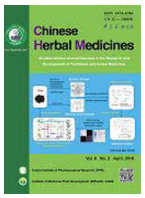

Available online at SciVarse ScienceDirect

Chinese Herbal Medicines (CHM)

ISSN 1674-6384

Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

Original article

Enzyme-assisted Extraction and Enrichment of Galanthamine from *Lycoris aurea*

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ARTICLE INFO

Article history

Received: November 21, 2015

Revised: January 5, 2016

Accepted: February 11, 2016

Available online:

April 8, 2016

DOI:

10.1016/S1674-6384(16)60028-X

ABSTRACT

Objective To explore the optimum condition for complex enzyme-assisted extraction of galanthamine from *Lycoris aurea* by $L_9(3^4)$ orthogonal array design and separation effect of cation exchange resin on galanthamine. **Methods** Cellulase and pectinase solution was used as the extraction solvent. The effects of pH value of enzyme, amount of complex enzyme, dissociation time, and enzymatic hydrolysis temperature on the extraction results were investigated. **Results** The optimal conditions were obtained as follows: ratio of solid to liquid (g: mL) 1:10, pH value 4.5, amount of complex enzymes 4%, enzymatic hydrolysis temperature 50 °C, and reaction time 2.0 h. Under these conditions, the extraction yield of galanthamine was 0.0294%. In addition, D-001 cation exchange resin was selected for separation of galanthamine. The separation conditions were that adsorption flow rate was 3 BV/h with reagent of pH 2 and the desorption flow rate was 3 BV/h with 70% ethanol solution containing 1.5 mol/L ammonia. After separation, the content of galanthamine was increased to 12.31%. **Conclusion** The results provide a reference for industrial production of galanthamine.

Key words

cation exchange resin; enzyme-assisted extraction; galanthamine; *Lycoris aurea*; separation

1. Introduction

Lycoris aurea (L'Hér.) Herb., also called golden spider lily, is a perennial plant in the Amaryllidaceae family and found in eastern Asia, mainly Korea, eastern and southern China, and Japan (Kang et al, 2012). Most plants in Amaryllidaceae are known to produce unique alkaloids with diverse bioactivities, such as acetylcholinesterase inhibitory, analgesic, antibacterial, antifungal, antimalarial, antitumor, antiviral activities, and so on (Wu et al, 2014). Especially, galanthamine exhibited the significant benefits in cognition behavior in rats, mice, and rabbits with cognitive deficits, which has been proven for the symptomatic treatment of Alzheimer's disease (Tsvetkova et al, 2014; Marco et al, 2006). Some extraction methods have been developed to obtain alkaloids from the plants of Amaryllidaceae family, such as solvent extraction, ultrasonic extraction, microwave extraction, supercritical fluid extraction, and so on (Li et al, 2008; Fan et al, 2006; Wang et al, 2013; Xiao et al, 2005; Du et al, 2007). However, due to these methods also existed some

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Funds: Hunan Provincial Science and Technology Department (2012TP4002-3); Zhangjiajie Science and Technology Bureau (2014YB17)

disadvantages like massive organic solvent consumption, high cost, while low yield, in recent years, enzyme-assisted extraction has attracted more attention relying on its advantages of efficiency, mild-condition, and pollution-free. According to Puri et al (2012), carbohydrate- hydrolyzing enzymes like cellulases, hemicellulases, and pectinases disrupt cell wall with the hydrolysis of its components are leading to a major permeability and allowing an easier release of the metabolites from plants. Until now, there is no related report on the utilization of carbohydrate-hydrolyzing enzymes in the extraction of galanthamine from *L. aurea*. We had also tried to utilize cation exchange resin for enhancing the separation efficiency of galanthamine. However, this technique has never been applied to galanthamine isolation.

In the present study, in order to improve the extraction rate of galanthamine and develop a greener extraction method, the enzyme-assisted extraction method was investigated for reaction and extraction variables, such as enzyme types, enzyme ratio, reaction temperature, pH value, time, and the solid to liquid ratio. In addition, the separation of galanthamine by cation exchange resin was also studied.

2. Materials and methods

2.1 Materials and instruments

The bulbs of *L. aurea* were collected from Huaihua City, Hunan province, China. The botanical authentication was carried out by researcher Bo-ru Liao, plant taxonomist at Jishou University.

Galanthamine was obtained from National Institute for Food and Drug Control with purity > 98%. The cellulase (2×10^4 U/g) was purchased from Baimai Green Bio-Energy Co., Ltd. (Huaian City, China). The hemicellulase (1×10^4 U/g) and pectinase (3×10^4 U/g) were from Ruji Biotech. Co., Ltd. (Shanghai, China). Cation exchange resins 732 (001 × 7), HD−8, HZ−008, D155, D001, and D152 were obtained from Zhengzhou Diligent Technology Limited (Zhengzhou, China). All organic solvents were of analytical grade, except for HPLC (Tedia, America).

HPLC (Agilent 1260, America) is used for the determination of galanthamine. Vacuum Freeze Dryer FD5-3 (SIM, America) is applied for drying.

2.2 Methods

2.2.1 Selection of enzyme types

The bulbs of *L. aurea* were washed, dried, and ground. For enzyme-assisted extraction, bulb powders were suspended in water (pH 4.5) with the solid to liquid ratio at 1:10. Later on, 3% of enzymatic preparations, such as cellulase, hemicellulase, pectinase, mixture of cellulase and hemicellulase, mixture of cellulase and pectinase, mixture of hemicellulase and pectinase, and mixture of these three enzymes were added to the bulb powders respectively. Then the samples were cultured at 50 °C for 2 h. Afterward, the cultured solution was centrifuged at 5000 r/min for 20 min. The supernatant was transferred into 50 mL volumetric flask and diluted to scale with water (pH 4.5). Obtained solutions were analyzed for their galanthamine content with HPLC after filtered with membrane (0.45 μm). All processes were repeated for three times.

2.2.2 Single factor experiment

The mixed enzymes of cellulase and pectinase were investigated. Reaction variables of enzyme to substrate ratio from 0−5%, reaction solution pH from 1.5 to 7.5, reaction temperature from 20 to 80 °C, and reaction time from 0.5 to 2.5 h were selected for single factor test, in addition, the solid to liquid ratio from 1:5 to 1:25 was also studied. All the determinations were made by triplicate and the means were reported.

2.2.3 Orthogonal test

The orthogonal test was carried out based on single factor experiment. Four variables of enzyme to substrate ratio, reaction pH, temperature, and time were investigated together as per L_9 (3^4) orthogonal arrays.

2.2.4 HPLC analysis on galanthamine in L. aurea

HPLC analysis on samples before and after the enzymatic treatment was performed to identify the galanthamine releasing during the enzyme-assisted extraction from *L. aurea*. The sample (20 μL) were injected into HPLC equipped with Kromasil ODS column (250 mm × 4.6 mm, 5 μm), in a mobile phase of 17% acetonitrile and 83% phosphate buffer, and monitored at 290 nm under 35 °C. Phosphate buffer was prepared by dissolving 2.72 g of monopotassium phosphate into 100 mL water added with 1.4 mL of triethylamine, diluting to a volume of 1 L with water. The pH value of phosphate buffer was 7.02.

2.2.5 Establishment of galanthamine standard curve

A galanthamine reference solution with the concentration of 1.0 mg/mL was prepared. Then 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 mL of this reference solution were transferred into 10 mL volumetric flask, respectively to obtain a series of diluted reference solutions with concentration of 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, and 0.5 mg/mL. After filtrated (0.45 μm), these solutions were injected into HPLC for galanthamine content analysis. The regression curve was plotted with content of galanthamine (x) against peak area (y) and the regression equation was $y = 1038x + 39\ 348$, $r = 0.9996$.

2.2.6 Calculation of galanthamine extraction rate

The extraction rate of galanthamine was calculated using the formula $y = cv / m_0$, Wherein c was the concentration of galanthamine. v was the final volume of extraction solution. m_0 was the weight of bulb powders.

2.2.7 Selection of cation exchange resins and optimization of chromatographic condition

The separation effect of two types of cation exchange resin, including strong acid cation exchange resins [732 (001 × 7), HD-8, HZ-008, and D001] and weak acid cation