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Pressurized Water Extraction of Hydrolysable Tannins from *Phyllanthus niruri* Linn

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Pressurized water extraction (PWE) was studied for the extraction of hydrolysable tannins from *Phyllanthus niruri* Linn. The effects of operating conditions (pressure, temperature, and the water flow rate) on the extraction yields were investigated. The results showed that the extraction yields increased with increasing temperature and with decreasing water flow rate, whereas pressure gave no significant effect. At 100 bar, 100°C and 1.5 ml/min, the extract had higher component contents (%g/g extract) of gallic acid (0.65%), corilagin (4.11%), and ellagic acid (8.91%) than a commercial HEPAR-P^{TM} extract (0.21%, 2.64%, 4.17%, respectively). It was also found that the dynamic PWE had a faster extraction rate and lower solvent consumption (0.018 m^3/kg) compared to the Soxhlet extraction and ultrasonication.

Keywords downstream processing; ellagitannins; phenolics; *phyllanthus niruri*; pressurized liquid extraction

INTRODUCTION

Phyllanthus niruri Linn. (Euphorbiceae) or locally known as Dukung Anak, is a medicinal herbal plant that has been traditionally used for treating kidney and gallbladder stones, jaundice, malaria, hepatitis, and liver-related diseases (1,2). The bioactive fractions of the plant were usually obtained by conventional solvent extraction method using water, alcohol, or aqueous solvents (3,4). A previous study of solvent screening using the Soxhlet extraction method showed that the desired hydrolysable tannins (gallic acid, corilagin, and ellagic acid) were best extracted using water and water-ethanol mixtures (5). Even though water is inexpensive, its removal from the extracts requires a lot of energy. Therefore, an alternative extraction method was investigated to possibly reduce the amount of water used and to enhance the extraction of the desired tannins.

Pressurized liquid extraction (PLE) or commercially known as accelerated solvent extraction (ASE) is a technique usually employed for the extraction of relatively high polar compounds by utilizing solvent extraction at elevated pressures and controlled temperatures (6,7). It serves as a substitute to overcome the drawbacks encountered in other extraction methods such as long extraction time and high solvent consumption in conventional solvent extractions, and low polarity of supercritical carbon dioxide in supercritical fluid extraction (SFE). Some work on the PLE or ASE of medicinal herbs has been carried out at pressures of 10–400 bars and temperatures of 50–200°C (7–10).

In the extraction of catechin and epicatechin from tea leaves and grape seeds, it was found that PLE (P = 100 atm atm and T = 100°–200°C) is a better method compared to magnetic stirring and ultrasonic if methanol and ethanol were used as solvents while ultrasonic is better when using water as solvent (7). It was also observed that the degradation of both catechins in PLE occurred at temperature above 130°C and above 10 min of extraction time. Benthin et al. (9) first reported on the comparative extraction study of five different medicinal herbs using PLE with different solvents. Comparison with Pharmacopeia methods such as soxhlet, reflux, maceration, and steam distillation showed better or comparable results and the desired components were exhaustively extracted within shorter extraction times (one to three cycles of 5 to 6 min) at temperatures above the boiling point of the respective solvent (9).

However, the extraction using pressurized or sub-critical water has not been widely explored. In comparison with other organic solvents and supercritical CO_2, the potential of sub-critical or pressurized water extraction is greater due to a wide range of polarities generated by heating liquid water from ambient to 300°C (11). As the temperature gets higher, water becomes less polar due to the lowering of its dielectric constant. At 200°C, the water polarity becomes similar to the less polar solvents such as methanol.

In this study, pressurized water extraction (PWE) was performed on the *P. niruri* and the effects of operating parameters such as pressure, temperature, and the water flow rate on total extraction and component yields were investigated. Extraction efficiency in terms of yield, solvent

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requirement, and the extraction time was presented in comparison with other extraction methods, namely Soxhlet (SE) and ultrasonication (US), and with a commercial standardized *P. niruri* product (HEPARP-P^{TM}).

MATERIALS AND METHOD

Plant Sample

Dried and ground *P. niruri* samples were obtained from Nova Laboratories Sdn. Bhd. (Malaysia). The particle size distribution determined after sieving was between 45–212 μm (8%), 212–600 μm (35%), 600 μm–1.18 mm (43%), and 1.18–3.35 mm (14%) were used.

Chemical and Standards

All chemical reagents such as ethanol, acetonitrile, and phosphoric acid were of analytical grades. Ultra-pure water was obtained using ultra-filtration system (USF ELGA, UK). Reference standards (gallic acid and ellagic acid) were both purchased from Sigma Chemicals (USA) at purity of 98%. Analysis of corilagin was carried out by Nova Laboratories Sdn. Bhd. (Malaysia) using their isolated and patented standard (purity of 98%). A commercial extract, HEPAR-P^{TM} (standardardized to 4% corilagin and 18% total flavonoid content) was obtained from the same company.

Pressurized Water Extraction (PWE)

In this study, the extraction of *P. niruri* using water at pressurized conditions of 50, 100, 150, and 250 bar, temperature of 40°, 60°, 80°, and 100°C, and water flow rate of 0 (static), 0.5, 1.5, 3, and 5 mL/min, were investigated using a similar high-pressure extraction system developed by Markom et al. (5). 5 g (±0.05 g) of a plant sample was used for each run. A 30-minute static extraction was allowed for the system to achieve equilibrium at the temperature and pressure studied, followed by a dynamic extraction until exhaustion. For the zero flow rate (static extraction), the extraction was carried out for 3 hours at 100 bar and 100°C using 150 mL water. Except for the static extraction, the overall component yields of all dynamic extractions were determined at 1 hour extraction time for the pressure and temperature effects since the extractions were already exhausted. For the flow rate effect, since the exhaustion time varied with the flow rates, the component yields were determined at 173 mL of water volume consumed.

The extract fractions were collected every 15 mL (except for the zero flow rate), filtered using a Whatman filter paper (no. 1), and the resulting extract solution was then dried in an air oven (Shel Lab, USA) at 70°C for about 15–30 hours. All extracts were cooled at room temperature in a desicator before weight determination by an analytical balance (±0.0001 g). The extracts were then stored in a cool room under freezing temperature (−4°C) for later use. The system was flushed and cleaned with water before starting the next run.

Soxhlet Extraction (SE)

5 g (±0.05) of a plant sample was placed in a Whatman 25 mm × 100 mm cellulose thimble. The extraction of standard Soxhlet method (BÜCHI Laboratechnik, Model B-811, Switzerland) was carried out using 150 mL of water. The heating power was set to two (2) cycles per hour so that six (6) cycles of extraction were achieved within 3 hours of extraction time.

The crude extract solutions obtained were concentrated and dried using vacuum rotary evaporator (BÜCHI Laboratechnik, Model R-144, Switzerland) at a temperature of 80°C or less to remove the solvents. Higher temperatures were avoided to minimize component degradation. All extracts were placed at a room temperature condition before weighing gravimetrically to determine the yields.

Untrasonication (US)

Ultrasonication extraction of 5 g (±0.05) plant sample was carried out three times using 50 mL of water at 60°C for 30 minutes each. The extract solutions were filtered using Whatman filter paper (no. 1) and combined before drying by a vacuum rotary evaporator (BÜCHI Laboratechnik, Model R-144, Switzerland) at temperature 80°C and placed at room temperature before being weighted.

HPLC Analysis

Component analysis was carried out using High Performance Liquid Chromatography (HPLC) technique equipped with an autosampler and a UV/vis detector (Agilent Technologies, Germany). The analysis method was modified from the method of catechin identification (11). The column used for the analysis was a reverse-phase C18 Genesis with 250 × 4.6 mm i.d. and 4 μm particle diameter (Jones Chromatography, UK). The chromatographic separation was carried out using a mobile phase of 0.1% phosphoric acid in water (solvent A) and acetonitrile (solvent B) with a gradient of solvent B: 8–22% (35 minutes), 22–8% (10 minutes) at flow rate of 1 mL/min. The injection volume was set at 20 μL and the detection was in UV absorbance at 270 nm.

Chromatographic peaks of gallic acid, ellagic acid, and corilagin were identified by comparison with the retention times of the external standards. Linear calibrations of the peak area versus the concentration of ellagitannin standards at an accuracy of more than 99.5% were obtained. A single injection of solvent (blank) was also made to determine the solvent retention time. Repeat analysis of the standards was done alternately with the extracts to determine reproducibility.

Prior to injection, all extracts were dissolved in 50% ethanol solutions at 1 mg/ml, ultra-sonicated at 60°C for 30 minutes to remove air bubbles, and to ensure all solids were completely dissolved, and finally pre-filtered using nylon micro membranes (0.45 μm).

RESULTS AND DISCUSSION

Effect of Pressure

Figure 1(a) presents the effect of pressure on the PWE yield as a function of extraction time. At a fixed temperature of 100°C, the extraction yield was slightly increased as the pressure increased from 50 to 150 bar but then was decreased as the pressure became higher (250 bar).

The solubility (kg extract/kg water), however, was not affected by the pressure increase. At a fixed temperature, vapor pressure and viscosity of water remained unchanged.

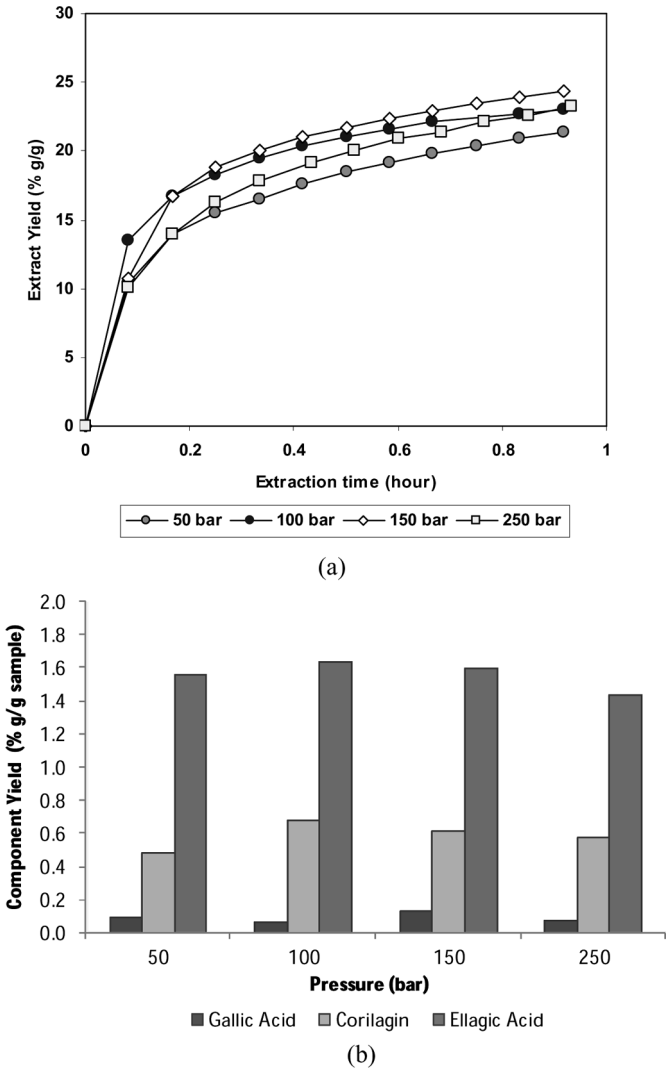

FIG. 1. Effects of pressure at 100°C and 3 mL/min on (a) extraction yield and (b) component yield.

As the cohesive energy of a liquid is expected not to change significantly with pressure, the solubility would also remain constant. This is experimentally observed as the initial slope of the extraction curve in Fig. 1(a). Typically, the pressurized liquid extractions of plants employ relatively low pressure, at about 10 to 150 bars (7,8,11).

Further investigation on the component yields indicated that they were also not that affected by the pressure change, as shown by Fig. 1(b). This showed that the polarity of water did not significantly change with pressure. However, it can be seen that the corilagin and ellagic acid yields were slightly higher at pressures of 100 and 150 bar. This could be possibly due to the modification of the plant matrix and the increase of component mass transfer from the solid phase at these pressures. Thus, a pressure of 100 bar was employed to further study the effects of temperature and water flow rate. A duplicate run at 100 bar, 100°C, and 3 mL/min resulted in the standard deviation errors for the extraction yield ($23.21 \pm 1.67\%$), gallic acid content ($0.33 \pm 0.005\%$), corilagin content ($2.35 \pm 1.04\%$), and ellagic acid content ($6.22 \pm 1.66\%$).

Effect of Temperature

Temperature, which affects water vapor pressure, viscosity, surface tension, and polarity, is significant in increasing both the extraction yield (Fig. 2(a)) and component yields (Fig. 2(b)) at pressure of 100 bar and water flow rate of 3 mL/min. At a higher temperature, the solute vapor pressure was increased, thus it was easier for the solute to free itself from the matrix. The reduced viscosity and surface tension of water at high temperatures also caused the solvent to be more easily diffused into the plant matrix and dissolved more solutes. Since the polarity of water is reduced with increasing temperature (11), the right solubility parameter for the extraction of tannins can be achieved without the addition of less polar solvent. It can be concluded that high overall component yields can be obtained at temperatures above 100°C. However, higher temperatures are not recommended to prevent the degradation of components.

Effect of Water Flow Rate

Figure 3(a) shows the effects of water flow rate (0.5, 1.5, 3, 5 mL/min) at 100 bar and 100°C on the extraction yield of *P. niruri* as a function of water volume. It was observed that a decreasing flow rate was capable of increasing the extraction yields and it was found highest at 0.5 mL/min. This was probably due to increased mass transfer rate, equilibrium, and component saturation at a lower flow rate.

Figure 3(b) shows that for the dynamic PWE, the maximum component yields were obtained at a water flow rate of 1.5 mL/min. At below or above this flow rate, component yields were reduced. Possible reasons for the lower component yields at higher flow rates (3 and 5 mL/min) were insufficient component-solvent contact and component

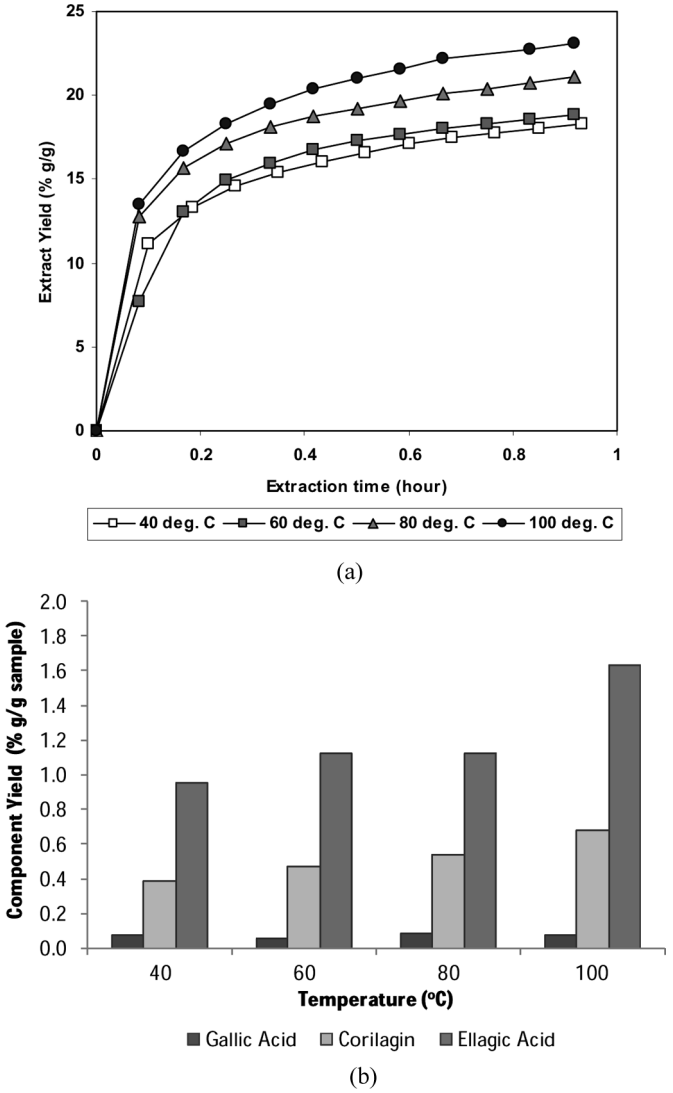

FIG. 2. Effect of temperature at 100 bar and 3 mL/min on (a) extraction yield and (b) component yield.

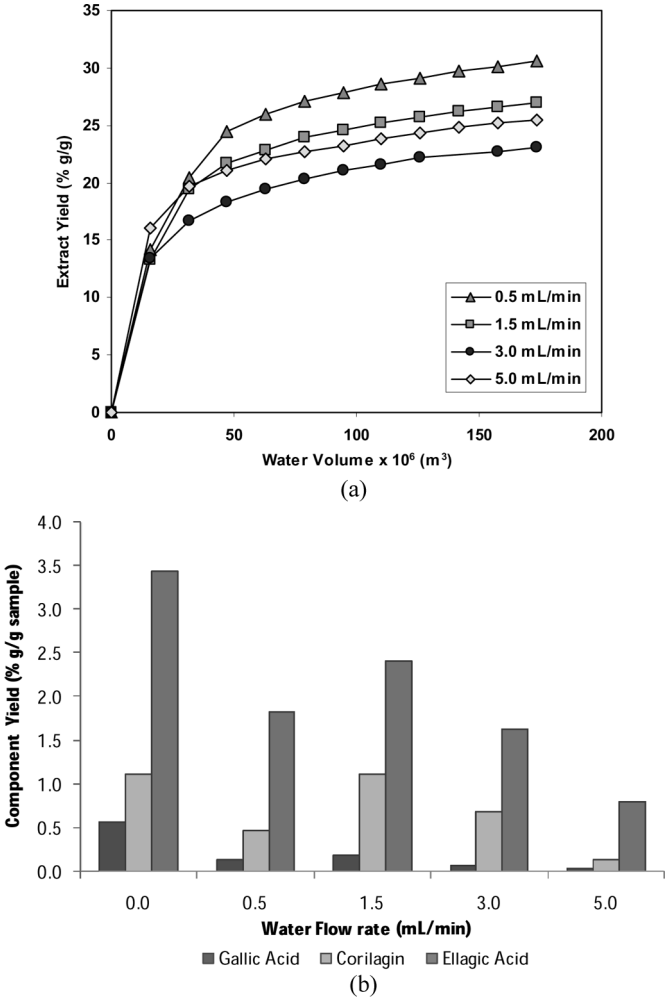

FIG. 3. Effect of water flow rate at 100 bar and 100°C on (a) extraction yield and (b) component yield.

saturation, and internal mass transfer in the solid matrices became the rate-controlling factor. Even though a lower flow rate (0.5 mL/min) was more efficient in terms of solvent consumption and obtaining higher extraction yield, longer extraction time was needed and component yields were reduced compared to 1.5 mL/min, as indicated in Fig. 3(b). This might be due to the entrainment of the heavier tannins extracted at a low flow rate of 0.5 mL/min. In contrast, a static extraction process (zero flow rate) resulted in a lower extraction yield (20%) but a higher component yields compared to the dynamic extractions. At these conditions, the yield of corilagin was almost similar to the dynamic extraction at 1.5 mL/min but the yields of the phenolic acids (gallic and ellagic) were much higher due to the longer hydrolysis process during static extraction at high temperature.

From the effects of operating conditions studied, the temperature and the water flow rate were the more significant factors compared to pressure. Even though the highest component yields were obtained at the static extraction (zero flow rate), the component profiles as a function of time could not be determined. Furthermore, the hydrolysis of heavier tannins (corilagin) to the simpler tannins (gallic and ellagic acids) should be minimized since the former has more glycosidic rings and is more pharmacologically active (4,13). Therefore, the best conditions for the dynamic extraction are chosen to be at 100 bar, 100°C, and 1.5 mL/min.

Comparison of PWE with Other Extractions

From the results of this study, the extraction yields were comparable between the dynamic PWE (27%) and SE (26%), followed by static PWE (20%) and US (15%). The method efficiency and component contents are shown in

TABLE 1
Comparison of efficiency and component content for different extraction methods

Method	Solvent-to-solid ratio (m^3/kg) $\times 10^3$	Residence time (hr)	Extraction yield (%g extract/g sample)	Component content (% g/g extract)		
				Gallic acid	Corilagin	Ellagic acid
US[a]	30	3.0	15	0.25	1.52	3.80
SE[b]	30	3.0	26	1.15	2.96	17.48
PWE[c]	30	3.0	20	2.87	5.54	17.21
PWE[d]	18	0.8	27	0.65	4.11	8.91
HEPAR-P™	–[e]	–[e]	–[e]	0.21	2.64[f]	4.17

[a]US – ultrasonication extraction at 60°C for 3 hours.
[b]SE – Soxhlet extraction at 100°C (b.p. of water) for 3 hours.
[c]PWE – pressurized water extraction at 100 bar, 100°C, static extraction for 3 hours.
[d]PWE – pressurized water extraction at 100 bar, 100°C, 1.5 mL/min for 1 hour.
[e]information is not available.
[f]the content analyzed was lower than the standardized corilagin content (4%) reported by Nova Laboratories Sdn. Bhd (14).

Table 1. Less water consumption was required for dynamic PWE (90 mL) compared to static PWE, SE, and US (150 mL each). This resulted in only 0.0018 m^3/kg solvent-to-solid ratio for the dynamic PWE, which was about half of those required by the other extractions. The extraction time in dynamic PWE was also reduced (30 min to 1 hour) depending upon the water flow rate used. It was somewhat longer than what was achieved by Benthin et al. (9) at 5–18 minutes, possibly due to different compounds extracted and different solvents used at the pressurized extraction. For the selected condition in this study, the residence time of dynamic PWE was the shortest (0.8 hour) compared to the other extractions (3 hours).

Component contents (% g/g extract) for the three hydrolysable tannins were found highest by the static PWE, followed by the dynamic PWE. The corilagin content of HEPAR-P™ was comparable to the Soxhlet extraction. Ultrasonication gave the lowest contents for all components. It was also found that high temperature used in Soxhlet (boiling point of water) resulted in high degradation of heavier tannins to the simpler ellagic and gallic acids due to the long contact with the hot solvent. A similar result was observed for the static PWE; however, the corilagin content was still relatively high. This degradation was not observed in the dynamic PWE even though the same boiling temperature was used. This indicated that the dynamic pressurized condition could limit component degradation at similar temperature.

Component profiles at the selected best condition of dynamic PWE are shown in Fig. 4. High extraction yield

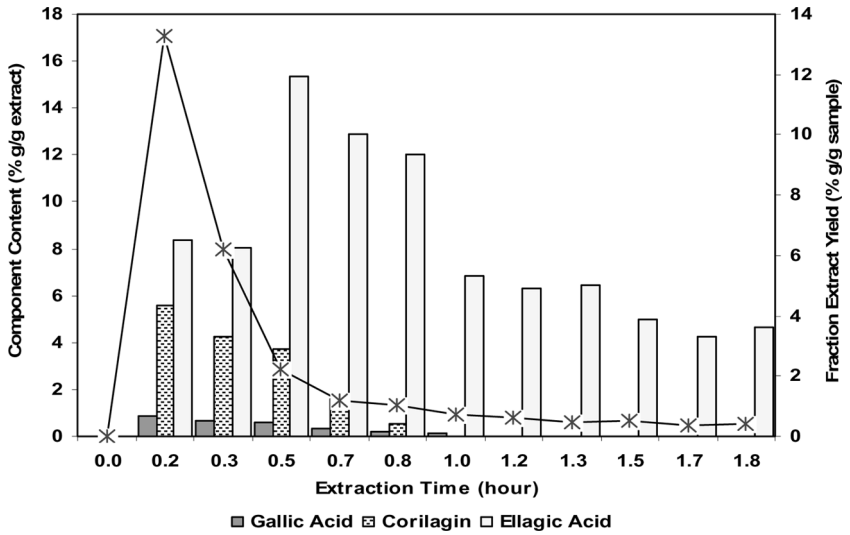

FIG. 4. Component profiles at 100 bar, 100°C and 1.5 mL/min as a function of time. Bar symbols are the component yields and curve symbols are the fraction extraction yields at a given extraction time.

with high gallic acid and corilagin contents could be accomplished in less than an hour of extraction, while ellagic acid could be selectively extracted after an hour of extraction. From Fig. 4, the high extraction yield and overall component contents could be achieved in less than 30 minutes of extraction. Therefore, the quality of the plant extract can be selectively controlled at the appropriate time by the dynamic PWE.

CONCLUSIONS

In this study, the effects of operating parameters such as pressure, temperature, and water flow rate in the pressurized water extraction (PWE) of the *P. niruri* on the total extraction and component yields were investigated. The results showed that the extraction and component yields in the PWE of *P. niruri* were more influenced by the temperature and water flow rate. The pressures investigated gave no significant effect on the yields. The study found that by using PWE at 100 bar, 100°C and 1.5 mL/min, it was possible to obtain higher contents of hydrolysable tannins than in the commercial *P. niruri* product, HEPAR-P^{TM}. In terms of the extraction efficiency (yield, solvent requirement, and extraction time), the PWE method was more efficient compared to the more conventional Soxhlet and ultrasonication methods since less water volume per kg of sample and a shorter residence/extraction time were required for a comparable or higher yield obtained. Thus, PWE is a potentially useful method for the quality extraction of hydrolysable tannins from plants.

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