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Extraction Kinetics of Ultrasound-assisted Extraction of Oleanolic Acid from Hedyotis corymbosa

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Abstract. This study investigated a hyphenated procedure of heat-reflux and ultrasound-assisted extraction (HUAE) to improve the extraction efficiency of classic solvent extraction techniques, such as heat-reflux extraction, to extract oleanolic acid (OA) from Hedyotis corymbosa. The superior yield was achieved at a a mean particle size of 0.355 mm, a duty cycle of 75%, a liquid/solid ratio of 12 mL/g, an ethanol concentration of 80% and with extraction temperatures of 56 °C. Compared with heat-reflux extraction method, the HUAE technique reduced the extraction time, extraction temperature and solvent consumption and afforded a higher extraction yield of the target compound from the plant matrix. A second-order kinetic model described the extraction process under different extraction temperatures and showed good agreement with the experimental results.

Keywords: *Hedyotis corymbosa*, oleanolic acid, heat-reflux extraction, ultrasound-assisted extraction.

1 Introduction

Hedyotis corymbosa (L.) Lam. (synonym Oldenlandia corymbosa (L.) Lam.) belongs to an evergreen Rubiaceae genus, and the medicinal herb is derived from the dried whole plants. The herb is widely used in China as a healthy tea [1] and as a traditional Chinese herbal medicine for the treatment of various diseases, such as skin disease, malaria, intestinal abscess, hepatitis and cancer [2]. Oleanolic acid (OA) is a natural pentacyclic triterpenoid carboxyl acid and is one of the most famous bioactive

plant compounds (Fig. 1). Many plants contain OA and have been reported to exhibit a wide range of biological functions, including anti-cancer, chemopreventive, hepatoprotective, antiviral, antibacterial, anti-diabetic, antioxidant, anti-inflammatory and gastroprotective properties [3, 4]. Furthermore, OA can inhibit proliferation and induce apoptosis in many types of cancer cells, including hepatocellular carcinoma, prostate carcinoma, colorectal cancer, acute myelogenous leukaemia, skin tumourigenesis, cervical carcinoma and lung carcinoma [5].

Several extraction techniques have been used to extract OA from plant matrix, including Soxhlet extraction, maceration [6], heat-reflux extraction (HRE) [7], shaking [8] (Yang and Wei, 2015b), ultrasound-assisted extraction (UAE) [9], microwave extraction [10] and supercritical carbon dioxide extraction (SC-CO2) [11]. Ultrasound-assisted extraction (UAE) is a potential alternative technology to the conventional techniques due to its reduced cost and instrumental requirements. Some characteristics of UAE are high extraction efficiency, good reproducibility, simplified manipulation, low consumption of organic solvents and time and lower energy input. Ultrasonic enhancement during extraction is attributed to the disruption of cell walls, particle size reduction, improved penetration, enhanced swelling and hydration processes and enhanced mass transfer of the cell content via cavitation bubble collapses [9]. Furthermore, the combination of energy sources by using conventional extractions under ultrasound irradiation can prevent the destruction of target components and improve the extraction of natural products.

Thus, ultrasound extraction can be combined with the other extraction procedures, such as heat-reflux extraction (HRE) [7], SC-CO2 extraction [11] and microwave-assisted extraction [12], to improve efficiency and yield. Previous studies [7] have shown that the simultaneous application of UAE and HRE can significantly improve the extraction efficiency, require less solvent, shorten the extraction time and extract thermally labile compounds under mild conditions compared with either operation. However, no references have been found in the literature reporting the simultaneous application of UAE and HRE for the extraction of OA from H. corymbosa.

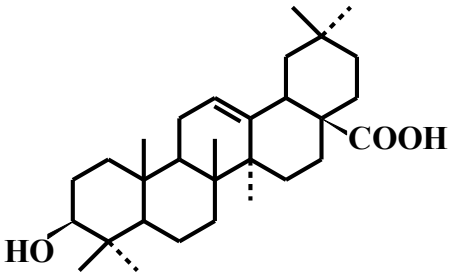

Fig. 1. Chemical structure of oleanolic acid (OA).

The extraction yield and efficiency of the target compounds from raw materials are strongly influenced by the operating conditions during extraction. For solid-liquid extraction processes, the kinetics of solute extraction from raw sources involves releasing the solute from porous matrices into the solvent phase by mass transfer. Therefore, understanding mass transfer at the solid-liquid interface plays a significant

role in scaling up the extraction process. However, no relevant kinetic model of ultrasound-assisted extraction of OA from *H. corymbosa* has been reported. Therefore, the objective of this work is to outline the feasibility of a hyphenated technique of HRE and UAE (HUAE) in the fast preparation of high-yield extracts rich in OA from *H. corymbosa*. Several parameters that could potentially affect the extraction efficiency were evaluated. Finally, the experimental kinetic data curves were validated using a second-order model for predicting the extraction processes and revealing the extraction mechanism.

2 Materials and Methods

2.1 Raw Material

Three sets of authentic plants of dried whole *H. corymbosa* (Sample HC1 to HC3) were procured from different local Chinese medicinal shops (Kaohsiung, Taiwan). All materials were identified and authenticated by professor Ming-Chi Wei with voucher specimens (Sample HC1 to HC3) deposited in the Department and Graduate Institute of Pharmacology, Kaohsiung Medical University (Kaohsiung, Taiwan). All samples were triturated in a knife mill, and were separated according to their sizes (standard testing sieve, series Tyler) with different mean particle sizes of 0.93, 0.73, 0.55, 0.36 and 0.11 mm. Subsequently, the moisture content (% dry weight basis) was determined using oven method for which the methodology has been discussed in detail elsewhere [7]. Briefly, the moisture content (% dry weight basis) was determined by drying at 105 °C to a constant mass and was 11.68, 13.21 and 10.93% for samples HC1 to HC3, respectively. All the yields were calculated based on a moisture-free basis and represent the mean values of at least six experiments. In the study, all the experiments for *H. corymbosa* were performed on sample HC2.

2.2 Chemicals and Reagents

Oleanolic acid (95%) is purchased as HPLC reference standards from Sigma Chemical Co. (St. Louis, MO, USA) and used without further purification. Methanol (99.9%), ethanol (99.9%), acetone (99.7%), acetonitrile (99.9%), ethyl acetate (99.9%), *n*-hexane (95%) and phosphoric acid (85%) were bought from Merck Co. (Darmstadt, Germany). Cyclohexane and sulfuric acid were supplied by Tedia (Fairfield, OH, USA) and Acros Organics (Morris Plains, NJ, USA), respectively. The deionized water was purified using a Milli-Q reverse osmosis unit (Millipore, Bedford, MA, USA).

2.3 Hyphenated Heat-reflux and Ultrasound-assisted Extraction (HUAE) Procedure

The HUAE procedure used in this work has been discussed in detail elsewhere [8] and is briefly described here. The experimental set-up consisted of two extraction stages: HRE (stirring at 300 rpm) and UAE (without stirring). For each experiment, a

5 min HRE was followed by a 2.5–30 min UAE. During the HUAE, 5 g of dried, powdered plant was placed in a 250 mL conical flask with the appropriate extraction solvent volume; this flask was immersed in a temperature-controlled ultrasonic bath (40 kHz working frequency and 185 W power, Branson B-33510E-DTH, USA) with stirring (immersion-stirring device, 300 rpm; Thermo Scientific Variomag Compact and Maxi, USA). The desired extraction temperature was adjusted, and a 5 min HRE was then used to obtain good solvent-to-plant material contact. The water in the ultrasonic bath was circulated and regulated at a constant temperature (Haake F3-K, Haake, Karlsruhe, Germany) to prevent any temperature increase from the ultrasonic waves. To avoid evaporating the extracting solvent, a condenser was connected to the conical flask. After the HRE, the immersion-stirring device was removed from the ultrasonic cleaning bath; UAE was then performed using an intermittent pulse mode. The UAE cycle time consisted of a pulse duration period and a pulse interval period. The ultrasound exposure duty cycle (intermittent sonication, expressed as a percentage) was the proportion of the cycle time spent as a pulse.

The HUAE experiments were performed using various solvent to raw material ratios (6–20 mL/g), duty cycles (0–100%), ethanol concentrations (0–99.5%, v/v), particle sizes (0.925, 0.725, 0.550, 0.355 and 0.106 mm), number of extraction cycles (1–3) and extraction temperatures (30–65 °C). The extraction times ranged from 2.5 to 30 min. All extractions were performed six times for each sample. After the contact time elapsed, the flask contents were immediately filtered before concentration using a rotary evaporator (Eyela Rotary Evaporator N-1000, Tokyo Rikakikai Co., Ltd, Japan).

2.4 Heat-reflux Extraction (HRE)

OA was subjected to HRE for different durations with a stirring speed of 300 rpm; this process was treated as the control. The HRE of OA from *H. corymbosa* was performed in a 250 mL conical flask using the same methods as previous described [11]. Six replicate experiments were performed with various extraction temperatures (30–80 °C), durations (30–120 min), solvent to material ratios (6–20 mL/g), ethanol concentrations (0–99.5%, v/v), mean particle sizes (0.925, 0.725, 0.550, 0.355 and 0.106 mm) and extraction cycles (1–4).

2.5 High-performance Liquid Chromatography (HPLC)

The extracts obtained from best HUAE condition and conventional extraction techniques were analyzed using a Jasco HPLC system which was composed of a column thermostat, a solvent degasser, a Jasco PU-986 intelligent HPLC pump, a Jasco MD-910 intelligent ultraviolet/visible (UV/vis) multi-wavelength detector, and a Jasco AS-2057 plus intelligent auto sampler (Jasco, Tokyo, Japan). Jasco Borwin 1.21 chromatography software (Jasco, Tokyo, Japan) was used for the manipulation of data processing.

All analyses were carried out using initial conditions chosen according to the literatures [9] and were modified to our purpose as follows. Chromatographic separation was carried out in a reverse-phase LiChrospher® C-18 column (250 mm × 4 mm i.d.,

5-μm particle size) connected to a LiChroCart guard column containing the same packing material (both Merck, Darmstadt, Germany) at 40 °C, using a mobile phase consisting of acetonitrile (solvent A) and 0.1% phosphoric acid in water (v/v) (solvent B) under the following gradient profile: 0–26 min, 22–24% (solvent A), flow rate of 1.0–1.5 mL/min; 26–42 min, 24% (solvent A), flow rate of 1.5–1.0 mL/min; 42–60 min, 24–92% (solvent A), flow rate of 1.0 mL/min. The injection volume for all samples was 20-μL, and UV detection was carried out at 210 nm for OA. Identification of OA was performed by comparing the retention times and UV–visible spectra of peaks in extracts to those of OA standards. Quantification of OA was achieved by the absorbance recorded in the chromatograms relative to external standards. The extraction yield of OA was calculated from peak area according to calibration curves and was expressed as milligrams per gram of dry plant (mg/g DW).

2.6 Statistical Analysis

All yields and composition analyses were calculated based on a moisture-free basis. The mean and standard deviation (SD) of the mean were calculated from six experiments. The results are expressed as the mean ± SD. Analysis of variance (ANOVA) was carried out using Tukey's method with a significance level of $P < 0.05$ using 2010 Microsoft Office Excel (Microsoft Co., USA) and Origin software version 6.1 (Origin Lab Co., Northampton, MA, USA). Furthermore, the concordance between experimental data and calculated value was established by the average absolute relative deviation (AARD) and the coefficient of determination (R2), which were established using the following equations:

$$AARD(\%) = \frac{100}{n}\sum_{i=1}^{n}\left|\frac{y_{p,i} - y_{e,i}}{y_{e,i}}\right| \tag{1}$$

$$R^2 = \frac{\sum_{i=1}^{n}(y_{p,i} - y_{e,i})^2}{(y_{p,i} - y_m)^2} \tag{2}$$

where $y_{p,i}$ and $y_{e,i}$ are the predicted and experimental values of the extraction yield, y_m is the mean value of the extraction yield, and n is the number of experimental runs.

3 Results and Discussions

3.1 Result of HPLC Analysis of Samples

The HPLC chromatograms of the OA standard and the aqueous ethanol extracts of HRE and HUAE are shown in Fig. 2. Fig. 2 A shows the standard substance with a retention time of 57.17 min for OA. Figs. 2 B and C show chromatograms of dried *H. corymbosa* HRE and HUAE extracts, respectively, and the other compounds in the samples did not interfere with the analysis of OA at the elution time of the analyte

(57.17 min). Peaks in the chromatograms obtained from *H. corymbosa* samples were identified by comparing the retention time with an authentic standard (OA). Therefore, the HPLC system successfully separated and identified OA in the *H. corymbosa* extracts (Figs. 2 B and C) and was used to quantify the content of OA. Therefore, OA was quantified in *H. corymbosa* extracts using a regression equation of the calibration curve of pure OA expressed as milligrams per gram of dry weight (mg/g of DW).

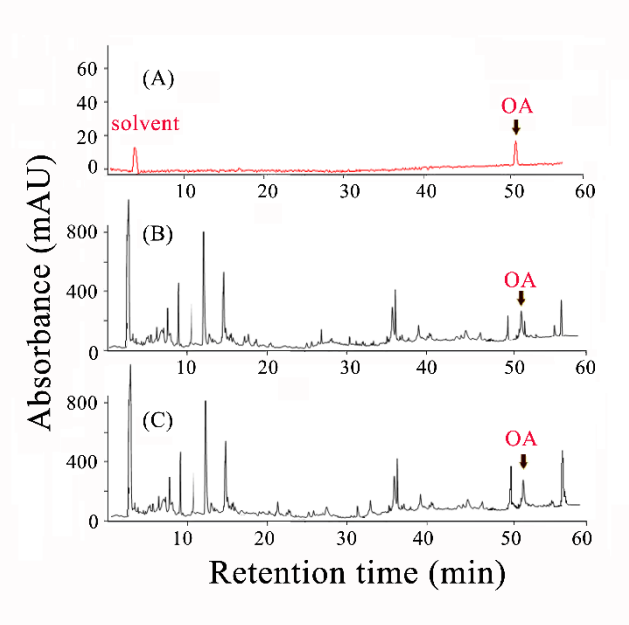

Fig. 2. Typical HPLC chromatograms obtained from a standard solution of OA (A) and *H. corymbosa* extracts obtained from HRE (B) and HUAE (C).

3.2 Effect of Extraction Temperature

The effects of extraction temperature on the extraction yield of OA were determined at a mean particle size of 0.355 mm, a duty cycle of 75%, a liquid/solid ratio of 12 mL/g, an ethanol concentration of 80% and various extraction times (7.5 to 35 min) with extraction temperatures of 31, 37, 44, 50, 56 and 65 °C, as shown in Fig. 3. The extraction yield of OA increased with increasing extraction temperature from 31 to 56 °C and approached a peak value at 56 °C due to the increased diffusion coefficient and increased solubility of OA. However, when the extraction temperature increased from 56 °C to 65 °C, the extraction yield started to decrease due to the increased volatility of UA at higher temperatures. The main effect of ultrasonication was cavitational bubble collapse. The effects of temperature appeared to be due to acoustic cavitation because higher efficiency was observed at 56 °C compared with 65 °C. At a lower temperature of 56 °C, the number of cavitation bubbles increases, and their collapse is powerful enough to effectively extract OA from *H. corymbosa*. Although the num-

ber of bubbles is higher, their collapse is less efficient at 65 °C, which causes the cavitational effects to exhibit a stronger intensity at 56 °C compared with 65 °C. Additionally, increasing extraction temperature might result in increased solvent volatilization, improved energy cost and enhanced impurity extraction; therefore, 56 °C was selected as the optimum temperature to obtain the highest extraction yield of OA.

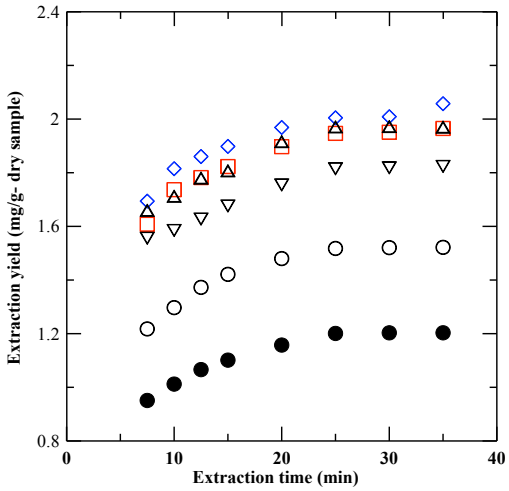

Fig. 3. Influence of temperature on the extraction yield of OA from H. corymbosa using HUAE process.

Extraction conditions: duty cycle: 75%; ethanol concentration: 80% (v/v); mean particle size: 0.355 mm; ratio of solvent to raw material: 12:1 (mL/g). (●: 31 °C, ○ : 37 °C, □ : 44 °C, △ :50 °C, □ :56 °C, □ :65 °C).[1]UA yield (mg/g) = weight of the extracted UA/ weight of feeding material.

3.3 Kinetics of Hyphenated Heat-reflux and Ultrasound-assisted Extraction

The typical kinetic models of solid–liquid extractions, such as Soxhlet extraction and heat-reflux extraction, were appropriately fitted using a second-order rate law [7] to experimentally evaluate the extraction rate constant and concentration at saturation. Because the HUAE process exhibited a similar shape compared with that of the solid–liquid extraction process and the enhancements by the cavitation effects of ultrasonic waves, the curves could be described by the same model [9]. According to a second-order rate law, the rate of dissolution of the OA from the plant cells into solution can be written as:

$$\frac{dC}{dt} = k(C_s - C)^2 \tag{3}$$

Where k is the second-order extraction rate constant (L/g min), *C* is the concentration of target compounds in the liquid extract at a given extraction time t (mg OA /g dry

plant) and *C*s is the extraction capacity (concentration of OA at saturation in mg OA /g dry plant). The integrated rate law for a second-order extraction under the initial and boundary conditions $t = 0$ to t and $C = 0$ to C, C was obtained from the following equations:

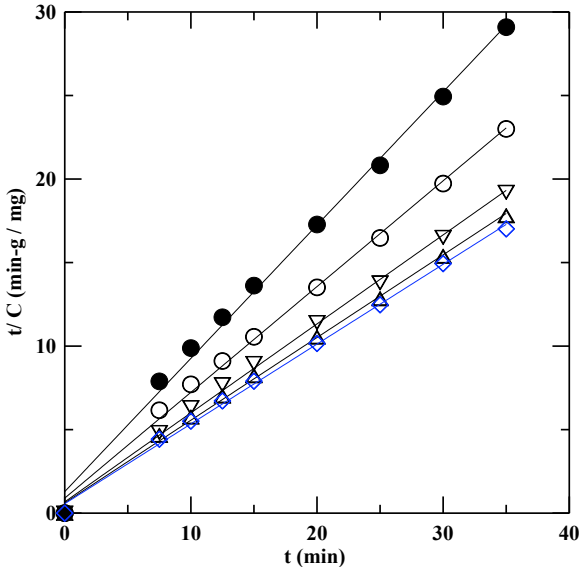

Fig. 4. Second-order extraction kinetics of OA from H. corymbosaat at various temperatures using the HUAE process. (●: 31 °C, ○ : 37 °C, □ : 44 °C, ∆ :50 °C, □ :56 °C,).

However, the saturated extraction capacity (Cs) and the initial extraction rate (h) all increased with temperature, which agreed with previous reports [7].

$$\int_0^C \frac{dC}{(C_s - C)^2} = \int_0^t k dt \tag{4}$$

$$C = \frac{C_s{}^2 kt}{1 + C_s kt} \tag{5}$$

By rearranging Eq. (5), its linear form would be as follows:

$$\frac{t}{C} = \frac{1}{kC_s{}^2} + \frac{t}{C_s} \tag{6}$$

When t approaches 0, the initial extraction rate, h, can be defined as follows:

$$h = kC_s{}^2 \tag{7}$$

The values for h, Cs and k were determined experimentally using the slope and intercept by plotting t/C versus t.

Based on Eq. (6), the t/C values were plotted over time, and the results were fitted as a straight line (Fig. 4) by a linear least squares regression method to estimate Cs and k. The values Cs and k were used to evaluate h values. However, because a slight decrease in the extraction yield was observed when increasing the temperature from 55 to 65 °C (Fig. 3), the experimental kinetic data curve at 65 °C was not included in Fig. 4. The parameters for the second-order rate law, the coefficient of determination (R_2) and the overall average absolute relative deviation (*AARD*) are listed in Table 1. The straight-line curve is shown in Fig. 4, and the high correlation coefficients at all of the temperatures (R_2 >0.996) and the low overall *AARD* (%) imply good agreement of the second order extraction model with the experimental results. Table 1 also showed that the extraction rate constant (k) decreased with increasing extraction temperature.

Table 1. Parameters of kinetic model for the extraction of OA from H. corymbosaat using HUAE technique.

Compounds	T (°C)[a]	h (mg/g-min)[b]	k (g/mg-min)[c]	Cs (mg/g)[d]	R_2 [e]
OA	31	0.775	0.493	1.254	0.995
	37	1.110	0.445	1.579	0.996
	44	1.482	0.421	1.877	0.997
	50	1.633	0.406	2.020	0.997
	56	1.769	0.400	2.093	0.998

[a] Extraction temperature.
[b] Initial extraction rate.
[c] Rate constant.
[d] Equilibrium concentration.
[e] Regression coefficient.

4 Conclusions

To effectively extract value-added oleanolic acid from *H. corymbosa*, the effects of HUAE processing parameters such as time and temperature on extraction performance and global kinetics were systematically studied. The extraction kinetics were investigated by varying time (7.5 to 35 min) and temperature (31 to 65 °C). The results showed that HUAE offers a promising alternative for the efficient extraction of OA from plant matrix. Furthermore, a second-order kinetics model was in good agreement with the experimental results.

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