

Study on the Separation and Preparation Technique of Phillyrin

Maoxun Yang^{1,a}, Bin Zhao¹, Qiaoli Lv², Wan Xie³ and Yaoguang Liang¹

¹Zhongshan Torch Polytechnic, Zhongshan 528436, China;

²Zhongshan Green-health Biotechnology Development CO., LTD, Zhongshan 528403, China;

³Zhongshan Yunqu Middle School, Zhongshan 528451, China

^agreenhealthbio@163.com

Abstract. Through the single factor experiment, this paper explore the best sample amount of phillyrin separation and preparation, using a combination method of positive phase silica gel chromatography and sephadex LH-20, in order to provide a reference for preparation of industrialization. According to the experimental results, comprehensive best quality and purity of phillyrin could be available when the sample amount was about 3 g.

Keywords: Phillyrin, Separation and preparation, Sample amount.

1. Introduction

Fructus forsythiae, dried fruit of Oleaceae plants Forsythiae suspense (Thunb.) Vahl, has antibacterial, anti-inflammatory, antipyretic, antiviral, weight loss, antidepressant, antioxidation, antiallergic, antitumor, neuroprotection, vasorelaxant and other pharmacological effects [1-10], is commonly used in the treatment of traditional Chinese medicine, distributed in Shandong, Shanxi, Henan and other regions [1].

In recent years, the gradual increase of the demand for fructus forsythiae extract and gradually strengthened control on natural products at home and abroad, accrete the demand for relevant reference material. As one of the main effective component in fructus forsythiae, phillyrin is quite harsh in its separation and preparation because of easy hydrolysis, large polarity, and so on. This paper explore the best sample amount of phillyrin separation and preparation, using a combination method of positive phase silica gel chromatography and sephadex LH-20, in order to provide a reference for preparation of industrialization.

2. Materials and Method

2.1 Experimental Materials.

Fructus forsythiae extract was from Fangsheng Biological Development Co., Ltd in Baoji City. The content of phillyrin was 2.04%, analyzed by HPLC.

2.2 Reagents and Instruments.

Standard substance of phillyrin (National Institute for the Control of Pharmaceutical and Biological Products), Chemical reagents were analytically pure, Silica gel (100–200 mesh) was purchased from Qingdao Ocean Chemical Co. (Qing-dao, China), High performance liquid chromatography (HPLC) grade acetonitrile was from Merck Chemical Co. (Darmstadt, Germany).

An LC-10Avp liquid chromatography (HPLC) system used was equipped with a CTO-10ASvp column oven, a manual sample injection valve (model 7725) with a 20- μ L loop, and an SPD-10Avp ultraviolet detector (Shimadzu, Kyoto, Japan), YMC-Pack ODS-A columns (5 μ m, 250 \times 4.6 mm I.D.) for analytic purposes (YMC, Kyoto, Japan).

2.3 Methods

2.3.1 Separation and enrichment of Fructus forsythiae extract

Fructus forsythiae extract (1.0 kg) were separated three times using methanol with the ratio of material to solvent of 1:10 for 30 min under ultrasonic. The combined layers of each organic solvent

were evaporated in vacuo to yield a phillyrin extract 896.5 g. The purity of phillyrin reach 17.89% (Fig.1) after enrichment by positive phase silica gel chromatography separation.

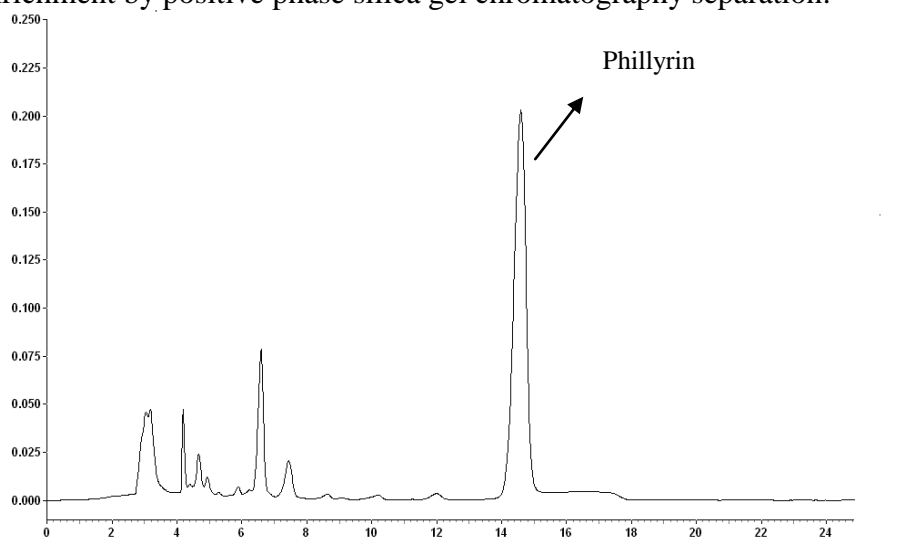


Fig. 1 HPLC chromatogram of Fructus forsythiae extract

The extract was further subjected to sephadex LH-20 using a isocratic elution, with chloroform/ MeOH (1:1, V/V) and for further purification.

2.3.2 Comparison of weight and purity of phillyrin by different sample amount

Single factor experiment with 1 g, 3 g, 5 g three levels of different sample amount was set to investigate the effect on weight and purity of phillyrin by different sample amount, using sephadex LH-20, each level set on the sample amount is 3 times repetition.

3. Result and discussion

The experimental result of effect on weight and purity of phillyrin by different sample amount can be seen in Table 1.

Table 1 Comparison of weight and purity of phillyrin by different sample amount

No	Sample Amount (g)	Weight (g)	Purity (%)
1	5.03	0.23	87.78
2	3.15	0.27	93.68
3	1.02	0.14	94.08

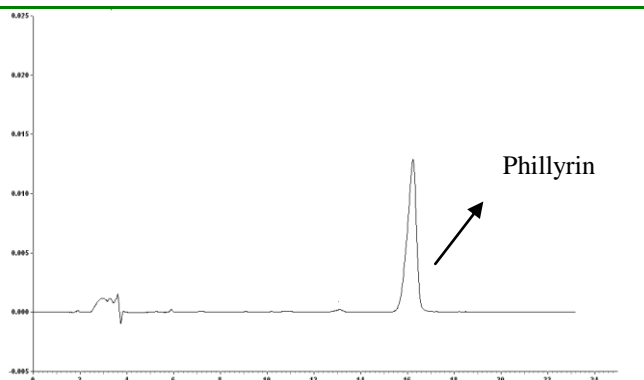


Fig. 2 HPLC chromatogram of pure Phillyrin with sample amount of 3.15g

From the above data, when average sample amount was 1.02g, relatively high purity, 94.08% of phillyrin was yielded with relatively low weight of 0.14g; When average sample amount was 5.03g, relatively high weight of phillyrin was yielded with relatively low purity; When average sample amount was 3.15g, high purity with high weight of phillyrin was yielded. Based on the analysis of the above, comprehensive best quality and purity of phillyrin could be available when the sample amount was about 3 g.

HPLC chromatogram of pure Phillyrin with sample amount of 3.15g can be seen in Fig. 2.

Acknowledgements

National Key Higher Vocational Colleges Construction Project “(Zhongshan Torch Polytechnic) ‘Biopharmaceutical technology Specialty and Specialty Group and professional group’ Construction Project”.

References

- [1] H.Y. Zhang: Research progress on chemical component and pharmacological action of Forsythia suspensa, Journal of Traditional Chinese Medicinal Materials, Vol. 23(2000) No.10, p.657-660.
- [2] G.Y.Wu: Research progress on pharmacological action of Forsythia suspensa, China Journal of Chinese Medicine, Vol. 28(2013) No. 185, p.1508-1509.
- [3] T. Lu, X. L. Piao, Q.Zhang, et al: Protective effects of Forsythia suspensa extract against oxidative stress induced by diquat in rats, Food Chem Toxicol, (2010) No. 48, p.764.
- [4] H. Qu, Y. Zhang, Y. Wang, et al.: Antioxidant and antibacterial activity of two compounds (forsythiaside and forsythin) isolated from Forsythia suspensa, J Pharm Pharmacol, Vol. 60 (2008) No. 2, p.261-266.
- [5] Y. Hao, D. F. Li, X. L. Piao, et al.: Forsythia suspensa extract alleviates hypersensitivity induced by soybean β -conglycinin in weaned piglets, J Ethnopharmacol, (2010) No.128, p.412.
- [6] H.M. Wang, L.W. Wang, X.M. Liu, et al.: Neuroprotective effects of forsythiaside on learning and memory deficits in senescence-accelerated mouse prone (SAM P8) mice, Pharmacol Biochem Behav, Vol. 105(2013) No.7, p.134-141.
- [7] J.M. Kim, S. Kim, D.H. Kim, et al.: Neuroprotective effect of forsythiaside against transient cerebral global ischemia in gerbil, Eur J Pharmacol, Vol.660(2011) No. 2-3, p.326-333.
- [8] T.Iizuka, M. Nagai: Vasorelaxant effects of forsythiaside from the fruits of Forsythia suspensa, Yakugaku Zasshi, Vol.125 (2005) No. 2, p.219-224.
- [9] Y.Lecrubier, G. Clerc, R.Didi, et al.: Efficacy of St. John's wort extract WS 5570 in major depression: a double-blind, placebo-controlled trial, Am J Psychiatry, (2002) No. 159: p.1361-1366.
- [10] T.Lu, X.L.Piao, Q.Zhang, et al.: Protective effects of Forsythia suspensa extract against oxidative stress induced by diquat in rats, Food Chem Toxicol, (2010) No. 48, p.764.