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Optimization of Extraction Conditions for Total Phenolics from *Sapium japonicum* Using a Pressurized Liquid Extractor

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Abstract *Sapium japonicum* was extracted by a pressurized liquid. Operating parameters such as the type and the ratio of solvent to water, temperature, pressure, and number of extractions were investigated as the main variables that influence the extraction efficiencies of total phenolics (TP). MeOH extracted the highest level of TP as 50.4 mg GAE/g compared to 48.8 and 27.2 mg GAE/g with H_2O and EtOH, respectively. EtOH:H_2O (40:60, v/v) was found to be the best solvent for TP extraction as 90.3 mg GAE/g compared to 85.0 and 84.3 mg GAE/g in 40:60 and 60:40 of MeOH:H_2O, respectively. TP were increased with the increase of the number of extraction steps. TP content was increased by 11% as the extraction temperature was increased from 40 (97.4) to 50°C (108.3 mg GAE/g). The optimum extraction conditions of TP were; extraction solvent, EtOH:H_2O (40:60, v/v); temperature, 50°C; pressure, 10.2 MPa; 2 extraction steps.

Keywords: total phenolic, total soluble solid, pressurized liquid extraction, *Sapium japonicum*

Introduction

Natural plant with antioxidant activity may be used to help the human body to reduce oxidative damage (1). Therefore, there is a growing interest in natural substances exhibiting antioxidant properties that are supplied to human and animals as food components or as specific preventive pharmaceuticals (2).

Phenolic compounds are a large class of secondary plant metabolites that are distributed widely in the plant kingdom and possess an aromatic ring with one or more hydroxyl substituents (3). They are known to exhibit various health benefits such as antioxidant, anti-inflammatory, antitumor, atherosclerosis, arthritis, diabetes, antimutagenic, anticarcinogenic, and antimicrobial activities (4-8). The chemical structure of the phenolic compounds varies from simple phenolics to complex polymerics that may possess multiple hydroxyl groups conjugated to sugars, acids, or alkyl groups. Thus, extraction of phenolic compounds from plant matrices is complex and challenging (3,9).

Traditionally, the techniques employed in the extraction of phenolic compounds from fruits and vegetables involve the use of organic solvents including methanol and ethanol (10). These traditional methods are time consuming and laborious. They also employ large amounts of organic solvents which are expensive and environmentally unfriendly (11). At present, there is a growing interest in developing new extraction methods of natural plants based on the use of small amounts of organic solvents. Pressurized liquid extraction (PLE) is an excellent alternative to conventional organic solvent extraction techniques that combines elevated temperature and pressures with liquid solvents to increase the extraction efficiency of phenolic compounds from natural plants (10). Since PLE is conducted at elevated pressures, it allows liquid extraction at temperatures above the boiling point of the solvent at atmospheric pressure, thereby improving analyte solubility and its desorption from the matrix (12). Temperature is used to break the analyte-matrix bonds and modify the relative permittivity of the extracting fluid. This technique also allows the required volume of extraction solvent to be reduced and the extraction time to be shortened. Light and oxygen in the air are two most important factors that facilitate degradation reaction of phenolic compounds. PLE offers the possibility of performing the extractions under an inert atmosphere and protected from light, which represents an attractive advantage (12,13).

Sapium japonicum was known to contain more than 55% oils in seed and its oil could be useful in cosmetics, detergents, and a few pharmaceuticals (14). The objective of this study was to determine the optimal extraction conditions for total phenolics from *S. japonicum* as a model substrate using a pressurized liquid extractor.

Materials and Methods

Plant material *Sapium japonicum* collected in Jeju, Korea was washed, dried, grinded (Ika Work, Inc., Wilmington, NC, USA), and passed through a standard sieve No. 30 (ChungGye Sanggongsa, Seoul, Korea). The sample was stored in freezer at −20°C until needed.

Pressurized liquid extraction *S. japonicum* powder (1 g) was extracted using enhanced solvent extraction system (SFX 3560; Isco Inc., Lincoln, NE, USA). The system was outfitted with 2 syringe pumps, one (B) for organic solvent (Model 260DX) and the other (A) for CO_2 (Model 100DX), and a variable restrictor (Fig. 1). The extraction involved first placing an extraction cartridge with the sample inside the extraction chamber. Next, the supply valve was switched to the open position which allowed organic solvent from pump B to enter the extraction cartridge. The extraction chamber was then pressurized to

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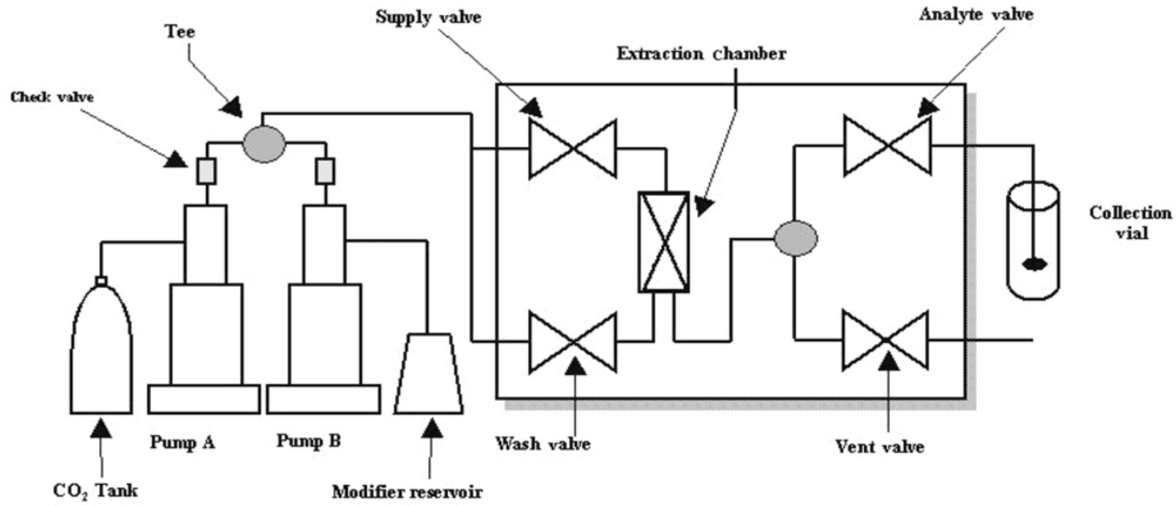

Fig. 1. Schematic diagram of pressurized liquid extraction system.

the desired pressure. After attaining equilibrium, a static extraction was initiated. After completion of the static period (3 min), the analyte valve was switched to the open position which allowed a certain volume (10 mL) of solvent from pump B to extract the sample out of the extraction cartridge at a flow rate of 1 mL/min. After completion of the dynamic extraction period, pump A which was filled with pressurized CO_2 flushed the remaining solvent out of the extraction chamber for 5 min. A 20-mL vial at ambient temperature was used to collect the extract. After completion of the CO_2 flush, both supply and analyte valves were closed and the system was vented. All extractions were done using 9-mL high temperature crystalline polymer cartridges. The cartridge was filled with a 15-20 mesh of inert sea sand (Junsei Chemical Co., Ltd., Tokyo, Japan) between the sample (2 and 5.2 g at the bottom and top, respectively) to prevent the clogging of the system. End caps have molded in 2-μm frits. Extraction was carried out using EtOH:H_2O (40:60, v/v) at 40°C and 10.2 MPa for 10 min. Extraction of the sample was carried out using 2 extraction steps, and the extracts were collected in 1 vial. After the extraction solvent was evaporated in a rotary vacuum evaporator at 40°C, it was adjusted to 10 mL with the extraction solvent and filtered through a 0.45-μm cellulose acetate filter (Advantec, Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The extract was kept in the dark in a freezer (−20°C) before analysis. Each extraction and analysis was carried out in triplicate.

To study the influence of different parameters on the extraction efficiency of total soluble solids and total phenolics, the type of extraction solvent, the ratio of solvent to water, number of extraction steps, temperature, and pressure were investigated as shown in Table 1.

Total solids and total phenolics assay Total solid content was measured by drying method at 105°C. The extraction yield of total soluble solids (TSS) was calculated from the solid content in the extract based on that in the dried sample. Total phenolics (TP) content in the extract was determined by the Folin-Ciocalteu method (15). The reaction mixture was composed of 0.1 mL extract, 7.9 mL distilled water, 0.5 mL of Folin-Ciocalteu's reagent (Fluka, Steinheim, Switzerland), and 1.5 mL of a 20% sodium carbonate solution (added 2 min after the Folin-Ciocalteu's reagent). After initial mixing, the flasks were allowed to stand for 2 hr. The optical density of the blue-colored samples was measured at 750 nm using the enzyme-linked immunosorbent assay (ELISA) reader (Multiskan EX; Thermo Electrom Corp., Vantaa, Finland). Results were expressed in mg gallic acid equivalents (GAE)/g dry sample by comparison with a calibration curve built with standard gallic acid (Sigma-Aldrich, St. Louis, MO, USA).

Statistical analysis Statistical analyses were performed using SAS V.8.2 software (16). Signification differences ($p<0.05$) among treatment means were determined by the Duncan's test.

Results and Discussion

Extraction solvent The plant material is frequently extracted using the organic solvents with different polarity,

Table 1. Details of the experimental parameters employed for optimization of the extraction procedure for phenolic compounds from *S. japonicum* using a pressurized liquid extractor

Extraction parameter	Solvent composition	Extraction step	Temperature (°C)	Pressure (MPa)
Solvent composition (20, 40, 60, 80%)	Variable	2	40	10.2
Extraction step (1, 2, 3)	EtOH 40%	Variable	40	10.2
Temperature (40, 50, 60, 70, 80°C)	EtOH 40%	2	Variable	10.2
Pressure (10.2, 13.6, 17.0, 20.4 MPa)	EtOH 40%	2	50	Variable

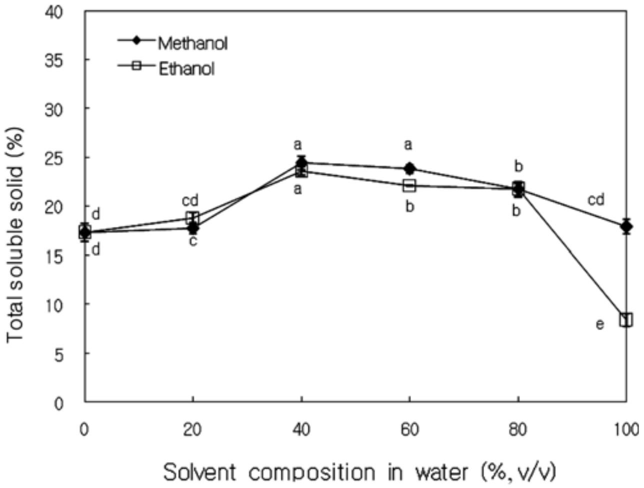

Fig. 2. Influence of solvent composition on the extraction yield of total soluble solids from *S. japonicum.* Extraction pressure, 10.2 MPa; temperature, 40°C; No. of extraction step, 2. Same letters are not significantly different at 5% level by Duncan's multiple test.

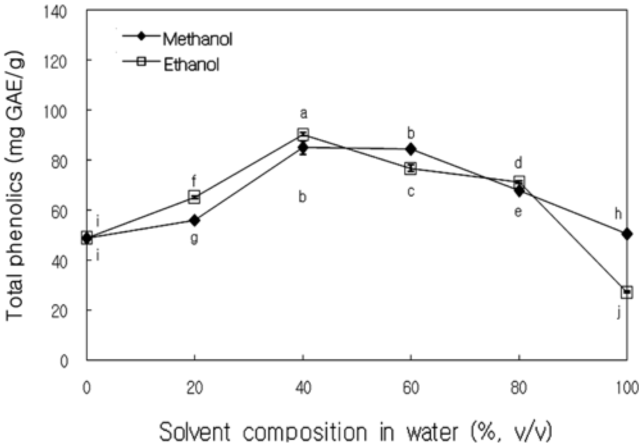

Fig. 3. Influence of solvent composition on the extraction efficiency of total phenolics from *S. japonicum.* Extraction pressurem 10.2 MPa; temperature, 40°C; No. of extraction step, 2. Same letters are not significantly different at 5% level by Duncan's multiple test.

and the extraction yield of the soluble solids strongly depends on the properties of the solvent because polyphenolic compounds may exist as free, conjugated, and polymeric forms, and complexes with carbohydrate, protein, or other plant components (9).

Effect of neat solvents such as MeOH, EtOH, and H_2O on the extraction yield of TSS from *S. japonicum* is shown in Fig. 2. The highest extraction yield of TSS was achieved with H_2O (17.3%) and MeOH (17.9%), while the lowest with EtOH (8.4%). Influence of neat solvents on the extraction efficiency of TP from *S. japonicum* is shown in Fig. 3. Use of neat MeOH resulted in the highest TP extraction yield (50.4) compared with H_2O (48.8) and EtOH (27.2 mg GAE/g).

As flavonoids and phenolic acids are more soluble in methanol than ethanol (17), it is reasonable to obtain a higher extraction yield when methanol is used as an extraction solvent as shown in Fig. 2. Chirinos *et al*. (18) have also reported that MeOH gave the highest TP values for 5 solvents such as water, methanol, ethanol, acetone, and hexane from mashua tubers.

The extraction yields of TSS and TP as a function of solvent-water ratio were also evaluated. The influence of solvent composition on the extraction yield of TSS from *S. japonicum* was shown in Fig. 2. The highest yield of TSS was obtained when extractions were carried out with MeOH:H_2O (40:60, v/v), EtOH:H_2O (40:60, v/v), and MeOH:H_2O (60:40, v/v) solvent mixtures.

The variation in the extraction efficiency of TP with different ratio of MeOH:H_2O and EtOH:H_2O solvent mixtures is shown in Fig. 3. The highest TP (90.3 mg GAE/g) was obtained with EtOH:H_2O (40:60, v/v). The extraction efficiency of TP with MeOH:H_2O (40:60, v/v) (85.0) was similar to that with MeOH:H_2O (60:40, v/v) (84.3 mg GAE/g).

Jayaprakasha *et al*. (19) reported that 40-50% ethanol has a greater effectiveness in extracting polyphenolic compounds compared with pure ethanol which was probably due to the increased solubility of flavonoids, phenolic compounds, hydrolysable tannins, and polysaccharides in the mixture of ethanol and water. Alonso-Salces *et al*. (20) also reported that mixture of methanol with water improved the extraction of polyphenols with several hydroxyl groups, such as glycosides which are hydrophilic than a pure alcoholic solvent. Mukhopadhyay *et al*. (3) reported that addition of water was found to swell the plant material and allow the solvent to penetrate to the solids matrix more easily. Thus, TP yields using solvent mixtures were higher than those using neat solvents only.

When the extract was used for medicinal or ingestion purposes, pure EtOH or a mixture of EtOH and H_2O has typically been used due to the toxicity of MeOH. Also, due to the experimental results obtained so far in this study, EtOH:H_2O (40:60, v/v) solvent mixture was selected for further experiments.

Extraction steps The influence of the number of extraction steps on the extraction yield of TSS and TP from *S. japonicum* using EtOH:H_2O (40:60, v/v) as the extraction solvent is shown in Fig. 4. Higher extraction yield of TSS was obtained in more than 1 extraction step. However, 3 extraction steps showed the same yield as 2 extraction steps. In terms of TP yield, 80.7, 88.8, and 97.0 mg GAE/g were obtained in 1, 2, and 3 extraction steps, respectively.

Even though the extraction yield of TP with 3 extraction steps was higher than that with 2 extraction steps, 2 extraction steps were chosen for clarifying the effect of additional PLE parameters for extracting TP from *S. japonicum*.

Temperature effect The influence of temperature on extraction efficiency of TSS and TP was investigated since it impact the equilibrium solubility, mass transfer rate, and the stability of phenolic compounds (9).

S. japonicum was extracted with EtOH:H_2O (40:60, v/v) solvent mixture at 5 different temperatures (40, 50, 60, 70, and 80°C) and 10.2 MPa with 2 extraction steps. Extraction temperature had very little effect on the extraction yields of TSS as shown in Fig. 5. However, the yield of TP was

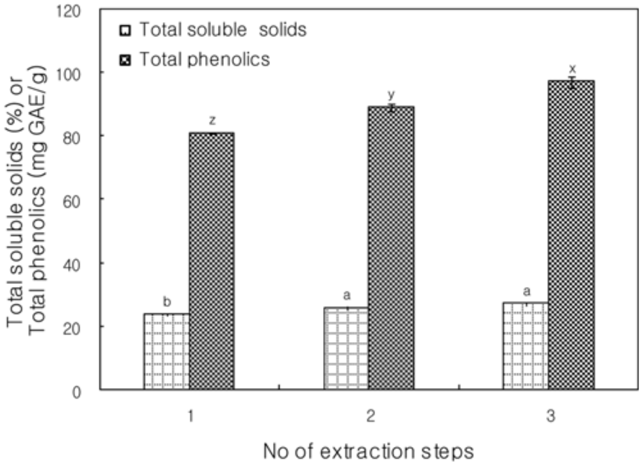

Fig. 4. Influence of extraction steps on the extraction yield of total soluble solids and total phenolics from *S. japonicum.* Solvent composition, EtOH:H_2O (40:60, v/v); extraction pressure, 10.2 MPa; temperature, 40°C. Same letters are not significantly different at 5% level by Duncan's multiple test.

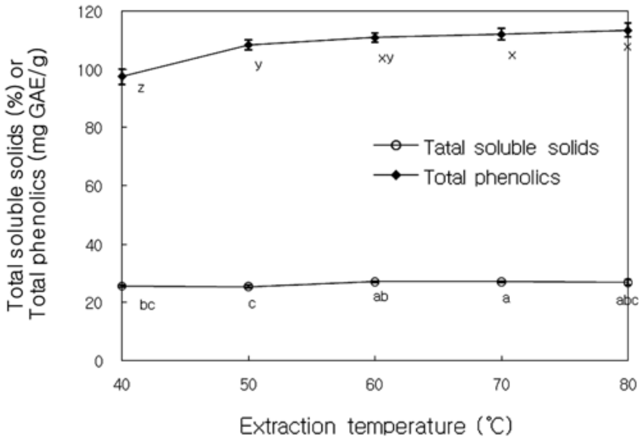

Fig. 5. Influence of temperature on the extraction yield of total soluble solids and total phenolics from *S. japonicum.* Solvent composition, EtOH:H_2O (40:60, v/v); extraction pressure, 10.2 MPa; No. of extraction step, 2. Same letters are not significantly different at 5% level by Duncan's multiple test.

increased by almost 11% as the temperature increased from 40 to 50°C. Higher TP yield at higher temperature is due to the breakage of bonds between various phenolics and the plant matrix (3).

However, TP contents were remained almost unchanged over 50°C such as 110.9 at 60°C, 112.0 at 70°C, and 113.4 mg GAE/g at 80°C. Alonso-Salces *et al.* (20) reported that the extraction yields of TP were slightly increased from 40 to 60°C and decreased at higher temperatures. In those experiments over 50°C, the formation of black colored precipitates in the extracts was observed. This phenomenon could be due to a possible degradation of polyphenols at high temperatures, caused by hydrolysis, internal redox reactions, and polymerizations (20).

On the basis of these results we chose to use 50°C as the extraction temperature for the rest of our experiments.

Pressure effect High pressure of PLE allows the use of temperature well above their atmospheric boiling point of the solvent and increases the diffusivity of the extraction solvent with the plant matrix (21).

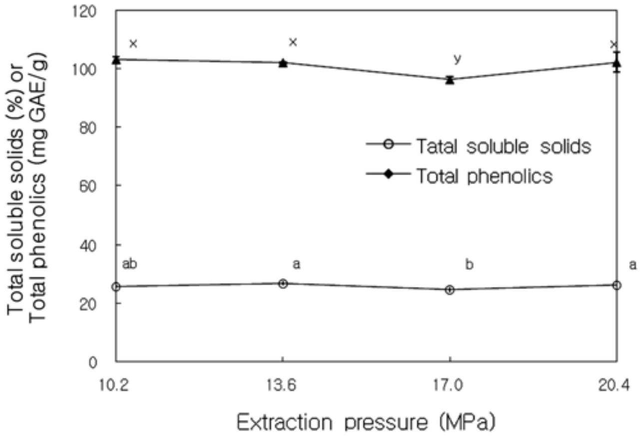

Fig. 6. Influence of pressure on the extraction yield of total soluble solids and total phenolics from *S. japonicum.* Solvent composition, EtOH:H_2O (40:60, v/v); extraction temperature, 50°C; No. of extraction step, 2. Same letters are not significantly different at 5% level by Duncan's multiple test.

Extractions were carried out at 4 different pressures (10.2, 13.6, 17.0, and 20.4 MPa) because the minimum operating pressure of the PLE machine used in this experiment was 10.2 MPa. The influence of pressure on the extraction yield of TSS from *S. japonicum* is shown in Fig. 6. Extraction pressure had no effect on the extraction yields of TSS. The extraction yields of TSS obtained at 10.2, 13.6, 17.0, and 20.4 MPa were 25.7, 26.6, 24.5, and 26.1%, respectively. An increase in extraction pressure also did not change the amount of TP. Values of TP obtained at 10.2, 13.6, 17.0, and 20.4 MPa were 103.1, 102.0, 96.3, and 102.1 mg GAE/g, respectively.

Similar results were observed during extraction of phenolic compounds from black cohosh at 3.4, 6.8, and 10.2 MPa (3) and from parsley at 6.8, 8.5, and 10.2 MPa (9). Actually the purpose of pressurizing the extraction chamber is to prevent the solvent from boiling at the extraction temperature and to ensure that the solvent remains in intimate contact with the sample (20).

In conclusion, the optimized operating conditions for extraction of TP from *S. japonicum* collected in Jeju were: EtOH:H_2O (40:60, v/v); temperature, 50°C; pressure, 10.2 MPa; 2 extraction steps.

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