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Seminar Nasional

Tumbuhan Obat Indonesia XXXVII

Pemanfaatan Tumbuhan Obat Indonesia untuk Peningkatan Derajat Kesehatan dan Ekonomi Masyarakat

Seledri (*Apium graveolens*)
Kwalot / buah makasar (*Brucea javanica* Merr.)

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PARAMETER EFFECTS IN THE PRESSURISED WATER EXTRACTION OF HYDROLYSABLE TANNINS FROM *Phyllanthus Niruri* Linn.

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ABSTRACT

Water is a safer and inexpensive extraction solvent than organic solvents for processing food and pharmaceutical products. However, high energy is required for its removal from the extracts, thus its usage should be minimized as possible. In this study, pressurized water extraction (PWE) was performed for the extraction of hydrolysable tannins of *Phyllanthus niruri* Linn. and the effects of its operating parameters on the extraction and component yields were investigated. The results showed that the 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™ extract (0.21%, 2.64%, 4.17%, respectively). PWE is also more efficient compared to the Soxhlet and ultrasonication since it requires less water volume and shorter extraction time.

Keyword s: downstream processing, ellagitannins, phenolics, pressurized liquid extraction

INTRODUCTION

Phyllanthus niruri Linn. (Euphorbiceae) or locally known as Dukung Anak, is a medicinal herbal plant that was traditionally used for treating kidney and gallbladder stones, jaundice, malaria, hepatitis and liver-related diseases (Jaganath and Teik 2000). The bioactive fractions of the plant were usually obtained by conventional solvent extraction method using water, alcohol or aqueous solvents (Huang *et al*., 1992; Notka *et al*., 2002). A previous study of solvent screening using Soxhlet extraction method showed that the desired hydrolysable tannins (gallic acid, corilagin and ellagic acid) were best extracted using water and waterethanol mixtures (Markom *et al*., 2007). Even though water is inexpensive, its removal from the extracts requires a lot of energy. Therefore, 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 (Piñeiro *et al*., 2004). 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 10-140 bars and temperatures of 80-160°C (Benthin et al. 1999; Ong and Len 2003). However, the extraction using pressurized or sub-critical water has not been widely explored.

In this study, pressurized water extraction (PWE) was performed on the *P. niruri* and the effects of operating parameters such as pressure, temperature, and water flow rate were investigated in terms of total extraction and component yields. Comparison of component contents with other extraction methods, namely Soxhlet (SE) and ultrasonication (US), and with a commercial product, was also carried out.

MATERIALS AND METHOD

Plant Sample

5 g (± 0.05) of dried and ground *P. niruri* samples with particle size distribution between 45 – 212 μm (8%), 212 – 600 μm (35%), 600 μm – 1.18 mm (43%) and 1.18 – 3.35 mm (14%) were used.

Extraction and Analysis

In this study, extraction of *P. niruri* using water at pressurized conditions: pressure 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 similar high-pressure extraction system developed by Markom et al. (2007). Comparison with Soxhlet (150 mL water at 3 hours) and ultrasonication (50 mL x 3 at 1 hour each) was carried out. All extracts were cooled at room temperature and placed in a dessicator before weighing gravimetrically using analytical balance (± 0.0001 g) to determine the yields.

Component analysis (detection and quantification) for gallic acid, corilagin, and ellagic acid in all extracts was then performed by high performance liquid chromatographic method, HPLC (Agilent, Germany). HEPAR-P™, a commercial *P. niruri* health maintenance product produced by Nova Laboratories Sdn. Bhd. (Sepang, Malaysia) standardized to 4% corilagin content, was obtained and analyzed for comparison.

RESULTS AND DISCUSSION

Effect of Pressure

Figure 1a 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 slightly increased as the pressure increased from 50 to 150 bar but then 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. 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 Figure 1a. Typically, the pressurized liquid extractions of plants employ relatively low pressures, at about 10 to 100 bars (Piñeiro *et al.*, 2004; Ong and Len 2003).

Further investigation on the component yields indicated that they were also not that affected by the pressure change, as shown by Figure 1b. This showed that the polarity of water did not change much 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.

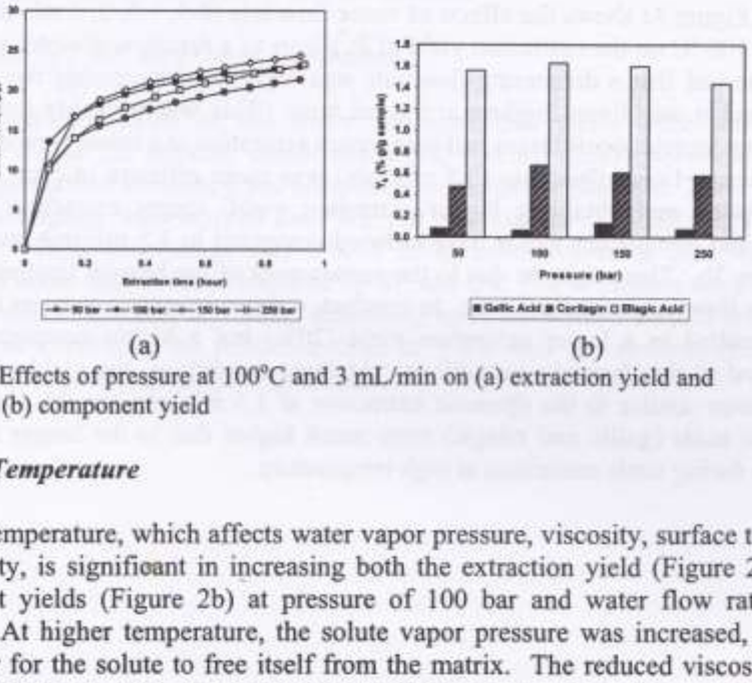
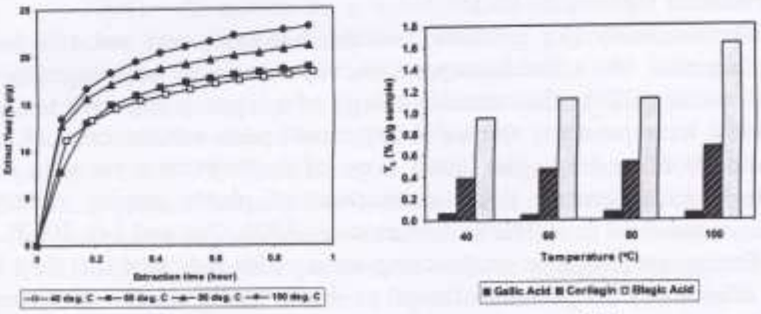


Figure 1. Effects of pressure at 100°C and 3 mL/min on (a) extraction yield and (b) component yield

Effect of Temperature

Temperature, which affects water vapor pressure, viscosity, surface tension, and polarity, is significant in increasing both the extraction yield (Figure 2a) and component yields (Figure 2b) at pressure of 100 bar and water flow rate of 3 mL/min. At 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 (Hawthorne *et al.*, 2000), 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 degradation of components.



(a) (b)

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

Effect of Water Flow Rate

Figure 3a 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 better mass transfer rate, equilibrium and component saturation at a lower flow rate.

Even though lower flow rate (0.5 mL/min) was more efficient in term 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 Figure 3b. 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.

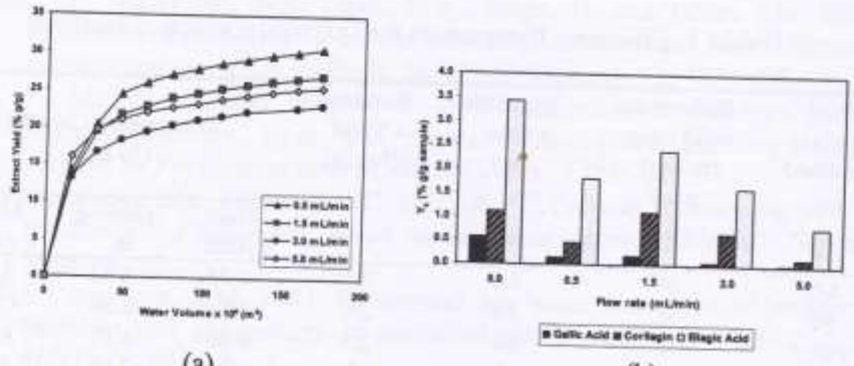


Figure 3. Effect of water flow rate at 100 bar and 100 °C on (a) extraction yield and (b) component yield

From the effects of operating conditions studied, temperature and 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 have better bioactivities (Ueno *et al.*, 1998; Notka *et al.*, 2002). 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 PWE at the selected conditions (27%) and SE (26%), followed by US (15%). The method efficiency and component contents are shown in Table 1. Less water consumption was required for PWE (90 mL) compared to SE and US (150 mL each). These resulted in only 0.0018 m^3/kg solvent-to-solid ratio for PWE, which was about half of those required by SE and US. The extraction time in PWE was also reduced (30 min to 1 hour) depending upon the water flow rate used. For the selected condition, the residence time of PWE was the shortest (0.8 hour) compared to the other extractions (3 hours).

Table 1. Efficiency Comparison for Different Extraction Methods

Method	Solvent-to-solid ratio (m^3/kg) x 10^3	Residence time (hr)	Extraction Yield (%g /g)	Component Content (% g/g)		
				Gallic Acid	Corilagin	Ellagic Acid
US	30	3.0	15	0.25	1.52	3.80
SE	30	3.0	26	1.15	2.96	17.48
PWE[a]	18	0.8	27	0.65	4.11	8.91
HEPAR-P™	-[b]	-[b]	-[b]	0.21	2.64 (4)	4.17

[a] PWE at 100 bar, 100 °C and 1.5 mL/min
-[b] information is not available
-Number in bracket is the standardized corilagin content reported by Nova Laboratories Sdn. Bhd (undated)

CONCLUSION

The results of this study 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™. This method was also more efficient compared to the more conventional Soxhlet and ultrasonication methods since less water volume per kg of sample and shorter residence/extraction time were required. Thus, PWE is a potential method for the extraction hydrolysable tannins from plants.

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