

RP-HPLC法研究前胡茎叶中的有效成分及其含量

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摘要 应用RP-HPLC法比较了前胡根和茎叶中的化学成分,发现白花前胡茎叶和根中的成分相似,且茎叶中有效成分含量比根中高,有可能做为中药前胡的代用品。而紫花前胡茎叶和根的化学成分相差较大,不能代替使用。

关键词 RP-HPLC法 白花前胡茎叶 紫花前胡茎叶 Pd-Ia

前胡为历次版本的《中华人民共和国药典》收载的常用中药,具有散风清热,降气化痰的功效。正品为伞形科植物白花前胡*Peucedanum praeruptorum*和紫花前胡*P. decursivum*的根^[1]。其有效成分和特征性化学成分分别为Pd-Ia和Pd-C-I^[2,3],主要化学成分为香豆素类化合物。作者已经建立了用RP-HPLC法分离白花前胡根和紫花前胡根中的香豆素类成分并分析白花前胡根中Pd-Ia和紫花前胡根中Pd-C-I含量的方法,而对其茎叶的化学成分及其含量的测定国内外均未见报道。为了充分利用白花前胡和紫前胡的地上部分,扩大药用植物资源,今应用RP-HPLC法对白花前胡茎叶和根的成分以及紫花前胡茎叶和根的成分进行了分析比较,并对白花前胡茎叶中有效成分Pd-Ia进行了含量测定。

1 仪器与试药

仪器:日本岛津LC-6A高效液相色谱仪;SPD-6AV紫外可见分光光度检测器;C-R3A数据处理机;LC-6A泵;微量进样器:10 μ l。试剂:甲醇为HPLC级,水为双蒸去离子水。对照品: Pd-Ia和Pd-C-I对照品均为作者从白花前胡和紫花前胡中分离得到,并经各种理化常数和波谱数据鉴定。样品:白花前胡植株和紫花前胡植株均采自南京中山植物园,其根和茎叶洗净后分别切碎于50℃干燥至恒重。

2 色谱条件

2.1 色谱柱:日本岛津Shim-pack SBC-ODS柱(5 μ m, 15cm $\times$ 2.5mmID)。

2.2 流动相的选择:经流动相组成改变后多次测试,选出流动相的最佳条件为:甲醇-水(71:29)0.4ml/min,检测波长320nm;柱温28℃;纸速2.5mm/min;进样量1 μ l。

3 对照品和待测液的制备

3.1 对照品贮备液的制备:精密称取2.0mg对照品Pd-Ia针晶和2.0mg对照品Pd-C-I针晶,各用甲醇溶解后定容50ml,其浓度为40 μ g/ml,即为对照品贮备液,备用。

3.2 待测液的制备:精密称取白花前胡根和茎叶各0.5g,紫花前胡根和茎叶各0.5g,分别用5ml氯仿加热回流提取2次,每次1h,合并提取液,蒸干氯仿,加甲醇溶解后定容至5ml容量瓶中;吸取1ml稀释后再定容至5ml容量瓶中,经0.20 μ m微孔滤膜过滤,即为试液待测。

4 样品测定

4.1 取Pd-Ia和Pd-C-I的对照液分别进样,确定保留时间,然后将白花前胡和紫花前胡茎叶的试液分别进样测试,其结果见图。

从以上测试结果可发现,白花前胡茎叶和根的化学成分相似,且有效成分Pd-Ia含量较高,而紫花前胡茎叶和根的化学成分相差较大,且特征性成分Pd-C-I的含量很低。

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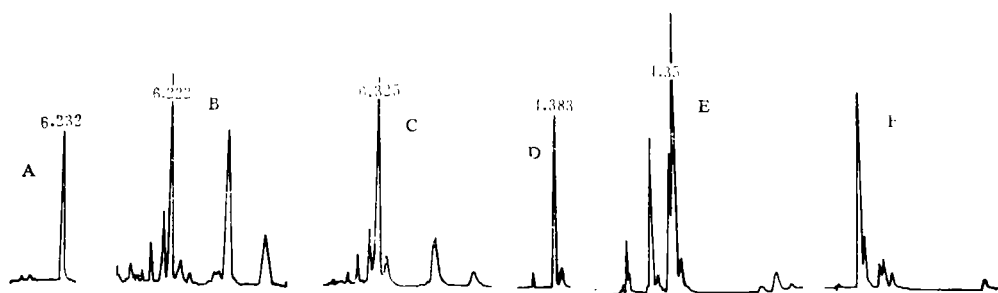


图 HPLC色谱图

A-Pd-Ia对照品 B-白花前胡根 C-白花前胡茎叶 D-Pd-C-I对照品 E-紫花前胡根 F-紫花前胡茎叶

取不同量的Pd-Ia对照液进样,测得Pd-Ia在 $0.02 \sim 0.08 \mu\text{g}$ 范围内峰面积与进样量(μg)的回归方程为: $A = -1.581 \times 10^3 + 2.690 \times 10^3 C$, $r = 0.9990$ 。据此对白花前胡茎叶中Pd-Ia进行含量测定,其含量与白花前胡根中Pd-Ia含量比较,结果见表。

5 精密度

白花前胡茎叶系列试液各进样 $1 \mu\text{l}$,测得其Pd-Ia含量,RSD为 5.26% ($n = 5$)。

6 稳定性

Pd-Ia及Pd-C-I对照品溶液放置30d后测得峰面积稳定。

7 回收率

取 2 ml Pd-Ia系列对照液分别加入白花前胡茎叶细粉中吹干,加入 5 ml 氯仿回流2次,每次 1 h ,提取液吹干后加甲醇定容于 2 ml 容量瓶中,经 $0.20 \mu\text{m}$ 微孔滤膜过滤,吸取滤液 $1 \mu\text{l}$ 进样,测得平均回收率为 99.1% ,RSD为 0.51% ($n = 5$)。

8 讨论

8.1 白花前胡根中的特征性成分为角型二氢吡喃香豆素类化合物,尤以Pd-Ia具有显著的钙离子拮抗活性,可代表前胡的功效^[2,3]。紫花前胡根中的特征性成分为线型二氢吡喃香豆素类化合物^[2,3],以Pd-C-I为其代表成分,故分析比较白花前胡根和茎叶以及紫花前胡根和茎叶的化学成分,测定茎叶中有效成分的含量,可为寻找前胡的代用品,扩大药用植物资源提供科学依据。

8.2 实验结果表明,白花前胡茎叶和根具有相似的化学成分,茎叶中有效成分Pd-Ia的含量比其根中还要高,这提示白花前胡茎叶有可能成为白花前胡根的代用品,而紫花前胡茎叶和根的化学成分相差较大,茎叶中特征成分Pd-C-I的含量也很低,故紫花前胡茎叶不能代替紫花前胡根使用。

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- 2 Shibata S, et al. Abstracts of Chinese Medicines, 1989, 3: 214
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ABSTRACTS OF ORIGINAL ARTICLES

Studies on the Chemical Constituents of Pedate

Pinellia (*Pinellia pedatisecta*)

Qin Wenjuan, Ma Libin, et al

From the alkaloid extraction of rhizoma of *Pinellia pedatisecta* schott, seven compounds were isolated and identified by chemical and spectroscopic methods. They are L-prolyl-L-alanine anhydride (XXV), 3-acetamino-2-piperidone (XXVI), adenosine (XXVII), L-phenylalanyl-L-seryl anhydride (XXVIII), L-tyrosyl-L-alanine anhydride (XXIX), pedatisectine D (XXX), pedatisectine E (XXXI). All above compounds were obtained from this plant for the first time, among which XXX and XXXI are new compounds.

(Original article on page 3)

Studies on the Glucoside Constituents of Shengengzhangyacai

(*Swertia elongata*)

Kong Deyun, Jiang Yi, Yao Ying, et al

Swertia elongata S. W. Lioa dt T. N. He (*Gentianaceae*) has been found to be effective in the treatment of liver disease. The present investigation resulted in the isolation and structure elucidation of four xanthone glycoside, two secoiridoid glycosides and a lignan glycoside in the plant. According to the chemical transformation, spectral (UV, IR, ^1H and ^{13}C NMR, MS) properties and comparison with reference samples, the structures of four xanthone glycosides were established as, 4- β -D-glucopyranosyl-1, 3, 6, 7-tetrahydroxyxanthone (VII), 2- β -D-glucopyranosyl-1,3,6, 7-tetrahydroxyxanthone (VI), 8-O- β -D-glucopyranosyl-1, 3, 5-trihydroxyxanthone (IV) and 8-O- β -D-glucopyranosyl-1, 5-dihydroxy-3-methoxyxanthone (V). The structures of two secoiridoid glycosides were identified as swertiamarin (II) and desacetylcentapicrin (I). The structure of lignan glycoside was identified as (+) hydroxypinoresinol-1- β -D-glucoside (III).

(Original article on page 7)

Studies on the Active Constituents and Their Contents of stem and Leaf of Qianhu by RP-HPLC

Li Yi, Kong Lingyi

Chemical constituents of the aerial parts of Qianhu were compared with those present in the roots by RP-HPLC. It was found that the constituents in aerial parts of *Peucedanum praeruptorum* are similar and higher in content than that in the roots. So it is possible that the aerial parts can be used instead of the roots of the plant. But the chemical constituents in the aerial parts and the roots of *P. decursivum* are quite different, rendering it impossible to use the aerial parts instead of the roots of the plant.

(Original article on page 11)

Studies on Processing Adhesive Rehmannia (*Rehmannia glutinose*)

I. Extraction, Separation, Identification and Assay of 5-Hydroxymethyl-Furfural

Liu Mei li, Bai Mei, Bai Rongzhi, et al

In the study on Processing of Dihuang, a traditional Chinese drug composed of the