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Short communication

Optimized conditions for the extraction of secondary volatile metabolites in *Angelica* roots by accelerated solvent extraction

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Abstract

Accelerated solvent extraction (ASE) of three common *Angelica* species found in Asia: *Angelica sinensis* (Oliv.) Diels from China, *Angelica acutiloba* (Sieb. et Zucc.) Kitagawa from Japan, and *Angelica gigas* Nakai from Korea was investigated. Preliminary experiments, including the selection of the solvent, extraction time, pressure, static cycle and time were investigated to optimize experimental parameters. Kováts indices and mass spectra were used to identify the components in the various fractions. These were then confirmed using gas chromatography–mass spectrometry (GC–MS). A total of 18 compounds were identified, with qualitative differences and similarities observed among the cultivars. From the 18 compounds found in the ASE extract of danggui cultivars, the major components were decursin, decursinol angelate (*A. gigas*); butylidene dihydrophthalide, 4-hydroxy-4-methyl-2-pentanone (*A. sinensis*); and 9,12-octadecanoic acid in *Angelica acutiloba*. The optimum ASE operating conditions were *n*-hexane as extraction solvent, extraction temperature and pressure of 80 °C and 1500 atm, respectively, static cycle of 2 min, and static time of 10 min. Under these conditions, the percentages of main analytes were increased.

Keywords: *Angelicae* radix; Chemical constituents; Accelerated solvent extraction; Gas chromatography; Mass spectrometry

1. Introduction

The chemical composition of extracts and essential oils from plant material is very complex. Any analytical procedure for the determination of its constituents involves the application of a sample preparation procedure that should permit to isolate the target substances from the plant matrix. A herbal extract could be defined as the compounds and/or mixtures of compounds obtained from fresh or dried parts of plants (leaves, flowers, seeds, roots, and barks) by different extraction procedures. In general, the active constituents are obtained together with other materials present in the vegetable sample. The extraction of bioactive components from vegetable materials is a part of phytopharmaceutical and food technology [1].

Several procedures have been proposed for isolating and concentrating the volatile compounds prior to gas chromatographic analysis in herbal medicines. These include the conventional methods such as steam distillation and hydrodistillation [2–4], and organic solvent extraction using percolation, maceration or soxhlet techniques. However, these methods require large volumes of solvents, which are often expensive or hazardous. The other disadvantages include long extraction time, labor-intensive procedures, and unsatisfactory extraction efficiency. The desire to reduce these disadvantages has led to the development of newer techniques for extraction of volatile compounds emitted from *Angelica* roots, such as solvent free solid injection (SFSI) [4], solid phase microextraction (SPME) and supercritical fluid extraction (SFE) [5]. A relatively new technique, known as pressurized liquid extraction or accelerated solvent extraction (ASE), has gained acceptance in recent years as an alternative to conventional solvent extraction for separation of compounds in many analytical and industrial processes. ASE has been proposed as an

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improved exhaustive extraction method that, although using the same solvents as soxhlet extraction, requires only small volumes of solvents and permits faster and thorough extractions of compounds [6,7]. ASE operates at high pressures and temperatures above the boiling point of the organic solvent. Therefore, by optimizing the ASE temperature and pressure, selective extraction of the compounds is possible. Here, we carried out for the first time the investigation of the ASE conditions needed to extract the chemical constituents from the three common *Angelica* species found in Asia: *Angelica sinensis* (Oliv.) Diels from China, *Angelica acutiloba* (Sieb. et Zucc.) Kitagawa from Japan, and *Angelica gigas* Nakai from Korea.

2. Materials and methods

2.1. Herb samples

A. gigas Nakai (Korean danggui), and *A. acutiloba* Kitagawa (Japanese danggui) were obtained from a traditional Korean drugstore (Yeosu Province, Republic of Korea) and the species verified by a Korean medical doctor, Dr. Seo Young Nam. *A. sinensis* (Chinese danggui) was purchased from Gansu Province, China and brought by a Chinese student who visited our laboratory in 2004. The dried rhizomes were used for analysis of the volatile components.

2.2. Accelerated solvent extraction

Accelerated solvent extraction was performed using a Dionex model ASE 100 equipped with a solvent controller (Dionex Corp, Sunnyvale, CA). Two circular cellulose filters 1.91 cm in diameter (Dionex Co.) were placed at each end of the standard stainless steel extraction cell (33 ml). Two grams of sea sand standard (Junsei Chemical Co. Ltd., Tokyo, Japan) were introduced into the cell, followed by 5 g of ground *Angelica* roots. After gently tapping the cell to settle the contents, the empty space (above the mixture) was filled with florisil (2 g), Chem tube hydromatrix (4 g) and sea sand (2 g). The cell was tightly closed and an extraction was performed under the optimized conditions. Optimal extraction conditions were achieved by sequentially varying the experimental parameters, one at a time, while all the other parameters remained fixed. The following extraction conditions were altered: extraction solvent acetonitrile, dichloromethane and *n*-hexane; temperature 40, 60, 80, 100 and 120 °C; pressure 500, 1000, 1500, 2000, and 2500 atm; and the duration of the static extraction time 5, 10 and 15 min, in order to determine the conditions that would allow for maximum extraction efficiency. The duration of static extraction time was then followed by 1 min of pre-heating and 5 min equilibration. Following the extraction, the thimble was flushed with solvent (60%) and purged with nitrogen. The solvent was collected in 60 ml vials with Teflon septa. Subsequently, each extraction cell containing the same sample went through another identical extraction cycle and the solvent was collected in the same vial. The total extract volume (60 ml) was transferred into 100 ml pear-shaped flasks and concentrated to about 3 ml using a rotary vacuum evaporator (Büchi Rotavapor R-114; Essen, Germany) at 40 °C. The remaining volume was evaporated to dryness at 55 °C using a gentle stream of nitrogen. The residues were dissolved in 2 ml of organic solvent and stored at 4 °C until injection at room temperature onto the gas chromatograph equipped with mass spectrometer.

2.3. Gas chromatography/mass spectrometry

The components were analyzed by GC–MS (Agilent 6890 gas chromatograph coupled to a 5973 N mass spectrometer, Palo Alto, CA, USA) using an HP-5MS column (30 m × 0.25 mm × 0.25 μm film thickness, Agilent Technologies, Palo Alto, CA, USA) coated with 5% phenyl and 95% methylpolysiloxane. Helium (99.999%) was run as a carrier gas at a constant flow rate of 1 ml/min, and the injector and transfer line temperatures were 250 and 300 °C, respectively. The oven temperature was maintained at 50 °C for 4 min and then increased to 280 °C at the rate of 5 °C/min, after which temperature the column was maintained for 10 min. Samples (1 μl) were injected using the split mode with a split ratio of 1:10 and the mass spectrometer was operated in an ionizing energy of 70 eV. The mass range scanned from 10 to 650 *m/z* at 2.33 s/scan for full-scan mode.

2.4. Identification of the volatiles

Peaks were identified according to Kováts indices [8], and mass spectra were compared either with data compiled in the Wiley 6th edition library (Wiley, New York, NY, USA [9]) or with published mass spectral data. Percent peak areas were calculated by dividing the ion counts for a particular peak (detected by MS) by the total ion counts for the entire chromatogram and expressing this value as a percent. Approximate concentrations of the compounds were calculated according to the external standard method using α-pinene (Sigma–Aldrich, St. Louis, MO) as a reference substance without considering calibration factors, that is, $F = 1.00$ for all compounds.

3. Results and discussion

Achieving maximum efficiency is the greatest concern in ASE method development. There are several parameters that influence the extraction efficiency. Selected compounds were decursin and decursinol angelate (*A. gigas*) and butylidene phthalide (A. *sinensis* and *A. acutiloba*). Because both *A. sinensis* and *A. acutiloba* gave the same results, the finding of *A. sinensis* will be presented throughout the text.

3.1. Effect of solvent

In the first part of this study, the effect of different solvents on the extraction efficiency of *Angelica* roots was evaluated. The proportions of decursin and decursinol angelate in the volatiles extracted by acetonitrile were very low compared to that of dichloromethane. However, when using *n*-hexane as the extraction solvent, the percent of decursin and decursinol angelate increased (Table 1). On the other hand, butylidene phthalide was

Table 1
Extraction efficiency of decursinol angelate and decursin emitted from *A. gigas* and butylidene phthalide emitted from *A. sinessis* via ASE expressed as GC peak area of three replicates $\times 10^{-3} \pm$ S.D.

Parameter	Decursinol angelate	Decursin	Butylidene phthalide
Solvent			
Acetonitrile	17.7 ± 0.07	13.0 ± 0.07	149.7 ± 0.80
Dichloromethane	77.3 ± 0.37	55.4 ± 0.28	150.9 ± 2.10
n-Hexane	150.8 ± 12.01	114.9 ± 8.97	155.0 ± 4.35
Pressures (atm)			
500	111.3 ± 1.91	83.5 ± 1.41	136.4 ± 2.29
1000	143.5 ± 3.32	106.5 ± 2.42	136.0 ± 0.61
1500	222.1 ± 6.55	165.8 ± 4.84	139.3 ± 2.57
2000	197.4 ± 3.95	149.7 ± 2.93	132.8. ± 0.12
2500	147.2 ± 4.21	110.8 ± 3.18	126.3 ± 3.10
Temperatures (°C)			
40	105.9 ± 0.64	81.8 ± 0.48	112.1 ± 2.71
60	127.0 ± 1.22	94.7 ± 0.89	117.7 ± 2.32
80	213.0 ± 2.51	158.7 ± 1.74	128.2 ± 1.68
100	183.7 ± 2.37	135.2 ± 1.69	119.9 ± 8.67
120	177.5 ± 1.10	133.9 ± 0.81	123.2 ± 8.51
Extraction times (min)			
5	194.3 ± 3.65	147.2 ± 2.68	124.0 ± 3.63
10	221.2 ± 6.38	165.8 ± 4.56	119.3 ± 4.46
15	194.3 ± 3.65	157.2 ± 2.68	119.7 ± 3.47

efficiently extracted by acetonitrile and dichloromethane, however, quite better by *n*-hexane (Table 1). In our previous study [4], we observed that hydrodistillation method failed to isolate the main components in *A. gigas*, a finding that supports the present study. Additionally, we found that decursin and decursinol angelate are the major compounds in *A. gigas*, but this was not the case in *A. sinensis* and *A. acutiloba*. In the present study, using *n*-hexane as an extraction solvent, decursin and decursinol angelate were also extracted from *A. sinensis* and *A. acutiloba*, though in very small quantities.

3.2. Effects of pressure and temperature

Pressure and temperature are the two most important physical parameters in ASE, and they have both theoretical and practical implications for the extraction process. In this investigation, the effect of pressure on the composition of extracted compounds was investigated at five different levels of pressure (500, 1000, 1500, 2000, and 2500 atm). The results showed that the proportions of decursin, decursinol angelate and butylidene phthalide in the volatiles, decreased when higher pressures were used. By considering the total GC peak area of decursin, decursinol angelate and butylidene phthalide under different conditions of pressure, the optimum value found for this variable was 1500 atm (Table 1). This pressure facilitates the extraction of secondary volatile metabolites from samples by improving the accessibility of the solvent to the compounds that are trapped in the pores of the matrix. A pressure higher than 1500 atm was excluded because of a safety issue with the equipment.

At a constant pressure (1500 atm), the effect of five different temperatures (40, 60, 80, 100, and 120 °C) on the extraction of compounds from *Angelica* roots was then investigated. Increasing the temperature from 40 to 80 °C at constant pressure drastically increased the proportions of decursin, decursinol angelate and butylidene phthalide in the volatiles, but increasing the temperature from 80 to 120 °C reduced the proportions of these components. The optimum temperature was found to be 80 °C (Table 1). This temperature increases the ability of the solvent to solubilize the compounds and, decrease the viscosity of liquid solvent, allowing better penetration of the solvent into the matrix. High temperature (more than 80 °C) will increase solubility and mass transfer, but decrease selectivity. It has to be noticed that there is no evidence of thermal degradation phenomena on representative thermally labile compounds extracted at higher temperature [7,10,11].

3.3. Effect of static cycle and time

The standard ASE method states that the static cycle is 2 min and the static time 10 min. It has been suggested that the second extraction step extracts most of the compounds of interest; the yield of the first step is much lower but is not negligible, and the yield of the third extraction is very low. Additionally, the yield of a 10 min extraction was higher than that of a 5 and a 15 min extraction for decursin and decursinol angelate, however, this is not the case for butylidene phthalide. Because two compounds were extracted in higher proportions, a static extraction time of 10 min was chosen for all further investigations (Table 1).

3.4. Extraction of secondary volatile metabolites by ASE

Table 2 lists the identified compounds emitted from *Angelica* roots by ASE followed by GC–MS. A total of 18 compounds were identified. The volatile profiles of danggui cultivar samples are shown in the GC–MS chromatograms in Fig. 1. Thirteen, six and seven compounds were characterized in *A. gigas*, *A. sinen-*

Table 2
Determination of secondary volatile metabolites in *Angelica* roots by ASE followed by GC–MS

No.	Secondary metabolites	RT	RI	*A. gigas*		*A. sinensis*		*A. acutiloba*	
				Area (%)	Amount (mg/g)	Area (%)	Amount (mg/g)	Area (%)	Amount (mg/g)
1	4-Hydroxy-4-methyl-2-pentanone	5.4	830	1.21	0.454	8.67	0.221	27.83	0.207
2	2-Methoxy-4-vinylphenol	5.4	1317	–	<0.001	1.64	0.042	–	<0.001
3	β-Farnesene	24.6	1460	–	<0.001	–	<0.001	2.62	0.020
4	α-Muurolene	25.8	1504	0.09	0.034	–	<0.001	–	<0.001
5	Elemol	27.3	1565	0.11	0.041	–	<0.001	–	<0.001
6	γ-Eudesmol	28.8	1631	0.31	0.116	–	<0.001	–	<0.001
7	α-Eudesmol	29.7	1656	0.66	0.247	–	<0.001	–	<0.001
8	Butylidene phthalide	30.1	1673	–	<0.001	4.09	0.104	3.80	0.029
9	Muskolactone	31.1	1740	0.1	0.034	–	<0.001	–	<0.001
10	Butylidene dihydrophthalide	31.5	1758	–	<0.001	85.38	2.165	58.74	0.436
11	9-Octadecanoic acid	36.5	2003	0.11	0.041	–	<0.001	–	<0.001
12	Methoxsalen	37.8	2034	0.05	0.019	–	<0.001	–	<0.001
13	9,12-Octadecanoic acid	38.4	2101	–	<0.001	–	<0.001	6.76	0.051
14	Seselin	38.4	2101	0.11	0.041	–	<0.001	–	<0.001
15	Columbianetin	42.9	2227	1.46	0.547	–	<0.001	–	<0.001
16	Lomatin	43.7	2365	0.42	0.157	–	<0.001	–	<0.001
17	Decursinol angelate	48.9	2757	36.27	13.60	0.09	0.04	0.1	0.04
18	Decursin	50.1	2792	59.10	22.16	0.13	0.045	0.15	0.051

RT: retention time (min); RI: retention index.

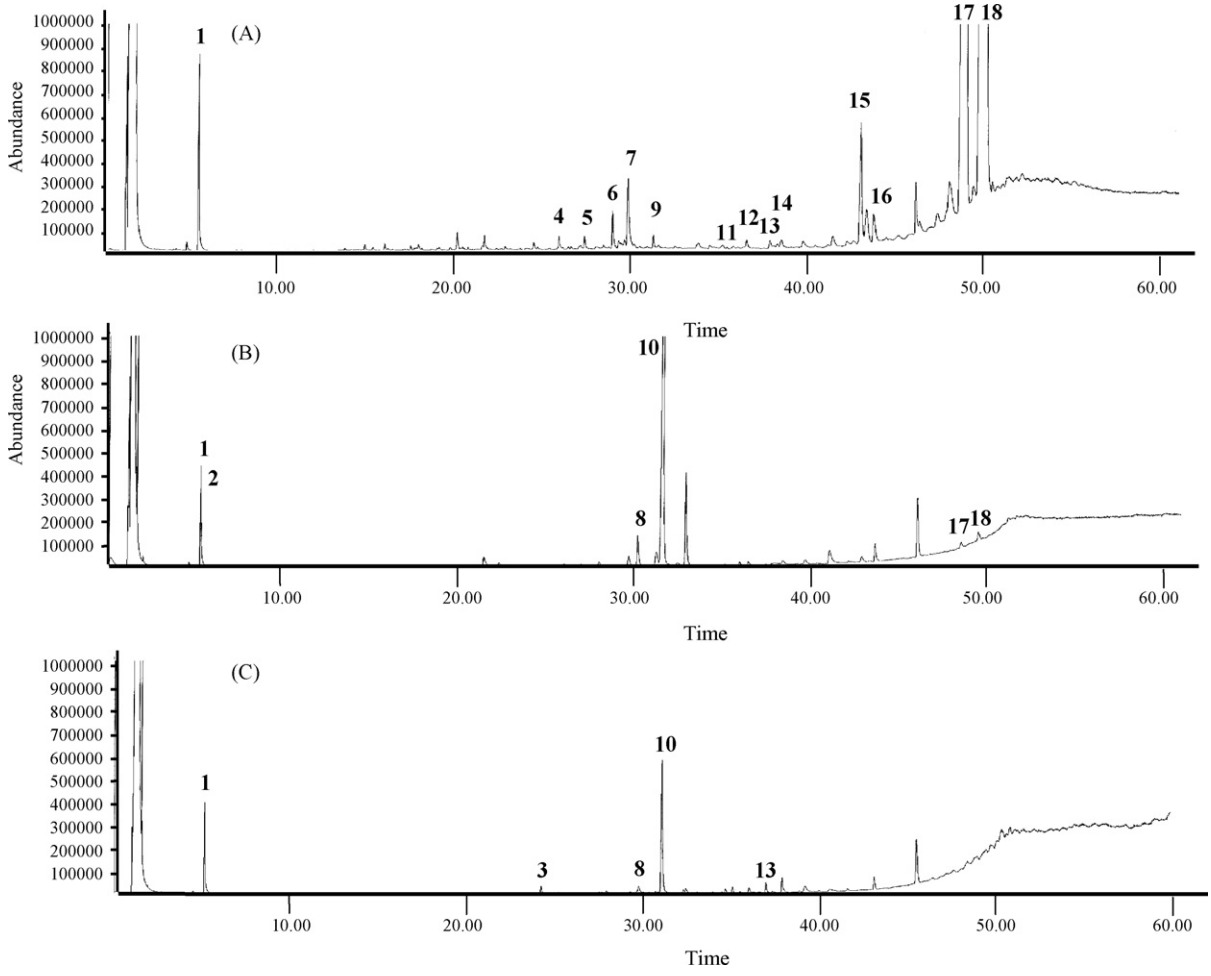

Fig. 1. Typical GC chromatograms of secondary volatile metabolites extracted by ASE from *A. gigas* (A), *A. sinensis* (B), and *A. acutiloba* (C). (See Table 2 for peak identification).

sis, and *A. acutiloba*, respectively. Coumarins were found to be the most abundant flavour compounds in *A. gigas*. Among the coumarins, decursin and decursinol angelate were found to be the most abundant [12–14], whereas butylidene dihydrophthalide was the main component found in *A. acutiloba* [15]. Lao et al. [16] identified 13 compounds including ferulic acid, 3-butylphthalide, *Z*-butylidenephthalide, 3-butylidene-4-hydroxyphthalide, *E*-butylidenephthalide, senkyunolide A, *Z*-ligustilide, *E*-ligustilide, 6,7-epoxyligustilide, senkyunolide F, senkyunolide H, senkyunolide I, and 6,7-dihydroxyligustilide in *A. sinensis* using GC–MS coupled with ASE. They showed that the contents of the investigated components in *A. sinensis* were higher than those in *A. acutiloba* and *A. gigas*, a finding that supports the present study. On the other hand, Song et al. [17] described a method based on GC–MS with the headspace solid phase microextraction (HS-SPME) technique for the analysis of the volatile constituents present in *Angelica pubescens* and *A. sinessis*. They identified 87 and 36 compounds from the essential oil of both routes, respectively. Our findings were not in a good agreement with them. We believe that SPME may effectively recover a greater quantity of volatile components in general without extracting the main components of the roots.

With the exception of the total compounds released from *Angelica* roots, the profile of the ASE is similar to that of the supercritical fluid extraction (SFE) and solvent free solid injection (SFSI) in extracting the main compounds; decursin, decursinol angelate, butylidene dihydrophthalide and butylidene phthalide [4,5]. In particular, ASE produced an enrichment of the active components decursinol angelate, decursin and butylidene dihydrophthalide compared to SFE and SFSI. The data of the present study illustrate the large compositional variations that different geo-botanical conditions can cause in terms of the fractions obtained from plants belonging to the same species. The variability of the qualitative composition of the compounds results from intrinsic features such as genetics and plant age, or to extrinsic factors such as climate, cultivation conditions, and isolation methods.

4. Conclusion

An optimized, small-scale ASE method selectively extracted decursin, decursinol angelate from *A. gigas* and also extracted butylidene dihydrophthalide from both *A. sinensis* and *A. acutiloba*. The ASE followed by GC–MS method is a convenient means by which the concentrations of decursin, decursinol angelate and butylidene dihydrophthalide, the important active components in *Angelica* roots, can be isolated and monitored. This method may be useful in further *Angelica* research.

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