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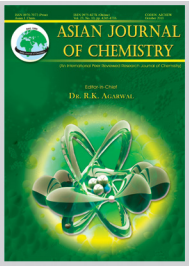

Extraction of Kurarinone and Leachianone A from *Sophora flavescens* Ait using Ultrasonic Wave

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Sophora flavescens Ait (Leguminosae) is a Chinese herbal medicine. Sophorae radix, the dried roots of *S. flavescens*, has been used for various diseases including atherosclerosis and arrhythmias. For the extraction of the kurarinone and leachianone A from Sophorae radix, different ultrasonic waves (40, 72 KHz) and extraction time (30, 60 min) were used with different extraction solvents such as water and ethanol. The extracted samples were analyzed by reversed-phase high performance liquid chromatography coupled with mass spectroscopy (RP-HPLC-MS). In HPLC condition, the mobile phase applied was linearly changed with A/B of 80/20-65/35 vol. % for 60 min (A: water/acetic acid, 99.9/0.1 vol. %, B: acetonitrile/acetic acid, 99.9/0.1 vol. %). From the experimental results, the highest yield of extraction amount 6.025 g was obtained by ultrasonic waves with a frequency of 72 KHz and an extraction time of 30 min. When the pre-irradiated time was increasing, the kurarinone and leachianone A yields decreased. This work would be useful for chemical and biological studies of *S. flavescens* Ait and its products.

Key Words: *Sophora flavescens* Ait, Kurarinone, Leachianone A, Ultrasonic wave, HPLC-MS.

INTRODUCTION

The dried root of *Sophora flavescens* Ait (Leguminosae), a typical traditional Chinese medicine, is commonly used for the treatment of viral hepatitis, cancer, viral myocarditis, gastrointestinal hemorrhage and skin diseases (such as colpitis, psoriasis and eczema)[1]. Previous phytochemical studies of *Sophora flavescens* Ait have reported the isolation of quinolizidine alkaloids, flavonoids and triterpenoids[2,3]. Of them flavonoids are well known for their antitumor activity, being able to bring about the differentiation and/or growth inhibition in various cancer cells, such as lung, esophageal, colourectal, breast and prostate cancers as well as osteosarcoma[3]. The dried roots of *Sophora flavescens* Ait contain flavones series such as kuraridin, kurarinone, isokurarinine, norkurarinone, pterocarpin, formononetin, trifolirhizin, daidzein, umbelliferone, maackiain, kuraridinol, kurarinol, neo-kurinol and norkurarinol[4]. Lee *et al.*[5] isolated kurarinone from *Sophora flavescens* Ait inhibited MCP-1 (Monocyte chemoattractant protein-1)-induced chemotaxis, which the migration of monocytes and would play a role in the development of atherosclerotic lesions. Furthermore, kurarinone has antiinflammatory and antiarthritic activity[6]. Some pterocarpans and flavanones such as kurarinone and leachianone A from *Sophora flavescens* Ait displaying potent neuraminidase inhibition[7].

In previous studies for the separation and identification of the bioactive compounds in *Sophora flavescens* Ait, several methods, including thin-layer chromatography, high-performance liquid chromatography (HPLC) and capillary electrophoresis, have been developed and used[8]. Among these analysis methods, HPLC is the most widely used method for determination of kurarinone and leachianone A in *Sophora flavescens* Ait. In recent years, ultrasonic-assisted extraction as a novel technique for extraction of pollutants has gained increasing attention[9]. Ultrasonic-assisted extraction has been found to have many benefits for extraction efficiency and extraction time. In addition, it can be carried out at lower temperature, which avoid thermal damage. Till now, ultrasonic-assisted extraction has not been used for extracting kurarinone and leachianone A from *Sophora flavescens* Ait. Therefore, we estimated the possibility of using ultrasonic treatment for extraction of kurarinone and leachianone A from *Sophora flavescens* Ait. in this study.

EXPERIMENTAL

The dried root of *Sophora flavescens* Ait was purchased from the Kyung-dong market, Seoul, Korea in October 2007. Acetonitrile and ethanol were all of HPLC grade and from Duksan Pure Chemical Co., Ltd., (Ansan, Korea). Acetic acid (analytical grade) was from Oriental Chemical Industries

(Incheon, Korea). Doubly distilled water was filtered by a decompressing pump (Division of Millipore, Waters) and filter (FH-0.2 μm, Waters, Milford, MA, USA). The compounds of kurarinone and leachianone A were identificated by HPLC online ABTS$^+$ and coupled with mass spectroscopy. The chemical structures of kurarinone and leachianone A is shown in Fig. 1. The molecular weight of kurarinone and leachianone A were 438.51 and 438.53, respectively.

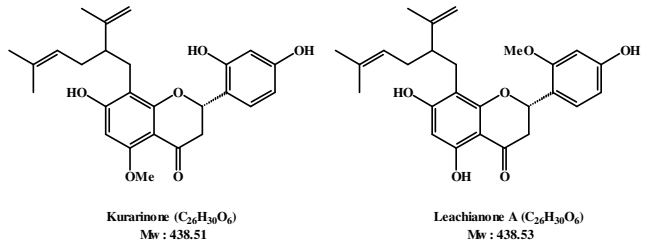

Fig. 1. Chemical structures of kurarinone and leachianone A.

The ultrasonic wave system was bath type with transfixing four-frequency ultrasonic outbreak instrument (220 V, Reactor : 1 L, Model No : Flexonic-500/100, specifications: W 382 × L 450 × H 150 mm, Frequency 40, 72 KHz, Intensity max 300 Watt, Mirae Ultrasonic Tech. Co., Korea). The experimental device for solid-liquid extraction with ultrasonic wave is shown in Fig. 2.

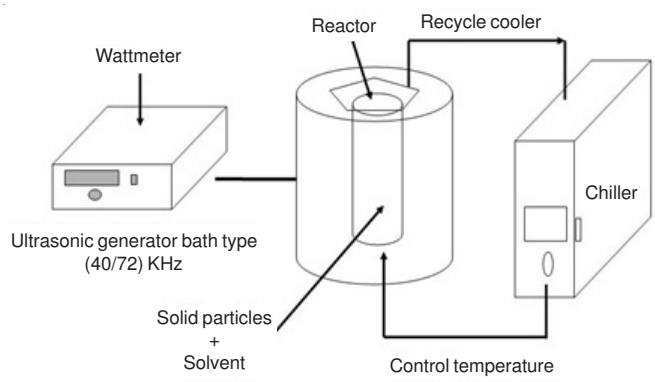

Fig. 2. Scheme of the experimental device in this work

The HPLC system was used a Agilent 1200 (Agilent Technologies, Waldbronn, Germany) with ChemStation (Agilent Technologies, Germany) program and a Rheodyne injection valve (20 μL sample loop). Millennium 3.2 (Agilent Technologies, Germany) was used for data acquisition. Quantitative determination was based on a C_{18} column (5 μm, 250 × 4.6 mm from RS-Tech Co., Korea), where the mobile phase applied was linearly changed with A/B of 80/20-65/35 vol. % for 60 min (A water/acetic acid, 99.9/0.1 vol. %, B acetonitrile/acetic acid, 99.9/0.1 vol. %). The flow rate of 1.0 mL/min with 20 % of the eluent being splitted into the inlet of the mass spectrometer and the column temperature was maintained at 35 ºC. The injection volume 20 μL and the UV spectra were recorded between 190 and 610 nm for peak characterization and the detection wavelength was set at 254 nm. UV spectra were measured with a UV-1600 visible spectrophotometer. A 10 min re-equilibration time was used between HPLC runs.

The mass spectra were acquired using an Agilent 1100 series SL ion trap mass spectrometer equipped with an electrospray ionization (ESI) interface. Nitrogen was used as the sheath and auxiliary gas and helium was used as the collision gas. The ESI-MS spectra were acquired in the positive ion mode recorded over a mass range of m/z 150-800. Capillary voltage was 3000V. Drying gas temperature was set at 350 ºC with a gas flow rate of 10.0 L/min and nebulising pressure was of 35 psi.

Extraction: Twenty grams of the dried root of *Sophora flavescens* Ait dissolved in 400 mL of 100 % ethanol or water were extracted by various ultrasonic waves (40, 72 KHz) and extraction time (30, 60 min). The ultrasonic-assisted extraction was compared with the dipping extraction method at same extraction times. After the extracted solution was filtered with paper filter, the extraction solutions were concentrated and adjusted to volume of 100 mL. Further, after the extracted solution was filtered with membrane filter, this solution were injected the HPLC coupled with mass spectroscopy. The extraction and purification process of kurarinone and leachianone A from *Sophora flavescens* Ait is shown in Fig. 3. Depending on the extraction method, each extract underwent decompression concentration to rotary evaporate the solvent. Then extract samples were expressed as a percentage of the weight. The extraction yield was measured using the following equation:

$$\text{Extraction yield}\,(\%, \text{wt/wt}) = \frac{\text{Extracts dry weight}}{\text{Sample dry weight}} \times 100\,\%$$

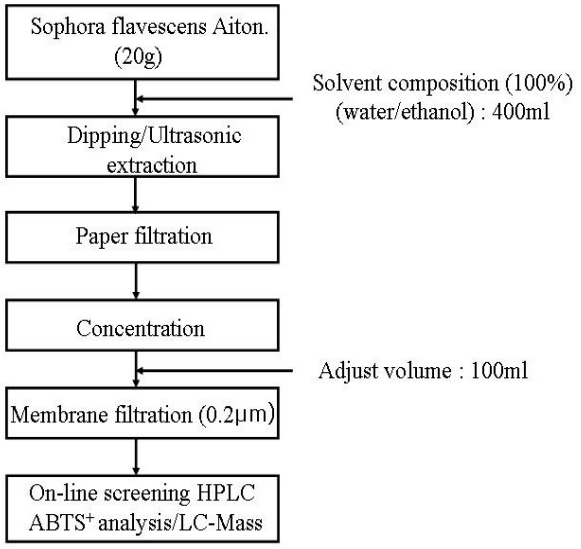

Fig. 3. Extraction and purification process of kurarinone and leachianone A from *Sophora flavescens* Ait

RESULTS AND DISCUSSION

In this study, 100 % ethanol and water were used to extract the kurarinone and leachianone A from *Sophora flavescens* Ait by using ultrasonic wave, respectively. The effects of variation in extraction methods such as dipping and ultrasonic wave were measured. For purification and separation the kurarinone and leachianone A from *Sophora flavescens* Ait, the HPLC-UV instrument was used. To determine the highest

TABLE-1
YIELD OF KURARINONE AND LEACHIANONE A FROM *Sophora flavescens* AIT

Extraction solvent	Extraction method		Extraction time (min)	Retention time (min)		Peak area (%)		Total extraction amount (g)
				Kurarinone	Leachianone A	Kurarinone	Leachianone A	
Water (100%)	Frequency (KHz)	40	60	32.331	41.543	1.0840	0.1157	5.465
			30	32.363	41.589	0.8384	0.1311	5.610
		72	60	32.308	41.523	0.9684	0.1248	5.817
			30	32.238	41.473	1.0693	0.1223	6.025
	Dipping	-	60	32.355	41.492	0.4812	0.0947	5.350
			30	32.254	41.531	0.9526	0.1171	4.857
EtOH (100%)	Frequency (KHz)	40	30	32.756	42.496	16.512	2.5316	0.684
		72	30	32.801	42.480	18.578	2.4705	0.966
	Dipping	-	30	33.074	42.795	16.006	2.4502	0.775

UV absorption, UV-1600 visible spectrophotometer was used. UV-VIS analysis showed that organic compounds had strong absorption peak near 254 nm (Fig. 4). Absorption within this zone indicated the presence of aromatic structure, aldehydes, ketones and conjugated unsaturated structure compounds in the samples. Since the UV-VIS scanning method has limitation in detecting all the contaminants and organic ingredients in the samples, further analytical measurements should be employed in order to provide more information. So, 254 nm of UV wavelength was used to detect the kurarinone and leachianone A in HPLC analysis. From the HPLC analysis, the retention times of kurarinone and leachianone A were 32-33 min and 41-43 min in this study, respectively (Fig. 5). The qualitative analysis of the solution collected from peak #1 and peak #2 by LC-ESI-MS is shown in Fig. 6. The molecular weights of the solution from the peak #1 and peak #2 were 438 (Fig. 6). Considering the molecular weights of kurarinone and leachianone A were 438, this implies that the collected solution from peak#1 and peak #2 were found as kurarinone and leachianone A, respectively. For extraction the kurarinone and leachianone A from *Sophora flavescens* Ait, different ultrasonic waves (40, 72 KHz) and extraction time (30, 60 min) were used. The experimental results, the highest yield of extraction amount 6.025 g was obtained by ultrasonic waves with a frequency of 72 KHz and an extraction time of 30 min (Table -1). It is known, when the ultrasonic frequency and pre-irradiated time were increasing, the kurarinone and leachianone A yield increased and decreased, respectively. However, conductivity increases at higher frequencies and higher ultrasonic intensities, suggesting that yield of urarinone and leachianone A were increased during the sonolysis. Ultrasound, having frequency between 20 and 100 kHz, is now well known to have significant effects on the rate of various processes in the analytical laboratory[10]. Previous workers studied the effect of the applied ultrasonic power on the degradation of ibuprofen and concluded that a higher applied power lead to higher degradation rate in almost linear relationship. Although more than 100 W of applied power should lead to faster degradation. It is not recommendable to exceed this value, because the piezoelectric generator of the ultrasound instrument could be damaged[11,12]. The result indicated that moderate ultrasound was helpful to release saponins from the fiber package, resulting in increase in diosgenin yield[13]. The enhancement on extraction efficiency by ultrasound can be explained that the collapse of cavitation bubbles during

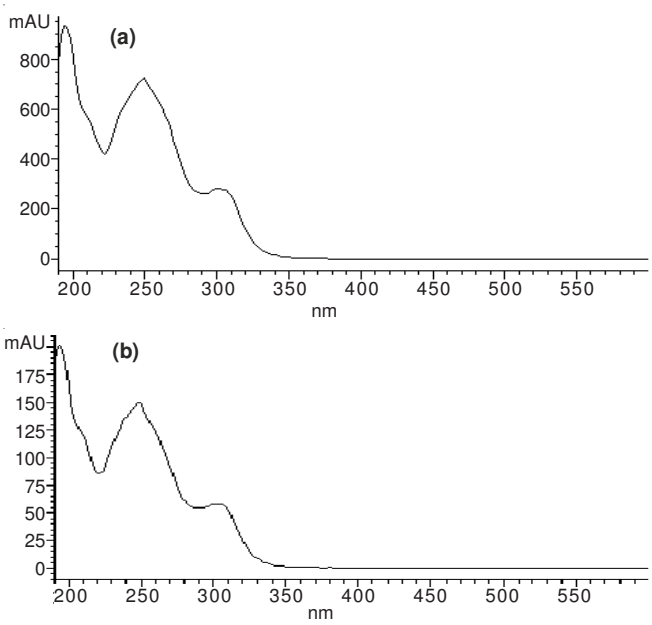

Fig. 4. UV spectrum of kurarinone (a) and leachianone A (b).

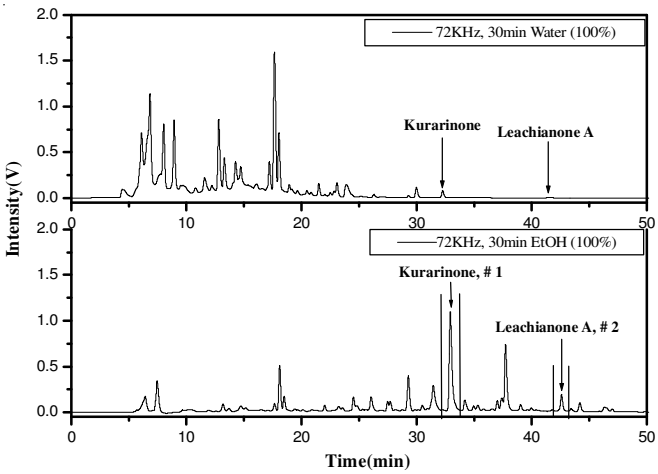

Fig. 5. HPLC Analysis of kurarinone and leachianone A from *Sophora flavescens* Ait.; (Mobile phase A: water 99.9 vol. % + AA 0.1 vol. %, B: ACN 99.9 vol. % + AA 0.1vol. %, gradient elution B : 20-35, run time : 50 min, flow rate : 1 mL/min, injection volume : 20 µL, wavelength : 254 nm)

ultrasonic pre-irradiation released enormous amount of energy, which was expected to damage cell walls and resulted in better contact with the extraction medium[14]. During sonication, the cavitation process caused the swelling of cells or the breakdown

of cell walls, which allowed high diffusion rates across the cell wall in the first case or a simple washing out of the cell contents in the second[15]. However, when the ultrasonic pre-irradiated time was too long, the kurarinone and leachianone A yield decreased. LC-MS analysis indicated the presence of kurarinone and leachianone A after ultrasonic pretreatment too long. These results confirmed that ultrasonic pretreatment led to formation of byproducts with low volatility and high hydrophilic character, migrating to the bulk of the solution and thus, they did not follow a degradation process by pyrolisis or ˆOH attack[12]. Form the identification by HPLC-MS, the extracts were proved that is Kurarinone and leachianone A. This method was also used to extract and identified more useful compound from other nature plants.

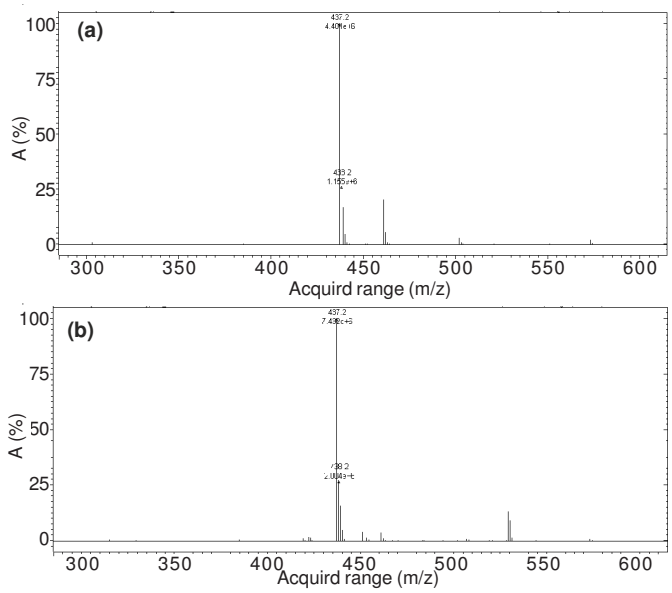

Fig. 6. Mass spectrum of kurarinone (a) and leachianone A (b) from *Sophora flavescens* Ait.

Conclusion

Kurarinone and leachianone A were extracted from *Sophora flavescens* Ait by using 100 % ethanol and water with different ultrasonic wave and pre-irradiated times, respectively. The effluents collected solutions from the RP-HPLC column were identified by LC-MS. From the experimental results, the highest yield of extraction amount 6.025 g was obtained by ultrasonic waves with a frequency of 72 KHz and an extraction time of 30 min. When the ultrasonic frequency and pre-irradiated time were increasing, the kurarinone and leachianone A yield increased and decreased, respectively. These results will form a database for investigating the constituents of natural products and the resources of pharmaceutical, nutrition and cosmetic products.

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