BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902023e21570

Article

Study on the effect of processing methods on the total polyphenol, 2,3,5,4′-tetrahydroxystilben-2-O-*β*-D-glucoside, and physcion contents in *Fallopia multiflora* Thunb. Haraldson root

Nguyen Thi Hong Thanh[1,2], Nguyen Thi Hong Tham[3], Tran Dinh Thang[4], Nguyen Thuy Chinh[5,6], Nguyen Dinh Luyen[1], Bui Thi Thuy Luyen[1,*]

[1] Department of Pharmaceutical Industry, Hanoi University of Pharmacy, Hoan Kiem, Ha Noi, Vietnam, [2]Vinh Medical University, Vinh, Nghe An, Vietnam, [3]Vietnam Military Medical University, Phuc La, Ha Dong, Ha Noi, Vietnam, [4]Ho Chi Minh University of Technology, Binh Thanh, Ho Chi Minh, Vietnam, [5]Institute for Tropical Technology, Vietnam Academy of Science and Technology, Cau Giay, Ha Noi, Vietnam, [6]Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Cau Giay, Ha Noi, Vietnam

This study investigated the changes in the ingredients in *Fallopia multiflora* Thunb. Haraldson (FMT) root after processing it with different methods such as soaking, stewing, and steaming or combined methods. The total polyphenol, 2,3,5,4′-tetrahydroxystilben-2-O-*β*-D-glucoside (THSG), and physcion contents in FMT products after processing were determined using high-performance liquid chromatography (HPLC) and ultraviolet-visible (UV-VIS) methods. The results demonstrated that the processing method and time significantly affected the contents of polyphenol, THSG, and physcion. The physcion and total polyphenol content increased or decreased during processing depending upon the processing time, while the THSG content gradually decreased with an increase in the processing time. The content of physcion (a substance that can cause liver toxicity) was analysed, and the suitable conditions for processing of the FMT products were determined as initial soaking in rice swill for 24 h and subsequent stewing with black beans and water for 12 h.

Keywords. *Fallopia multiflora* (Thunb.). THSG. Total polyphenol content. Physcion.

INTRODUCTION

Fallopia multiflora Thunb. Haraldson (synonym *Polygonum multiflorum*, FMT) is a herbal medicine that belongs to the Polygonaceae family, which has been widely used in Asia for centuries. According to traditional medicine, FMT root has various beneficial effects such as nourishment of blood, toning of kidney, healing of marrow, strengthening of bones and muscles, blackening of hair, treatment of neurasthenic, anaemia, dizziness, tinnitus, back pain, knee fatigue, somnolence, and early grey hair, and ensuring longevity. Modern pharmacological research has indicated that FMT root can prevent hair loss and premature greying, enhance anti-oxidation, anti-inflammatory, and anti-aging abilities, protect nerve cells, and improve memory and immunity (Li *et al*., 2017; Lin *et al*., 2015; Sun *et al*., 2013; Yu *et al*., 2011; Bounda, Feng, 2015).

FMT root is used in two forms, raw and processed FMT root. It has been used as a drug and food supplement. However, a few cases have reported that FMT root can cause toxicity when it is used directly (Dong *et al*., 2014; Jung *et al*., 2011; Wu *et al*., 2012). Additionally, FMT can cause liver damage. However, its toxic constituents and mechanism of liver toxicity have not been studied in detail (Dong *et al*., 2015; *Lei et al*., 2015; Li *et al*., 2017; Yun *et al*., 2019). A few studies have reported that stilbene glucoside

***Correspondence:** B. T. T. Luyen. Department of Pharmaceutical Industry. Hanoi University of Pharmacy. 13-15 Le Thanh Tong, Hoan Kiem District, Hanoi, Vietnam, 100000**.** Phone: (+84)96 1555 180. E-mail: luyenbtt@hup.edu.vn. ORCID: https://orcid.org/0000-0003-4298-1997

and anthraquinones are toxic to liver cells (Lv, *et al*., 2015; Ma, *et al*., 2015). A correlation between the processing time, content of substances, and liver toxicity of the processed FMT products was analysed. Ruo-Lan Li *et al.* reported that the hepatotoxicity caused by FMT was significantly decreased when FMT root was processed for 72 h. Stilbene glycoside might cause liver damage. It was confirmed that processed FMT products lead to lower liver toxicity than that of raw FMT (Li *et al*., 2020). Therefore, processing of FMT is crucial to increase its safe consumption. Yun-Xia Li *et al.* reported a correlation between the liver damage and dose of FMT (Li *et al*., 2017). Tests were performed on rats, and it was observed that raw FMT caused liver toxicity at a dose of 20 and 40 g/kg, and the toxicity was decreased at a dose of 10 g/kg. It was recommended that a high dose of FMT should not be consumed.

FMT contains multiple phenolic compounds such as 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside (THSG), physcion, emodin, and chrysophanol. THSG is the most valuable constituent because it enhances biological activities such as anti-inflammatory ability (Zeng *et al*., 2011; Wang *et al*., 2008; Zhang *et al*., 2007), anti-oxidation ability (Christian *et al*., 2015), anti-aging ability (Ling, Xu, 2016), enhancement of hair growth, reduction of premature grey hair (Han *et al*., 2015), prevention of insomnia (Qian *et al*., 2017), and limitation of hypopigmentation (Jiang *et al*., 2009). The content of the major chemical constituents in FMT were determined by HPLC-PAD-MS method. It was observed that the content of THSG, emodin-8-O-β-D-glucoside, physcion-8-O-β-D-glucoside emodin, and physcion were 35.75, 2.86, 0.64, 0.22, and 0.13 mg/g, respectively (Yi *et al*., 2007). It can be observed that a few studies have been conducted on the quantification of polyphenol (TP), THSG, and physcion contents in processed FMT products. Processing is necessary to eliminate or reduce the toxicity and side-effects, and modify the nature and action to enhance the therapeutic effects of the crude herb. Methods should be developed to control the quality of these products and affirm the effectiveness and safety of processed products (Tao *et al*., 2019). Moreover, the study on the effect of processing methods (soaking, stewing, and steaming methods) on the composition of processed FMT products has not been systematically performed. Therefore, the effect of the FMT processing methods on the contents of TP, THSG, physcion was investigated using high-performance liquid chromatography (HPLC) and ultraviolet-visible (UV-Vis) methods. The evaluation of the quality of processed FMT products was performed based on the results, which provided a basis for further studies on liver toxicity of processed FMT products.

Additionally, various factors such as geographical origin, harvesting, and processing can affect the quality of herbal materials, which can result in different pharmacological effects. Suitable climate and soil characteristics can ensure biodiversity and are potential factors for the development of medicinal herbs. Therefore, FMT roots were obtained from Ky Son district, Pu Hoat Nature Reserve, and Nghe An province. Vietnam is a crucial location for this study because it is characterized by Bazan Redland soils and sub-temperate region. Additionally, it is recognized by UNESCO as a natural biosphere reserve.

MATERIAL AND METHODS

Chemicals and Reagents

Acetonitrile, ethanol, methanol, and formic acid of HPLC grade were purchased from Merck (Germany). The standards consisting of 2,3,5,4'-tetrahydroxystilben-2-O-β-D-glucoside (THSG), physcion, gallic acid, and Folin-Ciocalteau reagent were purchased from Sigma-Aldrich (USA). Biobasic was procured from Canada. The chemicals had a purity higher than 98 %. The remaining chemicals used were of analytical reagent grade.

Plant materials

FMT roots were collected from Ky Son district, Pu Hoat Nature Reserve, and Nghe An province (Vietnam) (refer Figure 1). The average weight of fresh roots was approximately 98.6 – 200.0 g. The roots had a reddish-brown colour and were undamaged. The FMT roots were initially washed with clean water and cut into small pieces (thickness of 2 – 3 mm) before they were dried in an oven at 60^{o}C (until the moisture of the samples was lower than 12 %). Subsequently, the samples were packaged in vacuum conditions and stored at 25±0.1^{o}C.

FIGURE 1 - FMT tree and roots A: FMT tree, B: FMT roots, C: Cross section of FMT roots.

Processing FMT roots

Ruo-Lan Li *et al.* confirmed that the therapeutic effect of FMT can be enhanced after processing with black bean decoction. Moreover, the toxicity of FMT can be considerably decreased after processing with black bean decoction (Li *et al.*, 2020; Vietnamese Pharmacopoeia V, 2018). Therefore, the processing of FMT roots has been prepared by referring to Vietnamese Pharmacopoeia V and Li *et al.* with slight modifications. Initially, 50 g of black beans was cooked with 4 L of clean water for 3 h to obtain the black bean decoction for the test. The ratio of black beans: FMT root was 1:1 (wt/wt). Subsequently, 10 samples of FMT roots (50 g per each) were added into a 500 mL glass beaker. The FMT roots were processed using 10 different methods and were correspondingly denoted as follows:

P1: FMT roots were steamed in water for 12 h.
P2: FMT roots were steamed in black bean decoction for 12 h.
P3: FMT roots were soaked in rice swill for 12 h and then steamed in black bean decoction for 12 h.
P4: FMT roots were soaked in rice swill for 24 h and then steamed in black bean decoction for 12 h.
P5: FMT roots were soaked in rice swill for 24 h. Subsequently, 50 g of black beans and 200 mL of water was added and the mixture was stewed for 12 h.
T0: FMT roots were soaked in rice swill for 24 h.
T1: FMT roots were soaked in rice swill for 24 h. Subsequently, 50 g of black beans and 200 mL of water was added, and the mixture was stewed for 6 h.
T2: FMT roots were soaked in rice swill for 24 h. Subsequently, 50 g of black beans and 200 mL of water was added, and the mixture was stewed for 12 h (it is prepared using the method similar to that of the P5 sample).
T3: FMT roots were soaked in rice swill for 24 h. Subsequently, 50 g of black beans and 200 mL of water was added, and the mixture was for 18 h.
T4: FMT roots were soaked in rice swill for 24 h. Subsequently, 50 g of black beans and 200 mL of water was added, and the mixture was stewed for 24 h.
T5: FMT roots were soaked in rice swill for 24 h. Subsequently, 50 g of black beans and 200 mL of water was added, and the mixture was stewed for 30 h.

The samples were taken out after soaking in rice swill and dried before steaming or stewing.

The samples were dried at 40°C for 24 h after processing. The humidity in the samples was determined using an ADAM AMB 310 automatic moisture content meter. The moisture requirement of the processed samples was lower than 12 %. Figure 2 presents FMT roots before and after processing.

A B

FIGURE 2 - FMT roots before (A) and after (B) processing.

Preparation of Standard and Sample Solutions

Preparation of standard solutions:

The standards were accurately weighed using a Sartorius GP ISOBP electronic balance (Germany) before dissolving in the suitable solvent in a 20 mL volumetric flask. The physcion were dissolved in methanol 99.9 %, THSG were dissolved in ethanol 50 %, and gallic acid was dissolved in distilled water to prepare stock solutions (250.0 µg/mL physcion solution, 250.0 µg/mL THSG solution, and 1 mg/mL gallic acid solution, respectively). Subsequently, the stock solutions were diluted to different concentration solutions. For example, THSG solution: 25.0, 50.0, 75.0, 100.0, 125.0 µg/mL; physcion solution: 12.5, 25.0, 50.0, 75.0, 100.0 µg/mL, and gallic acid solution: 10.0, 20.0, 30.0, 40.0, 50.0 µg/mL.

Preparation of sample solutions:

The processed FMT products were ground into fine powder using a disintegrator and sieved through a 0.5-mm screen. The samples were accurately weighed (1.0 g/each) before soaking in 20 mL of solvent (ethanol 50 % for the quantification of THSG and methanol 99.9 % for the quantification of physcion) for 60 min at 25±0.1°C. Subsequently, the mixture was ultrasonicated for 60 min at 40^0C. Filtration through a PRP PTFE 0.45-µm membrane filter was performed, and 10 µL of the extract was added to a vial in the HPLC device for analysis (n=3). The sample injection program was set.

Determination of total polyphenol content in raw and processed FMT products using UV analysis

The total polyphenol content (TPC) in raw and processed FMT products was estimated by the Singleton method using Folin-Ciocalteau reagent with a slight modification (Singleton, Rosa, Raventos, 1999; ISO14502-1, 2005). The TPC was expressed as mg of gallic acid equivalents per gram of dry weight (*mg GAE/g DW*) based on a calibration curve of gallic acid. The absorbance was measured at a wavelength of 765 nm using a UV-1900 spectrometer (Shimadzu, Japan).

Determination of THSG and physcion content in raw and processed FMT products using the HPLC method

The analysis of THSG and physcion contents in raw and processed FMT extracts was performed using a Chromaster device (Hitachi, Japan) with DAD detector,

HiQ sil C18HS column (150 x 4.6 mm; 5 µm). The mobile phase consisted of acetonitrile (A) and distilled water containing 0.1 % formic acid (B) with a linear gradient elution at a flow rate of 1.0 mL/min.

THSG content analysis: the gradient program is as follows: 0-5 min, 23 % A; 5 – 10 min, 100 % A; 10 – 18 min, 100 % A; 18 – 20 min, 23 % A. The detection wavelength was 320 nm. The column temperature was maintained at 28°C (Cheng *et al.*, 2013; Liang *et al.*, 2010).

Physcion content analysis: the gradient program is as follows: 0 – 10 min, 44 % A; 10–25 min, 44–82 % A; 25 – 30 min, 82 – 90 % A; 30 – 35 min, 90 %. The detection wavelength was 275 nm. The column temperature was maintained at 30°C (Li *et al.*, 2017; Cheng *et al.*, 2013).

Statistical analysis

The data represented the mean ± S.D. of three independent experiments performed in triplicates. The statistical significance was determined using one-way ANOVA followed by Dunnett's multiple comparison test ($P<0.05$) using the GraphPad Prism 6 program (GraphPad Software Inc., San Diego, CA, USA).

RESULTS AND DISCUSSION

A standard curve for the quantification of total polyphenol, physcion, and THSG content

A standard curve for the quantification of TPC was constructed using the Singleton method (Singleton, Rosa, Raventos, 1999). The calibration equation of gallic acid was y = 9.063x - 0.0129 (y is the optical absorption and x is the concentration of gallic acid), and the regression coefficient (R^2) of this equation was 0.9935 (refer Figure 3A).

The linear regression analysis of physcion and THSG was performed using an HPLC device with a sample concentration in the range of 12.5 – 100.0 µg/mL and 25.0 – 125.0 µg/mL, respectively. The regression equations of physcion and THSG were y = 137060x - 51003, R^2 = 0.998, and y = 144619x + 63089, R^2 = 0.9966, respectively, wherein x is the concentration and y is the peak area of the sample. The value of R^2 was approximated 1 unit corresponding to these regression equations and used for the calculation of physcion and THSG contents in the test samples (refer Figure 3B and 3C).

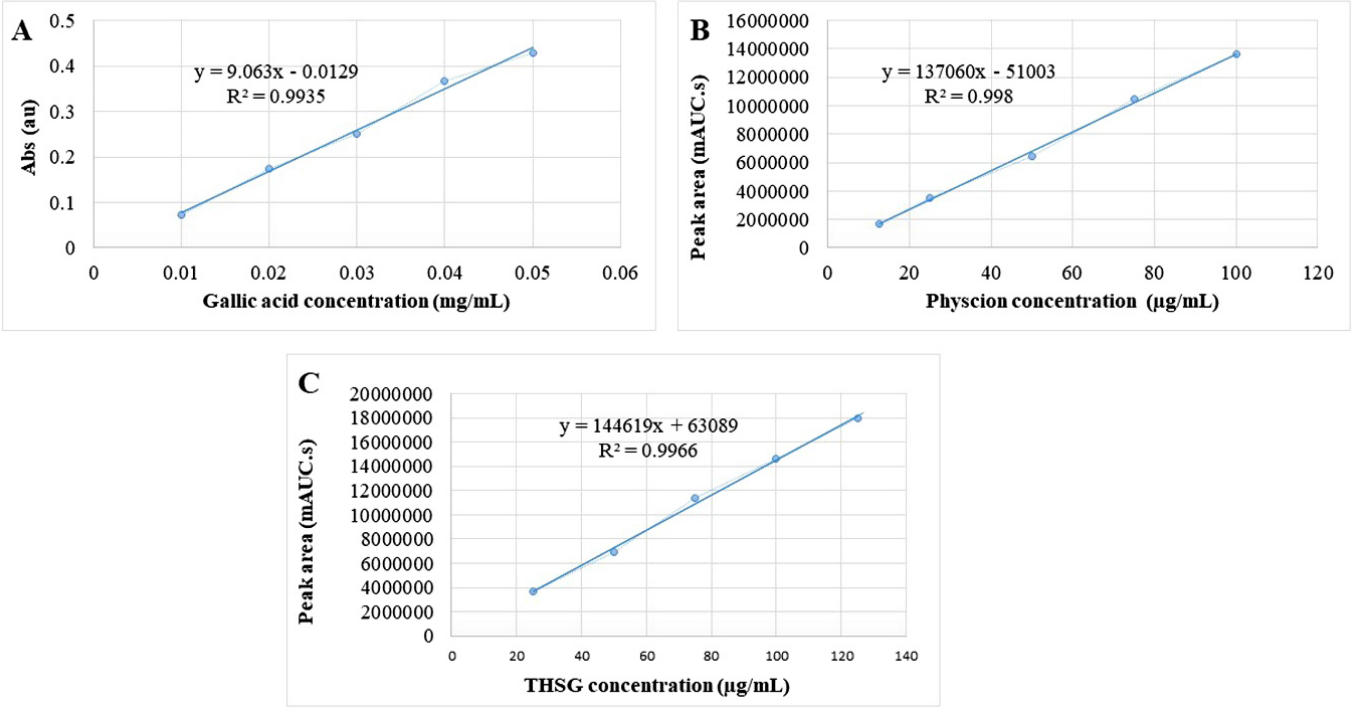

FIGURE 3 - The calibration curve of gallic acid (A), physcion (B), THSG (C).

The validation values for the analysis method of THSG and physcion have been reported in previous studies (Nguyen *et al.*, 2020; Nguyen *et al.*, 2021). The results demonstrated that the retention time in the standard and sample solutions of THSG were 10.053 and 10.013 min, respectively, and that of physcion were 30.653 and 30.980 min, respectively. The system compatibility test exhibited that the relative stand deviation (RSD) of retention time and peak area were 0.39 and 1.61 % for THSG, 0.33 and 0.92 % for physcion, respectively.

The same sample solution was analysed at designated time points in 48 h for stability testing. The results demonstrated that the RSD values of peak area for THSG and physcion were 1.51 and 1.05 %, respectively, which indicated the process for determining THSG and physcion content was stable.

The accuracy testing was performed by the standard addition method. The recovery testing for THSG was performed in the range of 94.59 – 98.33 % with an RSD value of 1.52 %. The recovery testing for physcion was 95.88 % with an RSD value of 1.51 %.

The repeatability test was performed using six sample solutions which were prepared using the similar method. The results demonstrated that the RSD value of peak area of THSG and physcion were 0.59 and 1.12 %, respectively, which indicated the repeatability of this method for the analysis of THSG and physcion.

The effect of processing methods and time on contents of THSG, physcion, and total polyphenols in processed FMT products

The HPLC method has been validated as a suitable method for the quantification of physcion and THSG in the FMT roots (Tao *et al.*, 2019; Nguyen *et al.*, 2020; Nguyen *et al.*, 2021). Therefore, HPLC method was used in this study to determine the physcion and THSG contents in the processed FMT products. UV-Vis method was used to determine TPC in the processed FMT products.

The TP, physcion, and THSG contents in the raw FMT roots were determined with the values of 89.59 ± 0.51, 0.56 ± 0.44, and 40.13 ± 0.009 mg/g, respectively. The TPC in the raw FMT roots in this study was two times higher than that in the FMT roots obtained from the Cao Bang province, Vietnam (38.6 mg/g) (Le, 2020). This result proved the effect of geographical origin on the compositions of material herbs. Multiple previous studies have demonstrated that the environmental conditions affect the production of secondary metabolites, which are important sources of bioactive natural components. The physiological and biochemical reactions are influenced directly by environment factors as they affect the phytochemistry composition (Tian *et al.*, 2008; Xu, Xu, 2009). Nguyen *et al.* (Nguyen *et al.*, 2018) performed the HPLC analysis to determine the content of phenolic compounds such as THSG, physcionin, emodin 8-glucoside, emodin, pleuropyrone A, torachrysone 8-O-*β*-D-glucopyranoside, 6-hydroxymusizin 8-O-*α*-D-apiofuranosyl- (1→6)-*β*-D-glucopyranoside. Additionally, statistical methods were applied to differentiate between natural roots and commercial medicinal slices of FMT. Six samples of 3- to 4-year-old natural FMT roots were obtained from six districts of Ha Giang, which is a northern mountainous province in Vietnam. The samples had different THSG content in the range of 26.211 – 55.010 mg/kg dry weight (Nguyen *et al.*, 2020). Samples of FMT were obtained from ten different locations in China and the correlations between 17 environmental factors and 5 bioactive components (THSG, emodin, emodin-8-O-*β*-D-glucoside, physcion and physcion-8-O-*β*-D-glucoside) were analysed. The results indicated that the highest contents of bioactive components were detected in samples from Deqing, and the lowest in samples from Tianyang (Yan *et al.*, 2010). Therefore, factors such as region, season, soil, moisture, and analysis method significantly affected the content of TP, physcion, and THSG in raw FMT roots.

In this study, the FMT roots were processed by steaming method, soaking combined with steaming method, and soaking combined with stewing method. The effect of processing conditions on THSG, physcion and TP contents was investigated.

The effect of solvents extraction

The FMT roots were processed by stewing in water (sample P1) or black bean decoction (sample P2). The results of TP, THSG, and physcion contents in the sample

P1 were 64.52±0.045, 21.58±0.303, 0.89±0.004 mg/g, respectively and those in the sample P2 were 81.30±0.228, 29.31±0.470, 0.92±0.005 mg/g, respectively (refer Table I). The content of physcion in the two samples was increased owing to the hydrolysis of dianthrone glucosides under high-temperature processing (Bartnik, Faccey, 2017).

TABLE I - TP, THSG, and physcion contents in the FMT products processed at different conditions. Data were represented as mean ± S.D. of three independent experiments performed in triplicates

Method	Content (mg/g)		
	TP	THSG	Physcion
P1	64.52±0.045	21.58±0.303	0.89±0.004
P2	81.30±0.228	29.31±0.470	0.92±0.005
P3	89.13±0.079	25.85±0.368	0.59±0.004
P4	97.63±0.284	24.17±0.271	0.57±0.008
P5	107.33±0.799	20.78±0.061	0.38±0.001

The results in Table I exhibited that the utilization of black bean decoction in the processing of FMT roots changed the contents of TP, THSG, and physcion in the processed FMT products. The THSG, physcion, and TP contents in the products processed with black bean decoction (sample P2) were higher than that processed with water (sample P1). Therefore, black bean decoction was selected for the processing of FMT roots.

According to the previous studies, the contents of stilbene glucoside and free anthraquinones in FMT decreased after processing in addition to five auxiliary materials including rice swill, ginger decoction, licorice decoction, prepared Radix Rehmanniae Preparata decoction, and black bean decoction (Li *et al.*, 2020). Several studies have reported that the toxicity of FMT gradually decreased when black bean decoction was used as an auxiliary material. Additionally, the extent of damage caused to the liver cells was lowest in the case of FMT steamed with black bean decoction for 16 h (Li *et al.* 2020.). Several potential toxic compounds such as emodin-8-O-glucoside and torachrysone-O-hexose were identified based on previous research on FMT. The content of emodin-8-O-glucoside and torachrysone-O-hexose which are of potential toxic ingredients were reduced after processing with black beans (Chen *et al.*, 2021). This indicated that black beans contain chemicals that can reduce the toxic components in FMT roots.

The effect of soaking time in rice swill

FMT roots were soaked in rice swill for different durations (12 h (sample P3), 24 h (sample P4)) before steaming in black bean decoction for 12 h to evaluate the effect of soaking time on the TP, THSG, and physcion contents in processed FMT products. The results are listed in Table I.

The soaking stage of FMT roots in rice swill before steaming affected the content of substances in processed products. The contents of THSG and physcion decreased with an increase in the soaking time of FMT roots in rice swill. The THSG and physcion contents decreased from 25.85 mg/g to 24.17 mg/g and from 0.59 mg/g to 0.57 mg/g, respectively, when FMT roots were soaked in rice swill for 12 and 24 h.

Several potential approaches have been proposed to pre-treat and extract active constituents from medicinal plants. These approaches include pre-soaking, liquid ammonia pre-treatment, and co-digestion. Ammonia-based pre-treatment was the common method used to reduce the lignin content in biomass based on breaking the lignin-carbohydrate ester linkages, depolymerizing

hemicellulose, and cleaving the crystalline region of cellulose. The early removal of lignin during the conversion process has multiple advantages because lignin significantly hinders enzymatic hydrolysis. This method was used widely in the agriculture and production of biofuel from crop residues, forestry residues, and herbaceous species (Zhao *et al*., 2017a; Zhao *et al*., 2017b). However, ammonia pre-treatment has been performed at various operating conditions such as temperature, pressure, solvent, reaction time, whereas pre-soaking is a simpler process. Therefore, the pre-soaking of raw FMT roots with rice swill was investigated to determine the suitable pre-soaking time required for processed products with higher TPC, and lower THSG and physcion contents. In addition, the determination of the water content in FMT roots is crucial to accurately evaluate the characteristics of products. However, this factor has been not extensively studied. The chemical analysis method is extensively used to determine the water content. However, it is time-consuming, labour-intensive, and cannot fulfil the requirements of online analysis (Büning-Pfaue, 2003). Near-infrared spectroscopy is used to determine the moisture in the plants because it is an efficient method, and it can reduce the analysis cost, and provide an efficient analysis platform (Zhang *et al*., 2019).

The reduction in contents of THSG and physcion after processing demonstrated the decrease in the toxicity of FMT roots as mentioned by Wu *et al.* (Wu *et al*., 2012). The TPC of the processed samples was significantly increased after 24 h of soaking in rice swill. Hence, soaking FMT roots for 24 h in rice swill before steaming in black bean decoction was selected for processing FMT roots.

The effect of preparing method

An investigation on the TPC, physcion and THSG contents in the processing FMT roots with the different preparing methods including soaking for 24 h combined with steaming for 12 h (sample P4) and soaking for 24 h combined with stewing for 12 h (sample P5) was performed. The results of TP, THSG, and physcion contents in processed FMT products indicated that the TPC increased from 97.63 to 107.33 mg/g, whereas the THSG and physcion contents decreased from 24.17 to 20.78 mg/g and from 0.57 to 0.38 mg/g, respectively (refer Table I). These results suggested that for FMT roots pre-soaked in rice swill, the next processing step, i.e., stewing with black beans was relatively effective than that of steaming with black bean decoration. This was explained by the difference between the steaming and stewing methods. The stewing processing is a type of cooking process wherein the ingredients are simmered inside a liquid, while steaming processing uses water vapour, heat, or moisture. The direct contact of FMT roots with black bean decoction led to a significant effect than that of indirect contact.

Previous studies have reported that physcion can cause live toxicity (Wu *et al*., 2012; Ma *et al*., 2015). Therefore, soaking combined with stewing method was selected for further studies based on the physcion content in processed products.

The effect of stewing time

The effect of stewing time (0, 6, 12, 18, 24, and 30 h) on TP, THSG, and physcion contents in the processed FMT products is depicted in Figure 4.

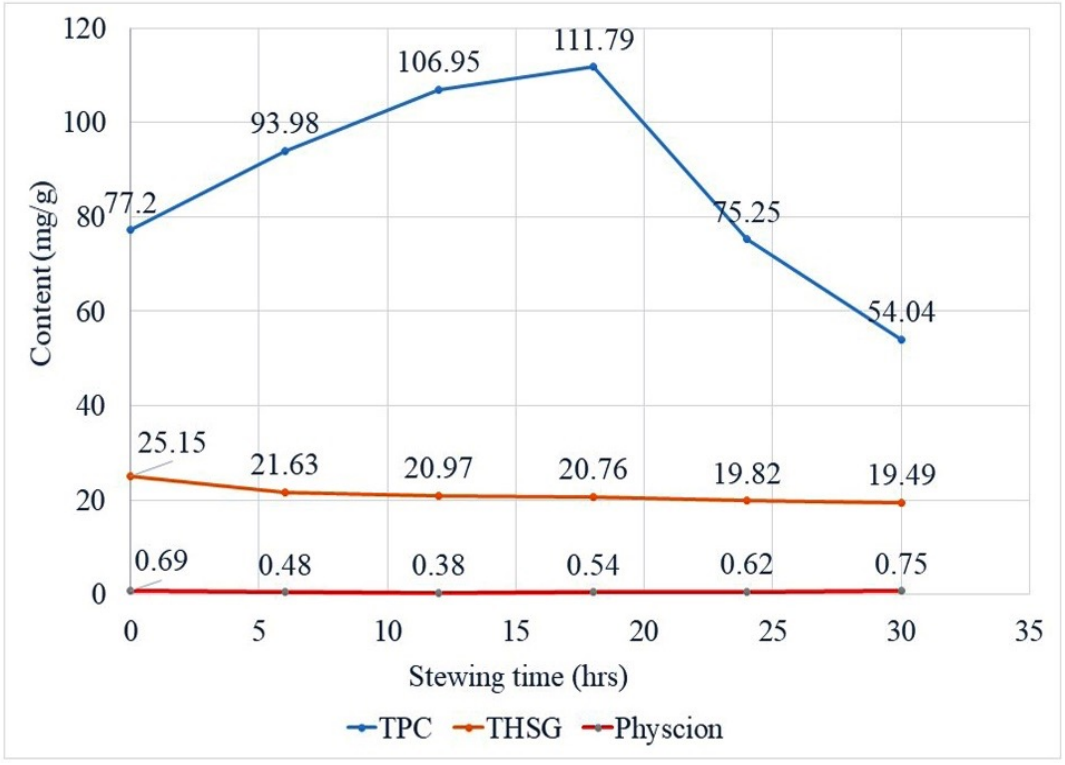

FIGURE 4 - Effect of stewing time on TP, THSG, and physcion contents in the processed FMT products.

The results demonstrated that the TPC in the processed FMT products increased with an increase in the stewing time from 0 to 18 h, and subsequently, TPC was decreased. This was due to the absorption of black bean decoction into FMT slices during the initial stewing hours and rupture of cell wall of FMT, which led to the increase in TPC. However, the polyphenols in FMT become unstable with an increase in the stewing time under high temperature. Therefore, the TPC of the products was decreased.

The content of THSG decreased as a function of stewing time. The THSG content in the processed FMT product was 21.63 and 19.49 mg/g after 6 and 30 h of stewing, respectively, which indicated a decrease of 13.99 % and 22.50 %, respectively, compared with that of the THSG content in the product that was not stewed. This was observed because THSG is a water-soluble active substance (Qian *et al.*, 2020; He *et al.*, 2021). Hence, it was extracted during the processing. Moreover, in the case of high-temperature processing, the active components usually become unstable, which leads to a decrease in their content (Tao *et al.*, 2019).

The change in the physcion content was not systematic with an increase in the stewing time. The physcion content in the products was gradually reduced with an increase in the stewing time during the initial 12 h of processing, and increased in the following hours. This was explained by rupture of the cell wall of herbs under the effect of high temperature during processing, which resulted in the release of physcion from cells (Tao *et al.*, 2019). The results of this study were consistent with that of the reports of Zhitao Liang *et al.* (decreased physiological concentration and increased physcion) and Xiaoqing Wu *et al.* (decreased 55.8 % of THSG, increased 34 % emodin) (Wu *et al.*, 2012; Liang *et al.*, 2010).

Jiang Ma *et al.* reported that the liver toxicity of stilbene glucoside is lower than that of anthraquinones and stilbene glucoside. Stilbene glucoside exhibited lipid-regulating and antioxidant activities, whereas anthraquinones are hepatotoxic substances (Ma *et al.*, 2015). Physcion is an anthraquinone and THSG is a stilbene glucoside. Therefore, these substances might be active and toxic. The suitable processing conditions

selected for further studies were soaking FMT roots in rice swill for 24 h before stewing with black beans and water for 12 h. This was selected because the obtained products had the lowest content of physcion and balance between the active and toxic components.

CONCLUSION

The effect of processing method on the content of active substances in *Fallopia multiflora* (Thunberg) Haraldson (FMT) roots was evaluated. The roots were obtained from Ky Son district and Nghe An province (Vietnam). The FMT roots were steamed in water or black bean decoction, soaked in rice swill, or stewed in black beans with water or these stages were combined for various periods. The decrease in the contents of 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (THSG) and physcion in the processed FMT products can reduce the toxicity of the FMT roots. The suitable processing conditions were selected for processing the FMT roots based on the physcion content. These conditions included soaking the FMT roots in rice swill for 24 h before stewing them with black beans and water for 12 h. The THSG, physcion, and TP contents in the FMT product processed at this condition reached 20.78, 0.38, and 107.33 mg/g, respectively. The processing ensured safe consumption of FMT.

COMPETING INTERESTS

The authors declare that there are no competing interests associated with this article.

REFERENCES

Bounda GA, Feng YU. Review of clinical studies of *Polygonum multiflorum* Thunb. and its isolated bioactive compounds. Pharmacog Res. 2015;7(3):225–236.

Büning-Pfaue H. Analysis of water in food by near infrared spectroscopy. Food Chem. 2003;82(1):107–115.

Chen W, Wang P, Chen H, Xing Y, Liu C, Pan G, et al. The composition differences between small black beans and big black beans from different habitats and its effects on the processing of *Polygonum multiflorum*. Phytochem Anal. 2021;32(5):767-779.

Cheng W, Gao J, Huang Q, Shi B, Zhang W. Simultaneous determination of five active ingredients in Huangshixiangsheng Pills by HPLC. Asian J Chem. 2013;25(14):7735-7737.

Christian B, Zhao L, Havermann S, Honnen S, Fritz G, Proksch P, et al. TSG (2,3,5,4′-tetrahydroxystilben-2-O-β-D-glucoside) from the Chinese Herb *Polygonum multiflorum* increases life span and stress resistance of caenorhabditis elegans. Oxid Med Cell Longev. 2015; Article ID124357.

Dong H, Slain D, Cheng J, Ma W, Liang W. Eighteen cases of liver injury following ingestion of *Polygonum multiflorum*. Complement Ther Med. 2014;22(1):70-74.

Dong Q, Li N, Li Q, Zang C, Feng WW, Li GQ, et al. Screening for biomarkers of liver injury induced by *Polygonum multiflorum*: a targeted metabolomic study. Front Pharmacol. 2015;6:217.

Han MN, Lu JM, Zhang GY, Yu J, Zhao RH. Mechanistic studies on the use of *Polygonum multiflorum* for the treatment of hair graying. BioMed Res Inter. 2015; Article ID 651048.

He X, Liu J, Long G, Xia XH, Liu M. 2,3,5,4′-Tetrahydroxystilbene-2-O-β-D-glucoside, a major bioactive component from *Polygoni multiflori* Radix (Heshouwu) suppresses DSS induced acute colitis in BALb/c mice by modulating gut microbiota. Biomed Pharmacother. 2021;137:111420.

ISO 14502-1, Determination of substances characteristic of green and black tea. Part 1: content of total polyphenols in tea. Colorimetric method using Folin-Ciocalteu reagent, 2005.

Jiang Z, Xu J, Long M, Tu Z, Yang G, He G. 2,3, 5, 4′-tetrahydroxystilbene-2-O-β-D-glucoside (THSG) induces melanogenesis in B16 cells by MAP kinase activation and tyrosinase upregulation. Life Sci. 2009;85(9-10):345–350.

Jung KA, Min HJ, Yoo SS, Kim HJ, Choi SN, Ha CY, et al. Drug-induced liver injury: twenty-five cases of acute hepatitis following ingestion of *Polygonum multiflorum* Thunb. Gut Liver. 2011;5(4):493–499.

Le PTQ. Research on polyphenols extraction from *Polygonum multiflorum* Thunb. roots. Herba Pol. 2020;66(1):9-17.

Lei X, Chen J, Ren JT, Li Y, Zhai J, Mu W, et al. Liver damage associated with *Polygonum multiflorum* Thunb.: a systematic review of case reports and case series. Evid Based Complement Alternat Med. 2015; Article ID 459749.

Li RH, Gan GX, Long GB, Xiang YF, Zhu WX. Effects of different processing excipients on contents of effective components in *Polygonum Multiflorum* Thunb. Pharm Today. 2020;30(04):255-258.

Li RL, Gao F, Yan ST, Ou L, Li M, Chen L, et al. Effects of different processed products of *Polygonum multiflorum* on the liver. Evid Based Complement Alternat Med. 2020; Article ID 5235271.

Li Y, Li Y, Gong XH, Liu MC, Peng C, Li P, et al. A New Strategy for quality evaluation and of representative chemical components in *Polygonum multiflorum*. Thunb. Evid Based Complement Alternat Med. 2017; Article ID 6238464.

Li YX, Gong XH, Liu MC, Peng C, Li P, Wang YT. Investigation of liver injury of *Polygonum multiflorum* Thunb. in Rats by metabolomics and traditional approaches. Front Pharmacol. 2017;8:Article 791.

Liang Z, Chen H, Yu Z, Zhao Z. Comparison of raw and processed Radix Polygoni Multiflori (Heshouwu) by high performance liquid chromatography and mass spectrometry. Chin Med. 2010;5:29.

Lin L, Ni B, Lin H, Zhang M, Li X, Yin X, et al. Traditional usages, botany, phytochemistry, pharmacology and toxicology of *Polygonum multiflorum* Thunb.: a review. J Ethnopharmacol. 2015;159:158–183.

Ling S, Xu JW. Biological activities of 2,3,5,4′-tetrahydroxystilben-2-O-β-D-glucoside in antiaging and antiaging-related disease treatments. Oxid Med Cell Longev. 2016; Article ID 4973239.

Lv GP, Meng LZ, Han DQ, Li HY, Zhao J, Li SP. Effect of sample preparation on components and liver toxicity of *Polygonum multiflorum*. J Pharm Biomed Anal. 2015;109:105–111.

Ma J, Zheng L, He YS, Li HJ. Hepatotoxic assessment of Polygoni Multiflori Radix extract and toxicokinetic study of stilbene glucoside and anthraquinones in rats. J Ethnopharmacol. 2015;162:61–68.

Nguyen THT, Nguyen TP, Doan VT, Bui TTL, Nguyen DL. Determination of 2,3,5,4'-tetrahydroxystilben-2-O-β-D-glucosid (THSG) in the *Fallopia multiflora* Thunb. root by HPLC. J Med Pharm. (ISSN 2734-9209). 2020;10:71-76.

Nguyen THT, Doan VT, Tran DT, Bui TTL, Nguyen DL. Qualitative and quantitative analysis of physcion in the *Fallopia multiflora* Thunb. root using HPLC method. J Med Pharm. (ISSN 2734-9209) 2021;9:44-50.

Nguyen TT, Nguyen HD, Do HG, Nguyen TTM, Nguyen TD. Metabolomics approach for discrimination and quality control of natural and commercial *Fallopia multiflora* products in Vietnam. Int J Anal Chem. 2020;2020:8873614.

Nguyen TT, Pham TB, Nguyen PT, Nguyen HD, Nguyen VH, Pham VC, et al. Phenolic constituents from *Fallopia multiflora* (Thunberg) Haraldson. J Chem. 2018;2018:4851439.

Qian J, Hou M, Wu X, Dai C, Sun J, Dong L. A review on the extraction, purification, detection, and pharmacological effects of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside from *Polygonum multiflorum*. Biomed Pharmacother. 2020;124:109923.

Qian W, Guang T, Wenjing H, Wei W, Qiucheng W. Stilbene glucoside, a putative sleep promoting constituent from *Polygonum multiflorum* affects sleep homeostasis by affecting the activities of lactate dehydrogenase and salivary alpha amylase. Chem Pharm Bull (Tokyo). 2017;65:1011.

Singleton VL, Rosa RO, Raventos ML. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999;299:152-178.

Sun YN, Cui L, Li W, Yan XT, Yang SY, IlKang J, et al. Promotion effect of constituents from the root of *Polygonum multiflorum* on hair growth. Bioorganic Med Chem Lett. 2013;23(17):4801–4805.

Tian M, Tanaka H, Shang MY, Karashima S, Chao Z, Wang X, et al. Production, characterization of a monoclonal antibody against aristolochic acid-II and development of its assay system. Am J Chin Med. 2008;36(2):425–436.

Tao Y, Zhou X, Li W, Cai B. Simultaneous quantitation of five bioactive ingredients in raw and processed *Fallopia multiflora* by employing UHPLC-Q-TOF-MS. J Chromatogr Sci. 2019;57(7):618-624.

Vietnamese Pharmacopoeia V, 2018. Available from: https://duocdienvietnam.com/ha-thu-o-do-re/.

Wang X, Zhao L, Han T, Chen S, Wang J. Protective effects of 2,3,5,4′-tetrahydroxystilbene-2-O-beta-D-glucoside, an active component of *Polygonum multiflorum* Thunb. on experimental colitis in mice. Eur J Pharmacol. 2008;578(2-3):339–348.

Wu X, Chen X, Huang Q, Fang D, Li G, Zhang G. Toxicity of raw and processed roots of *Polygonum multiflorum*. Fitoterapia. 2012;83(3):469-475.

Xu H, Xu HE. Analysis of trace elements in Chinese therapeutic foods and herbs. Am J Chin Med. 2009;37(4):625–638.

Yan HJ, Fang ZJ, Fuy J, Y SX. The correlation between bioactive components of *Fallopia multiflora* root and environmental factors. Am J Chin Med. 2010;38(3):473–483.

Yi T, Leung KSY, Lu GH, Zhang H, Chan K. Identification and determination of the major constituents in traditional chinese medicinal plant *Polygonum multiflorum* Thunb by HPLC coupled with PAD and ESI/MS. Phytochem Anal. 2007;18(3):181–187.

Yu J, Xie J, Mao XJ, Wang MJ, Li N, Wang J, et al. Hepatoxicity of major constituents and extractions of radix Polygoni multiflori and radix Polygoni multiflori praeparata. J Ethnopharmacol. 2011;137(3):1291–1299.

Yun W, Liu M, Liu J, Li H. Influence factors on the hepatotoxicity of *Polygoni multiflori* radix. Evid Based Complement. Alternat Med. 2019; Article ID 5482896.

Zeng C, Xiao JH, Chang MJ, Wang JL. Beneficial effects of THSG on acetic acid-induced experimental colitis: involvement of upregulation of PPAR-γ and Inhibition of the Nf-Kb Inflammatory Pathway. Molecules. 2011;16(10):8552-8568.

Zhao C, Cao Y, Ma Z, Shao Q. Optimization of liquid ammonia pretreatment conditions for maximizing sugar release from giant reed (*Arundo donax* L.). Biomass Bioenergy. 2017a;98:61-69.

Zhao C, Qiao X, Cao Y, Shao Q. Application of hydrogen peroxide presoaking prior to ammonia fiber expansion pretreatment of energy crops. Fuel. 2017b;205:184-191.

Zhang M, Zhao C, Shao Q, Yang Z, Zhang X, Xu X, et al. Determination of water content in corn stover silage using near-infrared spectroscopy. Int J Agric Biol Eng. 2019;12(6):143.

Zhang YZ, Shen JF, Xu JY, Xiao JH, Wang JL. Inhibitory effects of 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside on experimental inflammation and cyclooxygenase 2 activity. J Asian Nat Prod Res. 2007;9(3-5):355-363.

Received for publication on 06th August 2021
Accepted for publication on 04th April 2022