Journal of Multidisciplinary Engineering Science and Technology (JMEST)
ISSN: 2458-9403
Vol. 3 Issue 5, May - 2016

Validated High Performance Liquid Chromatography Method for Quantification of a Major Saponin in *Polyscias fruticosa*

Tran Van Thai, Tran Thi Hong Hanh, Nguyen Tien Dat
Institute of Marine Biochemistry (IMBC), Vietnam Academy of Science and Technology (VAST), 18-Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

*Corresponding Author: ngtiend@imbc.vast.vn

Nguyen Hai Dang
Advanced Center for Bio-organic Chemistry, IMBC, VAST,

Dao Thi Thanh Hien
Hanoi University of Pharmacy, 15-Le Thanh Tong, Hoan Kiem, Hanoi, Vietnam

Ho Luong Nhat Vinh
Thai Nguyen Medical College, Thinh Dan, Thai Nguyen, Vietnam

***Abstract*—A method, high-performance liquid chromatography coupled with diode array detector (HPLC-DAD), was developed to quantification of the major saponin 3-O-[β-D-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl] oleanolic acid 28-O-β-D-glucopyranosyl ester (PFS) in *Polyscias fruticosa* samples. The analysis was performed on a Zorbax Eclipse XDB C18 column, in which the mobile phase consisted of 2% acetonitrile in 0.1% aqueous acetic acid. The calibration curve showed good linear regression (r2 0.9996) within test range. The limit of detection and limit of quantification were 2.5 and 7.0 µg/mL. This method was successfully applied to quantify PFS in *P. fruticosa* leaves and roots. The present paper is the first report for the quality control of *P. fruticosa* via determination of its main active component.**

***Keywords*—Polyscias fruticosa; HPLC-DAD; saponin**

I. INTRODUCTION

Polyscias fruticosa (L.) Harms (syn. *Nothopanax fruticosus* (L.) Miq., *Panax fruticosus* L.), belonging to the family Araliaceae, is widely used in Vietnam as a tonic agent for the treatment of ischemia and inflammation, and to increase blood flow in the brain [1]. Previous studies showed that *P. fruticosa* contained oleanolic acid saponins as main constituents [2]. The saponin 3-O-[β-D-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl] oleanolic acid 28-O-β-D-glucopyranosyl ester (PFS, Figure 1) is a major compound isolated from the leaves and roots of *P. fruticosa* [2, 3]. This compound showed strong inhibitory activity against α-amylase and α-glucosidase and has been considered as a promising antihyperglycemic agent [3, 4]. Therefore, PFS can be used as a marker for quality control of *P. fruticosa*. It has reported that various chromatographic methods such as high performance thin-layer chromatography (HPTLC), high performance liquid chromatography (HPLC), capillary electrophoresis (CE)… can be used for analyze saponin in plant extract [5, 6]. In this study, we developed and validated an HPLC-DAD method for the quantification of PFS in *P. fruticosa* leaves and roots.

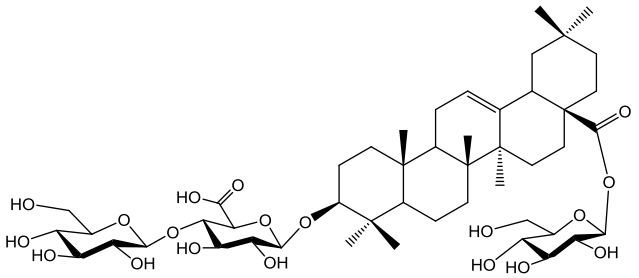

Fig. 1. Structure of 3-O-[β-D-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl] oleanolic acid 28-O-β-D-glucopyranosyl ester

II. MATERIALS AND METHODS

A. Chemicals and instruments

HPLC grade acetonitrile, water and analytical grade acetic acid were purchased from Merck. The HPLC analysis was performed using an Agilent 1260 Series (Agilent Technology, USA). 3-O-[β-D-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl] oleanolic acid 28-O-β-D-glucopyranosyl ester (PFS, 98% purity by HPLC) was isolated and purified as previously described [3].

B. Preparation of standard and sample solutions

Stock solutions of PFS were prepared by dissolving the compound in methanol at 1 mg/ml. The stock solution was further diluted to make different concentration ranges, which were filtered through a 0.45 µm membrane filter before HPLC analysis.

Five grams of dried and powdered *P. fruticosa* leaves or roots were extracted with 25 ml methanol three times in a sonic bath for 10 min. The combined extracts were concentrated under reduced pressure and adjusted to a volume of 50 ml and then filtered through a 0.45 µm membrane filter before injected to the HPLC system.

C. HPLC analysis

HPLC separation was carried out in a Zorbax Eclipse XDB C18 column (4.6 x 250 mm, 5 μm) with a C18 guard column maintained at 24 ºC. The elution was performed with a 30 min. isocratic 2% acetonitrile in water containing 0.1% acetic acid at flow rate of 0.5 ml/min. The injection volume was of 5 μL. The DAD acquisition wavelength for PSF was set at 205 nm, respectively.

D. Calibration curves, limits of detection and quantification

The calibration curve was performed with five appropriate concentrations of PFS. The regression equation was calculated in the form of y = ax + b, where y and x were peak area and compound concentration. The dilute solution of the reference compound was further diluted to a series of concentrations with methanol for the gain of the limits of detection (LOD) and quantification (LOQ). The LOD and LOQ under the present chromatographic conditions were determined at a signal-to-noise (S/N) ratio of 3 and 10, respectively.

III. RESULTS AND DISCUSIONS

A. Optimization of chromatographic conditions

The chromatographic conditions were optimized to obtain chromatograms with good resolution. For stationary phase, various HPLC columns including Zorbax Eclipse XDB C18 column (4.6 x 150 mm, 5 μm), Zorbax 300SB-C18 (4.6 x 250 mm, 3.5 μm), Zorbax Extend-C18 (4.6 x 250 mm, 5 μm), and YMC-Pack ODS-A 4.6 x 150 mm, 5 μm) were tested. The preferred chromatographic separation was achieved using a Zorbax Eclipse XDB C18 column (4.6 x 250 mm, 5 μm).

The mobile phase optimization was done in terms of stability of retention time, as well as achieving correct order of elution and good baseline separation. Various mixtures of water and acetonitrile in combination with several acids including trifluoroacetic acid, phosphoric acid, and acetic acid were tested. As the results, an isocratic 2% acetonitrile in water provided good separation. The addition of 0.1% acetic acid in water increased the resolution of the peaks, whereas the addition of other acids resulted in unsatisfying resolution and peak broadening.

With the selected conditions, PFS was well separated and detected at the retention time of 16.6 min (Figure 2).

B. Method validation

In this study, linearity of peak area was investigated with the PFS concentrations ranging from 10 up to 300 μg/mL. Each concentration of each standard solution set was injected into HPLC in triplicates. The obtained calibration curve was linear in relatively wide range of amounts and all showed good linear regressions with high correlation coefficient value (r^2 0.9996) between the peak area and amount. The equation of the regression line was y = 30214.1x – 33.5. The limit of detection (LOD) and limit of quantification (LOQ) were determined by analyzing decreasing concentrations of PFS standard spiked in blank sample. Thus LOD and LOQ for PFS were 2.5 and 7.0 μg/mL, respectively.

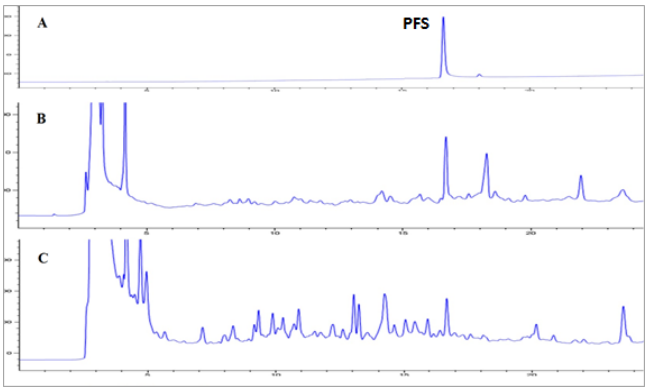

Fig. 2. HPLC chromatograms of PFS (A), extract of *P. fruticosa* leaves (B) and roots (C).

C. Extraction method development

In order to achieve quantitative extraction, variables involved in the procedure such as solvent, extraction method and extraction time were optimized. The extracts were checked by HPLC analysis for detection of PFS level. Pure or aqueous methanol and ethanol solutions were tried as the extraction solvents, the best solvent was found to be methanol. Compared to the refluxing extraction and Soxhlet extraction, the ultrasonic treatment was found to be the best extraction method due to its simplicity and rapidity. In order to investigate extraction time, the extraction was tested for 5, 10, 15, and 20 min, respectively. The results indicated that PFS was almost completely extracted after 3 times with 10 min each.

D. Quantifcation of PFS in P. fruticosa samples

The established HPLC method was applied for quantification of PFS in P. fruticosa leaves and roots (Figure 2). The result showed that PFS content was highest in the leaves (1300 μg/g dry weight). In the root parts, PFS content (570 μg/g) was 2.28 times lower than in the leaves.

IV. CONCLUSIONS

It is evident from previous reports that the main saponin 3-O-[β-D-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl] oleanolic acid 28-O-β-D-glucopyranosyl ester (PFS) is responsible for the biological activities of *Polyscias fruticosa*. In this study, HPLC–DAD conditions were optimized for the quantitative and qualitative determination of PFS in *P. fruticosa.* The method was validated for good linearity and limit of detection. The proposed method makes it possible to evaluate the quality of *P. fruticosa* material as well as related products.

ACKNOWLEDGMENT

This work is supported by a grant of the Vietnam Academy of Science and Technology (VAST.DLT.06/14-15).

REFERENCES

[1] Do TL. *Vietnamese Medicinal Plants and Remedies. Hanoi*. Medicine Publisher, p. 828-830 (2000).
[2] Vo DH, Yamamura S, Ohtani K, Kasai R, Yamasaki K, Nguyen TN, Hoang MC. Oleanane saponins from Polyscias fruticose. Phytochemistry, 47, 451-457 (1998).
[3] Tran Thi Hong Hanh, Nguyen Hai Dang, Nguyen Tien Dat. α-Amylase and α-glucosidase inhibitory saponins from *Polyscias fruticosa* leaves. J. Chem. 2016, article ID 2082946.
[4] Guo T, Wu S, Guo S, Bai L, Liu Q, Bai N. Synthesis and evaluation of a series of oleanolic acid saponins as α-glucosidase and α-amylase inhibitors. Archiv der Pharmazie, 348(9), 615-628 (2015).
[5] Liang XM, Jin Y, Wang YP, Jin GW, Fu Q, Xiao YS. Qualitative and quantitative analysis in quality control of traditional Chinese medicines. Journal of Chromatography A, 1216(11), 2033-2044 (2009).
[6] Xie P, Chen S, Liang YZ, Wang X, Tian R, Upton R. Chromatographic fingerprint analysis--a rational approach for quality assessment of traditional Chinese herbal medicine. Journal of Chromatography A, 1112(1-2), 171-80 (2006).