JOURNAL OF FOOD AND DRUG ANALYSIS XXX (2017) 1–7

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

# Convenient isolation of strictinin-rich tea polyphenol from Chinese green tea extract by zirconium phosphate

Yilong Ma [a,*], Yafang Shang [a], Fengru Liu [a], Wenqing Zhang [b], Caihong Wang [a], Danye Zhu [a]

[a] Department of Chemical Engineering and Food Processing, Hefei University of Technology, Xuancheng Campus, Xuancheng, PR China

[b] School of Food Science and Engineering, Hefei University of Technology, Hefei Campus, Hefei, PR China

ARTICLE INFO

*Article history:*
Received 18 August 2016
Received in revised form
28 October 2016
Accepted 2 November 2016
Available online xxx

*Keywords:*
green tea
separation
strictinin
zirconium phosphate

ABSTRACT

Zirconium phosphate (ZrP) was prepared and employed to separate strictinin-rich tea polyphenol from Chinese green tea extracts. The influences of ZrP calcination temperatures, green tea extraction conditions, and the amounts of ZrP on the isolation of strictinin-rich tea polyphenol were evaluated; the absorption and desorption dynamics of strictinin on ZrP were also determined. Our results revealed that the HPLC content of strictinin increased from 4.96% in 70% ethanol extract of green tea to 58.2% in isolated strictinin-rich tea polyphenol obtained by ZrP-900 (ZrP calcined at 900°C). Furthermore, the suitable time for both strictinin absorption and desorption was 4 hours at 37°C. The method developed here consisted of easy steps such as ZrP absorption, water washing, and 0.4% phosphoric acid solution desorption, which may facilitate the detection and isolation of strictinin from different samples.



## 1. Introduction

Green tea produced from the buds of the *Camellia Sinensis* is one of the most popular beverages around the world [1]. Due to the abundance of phenolic compounds (known as tea polyphenol), green tea has plentiful health functions, including antioxidant, antiallergic, and antiviral effects [2–4]. Among these functions, tea polyphenol has received increasing attention because of its antiviral effects [5–7]. Strictinin (Figure 1), an important polyphenol found in green tea and other plants [8–10], has been proved to show special antiviral effect on influenza virus [11,12], making it a potential functional food additive. However, strictinin is one of the minor tea polyphenol in green tea [11], and the extremely low content may greatly limit its antiviral effect. Thus, increasing the content of strictinin in tea polyphenol is of great importance. Traditional methods to obtain strictinin-rich tea polyphenol include fresh silica gel

* *Corresponding author*. Department of Chemical Engineering and Food Processing, Hefei University of Technology, Xuancheng Campus, Xuancheng 242000, PR China.
E-mail address: yilong.ma@hfut.edu.cn (Y. Ma).

http://dx.doi.org/10.1016/j.jfda.2016.11.013

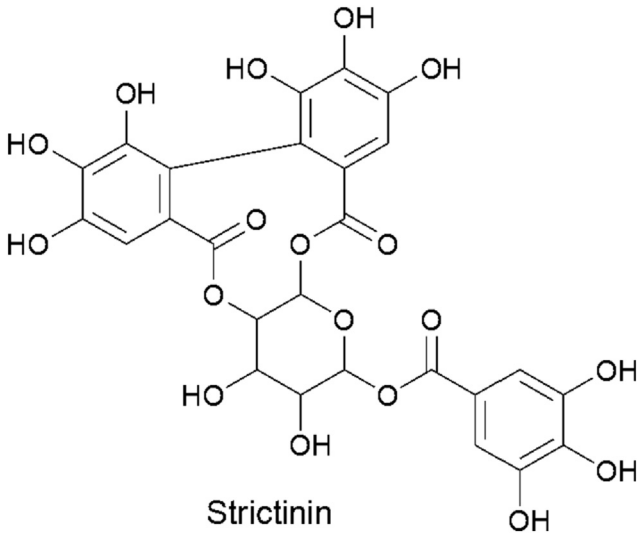


**Figure 1 – Chemical structure of strictinin.**

chromatography and high-performance liquid chromatography (HPLC) [11,13], either of which is suffered from limitations as organic solvent consuming or instrument dependent. Therefore, more facile and green method should be developed to produce strictinin-rich tea polyphenol.

Zirconium phosphate (ZrP), one of the layered materials with acidic property, has been widely applied in catalysis, ion exchange, and adsorption [14–16]. Because of its nanoscaled structure and the positive charged zirconium (IV), ZrP and their analogs (e.g., zirconium silicate) were recently proved to be special absorbents for various phenolic compounds, including 2-chlorophenol from waste water [17], galloyl- and caffeoylquinic acids from *Galphimia glauca and Arnicae flos* [18], and 5-O-galloylquinic acid (a polyphenol with leishmanicidal activity) from green tea (unpublished data, Figure S1). And the latter application provides a new way to separate bioactive phenolic compounds from various plants. Similar to 5-O-galloylquinic acid, the structure of strictinin contains several galloyl groups; therefore, we assume that ZrP with special structures may be a selective absorbent for separation of strictinin-rich tea polyphenol.

In this study, strictinin-rich tea polyphenol was conveniently isolated from green tea extracts by ZrP. The effects of material calcination temperatures, green tea extraction conditions, and material amounts on strictinin-rich tea polyphenol isolation were evaluated; the adsorption and desorption dynamics of strictinin on ZrP were also determined. A facile method for isolation of strictinin-rich tea polyphenol from green tea extract was developed.

## 2. Materials and methods

### 2.1. Materials

Green tea was bought from the local tea market (Xuancheng, China), and the standard compounds such as 5-O-galloylquinic acid, caffeine, strictinin, epigallocatechin gallate (EGCG), and epicatechin gallate (ECG) were purchased from Nanjing Spring & Autumn Biological Engineering Co., Ltd. (Nanjing, China). Syringe filters (0.45 μm, 13 mm) were supplied by Pall (Beijing, China), and deionized water was obtained from a water purifier system (Milli-Q, Millipore Corp., MA, USA). All chemicals and solvents were of analytical or HPLC grade.

### 2.2. Synthesis of ZrP

ZrP were prepared by direct precipitation method. In a typical synthesis, zirconium oxychloride octahydrate (1.79 g) was dissolved in 100 mL deionized water, followed by adding of concentrated phosphoric acid (85%, 0.76 mL) drop wise in 15 minutes, and the resultant solution was stirred at room temperature for another 2 hours. The white precipitate was then obtained and thoroughly washed with deionized water and ethanol by centrifugation. The precipitate was dried at 80°C in an oven for 12 hours and subsequently calcined at 500°C for 1 hour to give ZrP, which was named ZrP-500. ZrP dried or calcined under various temperatures were denoted as ZrP-n (n: temperature); they were called ZrP-80, ZrP-400, ZrP-600, ZrP-700, ZrP-800, and ZrP-900, according to the various temperatures. The structures of the materials were then characterized by X-ray diffraction (XRD; DX-2700B, Haoyuan Instrument Co., LTD, Dandong, China) or Fourier-transform infrared spectroscopy (FT-IR; Cary630, Agilent Technologies, CT, USA). XRD were determined in the 2θ range of 10° to 80° with Cu Kα radiation; the FT-IR spectrum was recorded in the range 400–4000 $cm^{-1}$ using a potassium bromide technique.

### 2.3. Preparation of green tea extracts

The ground green tea was extracted with ethanol–water mixtures at 60°C for 1 hour in an ultrasonic cleaner (KQ50B, Kunshan Ultrasonic Instrument Co., China). In a typical extraction, ground green tea (1 g) was mixed with 70% ethanol (ethanol/water, v/v, 10 mL) and sonicated for 1 hour. The resultant solution was separated using a 0.45-μm filter and further analyzed with HPLC or liquid chromatography-mass spectrometry (LC-MS).

### 2.4. Isolation of strictinin-rich tea polyphenol

The general procedures for isolation of strictinin-rich tea polyphenol are as follows: the mixtures of ZrP and green tea extracts were shaken for several hours on a shaker (150 rpm, 37°C); after the removal of supernatants by centrifugation, different ZrP were washed with deionized water (10 mL × 5) and subsequently mixed with the phosphoric acid solution (0.4% in water, v/v, 1 mL); the mixtures were shaken for several hours; finally, different ZrP were removed by centrifugation, and desorption solutions were obtained. In a typical separation, the ratio of ZrP and green tea extract was 1:10 (0.1 g: 1 mL), and the shaking time for each section was 24 hours. To study the adsorption/desorption dynamics of strictinin on ZrP, the supernatants from each section were extracted at different time points. For the recovery study of strictinin, the final supernatants after adsorption or desorption were collected. Each experiment was repeated three times, and the corresponding samples were analyzed by HPLC. The structure of the separated strictinin was further confirmed by nuclear magnetic resonance spectroscopy

Please cite this article in press as: Ma Y, et al., Convenient isolation of strictinin-rich tea polyphenol from Chinese green tea extract by zirconium phosphate, Journal of Food and Drug Analysis (2017), http://dx.doi.org/10.1016/j.jfda.2016.11.013