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# 树脂法提取纯化白藜芦醇的新工艺研究

徐月灿\*,马德新

湖南希尔天然药业有限公司,永州 425000

**摘要:**对虎杖中的白藜芦醇先酶解再提取,提取液采用一种新树脂进行纯化分离。考察了酶解物、不同树脂、上柱量、洗脱量等因素对白藜芦醇提取纯化的影响,确立了白藜芦醇的最佳提取纯化条件:用酿酒曲于35~40℃将原药材先酶解24 h,再用75%乙醇提取,提取液经回收乙醇后上XC-7大孔树脂柱,先加10%乙醇洗脱,然后再用60%乙醇洗脱并收集洗脱液,浓缩,真空干燥即可,产品中白藜芦醇含量达85%,白藜芦醇转化率达85%以上。

**关键词:**白藜芦醇;虎杖;酶解;大孔树脂

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## A New Method Developed for the Extraction and Purification of Resveratrol

XU Yue-can\*, MA De-xin

Hill Pharmaceutical Co., Ltd., Hunan Yongzhou 425000, China

**Abstract:** In this study, resveratrol was extracted from the fermented giant knotweed. A new resin was applied to purify the extracted solution. The influencing factors of resveratrol extraction and purification results, including enzyme, resin, sample amount used for column chromatography and elution volume were investigated. The optimal conditions were determined to be: enzymolysis of raw material with distiller's yeast for 24 h under 35~40 °C, extract with 75% ethanol, purify with XC-7 macroporous resin column chromatography, use 10% to 60% ethanol to elute. After concentrating, vacuum drying, the content of resveratrol in product reached 85%. The conversion rate of resveratrol was hence higher than 85%.

**Key words:** resveratrol; giant knotweed; enzymolysis; macroporous resin

白藜芦醇(resveratrol, Res.) ,化学名称为3,4',5-三羟基-二苯乙烯(3,4',5-trihydroxystilbene)。其别名为虎杖甙元,又称芪三酚,分子式为C<sub>14</sub>H<sub>12</sub>O<sub>3</sub>,其相对分子质量为228.25,纯品为无色针状结晶,熔点为256~258℃,在261℃以上易升华。其药理作用为:抗菌、抗氧化、预防心脏病、抗癌、抗血小板凝聚、保护肝脏、雌激素作用、防辐射、免疫调节、抗艾滋病活性等<sup>[1]</sup>。目前,白藜芦醇的市场竞争非常激烈,提取纯化白藜芦醇的方法很多,但都采用先酶解再有机溶剂萃取或者柱层析提纯工艺<sup>[2,3]</sup>,生产成本高,产品收率低,不适用于工业化大生产。为了解决提取白藜芦醇工艺中存在的上述问题,提高市场的竞争力,该文采用树脂<sup>[4]</sup>法从虎杖中提取高含量的白藜芦醇,工艺简单,生产周期短,生产成本低,更适合于工业化大生产。

## 1 材料与仪器

### 1.1 实验材料

虎杖原药材购自湖北恩施;白藜芦醇对照品购自中国药品生物制品检定所;各种大孔树脂购自西安蓝晓科技有限公司;酿酒曲购自安琪酵母股份有限公司;其余试剂均为分析纯。

### 1.2 实验仪器

玻璃树脂柱尺寸:20 mm×420 mm(自制);安捷伦高效液相色谱仪(HPLC,美国安捷伦公司);N-1001旋转蒸发仪(上海爱郎仪器有限公司);真空干燥箱(上海新苗医疗器械制造有限公司);四孔水浴锅(金坛市大地自动化仪器厂);AG135分析电子天平(梅特勒托利多仪器有限公司)。

## 2 实验方法

### 2.1 白藜芦醇的提取纯化

取200 g原料粗粉,加入1 g酿酒曲,再加入750 mL水充分摇匀,置于35~40℃水浴中浸泡过夜,接

近24 h后,将水酶解液放下来,然后将药渣加75%乙醇回流提取,提取三次,每次1 h,合并三次提取液,经回收乙醇,浓缩至500 mL,离心过滤后,滤液上已处理好的XC-7大孔树脂柱,柱体积100 mL,上完药液后,用3倍量树脂体积的10%乙醇洗脱,最后用5倍量树脂体积的60%乙醇洗脱,收集60%乙醇洗脱液,经回收乙醇减压浓缩至稠膏,真空干燥即得产品。

## 2.2 HPLC检测白藜芦醇含量

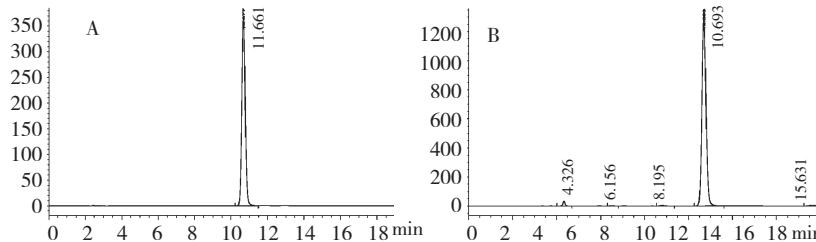


图1 白藜芦醇标准品(A)及白藜芦醇样品(B)的HPLC色谱图

Fig. 1 HPLC chromatograms of resveratrol standard (A) and resveratrol sample (B)

### 2.2.3 线性关系考察

精密吸取上述对照品溶液2、4、6、8和10 μL,注入液相色谱仪。按上述色谱条件测定,以对照品峰面积Y为纵坐标,以对照品进样量X为横坐标,绘制标准曲线,得回归方程 $y = 238722x - 4597$ , $r = 0.9997$ 。线性范围为白藜芦醇0.03~0.15 μg。

### 2.2.4 精密度实验

精密吸取对照品溶液,按上述色谱条件,同法连续测定6次,测得白藜芦醇峰面积的RSD为0.94%( $n=6$ ),表明仪器精密度良好。

### 2.2.5 稳定性实验

取同一白藜芦醇供试品溶液,按上述检测条件,分别于0、4、8和12 h时间间隔进行测定,分别测定峰面积,计算得白藜芦醇峰面积的RSD为1.12%,结果表明供试品溶液在12 h内稳定。

### 2.2.6 加样回收率实验

分别称取已知白藜芦醇含量的供试样品共6份,照供试品溶液制备,分别加入白藜芦醇对照品溶液4 mL,测定结果平均回收率为101.23%,RSD为1.15%( $n=6$ )。

### 2.2.7 重复性实验

取同一批次的虎杖原药材粗粉,分为5份,按2.1项下方法制备供试品溶液共5份,并在上述色谱条件下进行测定,测定结果为样品中白藜芦醇含量平均值为85%,RSD为0.95%,结果表明方法重

### 2.2.1 色谱条件

色谱柱为ODS C<sub>18</sub>柱;流动相为乙腈-水(25:75),流速1.0 mL/min,检测波长307 nm,进样量为10 μL。

### 2.2.2 系统适用性实验

分别取对照品溶液、供试品溶液,按上述色谱条件分别进样,如图1,理论板数按白藜芦醇峰计不低于3000。

复性较好。

### 2.2.8 对照品溶液的制备

取白藜芦醇对照品适量,精密称定,加稀乙醇制成每1 mL含15 μg的溶液,即得。

### 2.2.9 供试品溶液的制备

精密称取按2.1方法试验下来的产品5 mg,精密加入稀乙醇50 mL,超声溶解,滤过,取续滤液,即得。

### 2.2.10 样品测定

分别精密吸取对照品溶液与供试品溶液各10 μL,注入液相色谱仪,测定,即得。

## 3 结果与讨论

### 3.1 酶解物的选择

不同酶解物对虎杖原药材进行对比酶解实验,结果表明,在24 h内酶解最完全的为酿酒曲,见表1。

### 3.2 纯化白藜芦醇的不同树脂实验

不同树脂对虎杖提取药液中白藜芦醇的除杂纯化比较,结果表明,XC-7大孔吸附树脂对白藜芦醇纯化最好,纯化的白藜芦醇含量远远超过其它树脂的纯化结果。结果见表2。

### 3.3 XC-7大孔树脂对白藜芦醇的动态吸附

准确量取XC-7大孔树脂100 mL,湿法装柱(Φ2 cm × 50 cm),用95%乙醇浸泡24 h,并加水淋

表 1 不同酶解物酶解情况

Table 1 Enzymolysis results with different enzyme substrate used

酶解物 Enzyme substrate	酶解时间 Enzymolysis time (h)	酶解温度 Enzymolysis temperature	酶解后原药材 中虎杖苷含量 Content of giant knotweed glycosides in raw material after enzymolysis	酶解后原药材中 白藜芦醇含量 Content of resveratrol in raw material after enzymolysis
酵母 Yeast	24	38 ℃	0.25%	1.12%
酿酒曲 Distiller's yeast	24	38 ℃	0.03%	1.23%
纤维素酶 Cellulase	24	38 ℃	0.85%	0.82%
植物水解酶 Plant hydrolysis enzyme	24	38 ℃	0.54%	0.96%

表 2 不同树脂对白藜芦醇纯化结果

Table 2 Resveratrol purification results with different resins

树脂型号 Resin model	药液量 Solution quantity	树脂体积 Resin volume	上柱流速 Flow rate	白藜芦醇含量 Resveratrol content
D-101	500 mL	100 mL	2BV/h	28.25%
AB-8	500 mL	100 mL	2BV/h	32.17%
XC-7	500 mL	100 mL	2BV/h	85.58%
LX-17	500 mL	100 mL	2BV/h	20.46%

洗至无醇味。将白藜芦醇质量浓度为 4.48 mg/mL 的样液,以 10 mL/min 上样,同时控制洗脱液体积流量 10 mL/min,每 100 mL 接一管,进行 HPLC 法检测,结果见图 2。当上样量达到 800 mL 时,流出液中白藜芦醇的质量浓度与样液中的一致,表明此大孔树脂的吸附已经达到饱和。计算得出此大孔树脂对白藜芦醇的动态吸附量为 22.4 mg/mL 湿树脂。

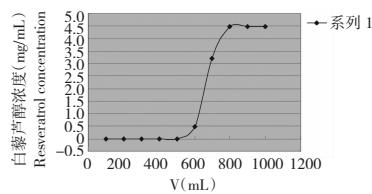


图 2 XC-7 大孔树脂对白藜芦醇的动态吸附曲线

Fig. 2 Dynamic adsorption curve of resveratrol on XC-7 macroporous resin

### 3.4 白藜芦醇的洗脱

为研究乙醇对白藜芦醇的洗脱性能,取白藜芦醇质量浓度为 4.48 mg/mL 的提取液 500 mL 进行大孔树脂分离,树脂柱规格同 3.3 项下的。分别用 10%、30%、50%、60%、70% 乙醇各 3BV 洗脱,洗脱流速为 10 mL/min,每 100 mL 接一管,进行 HPLC 法检测,结果见图 3。300 ~ 600 mL 洗脱液为 30% 乙醇,600 ~ 900 mL 洗脱液为 50% 乙醇,900 ~ 1200

mL 洗脱液为 60% 乙醇,可见白藜芦醇主要被 30% ~ 60% 乙醇洗脱。

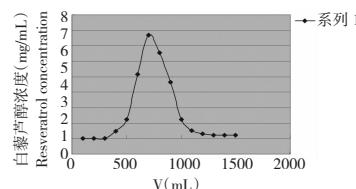


图 3 乙醇对 XC-7 大孔树脂上的白藜芦醇洗脱

Fig. 3 Elution of resveratrol on XC-7 macroporous resin with ethanol

### 4 结论

先将原药材酶解,再利用树脂对白藜芦醇的吸附,只需简单设备几步就可以将白藜芦醇含量提高到 85% 以上,与传统的萃取重结晶纯化工艺相比,该法具有工艺简单易行,操作安全方便,产品收率高,生产成本低的特点。

树脂法从虎杖中提取高含量白藜芦醇的最佳工艺:用酿酒曲于 35 ~ 40 ℃ 将原药材先酶解 24 h,再用 75% 乙醇提取,提取液经回收乙醇后上 XC-7 大孔树脂柱,先加 10% 乙醇洗脱,然后再用 60% 乙醇洗脱并收集洗脱液,浓缩,真空干燥即可,产品中白藜芦醇含量达 85%,白藜芦醇转化率达 85% 以上。

## 参考文献

- 1 Zeng CZ(曾超珍), Liu ZX(刘志祥), Wu YH(吴耀辉), et al. Research developments in extraction technology and determination of resveratrol from *Polygonum cuspidatum*. *Lishizhen Med Mater Med Res*(时珍国医国药), 2007, 18:2992-2993.
- 2 Deng MR(邓梦茹), Liu S(刘韶), Zhu ZJ(朱周觐). Optimization of resveratrol from *Polygonum cuspidatum* by enzyme-assisted extraction. *Central South Pharm*(中南药学),

2011, 9:699-672.

- 3 Xing H(邢花), Tan Q(谭琴). Development research in resveratrol extraction and purification and its biological activity. *Culinary Sci J Yangzhou Univ*(扬州大学烹饪学报), 2011, 28(2):57-60.
- 4 Wang H(王辉), Dong RS(董悦生), Qin JQ(秦建全), et al. Isolation and purification of resveratrol from fermentation broth of *Polygonum cuspidatum*. *Chin Tradit Herb Drugs*(中草药), 2010, 41:223-227.

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- 32 Peng J, et al. Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: metabolism and bioactivities relevant to human health. *Mar Drugs*, 2011, 9:1806-1828.
- 33 Xia S, et al. Production, characterization, and antioxidant activity of fucoxanthin from the marine diatom *Odontella aurita*. *Mar Drugs*, 2013, 11:2667-2681.
- 34 Rosa AP, et al. Effect of canthaxanthin on the productive and reproductive performance of broiler breeders. *Poultry Sci*, 2012, 3:660-666.
- 35 Gradelet S, et al. Dietary carotenoids inhibit aflatoxin B1-induced liver preneoplastic foci and DANN damage in the rat: role of the modulation of aflatoxin B1 metabolism. *Carcinogenesis*, 1998, 19:403-411.
- 36 Baker RTM. Canthaxanthin in aquafeed application: is there any risk? *Trends Food Sci Tech*, 2002, 12:240-243.
- 37 Sujak A. Exceptional molecular organization of canthaxanthin in lipid membranes. *Acta Biochim Pol*, 2012, 59:31-33.
- 38 Fu H, et al. Evaluation of antioxidant activities of principal carotenoids available in water spinach (*Ipomoea aquatica*). *J Food Compos Anal*, 2011, 24:288-297.
- 39 Pasquet V, et al. Antiproliferative activity of violaxanthin isolated from bioguided fractionation of *Dunaliella tertiolecta* extracts. *Mar Drugs*, 2011, 9:819-831.
- 40 Soontornchaiboon W, et al. Anti-inflammatory effects of violaxanthin isolated from microalga *Chlorella ellipsoidea* in RAW 264.7 macrophages. *Biol Pharm Bull*, 2012, 35:1137-1144.
- 41 Viskari PJ, Colyer CL. Rapid extraction of phycobiliproteins from cultured cyanobacteria samples. *Anal Biochem*, 2003, 319:263-271.
- 42 Bermejo Roman R, et al. Recovery of pure B-phycoerythrin from the microalga *Porphyridium cruentum*. *J Biotechnol*, 2002, 93:73-85.
- 43 Takaichi S. Carotenoids in algae: distributions, biosyntheses and functions. *Mar Drugs*, 2011, 9:1101-1118.
- 44 Pereira H, et al. Polyunsaturated fatty acids of marine macroalgae: Potential for nutritional and pharmaceutical applications. *Mar Drugs*, 2012, 10:1920-1935.
- 45 Hu C, et al. Variation of lipid and fatty acid compositions of the marine microalga *Pavlova viridis* (Prymnesiophyceae) under laboratory and outdoor culture conditions. *World J Microbiol Biotechnol*, 2008, 24:1209-1214.
- 46 Zhang CW, et al. An industrial-size flat plate glass reactor for mass production of *Nannochloropsis* sp. (Eustigmatophyceae). *Aquaculture*, 2001, 195:35-49.
- 47 Vazhappilly R, Chen F. Eicosapentaenoic acid and docosahexaenoic acid production potential of microalgae and their heterotrophic growth. *J Am Oil Chem Soc*, 1998, 75:393-397.
- 48 Jiang Y, et al. Fatty acid composition and squalene content of the marine microalga *Schizochytrium mangrove*. *J Agr Food Chem*, 2004, 52:1196-1200.
- 49 Kusaikin MI, et al. Structural characteristics and antitumor activity of a new chrysolinan from the diatom alga *Synechidra acus*. *Chem Nat Compd*, 2010, 46:1-4.
- 50 Arad SM, Levy-Ontman O. Red microalgal cell-wall polysaccharides: biotechnological aspects. *Curr Opin Biotechnol*, 2010, 21:358-364.
- 51 Apt KE, Behrens PW. Commercial developments in microalgal biotechnology. *J Phycol*, 1999, 35:215-226.
- 52 Faulkner DJ. Marine natural products. *Nat Prod Rep*, 1998, 16:113-158.
- 53 Morsy N, et al. Isolation and structure elucidation of a new amphidinol with a truncated polyhydroxyl chain from *Amphidinium klebsii*. *Tetrahedron*, 2005, 61:8606-8610.
- 54 Bhakuni DS, Rawat DS. Bioactive Marine Natural Products. New York: Springer, 2005.
- 55 Wright JLL, et al. Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from Eastern Prince Edward Island. *Can J Chem*, 1989, 67:481-490.