表5 塞隆骨和虎骨钙、磷含量及比值

Table 5 Content of Ca and P, and their ratio in bone of Sailong and tiger

名 称		Ca/%	P/%	Ca/P
塞隆骨	全骨(n=18)	15.27	7.25	2.102
	头骨(n=5)	15.67	7.44	2.106
	脊梁骨(n=5)	14.46	6.80	2.106
	腿骨骨(n=5)	16.89	7.69	2.196
虎 骨	全骨(n=18)	19.85	9.09	2.184
	头骨(n=5)	19.47	9.04	2.154
	脊梁骨(n=5)	19.13	8.88	2.154
	腿骨(n=5)	20.36	9.36	2.175

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Influence of producing area and plant age on oxymatrine content in root of *Sophora flavescens*

LI Hong-min[1], HUANG Ren-quan[2], HAO Jian-guo[1], JIA Jing-fen[1*]

(1. College of Life Science, Northwest University, Xi'an 710069, China; 2. Tiancheng Drug and Pharmaceutical Division, Xi'an High Tech Industries Co., Xi'an 710075, China)

Abstract: **Object** The influence of producing area and plant age on oxymatrine levels in the dried root of *Sophora flavescens* Ait. has been investigated to provide useful information to optimize the areas for mass scale propagation, proper management and species conservation. **Methods** The oxymatrine was quantified by HPLC and various statistical treatments were carried out following SPSS 9.0 and Microsoft Excel. **Results** The oxymatrine concentrations in the root samples collected from 17 provinces and regions differed from 0.494% to 4.127% and the maximum oxymatrine content (3.493%) was recorded in the root sample collected from Heilongjiang Province. Analysis of variance indicates a highly significant difference in the oxymatrine content of roots collected from different provinces, and the samples from the cold arid northern high-latitude areas had higher oxymatrine than those from warm humid southern low-latitude areas in the mainland China. Plant age is positively related to the total root biomass and oxymatrine content. **Conclusion** The oxymatrine content in the roots of *S. flavescens* is quite different between different areas and plant ages. The *S. flavescens* growing in the cold arid northern high-latitude areas had higher oxymatrine concentration.

Key words: *Sophora flavescens* Ait.; oxymatrine; HPLC; producing area; plant age

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作者简介: 李红民(1972-), 女, 陕西泾阳人, 1997年毕业于华中农业大学微生物专业, 获理学硕士学位, 现为西北大学生命科学学院讲师、植物细胞工程方向在职博士研究生。 E-mail: hlhm2002@yahoo.com.cn
* 通讯作者

产地和植株年龄对苦参干燥根中氧化苦参碱含量的影响

李红民[1], 黄仁泉[2], 郝建国[1], 贾敬芬[1*]

(1. 西北大学生命科学学院,陕西 西安 710069; 2. 西安高科集团天诚药业分公司,陕西 西安 710075)

摘 要: 目的 考察不同产地和植株年龄对苦参干燥根中氧化苦参碱含量的影响,为选择合适的大规模人工种植场地、合理的管理及物种保护提供可考的依据。方法 采用 HPLC 法测定苦参干燥根中氧化苦参碱的含量并采用 SPSS 9.0 及 Microsoft Excel 软件进行数据统计和分析。结果 从 17 个省、自治区采集的苦参根样品中氧化苦参碱的含量差异很大(0.494%～4.127%),其中以黑龙江省采集的样品中氧化苦参碱的平均含量最高(3.493%)。方差分析表明采自北方寒冷干燥的高纬度地区的样品中氧化苦碱的含量明显高于采自南方温暖潮湿的低纬度地区的样品。而且,植株年龄与苦参干燥根中氧化苦参碱含量呈相关。结论 不同产地和植株年龄均会明显影响苦参根中氧化苦参碱的含量;生长在寒冷干燥的高纬度地区的苦参根中氧化苦参碱含量较高。

关键词: 苦参; 氧化苦参碱; HPLC; 产地; 植株年龄

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Sophora flavescens Ait. is an indigenous perennial herb in China. It is distributed throughout the mainland China, ranging from low to high latitudes. The roots, as a typical traditional Chinese herb, are mainly used in the treatment of fever, peptic ulcer, eczema, hemorrhoids, asthma, tumors, bacillary dysentery and as analogy[1,2]. The herb is known to contain more than ten kind of quinolizidine alkaloids[3], among which oxymatrine is one of the major bioactive components[4]. Due to its highly pharmacological activities, such as antipyretic, antiulcer, nonsteroidal anti-inflammatory and antitumor[5-10], it has been studied greatly and deveolped into several patent medicines and some kind of medicinal herb pesticides in the past ten years[11-14]. At present, complete chemical synthesis of oxymatrine has not been reported and its commercial supply still depends on the isolation from the roots of natural sources. In recent years, uncontrolled continuous collection of plant material from natural habitat for the extraction of alkaloids has led to the sharp depletion of wild *S. flavescens* population. It is essential to develop the large-scale cultivation of this plant. However, a few studies has been conducted to evaluate the effect of producing area and plant age on oxymatrine levels in the root of *S. flavescens* growing in different provinces in China, and to estimate the total root biomass and oxymatrine content. In this paper, variation in the oxymatrine content of the roots from different areas and different ages growing in a homogenous environment has been determined.

1 Materials and methods

1.1 Chemical agents

The standard oxymatrine was purchased from Sigma Chem. Co. (USA). Sodium dihydro-phosphate, phosphoric acid, methanol, and sodium perchlorate were all HPLC grade. Water was double-distilled.

1.2 Plant material and root collection

The root samples were bought from medicinal markets within different provinces in October, 2001, which were collected from different regions in September in the identical year and air dried. The air dried root samples were dried in an oven at 70 ℃ up to constant weight, then were finely powdered in a blender.

1.3 Determination of oxymatrine

Determination of oxymatrine in the root sample was carried out by the method of quantification against external standard[15].

1.4 Solution preparation of authentic oxymatrine

Standard oxymatrine (5 mg) was weighed accurately and put into volumetric flask of 50 mL, and then dissolved and identified the volume with mobile phase used in HPLC analysis.

1.5 Solution preparation of sample oxymatrine

Powder of dried root sample (500 mg) was weighed accurately and put into volumetric flask of 50 mL and 30 mL mobile phase was added, then the volumetric flask was vibrated in an ultrasonic generator for 30 min, laid aside until the temperature of the solution was down to room temperature. To filter the solution using 0.45 μm filter membrane after fixing the volume with mobile phase used in HPLC analysis, the filtrate was used as the solution of oxymatrine sample for HPLC analysis.

1.6 HPLC analysis

Quantification of oxymatrine content in the root sample was carried out by subjecting solution of oxymatrine samples (10 μL) to HPLC analysis equipped with a reverse phase column [Zorbax SB-C_{18}, 150 mm×4.6 mm, 5 μm), Agilent, USA]. The sample was eluted in an isocratic mode with NaH_2PO_4-H_3PO_4-$NaClO_4$-MeOH-H_2O (3.3 g ∶ 0.85 mL ∶ 10 g ∶ 75 mL ∶ 425 mL) at a flow rate of 1.0 mL/min and the column elute was monitored at 220 nm with a UV deterctor (Waters 600 photodiode array detector, Millipore, USA) and Waters printer plotter (Millipore, Waters). Oxymatrine content was quantified on the basis of standard curve prepared by injecting known quantities of authentic oxymatrine.

1.7 Plant age and calculation of root biomass

The plant age was calculated on the basis of seeding and the root biomass was obtained by weighing the intact root which was dried in an oven at 70 ℃ up to constant weight after being collected from the soil very carefully.

1.8 Statistical treatments

The various statistical treatments were carried out following Microsoft Excel programme and SPSS 9.0 for Windows.

2 Results and discussion

Significant variation in the oxymatrine content was found in the root samples within the natural population of *S. flavescens* growing in different provinces in China (Table 1 and 2). Based on ANOVA (Analysis of variance), a highly significant difference at 0.01 level can be observed in oxymatrine content of root collected from different provinces. The values range from 0.494% to 4.127% (coefficien of variation= 53.96%) and the maximum (avg. 3.493%) oxymatrine level was recorded in the root from Heilongjiang province. Further, considerable differences were also observed in oxymatrine content in the root samples from different geographical locations in the identical province. These differences may indicate the effect of genetic and epigenetic variation on oxymatrine content in the root of *S. flavescens*, although little or nothing was known about it. Furthermore, the result suggests that there be opportunities to select superior areas for high-yielding plant cultivation by applying strong selection pressure.

Table 1 Oxymatrine content in root of *S. flavescens* (*n*= 8)

Provinces	Oxymatrine content/%	Provinces	Oxymatrine content/%
Heilongjiang	3.493	Sichuan	1.087
Inner Mongolia	3.129	Ghuzhou	1.058
Qinghai	2.619	Henan	1.054
Shanxi	2.579	Jiangsu	0.996
Jilin	2.557	Shandong	0.192
Gansu	2.31	Hubei	0.814
Liaoning	2.249	Zhejiang	0.764
Ningxia	2.203	Hunan	0.662
Shaanxi	1.452		

[a] The root samples were purchased from medicinal markets within different provinces in October, 2001, which were collected from different regions in September in the identical year

Table 2 Analysis of variance (ANOVA)

Source of variation	SS	df	MS	*F*	Sig
Between groups	109.23	16	6.827	62.068	0.000
Within groups	13.089	119	0.110		
Total	122.323	135			

More interestingly, this study also indicates that the samples from the cold arid northern high-latitude areas had higher oxymatrine concentration than those from warm humid southern low-latitude parts of the mainland China. To investigate the effect of environmental variation on oxymatrine content in the roots of *S. flavescens*, the root samples from 14 provinces (Their exact geographical locations are shown in Table 3.) were picked out and separated into three groups according to their longitudes. From south to north, the first group consisted of the root samples from Zhejiang, Shandong, Liaoning, Jilin and Heilongjian Provinces, respectively; the second group samples were from Hunan, Hubei, Henan, Shanxi and Inner Mongolia, respectively; the third group included Guizhou, Sichuan, Gansu, Ningxia and Inner Mongolia. Fig. 1. showed clearly that the average oxymatrine content in the roots of *S. flavescens* was going up increasingly with geographical locations migrated from South to North, and high significant difference at 0.01 level was observed in the mean of oxymatrine content in every groups based on ANOVA (Table 4) and LSD.

Table 3 Identical geographic locations of sampled provinces

Provinces	East longitude	North latitude
Heilongjiang	121°11′–135°05′	43°26′–53°33′
Jilin	121°38′–131°19′	40°52′–46°18′
Liaoning	118°50′–125°47′	38°43′–43°29′
Shandong	114°36′–122°43′	34°25′–38°28′
Zhejiang	118°02′–119°05′	27°08′–31°10′
Inner Mongolia	97°12′–126°04′	37°24′–53°23′
Shanxi	110°14′–114°33′	34°34′–40°43′
Henan	110°21′–116°39′	31°23′–36°22′
Hubei	108°21′–116°07′	29°05′–33°20′
Hunan	108°47′–114°45′	24°38′–30°08′
Inner Mongolia	97°12′–126°04′	37°24′–53°23′
Ningxia	104°17′–107°39′	35°14′–39°23′
Gansu	92°13′–108°46′	32°31′–42°57′
Sichuan	97°21′–110°12′	26°03′–34°19′
Guizhou	103°36′–109°35′	24°37′–29°13′

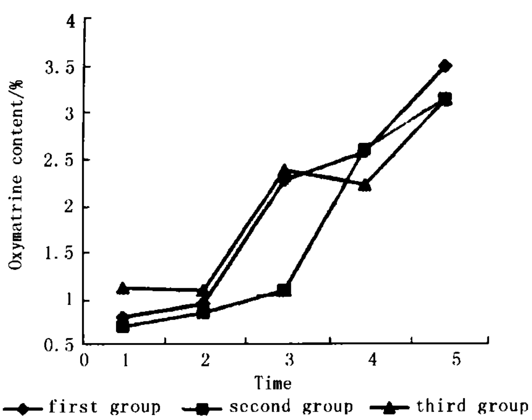

Fig. 1 Variation of oxymatrine content from South to North

Table 4 ANOVA for three selected groups

Source of variation		SS	df	MS	*F*	Sig
First group	Between groups	42.512	4	10.628	73.193	0.000
	Within groups	5.082	35	0.145		
	Total	47.594	39			
Second group	Between groups	40.649	4	10.162	97.780	0.000
	Within groups	3.638	35	0.104		
	Total	44.286	39			
Third group	Between groups	25.300	4	6.325	46.237	0.000
	Within groups	4.788	35	0.137		
	Total	30.088	39			

This study also indicates that plant age be positively related to the total root biomass and oxymatrine content. The root biomass and oxymatrine content were going up year by year. However, the rate of increase was higher in the first three years (Table 5) than that in the years soon atferwards.

Table 5 Root biomass and oxymatrine content in relation to tree age

Age/ year	Root biomass/ ($g \cdot g\ plant^{-1}$)	Average rate of increase/%	Oxymatrine content ($g \cdot g\ plant^{-1}$)	Average rate of increase/%
1	49	–	0.279	–
2	78	59.19	0.679	143.3
3	101	29.49	1.080	59.06

In conclusion, a significant variation in the root oxymatrine content exists in *S. flavescens* plants depending on the geographical locations. The difference would appear to be related to genetic variation amongst plants growing in specific natural habitat. Oxymatrine content was increasing with the increase in latitude. On one hand, the result of this work may be useful for developing simple, inexpensive and effective large-scale propagation of *S. flavescens* to meet the demand of oxymatrine. On the other hand, this information can benefit the conservation of this species. Further, *S. flavescens* plant had long root and they are likely to grow in sandy loam and they were regarded to be good at water and soil conservation[16,17], so this work would also help to conserve the genetic diversity at ecosystem level.

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西洋参、紫苏籽和薏苡根水提物的化感作用

赵杨景, 杨峻山, 王玉萍, 刘 东°

(中国医学科学院 中国协和医科大学药用植物研究所, 北京 100094)

摘 要: 目的 研究西洋参、紫苏籽和薏苡根水提物的化感作用。方法 以白菜和西洋参为测试植物, 观察西洋参、紫苏籽和薏苡根水提物对其种子萌发和活力、存苗率、幼苗初期生长的影响。结果 西洋参茎叶水提物与高于 1% 浓度的须根水提物严重抑制白菜和西洋参的种子萌发及活力、存苗率、幼苗初期生长, 而紫苏籽和薏苡根的水提物则促进实验植物幼苗根和芽的生长。高浓度的薏苡根水提物可使西洋参的存苗率显著降低。结论 3 种药用植物中存在活性较强的化感物质, 其化感作用的正负效应和强度与它们的水提物浓度及植物受体种类有关。

关键词: 西洋参; 紫苏籽; 薏苡根; 水提物; 化感作用

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Allelopathy of water extract from *Panax quinquefolium*, *Perilla frutescens*, and *Coix Lacryma-jobi*

ZHAO Yang-jing, YANG Jun-shan, WANG Yu-ping, LIU Dong

(Institute of Medicinal Plant Development, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100094, China)

Key words: *Panax quinquefolium* L.; the seed of *Perilla frutescens* (L.) Britt.; the root of *Coix Lacryma-jobi*; water extract; allelopathy

植物的化感作用在自然界中广泛存在, 而且与植物间的光、水分、养分和空间的竞争一起构成了植物间的相互作用, 这种作用包括促进和抑制, 即 "相生" 或 "相克"。而农作物栽培中的连作障碍即为植物间的 "相克" 作用, 这一现象在药用植物栽培中尤为常见。西洋参为五加科多年生宿根药用植物, 其连作障碍突出。为得知连作障碍的物质, 进行了西洋参化感作用的研究, 现将西洋参 *Panax quinquefolium* L.、紫苏 *Perilla frutescens* (L.) Britt. 籽和薏苡 *Coix Lacryma-jobi* L. 根水提物的化感作用报道如下。

1 材料和方法

1.1 供试材料: 西洋参为 4 年生根和茎叶, 种根来源于

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基金项目: 国家自然科学基金资助项目(30070437)
作者简介: 赵杨景(1945-), 女, 研究员, 主要从事植物与其生态环境关系研究。近十几年先后主持和参加了西洋参、人参、地黄、甘草、薏苡等多种药用植物栽培研究, 曾获国家级、省部级科技进步成果奖 4 项, 发表研究论文 50 余篇, 出版专著《药用植物营养与施肥技术》, 参编《中国药用植物栽培学》等, 1996 年开始研究药用植物的化感作用。
Tel: (010) 62898219, 62899739 E-mail: zhaoyangjing@hotmail.com