HOT PRESSURIZED WATER EXTRACTION OF GYPENOSIDES FROM *Gynostemma Pentaphyllum*

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ABSTRACT

This study investigated the feasibility of extracting gypenosides from *Gynostemma pentaphyllum* (GP) by using batch and semi-continuous hot pressurized water extractions. Extraction conditions ranged from 100 to 1000 psig and 333 to 433 K. Experimental results indicated that hot water under suitable pressures enhanced the extracted amount of gypenosides, but temperature was the major effect on extracting gypenosides from GP. When semi-continuous hot pressurized water at 200 psig, 373 K, 10 mL/min, 3 hours, and a feed ratio of 200 g/L (GP/ Extractor Volume) was used, the recovery of the gypenosides attained 91.8 % of what was obtained by using EtOH Soxhlet extraction for 6 hours, and it reached 150 % of that by using the atmospheric water Soxhlet extraction for 11 hours.

Keywords: Hot pressurized water, *Gynostemma pentaphyllum*, gypenosides, semi-continuous extraction

INTRODUCTION

Gynostemma pentaphyllum (GP) is a perennial liana of Cucurbitaceae, growing wild throughout China, Japan, Korea, India, and Taiwan [1]. This liana has been used extensively in Chinese traditional medicine to treat maladies such as bronchitis. Previous studies have found and isolated more than 90 dammarane-type saponins (called gypenosides: GYP) from some GP species, structurally related to ginseng saponins [2]. A few GYP have the same structures with those from *P. ginseng*, *P. quinquefolium*, and *P. notoginseng* [3-8]. Pharmacological studies have revealed that among many bioactivities of GYP, these include anti-cancer, anti-aging, anti-fatigue, anti-ulcer, hypolipidemic, and immuno-modulatory qualities [9-15]. Owing to its lower retail price and easier availability than *P.Ginseng*, GP

has been widely commercialized in such products as herbal teas and extracts [16-17]. A few literatures reported that the extraction of ginsenosides from *P.Ginseng* required an enormous amount of organic solvents, resulting in environmental pollution, the residue of solvents [18]. Compared to that solvent extraction, atmospheric hot water extraction obtains less production in terms of ginsenosides. Recent studies showed that hot pressurized water could modulate dielectric constant of water, even lower down to that of EtOH at 1 atm and 25 ？, and was able to easily extract organic-affinity compounds [19, 20]. Therefore, this study investigated the feasibility of water extraction at elevated pressures for recovering gypenosides from GP.

MATERIALS AND METHODS

(1) Plant materials

Gynostemma pentaphyllum, collected from Guilin, Guangxi Province of China, was purchased from a Taiwan herbal supplier. Dried GP whole bodies were ground by using a mixer, and to the standard size of #60 mesh (about 250 μm). It was then stored in a vacuum container before use.

(2) Chemicals

Methanol (99%), ethanol (95%), *n*-butanol, ethyl acetate (99.5%), perchloric acid (70-72%), and glacical acetic acid (99.8%) were of analytical reagent grade (Merck, Germany). Standard Gypenoside (80%) (Luster, Taiwan). All are used without further purification.

(3) Extraction and Analysis

The GP samples were extracted by organic solvent extraction (OSE), water extraction at atmospheric pressure (WE), batch hot pressurized water extraction (B-HPWE) and semi-continuous hot pressurized water extraction (SC-HPWE), respectively. All hot pressurized water extractions were conducted on a 0.6-liter stirred pressure autoclave (Parr Instrument Company, Moline Illinois 61265, United States).

The colorimetric content of the gypenosides in the extract was determined according to the method of Ding et al. [21], using a UV-3000 spectrophotometer (Hitachi, Japan). The standard calibration curve of gypenosides was:

$$Y = 2381.1\ x + 8.1911\ (R^2=0.9996,\ \text{linear range: } 250\text{-}2000\mu g/g)$$

RESULTS AND DISCUSSION

Figures 1 and 2 described how pressure, temperature and time affect the contents of gypenosides. Obviously, suitable pressure higher than 100psig enhanced the extracted

amount of gypenosides, but it was temperature (60? to 120?) that was the major factor for extracting gypenosides from GP. The appropriate B-HPWE process was found at 200 psig, 373 K, and 1hour, respectively. Following the condition given above, a few different feed ratio experiments were discussed later. The flow rate of all SC-HPWE experiments was set at 10 mL/min.

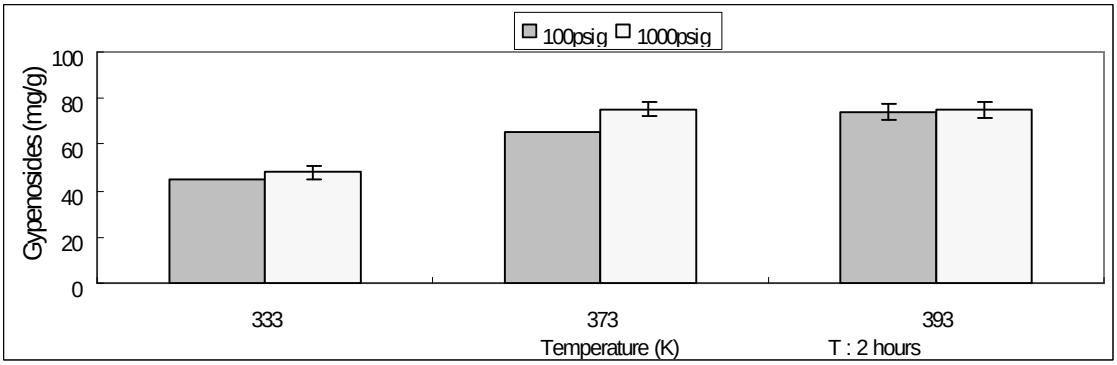

Figure 1: The effect of pressure on gypenosides extracted in B-HPWE

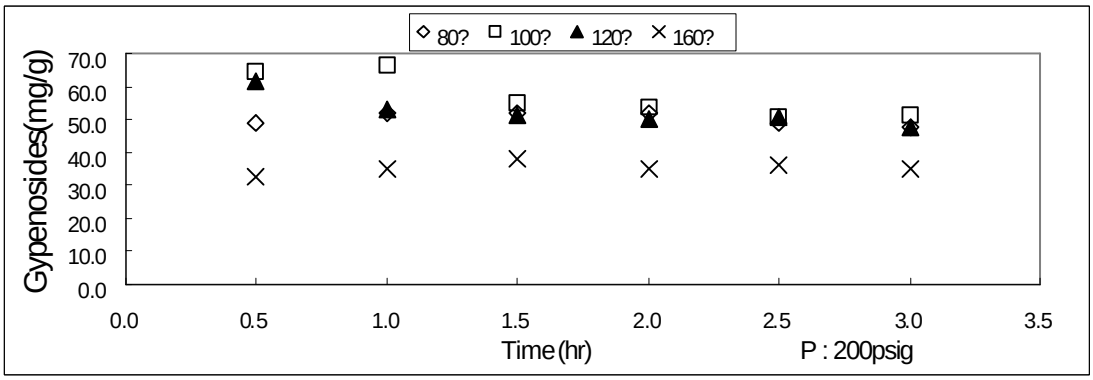

Figure 2: The effect of temperature and time on gypenosides extracted in B-HPWE

Figures 3 and 4 showed that the concentration of gypenosides in both B-HPWE and SC-HPWE extractions increased with the increasing feed ratio from 50 g/L to 200 g/L. However, an excessive feed ratio resulted in wasting plant materials. In B-HPWE, the extracted amount of gypenosides decreased with the increasing feed ratio due to the quantity of water in the autoclave was limited, shown in Fig. 5. The result showed that the optimum feed ratio in B-HPWE was 50 g/L. Figure 6 showed that the optimal feed ratio in SC-HPWE is different from that in B-HPWE. In SC-HPWE the quantity of pumping fresh water increased as time progressed. In this situation, a higher outlet concentration was

continuously obtained with increased amounts of gypenosides in the extract. SC-HPWE could accumulate 106.69 GYP mg/g GP in the extract. This amount of gypenosides is 1.7 times that of B-HPWE (66.60 GYP mg/g GP). When the same autoclave was used, SC-HPWE proved to be a better process than B-HPWE to extract GYP from *Gynostemma pentaphyllum*.

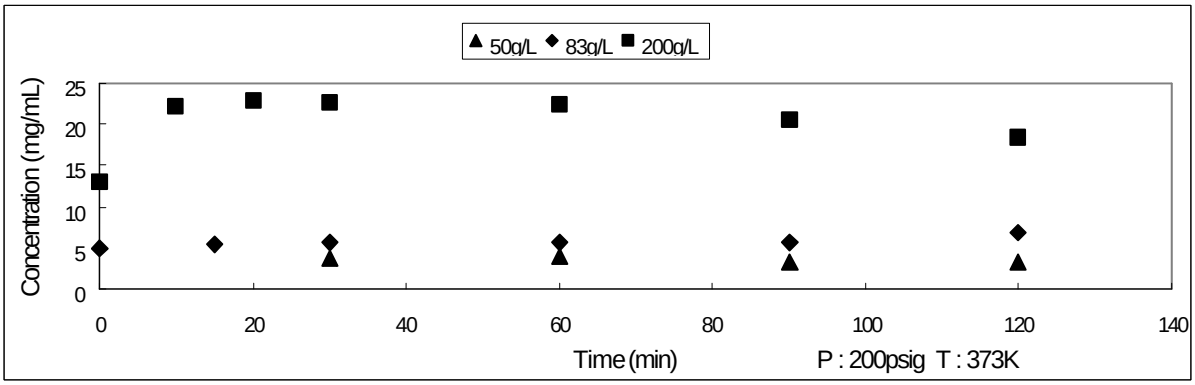

Figure 3: The effect of feed ratio on gypenosides concentration in B-HPWE

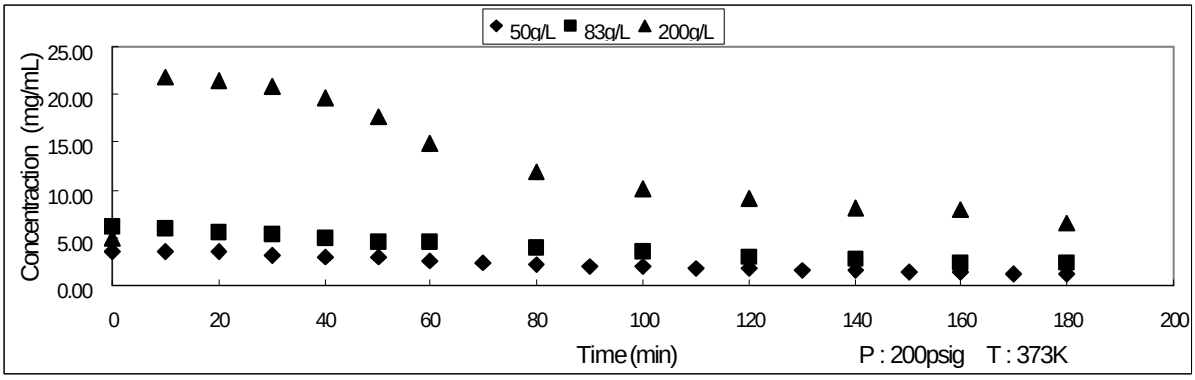

Figure 4: The effect of feed ratio on gypenosides concentration in SC-HPWE

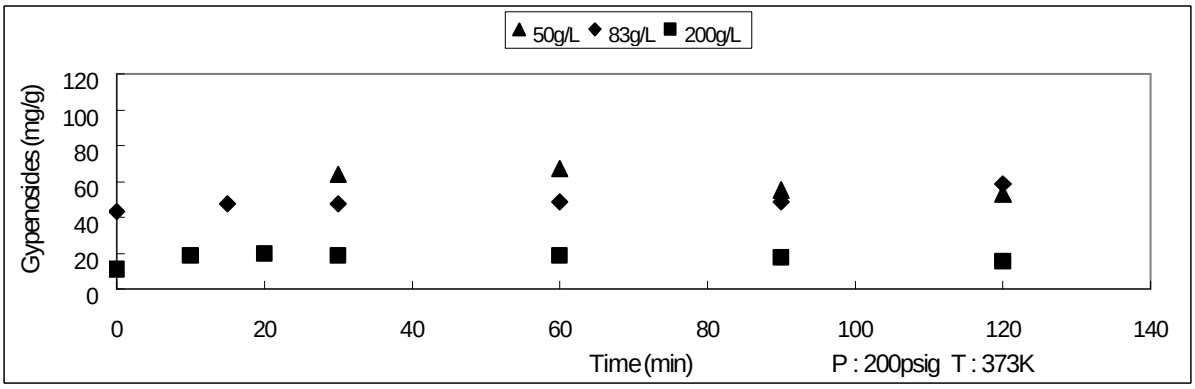

Figure 5: The effect of feed ratio on gypenosides extracted in B-HPWE

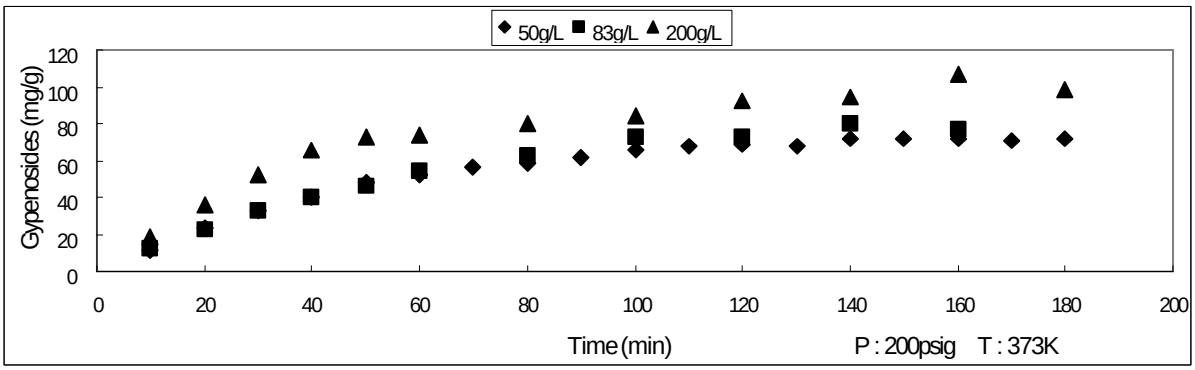

Figure 6: The effect of feed ratio on gypenosides extracted in SC-HPWE

CONCLUSIONS

This study has demonstrated that semi-continuous hot pressurized water extraction is a better process than batch extraction and can be a viable alternative for extracting gypenosides from *Gynostemma pentaphyllum*. Our results have shown that the suitable condition of SC-HPWE for extracting gypenosides was found to be 200 psig, 373 K, 10 mL/min, and 3 hours with a feed ratio of 200 g/L (GP / Extractor volume). It produced 106.69 GYP mg/g GP. The amount of gypenosides is equivalent to 91.8 % of Soxhlet EtOH extraction (116.25 GYP mg/g GP) for 6 hours and 150 % of Soxhlet H_2O extraction (71.27 GYP mg/g GP) for 11 hours.

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