 16 May 2023

[8]. Alkaloids are the main active pharmaceutical ingredients isolated from *Stephania* plants [9,10]. It has been reported that *Stephania* tubers contain approximately 3–4% alkaloids [11]. Several alkaloids, such as palmatine, sinomenine, stephanine and tetrahydropalmatine, have been isolated from plants of this genus [10], and many of these have been evaluated for biological activity. However, the utilization of *Stephania* resources is limited due to the superficial understanding of the genetic variation among *Stephania* species.

In China, the wild resources of the genus *Stephania* are widely distributed in tropical and subtropical regions, generating potential genetic diversity in pharmacologically active substances. Commonly cultivated *Stephania* species such as *Stephania epigaea* H.S. Lo. and *Stephania yunnanensis* H.S. Lo. are mainly distributed in Yunnan Province, China, and *Stephania kwangsiensis* Lo. is found in Guangxi Province, China [1,6,12]. The geographical origin of the genus *Stephania* is an important factor influencing its quality. However, the variation within the *Stephania* species used in TCM has rarely been evaluated.

The present study thus performed a preliminary evaluation of the variations in the main pharmacological active substances, i.e., alkaloid contents, in commonly used *Stephania* sources from Guangxi and Yunnan Provinces, China. The objective of this study was to deepen the understanding of the variations in *Stephania* plants commonly used in TCM, which could provide insights for screening ideal germplasms of the genus *Stephania*.

2. Materials and methods

2.1. Plant materials and sampling

Four commonly cultivated *Stephania* genotypes, i.e., *Stephania kwangsiensis* Lo. from Guangxi Province, China (SK-guangxi), and three *Stephania yunnanensis* H. S. Lo. genotypes from Yunnan Province, China (SY-xueteng, SY-hongteng, and SY-lvteng), were used in the present study. Pot experiments were conducted during Mar. 2020–Mar. 2022; during this period, the three *Stephania* genotypes SY-xueteng, SY-hongteng and SY-lvteng were cultivated at Kunming Agricultural University, Kunming, Yuannan (25°5′ N, 102°40′ E, 1890 m above sea level), and the *Stephania* genotype SK-guangxi was cultivated at Guangxi University, Nanning, Guanxi (22°49′ N, 108°19′ E, 79 m above sea level). All of the plants were transported to Guangxi Institute of Botany, Chinese Academy of Sciences, Guilin, China (25°4′N, 110°18′E, 175 m above sea level) for sampling and assays, and four replications of each genotype were included in the experiments.

2.2. Extraction and determination of alkaloids in tubers

The reagents used for analytical determination were of analytical or high-performance liquid chromatography (HPLC) grade. Four standards of alkaloids (palmatine, sinomenine, stephanine and tetrahydropalmatine), which have a purity higher than 98%, were obtained from Macklin (Shanghai Macklin Biochemical Co., Ltd., Shanghai, China). The chromatographic-grade solvents (acetonitrile, phosphoric acid and alcohol) for extraction and elution were obtained from Tedia (Fairfield, OH, USA). Alkaloids were extracted from the tubers of *Stephania* plants and quantified using an HPLC system equipped with a UV detector (Shimadzu LC Prominence-i 2030C model, Shimadzu, Japan) according to the methods described by Dai et al. (2012) [13] and Huang et al. (2018) [14] with minor modifications.

The tubers were cut into thin slices, dried at 100 °C in a dryer for 24 h and broken into pieces before being ground into a powder. Then, 2.0 g of powder was weighed and extracted with 100 mL of 50% alcohol (HPLC-grade alcohol: double-distilled H_2O = 1:1, V:V) by ultrasonic-assisted extraction for 45 min. Next, a volume of 2.0 mL of extract was pipetted into a centrifuge tube and centrifuged at 12,000×*g* for 3 min, and 1.0 mL of supernatant was then collected as crude extract for the determination of alkaloids.

The obtained crude extracts were purified by a C18 Sep-Pak cartridge (Waters Corporation, Millipore, MA, USA). The purified samples were filtered using a 0.22-μm nylon filter prior to sample analysis. The concentrations of the alkaloids were measured using an HPLC-UV instrument equipped with a C_{18} column (Hypersil ODS2, 4.6 mm × 250 mm, 5 μm) by multistep isocratic elution (40 min) at a flow rate of 1.0 mL min^{-1}. The 40-min mobile phase included 3 isocratic elutions: isocratic elution with 10% acetonitrile and 90% phosphoric acid solution from 0 to 10 min, isocratic elution with 20% acetonitrile and 80% phosphoric acid solution from 10 to 30 min, and isocratic elution with 30% acetonitrile and 70% phosphoric acid solution from 30 to 40 min (Table 1). The injection volume was 20 μL. The UV detection wavelength was 282 nm, and the column temperature was maintained at 30 °C. The HPLC chromatograms and separation of alkaloid standards (Fig. S1) and alkaloids in samples (Fig. S2) are presented in the Supplementary Materials.

Calibration was carried out by 3 independent injections (three repetitions) of sinomenine, palmatine, tetrahydropalmatine and stephanine standards. The stock solution of alkaloids (palmatine, sinomenine, stephanine and tetrahydropalmatine, 2 g L^{-1}) contained a total of 20 mg of certain alkaloids dissolved in 10 mL of HPLC-grade alcohol. The calibration standards for each alkaloid standard

Table 1
Linearity, detection limits, recovery and precision (RSD%).

Compound	Calibration equation	r	Recovery (%)
Sinomenine	Y = 62.09x	0.997	99.4%
Palmatine	Y = 71.07x	0.999	92.9%
Tetrahydropalmatine	Y = 206.69x	0.998	97.3%
Stephanine	Y = 14.46x	0.999	101.2%

were prepared at concentrations of 0.025, 0.05, 0.10, 0.15 and 0.20 mg L^{-1}, which were generated from the stock solution. The standard curve of each compound was calculated (Table 1). To evaluate the recovery of the alkaloid extractions, another 2-g sample of tuber tissue was extracted with 1 mg of each alkaloid standard. The extraction procedure was performed as mentioned above. The recovery was calculated by comparing the results from a tuber tissue sample with an internal standard (S_1) referring to another equal sample without alkaloid standards (S_2) (Percentage recovery % = ($S_1 - S_2$) mg/1 mg × 100%). The percentage recoveries of sinomenine, palmatine, tetrahydropalmatine and stephanine were 99.4%, 92.9%, 97.3 and 101.2%, respectively. The content of each alkaloid was calculated based on the standard curves and percentage recovery and expressed in units of mg per g dry weight. The proportion of certain alkaloids is the ratio of the alkaloid concentration to the total concentration of the four alkaloids (sinomenine, palmatine, tetrahydropalmatine and stephanine).

2.3. Statistical analysis

Analysis of variance (ANOVA) was performed to test the significance of the variation in parameters within the genus *Stephania* using Statistix 9.0 (Analytical Software, Tallahassee, FL, USA). The ANOVA results for each variety were assessed by the F test. The least significant difference (LSD) test at a probability level of 5% was used to compare the means.

3. Results

Significant variations in the concentration of alkaloids were found within the genus *Stephania* (Table 2). In general, the *Stephania* genotype SY-xueteng showed the highest concentrations of alkaloids (sinomenine, palmatine, tetrahydropalmatine and stephanine), followed by the *Stephania* genotype SY-hongteng, and relatively low levels of alkaloids were observed in the *Stephania* genotypes SY-lvteng and SK-guangxi. Specifically, the *Stephania* genotype SY-xueteng had the highest concentration of palmatine, and this concentration was significantly higher than those in the *Stephania* genotypes SY-lvteng, SK-guangxi and SY-hongteng. The *Stephania* genotype SY-hongteng showed the highest concentration of stephanine, and this concentration was significantly higher than that in the *Stephania* genotypes SY-xueteng, SK-guangxi and SY-hongteng. The concentrations of sinomenine in the *Stephania* genotypes SY-xueteng and SY-hongteng were significantly higher than those in the *Stephania* genotypes SY-lvteng and SK-guangxi, but no significant variation in the sinomenine content was detected between SY-xueteng and SY-hongteng or between SY-lvteng and SK-guangxi. The concentration of tetrahydropalmatine in the *Stephania* genotype SY-hongteng was significantly higher than that in the *Stephania* genotype SY-lvteng, and lower concentrations were found in the *Stephania* genotypes SY-xueteng and SK-guangxi.

The *Stephania* genotype SY-xueteng showed the highest proportion of palmatine (83.4%) but quite low proportions of stephanine (7.9%), sinomenine (3.2%) and tetrahydropalmatine (5.4%). In contrast, SY-hongteng showed the highest proportion of stephanine (78.2%) but low proportions of palmatine (2.5%), sinomenine (4.1%) and tetrahydropalmatine (15.2%), and SY-lvteng exhibited relatively high proportions of tetrahydropalmatine (47.3%) and palmatine (30.7%) and lower proportions of sinomenine (12.7%) and stephanine (9.3%). Moreover, the genotype SK-guangxi showed relatively high proportions of palmatine (51.5%) and tetrahydropalmatine (23.7%) and lower proportions of sinomenine (15.2%) and stephanine (9.7%) (Fig. 1).

4. Discussion

Rich chemical diversity exists in higher plants [15]. In *Stephania* plants, alkaloids are the main and common active pharmaceutical compounds [9]. In the present study, we found that the abundances of alkaloids varied significantly among *Stephania* genotypes (Table 2; Fig. 1). Similarly, Zhao et al. (2020) [1] reported the differentiation, chemical profiles and quality evaluation of five medicinal *Stephania* species but did not emphasize the variation in alkaloid abundance within *Stephania* species. Here, we found that the *Stephania* genotype SY-xueteng had the highest abundance of total alkaloids, followed by the *Stephania* genotype SY-hongteng, and the *Stephania* genotypes SY-lvteng and SK-guangxi showed the lowest alkaloid abundance (Table 2). The types of alkaloids also vary within the genus *Stephania* (Fig. 1). In summary, the *Stephania* genotypes SY-xueteng and SY-hongteng are good germplasms to be utilized for alkaloids.

Pharmacological studies have revealed that palmatine exerts neuroprotective, anticancer, antibacterial, antiviral, antioxidation, anti-inflammatory, and blood lipid regulatory effects [16], that stephanine has effective antiplasmodial and anticancer activities [17],

Table 2
Variation in the concentration of alkaloids among *Stephania* genotypes.

Stephania genotype	Sinomenine (mg g^{-1})	Palmatine (mg g^{-1})	Tetrahydro-palmatine (mg g^{-1})	Stephanine (mg g^{-1})	Alkaloids (mg g^{-1})
SK-guangxi	0.072 ± 0.003^{b}	0.244 ± 0.005^{b}	0.112 ± 0.008^{c}	0.046 ± 0.003^{b}	0.474 ± 0.012^{b}
SY-xueteng	0.130 ± 0.024^{a}	3.741 ± 1.527^{a}	0.207 ± 0.081^{bc}	0.336 ± 0.118^{b}	4.414 ± 1.503^{a}
SY-hongteng	0.132 ± 0.027^{a}	0.078 ± 0.036^{b}	0.503 ± 0.066^{a}	2.619 ± 0.562^{a}	3.332 ± 0.549^{a}
SY-lvteng	0.071 ± 0.013^{b}	0.185 ± 0.048^{b}	0.291 ± 0.111^{b}	0.055 ± 0.014^{b}	0.603 ± 0.157^{b}
F value	9.71**	16.42**	14.36**	56.22**	18.12**

The data are presented as the means ± SDs ($n = 4$). * and ** indicate significance at the $P < 0.05$ and $P < 0.01$ levels, respectively. Different letters indicate significant differences among the genotypes at the $P < 0.05$ level, as revealed by a least significant difference test. The F value was obtained by an ANOVA F test.

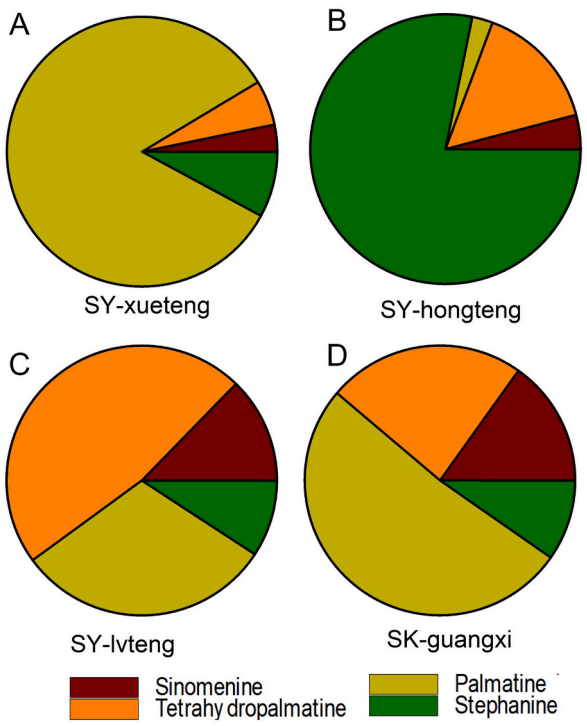

Fig. 1. Variation in the proportion of alkaloids within the genus *Stephania*. A, proportion of alkaloids in the *Stephania* genotype SY-xueteng; B, proportion of alkaloids in the *Stephania* genotype SY-hongteng; C, proportion of alkaloids in the *Stephania* genotype SY-lvteng; D, proportion of alkaloids in the *Stephania* genotype SK-guangxi.

and tetrahydropalmatine shows pharmacological effects, including analgesia, sedation and antiarrhythmia effects [18,19], and that sinomenine exhibits significant immunosuppressive, anti-inflammatory, and antiarthritic properties [20]. Recently, stephanine was found to be effective in inhibiting the replication of COVID-19 [7]. The present study found that the *Stephania* genotype SY-xueteng had the highest content and proportion of palmatine, whereas the *Stephania* genotype SY-hongteng showed a large content and proportion of stephanine (Table 2; Fig. 1). Thus, the *Stephania* genotypes SY-xueteng and SY-hongteng would be good germplasms for the extraction of palmatine and stephanine, respectively.

5. Conclusion

The present study reveals significant variations in the abundance of alkaloids in tubers within the genus *Stephania*. The *Stephania* genotypes SY-xueteng and SY-hongteng, which were obtained from Yunnan Province, China, show the highest abundances of total alkaloids and are thus good germplasms for the utilization of alkaloids. Among these, the *Stephania* genotype SY-xueteng has a relatively high abundance of palmatine in tubers, and the *Stephania* genotype SY-hongteng has a high stephanine abundance in tubers. Our study provides foundations for further utilization of the ideal *Stephania* genotype by clarifying the variations in the abundances and types of alkaloids between species of the genus *Stephania* from different sources in China.

Author contribution statement

Beibei Qi, Liangbo Li and Rongshao Huang: Conceived and designed the experiments; Performed the experiments, Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data and Wrote the paper.

Funding statement

This work was supported by the Scientific Research Foundation of Guangxi University of Chinese Medicine (2020BS014); Inheritance and innovation team of Guangxi Traditional Chinese Medicine (2022B005); Guilin Innovation Platform and Talent Plan (20210102-3).

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16344.

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