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Original Article

Antioxidant and flavor properties of *Angelica sinensis* extracts as affected by processing

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ABSTRACT

Angelica sinensis (AS) was extracted with water or 20% ethanol for different time periods, and the antioxidant activity as well as flavor quality of the extracts were investigated. The AS extracts contained significant amount of phenolic acids, including nicotinic acid, phthalic acid, *p*-coumaric acid, and ferulic acid. Regardless the water or alcohol extraction, most of the phenolic acids reached their maximum values in 15 min. Assays including inhibition of 1,1-diphenylpicrylhydrazyl (DPPH), lipid peroxidation, and DNA relaxation activities also indicated that 15 min extraction resulted in a product with the highest antioxidant activity. The 15 min AS extracts in the concentration range of 20–200 μg/ml also showed inhibitory effects on NO production in LPS-activated RAW 264.7 macrophage in a dose-dependent manner. Statistical analysis revealed that the antioxidant activity and phenolic acid concentration for all AS extracts exhibited a positive and significant linear correlation, suggesting that the phenolic acids are the important contributors for the antioxidant activity of the AS extracts. The contents of volatile compounds of AS were much higher in the 20% ethanol extracts than those in water extracts. In the 20% ethanol extracts, the amount of ligustilide, butylidene phthalide and butyl phthalide were higher in the 30-min extracts than that prepared for longer time. Considering both of antioxidant activity and flavor quality, the AS extract should be prepared with 20% ethanol with extraction time less than 30 min.

1. Introduction

The rhizome of *Angelica sinensis* (Oliv.) Diels (Umbelliferae), known as Dong-gui in Chinese herb, is one of the most important traditional Chinese herbs used as a sedative or a tonic agent (Hsu and Peacher, 1976). Its varieties of pharmacological effects include anti-oxidative, anti-inflammatory, and immunomodulatory activities (Wu et al., 1999; Liu et al., 2003). The active components of *Angelica sinensis* (AS) include ferulic acid and polysaccharides, the main components found in the non-aromatic fractions. The ligustilide and phthalides are found in the volatile aromatic oil.

Ferulic acid (4-hydroxy-3-methoxycinnamic acid), a phenolic compound, has antioxidant and anti-inflammatory activities and chemopreventive effect on carcinogenesis (Lu et al., 2005; Ou and Kwok, 2004). Ferulic acid has been demonstrated to protect against free radical-mediated changes in conformation with synaptosomal membrane proteins (Kanski et al., 2002). Long-term oral administration of ferulic acid significantly reduced $A\beta_{1-42}$-induced memory impairment in mice (Yan et al., 2001). Ligustilide is a volatile and unstable compound, which can change to other phthalides through oxidation and dimerization (Lu et al., 2004; Doneanu and Anitescu, 1998; Song et al., 2004). Ligustilide and butylidene phthalide possess the suppressive and modulatory activities to the central nervous system (Matsumoto et al., 1998). ROS are capable of causing lipid peroxidation, the oxidation of amino acid residues, the formation of protein–protein cross-links, and DNA oxidative damage. Under normal physiological conditions, ROS can be scavenged by the cellular defending systems, but under certain pathological conditions, the dynamic balance between the generation and elimination of ROS may be broken, causing increases in cellular ROS levels. High levels of ROS may do oxidative damage to various cellular components, and result in cell death. It is believed that ROS are associated with various diseases such as cancer, arteriosclerosis, diabetes, and Alzheimer's disease (Khan, 2002; Johar et al., 2004; Varadarajan et al., 2000; Markesbery, 1997).

ROS can also function as signaling molecules and inducers of signaling pathways in many cell types (Lander et al., 1996).

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Increases in oxidative stress may promote inflammatory cytokine production by the activation of redox-sensitive signal transduction pathways such as the mitogen-activated protein kinase (MAPK) and NF-κB (Suzuki et al., 1997). These signaling pathways control the expression of many genes involved in the innate inflammatory response, cellular proliferation, and apoptosis (Tse et al., 2004). Nitric oxide production has been implicated in the process of carcinogenesis and inflammation. Excessive iNOS (inducible nitric oxide synthase)-mediated NO generation has been reported to cause mutagenesis and deamination of DNA bases and to form carcinogenic *N*-nitrosoamine (Huang et al., 2005). Antioxidant, such as docosahexaenoic acid, has been proven to be able to inhibit the accumulation of intracellular peroxides, and indirectly result in the inhibition of NO production and iNOS expression (Komatsu et al., 2003). And inhibitors of iNOS may have a therapeutic role in certain cancers (Lala and Chakraborty, 2001).

Because of the health-promoting function and its unique pleasant flavor, *Angelica sinensis* is frequently used as an ingredient for preparing medicinal Chinese foods, such as Dong-gui Duck, Dong-gui Chicken, and Dong-gui Mutton. Traditionally, the Dong-gui foods is often prepared by mixing these food materials with slices of Dong-gui, covering with water or rice wine (containing alcohol 20%), and simmering for several hours. It is assumed that longer cooking time would extract more active components from AS. However, some of the active compounds may decompose during the long processing time involving high heat (Lu et al., 2004; Song et al., 2004; Gadow et al., 1997). Many antioxidants in foods are significantly lost as a consequence of processes such as sterilization, pasteurization, and dehydration as well as prolonged storage (Manzocco et al., 1998). Long-term cooking may also cause the loss of volatile compounds and affect the flavor quality of the foods. Therefore, this study was carried out to investigate the changes of non-volatile components and their effects on the antioxidant activity of AS extracts. In addition, the changes of volatile components were monitored in order to evaluate the effect of extraction at elevated temperatures on the flavor property of *A. Sinensis*.

2. Materials and methods

2.1. Chemicals and materials

LPS (*Escherichia coli* O127:B8) and other chemicals were from Sigma chemical Co. (St. Louis, MO). All of the solvents used in this study were from E. Merck (Darmstadt, Germany). The DMEM medium, fetal bovine serum, penicillin, and streptomycin were obtained from Gibco BRL (Grand Island, NY). *A. Sinensis* (Oliv.) Diels (AS) were purchased from a Chinese medicine shop (Jung-hwa, Taipei, Taiwan).

2.2. Preparation of A. sinensis extracts

A. sinensis (AS) was identified by the Center of Herbal Authentication of Taipei Medical University and stored at −20 °C To simulate the preparation of medicinal Chinese foods, 40 g of sliced AS was extracted in 2 l of boiling water, and another 40 g were extracted using 2 l of 20% boiling ethanol solution for various periods of time (15, 30, 60, 90 min). The extract was cooled and centrifuged at 5000 rpm for 10 min, and filtered to remove insoluble materials. The supernatants were adjusted to the original volume (2 l), and the obtained preparations were designated ASW (*A. sinensis* water extractions) and ASalc (*A. sinensis* alcohol extractions) and stored at −20 °C.

2.3. Determination of phenolic acids

The phenolic acids were analyzed by HPLC (Agilent 1100 HPLC system, Palo Alto, CA) equipped with a diode-array detector (DAD). The separations were performed on a HS ODS column (5 μm, 250 × 4.6 mm, Torrance, CA) at 25 °C. Aliquots of 20-μL were injected and eluted at flow rate 0.8 mL/min with a mobile phase composed of solution A (30 mM potassium dihydrogen phosphate at pH 3.50) and solution B (acetonitrile/H_2O = 80/20). Phenolic compounds in the AS extracts were eluted with gradient from 100% A initially to 90% A and 10% B at 20 min, 88% A and 12% B at 30 min, 35 min, 70% A and 30% B, 40% A and 60% B at 45 min, and 100% B at 50 min. Detection was at 320 nm for ferulic acid and 210 nm for other phenolic acids. The limit of detection (LOD) has been estimated as 3 SD. The total peak area of phenolic acids (TPA) was also used for determining the total phenolic acids content, which is defined as the sum of peaks area ≧3 SD. For the calibration of phenolic acids, the standards were weighed and dissolved in methanol to give serial concentrations and three injections onto HPLC were performed for each dilution. The concentrations of phenolic acids in the samples were calculated according to the standard curve. The results were expressed as microgram per milliliter of AS extracts.

2.4. Quantification of volatile compounds

The liquid–liquid extraction method was used to extract the volatile compounds of AS extracts (Charles et al., 2000). An aliquot of 100 mL AS extracts, 200 mL dichloromethane, and 10 mg/L *n*-decanol as internal standard, was mixed in a flask cooled by crushed ice for 30 min. After settling for ca. 30 min, the bottom layer (dichloromethane) was collected using a separator funnel. The dichloromethane extracts were dried over anhydrous sodium sulfate, and concentrated by a vacuum evaporator at 30 °C and 30 cm-Hg vacuum. Then, the residue was re-dissolved in methanol, filtered with 0.22 μM PVDF, and stored at −80 °C for analysis. Volatile compounds were identified and quantified by GC–MS (Doneanu and Anitescu, 1998) using a Hewlett-Packard 5890 Series II chromatograph coupled to a Hewlett-Packard 5890A MSD mass spectrometer, and equipped with Carbowax 20 M column (30 m × 0.32 mm i.d., film thickness = 0.25 μM) (J & W Scientific Inc., Folsom, CA, USA). The injector temperature was 220 °C, detector temperature 260 °C, and the oven programmed from 50 to 220 °C at an increasing rate of 5 °C/min. The carrier gas was helium at a flux of 0.8 mL/min. Compounds were identified by the comparison of GC–MS spectra to their characteristic spectra from a database. Ligustilide, butyl phthalide, and butylidene phthalide were determined on the basis of the internal standard. The results were expressed as milligram per 100 mL of AS extracts.

2.5. Antioxidant activities of AS extracts

2.5.1. DPPH radical scavenging assay

The reaction was performed in 3 mL of methanol containing 250 μM of freshly prepared 1,1-diphenylpicrylhydrazyl (DPPH) and 1 mL of the AS extracts. Reaction mixtures were protected from light and incubated for 90 min at room temperature, after which the absorbance of the remaining DPPH was determined colorimetrically at 517 nm. The scavenging activity of AS extracts were measured as the decrease in absorbance of the DPPH expressed as a percentage of the absorbance of a control DPPH solution without AS extracts (Chen et al., 2000). All analyses were carried out in triplicate.

2.5.2. Inhibition of lipid peroxidation

Lipid peroxidation was assayed by measuring malondialdehyde (MDA) according to the method of Sánchez-Moreno et al. (1999). Arachidonic acid was oxidized by the Fenton reaction (Fe^{2+}/H_2O_2). The AS extracts were added to 0.5 mL of an aqueous solution containing arachidonic acid (2.5 mM), 0.25 mmol of Trizma-HCl/0.75 mmol of potassium chloride buffer (pH7.4), 0.2% *N*-lauroyl sarcosine, 1 μM of ferrous sulfate, and 0.5 μM of hydrogen peroxide in a 2 mL micro-tube. The mixture was agitated for 16 h at 37 °C The degree of oxidation was measured by the thiobarbituric acid method. An aliquot of 20 μL testing solution was mixed in a microtube with 20 μL aqueous 8% sodium dodecyl sulfate (SDS) 150 μL of 1 M acetate buffer, and 150 μL of aqueous 0.67% 2-Thiobarbituric acid (TBA). The mixture was heated at 90 °C for 60 min. After cooling, 0.5 mL of butanol was added and mixed. The solution was centrifuged, and the absorbance of the upper butanol layer was measured at 532 nm. The antioxidant activity of the tested sample for lipid peroxidation was expressed by the % inhibition, which was calculated as: % inhibition = [(absorbance of control−absorbance of test sample)/absorbance of control] × 100%. A test solution without AS extracts was used as control.

2.5.3. Inhibition of plasmid DNA relaxation

The inhibitory effect of AS extracts on super-coiled DNA strand breakage nicking caused by the Fenton reaction was evaluated (Ermilov et al., 1999). An amount of 200 ng of pUC-19 plasmid DNA was incubated at 37 °C for 30 min in TE buffer (10 μM Tris/1 μM EDTA, pH = 8.0) containing 100 mM hydrogen peroxide and 100 μM ferrous sulfate. This was done in the presence or absence of AS extracts with a final volume of 20 μL. Then, the samples were loaded onto 1% agarose gel, and electrophoresis was performed in a TAE buffer (40 mM of Tris-acetate and 1 mM of EDTA) in the presence of 0.5 μg/mL of ethidium bromide. After electrophoresis, the gel was photographed under transmitted ultraviolet light.

2.6. Nitric oxide (NO) assay

2.6.1. Cell culture

The mouse monocyte-macrophage cell line RAW 264.7 (ATCC TIB-71) was cultured in Dulbecco's modified Eagle's (DMEM) medium supplemented with 10% heat-inactivated fetal bovine serum. Cells were plated in at a density of $1.0 \times 10^6\ mL^{-1}$ for 18–24 h before activation by lipopolysaccharide (LPS) (50 ng/mL). AS extracts dissolved in the DMEM medium were administered with LPS.

2.6.2. Determination of nitrite

The nitrite accumulating in the culture medium was measured as an indicator of NO production according to the Griess reaction (Kim et al., 1995). Cells were plated in 24-well culture plate and stimulated with LPS (50 ng/mL) in the presence or absence of various concentrations of AS extracts for 24 h. An aliquot of 100 μL of the culture medium (cell free) was mixed with 50 μL 1% sulfanilamide (in 5% phosphoric acid) and 50 μL 0.1% naphthylethylenediamine dihydrochloride (in distilled H_2O) at room temperature. Absorbance at 550 nm was measured with a sodium nitrite serial dilution standard curve, and nitrite production was determined.

2.7. Statistical analysis

The results were represented as the means ± standard deviation from three independent experiments ($n = 3$). Statistical analysis was performed using ANOVA. The difference between the means was determined by Duncan's multiple range test.

3. Results and discussion

3.1. Non-volatile components of AS extracts

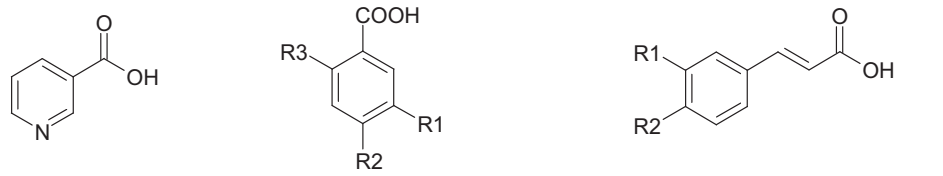
The amounts of phenolic acids from AS subjected to different extraction conditions were examined because these non-volatile compounds are considered to be the biologically active components of AS (Graf, 1992; Huang and Sheu, 2006). Fig. 1 shows the chemical structure of phenolic acids, a major class of phenolic compounds that occur widely in the plant kingdom (Proestos et al., 2006). Predominant phenolic acids include hydroxybenzoic acids (e.g. protocatechuic acid, vanillic acid) and hydroxycinnamic acids (e.g. ferulic acid, caffeic acid, *p*-coumaric acid, cinnamic acid). Although the nicotinic acid does not belong to the family of phenolic acids, it is an important chemical constituents of *A. sinensis* (Hsu, 1968; Huang and Sheu, 2006). The phenolic compounds were readily identified using HPLC-DAD by comparison with authentic standards. Fig. 2 displays a typical HPLC chromatogram of phenolic standards. The HPLC profiles in Fig. 2B show that AS extract (20% ethanol extraction) contained significant amount of nicotinic acid, phthalic acid, *p*-coumaric acid, and ferulic acid.

The effects of extraction time and types of solvent on the phenolic acid contents of AS extracts are shown in Table 1. The ferulic acid contents were the highest among various phenolic acids in AS extracts. Cai et al. (2004) summarized the major types of phenolic acids from most medicinal herbs, and indicated that ferulic acid was the major phenolic compound in AS. In the water extraction, most of the phenolic acids reached their maximum values in 15 min except the ferulic acid content reached a high point in 30–60 min (18.9 μg/mL), and decreased once the time surpassed 60 min. In the 20% ethanol extraction, the ferulic acid, nicotinic acid, *p*-coumaric acid, and phthalic acid contents all displayed the highest value within 15 min. It should be noted that the contents of phenolic acids in the ethanol extracts decreased after 15 min of extraction, but increased again when the extraction time extended to 90 min. There are several possibilities. First, some of the phenolics might be destructed during thermal processing. Second, the major phenolic compound, ferulic acid, is readily oxidized under heat, and therefore could be degraded due to the boiling (Song et al., 2004; Zhao et al., 2003). Third, it has been found that beside the natural content of ferulic acid in herbs, the free ferulic acid could be obtained during processing through hydrolyzing compounds such as coniferyl ferulate (Lu et al., 2005). It was therefore suspected that changes of phenolic acids contents in the AS extracts were most likely affected by the combined effects of the phenomena described above. Nevertheless, it appeared that 15 min, in general, was enough to extract most of the phenolic acids from the AS, and the 20% ethanol solution might be the preferred solvent.

Because of the diversity and complexity of the natural mixtures of phenolic compounds in AS extracts, characterizing every compound is rather difficult. In this study, the total peak area of phenolic acids (TPA) as determined by HPLC was also used for determining the total phenolic acids content in the extract (Table 1). It appeared that 15 min, in general, was enough to extract most of the phenolic acids from the AS.

3.2. Antioxidant activity of A. sinensis extracts

The inhibition of 1,1-diphenylpicrylhydrazyl (DPPH), lipid peroxidation, and DNA relaxation activities by various AS extracts



Nicotinic acid

Phthalic acid, R1=R2=H, R3=COOH
Protocatechuic acid, R1=R2=OH, R3=H
Vanillic acid, R1=OMe, R2=OH, R3=H

Caffeic acid, R1=R2=OH
Ferulic acid, R1=OMe, R2=OH
p-coumaric acid, R1=H, R2=OH
trans-cinnamic acid, R1=R2=H

Fig. 1. Chemical structures of phenolic acids and nicotinic acid.

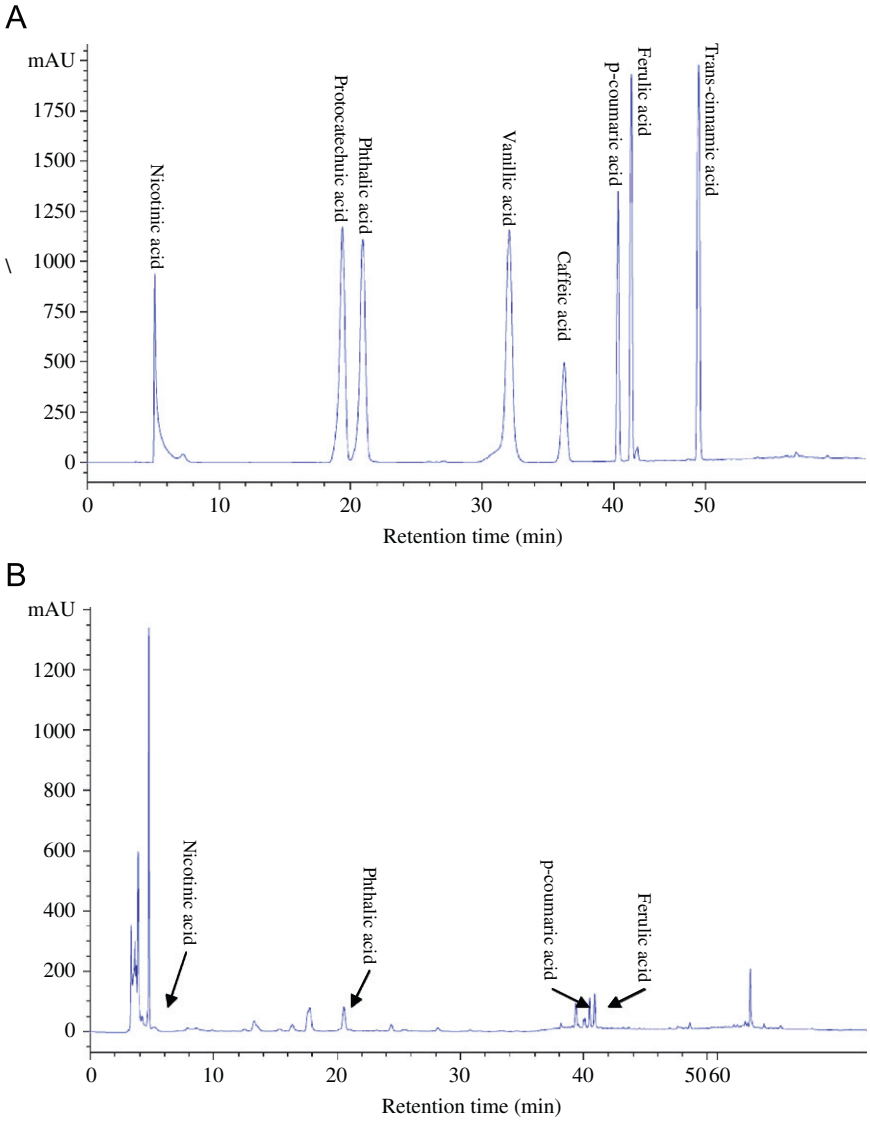


Fig. 2. Chromatogram of standard phenolic compounds (A) and ASalc15 extract of the root of *Angelica sinensis* (Oliv.) Diels (B) measured at 210 nm. Analytical column: HS ODS column (5 μm, 250 × 4.6 mm) with ODS guard column (5 μm, 4 × 4 mm); Sample injection volume: 20 μL; Mobile phase: solution A, 30 mM potassium dihydrogen phosphate, pH = 3.5, and solution B, acetonitrile/H_2O = 80/20, flow rate: 0.8 mL/min; temperature: ambient.

were assessed for their antioxidant activity. DPPH radicals were routinely used for evaluating the antioxidant activity of plants, red wine, and tea extracts. The DPPH scavenging activities were not significantly different among 15-, 30-, and 90-min AS water extracts (ASW), but the 15- and 60-min water extracts (ASW15 and ASW60) showed a higher scavenging effect (Table 2). For the ethanol extracts (ASalc), the highest scavenging activity appeared at 15 min, and decreased as the extraction time was prolonged.

Table 1
Phenolic acid contents of *Angelica sinensis* with different extraction conditions

Treatment of AS	Nicotinic acid (μg/mL)	Phthalic acid (μg/mL)	P-coumaric acid (μg/mL)	Ferulic acid (Mg/mL)	TPA (mAU s)
ASW15	2.46±0.64 a	0.44±0.03 ab	1.49±0.24 a	15.8±1.7 bc	25378±1408 ab
ASW30	2.23±0.66 a	0.51±0.03 a	1.59±0.16 a	16.7±2.0 ab	25552±2457 ab
ASW60	1.91±0.07 ab	0.43±0.02 ab	1.84±0.33 a	18.9±2.7 a	27133±3571 ab
ASW90	1.69±0.21 ab	0.38±0.1 b	1.64±0.85 a	15.1±1.2 bc	23913±3649 b
ASalc15	2.14±0.18 a	0.44±0.03 ab	1.68±0.35 a	17.1±1.5 ab	29489±2551 a
ASalc30	2.27±0.39 a	0.41±0.07 ab	1.51±0.51 a	15.6±3.0 bc	23700±3942 b
ASalc60	1.30±0.10 b	0.21±0.01 c	1.03±0.56 a	13.9±4.1 c	16985±5934 c
ASalc90	1.70±0.48 ab	0.43±0.11 ab	1.31±0.86 a	15.8±1.9 bc	25261±2211 ab

ASW represents water extraction.
ASalc represents alcohol extraction.
Mean±SD ($n = 3$), mean within a column followed by the same lowercase letter are not significantly different at $p > 0.05$.
The total peaks area of phenolic acids (TPA): the sum of phenolic acids peak areas ≧ 3 SD.

Table 2
Comparison of DPPH free radical scavenging activities and lipid peroxidation inhibitory activities of *A. sinensis* extracts

Treatment of AS	DPPH scavenging activity (% of control)	Lipid peroxidation inhibitory activity (% of control)
ASW15	75.7±1.6 abc	85.1±4.4 a
ASW30	72.3±1.2 dc	84.5±2.2 a
ASW60	79.0±0.6 a	85.6±1.6 a
ASW90	73.0±0.4 bcd	84.3±2.8 a
ASalc15	76.8±2.3 ab	87.9±3.1 a
ASalc30	70.3±2.4 d	77.0±4.4 ab
ASalc60	66.0±3.3 e	72.7±8.9 b
ASalc90	76.5±2.2 ab	76.9±1.6 ab
Vitamin E (200 μg/mL)[a]	77.1±1.3	51.2±6.8
Vitamin C (200 μg/mL)[a]	94.2±2.6	0.0±0.0

ASW represents water extraction.
ASalc represents alcohol extraction.
Mean±SD ($n = 3$), mean within a column followed by the same lowercase letter are not significantly different at $p > 0.01$.
[a] Positive control.

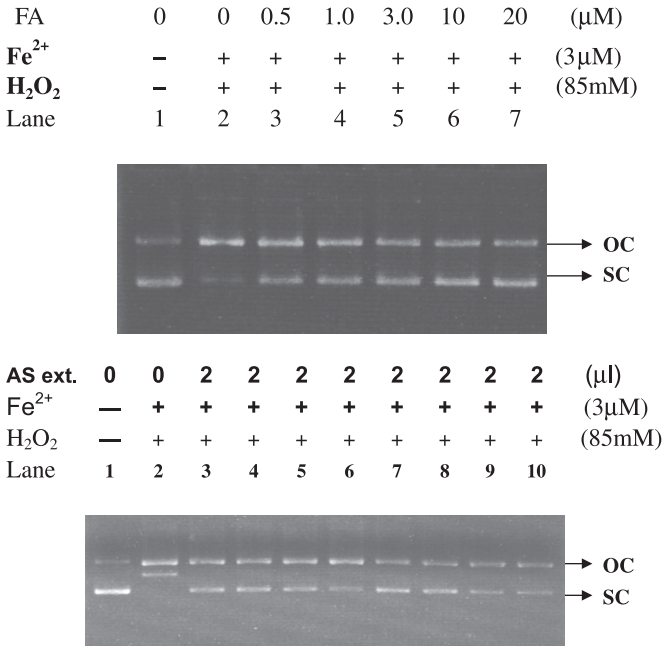


Fig. 3. Comparison of the AS extracts suppressed ROS induced DNA strand breakage. Upper: various ferulic acid (FA) concentrations (0.5–20 μM); lower: AS extracts. SC, super-coiled form DNA; OC, open circular form DNA. Lane 1: only plasmid DNA, Lane 2: plasmid DNA with H_2O_2 and ferrous sulfate, Lane 3-10: plasmid DNA with H_2O_2, ferrous sulfate and AS extracts. Lane 3: ASW15, Lane 4: ASW30, Lane 5: ASW60, Lane 6: ASW90; Lane 7: ASalc15, Lane 8: ASalc30, Lane 9: ASalc60, Lane 10: ASalc90.

However, the DPPH scavenging activity of the AS ethanol extract increased again when the extraction time extended to 90 min. The AS extracts were also evaluated for their ability to reduce lipid peroxidation induced by the Fenton reaction. The water extracts showed only slight variation in lipid peroxidation inhibitory activities with $p > 0.05$. ASalc extract of 15 min exhibited a higher inhibitory activity on lipid peroxidation. Similar to the DPPH scavenging activity, the 60-min extraction with 20% ethanol also showed a relatively lower inhibitory activity on lipid peroxidation.

Hydroxyl radicals (•OH) generated by the Fenton reaction are known to cause DNA strand breaks to yield open circular DNA (relaxed circular DNA). Hydroxyl radical scavengers can protect DNA from strand breaks induced by hydroxyl radicals. The hydroxyl radical scavenger ability of AS extracts was evaluated. Super-coiled DNA migrated faster than relaxed open circular DNA on an agarose gel electrophoresis, and DNA strand breakage was induced in vitro in the presence of H_2O_2 and Fe^{2+} (lane 2, Fig. 3). DNA in the presence of H_2O_2 or Fe^{2+} alone did not show significant strand breakage (data not shown). Treatment of DNA with ferulic acid (Fig. 3, upper) or AS extracts (Fig. 3, lower) reduced the concentration of relaxed circular DNA. In addition, it appeared that the most protective effect came from ASalc15 (lane 7, Fig. 3, lower).

The results of antioxidant evaluations suggest that a short AS extraction time (15 min) is enough to produce a product with favorable antioxidant effect. All hydroxyl radicals in this study were generated by Fenton reaction, which involved Fe^{2+} as radical initiator. The iron chelating ability of natural phytochemicals had been reported elsewhere to be an important criterion of antioxidation (Hagerman et al., 1998). This study further implies that the AS extracts enhance antioxidant activity through their iron chelating ability, in addition to being ROS scavengers.

3.3. Inhibition of NO production by AS extracts

The overproduction of NO is a key biochemical event during inflammation. Many anti-inflammatory agents possess effectiveness in suppressing NO production in vivo. In this study nitrite production was used as an indicator of NO released in the LPS-activated macrophage. The nitrite concentrations in culture

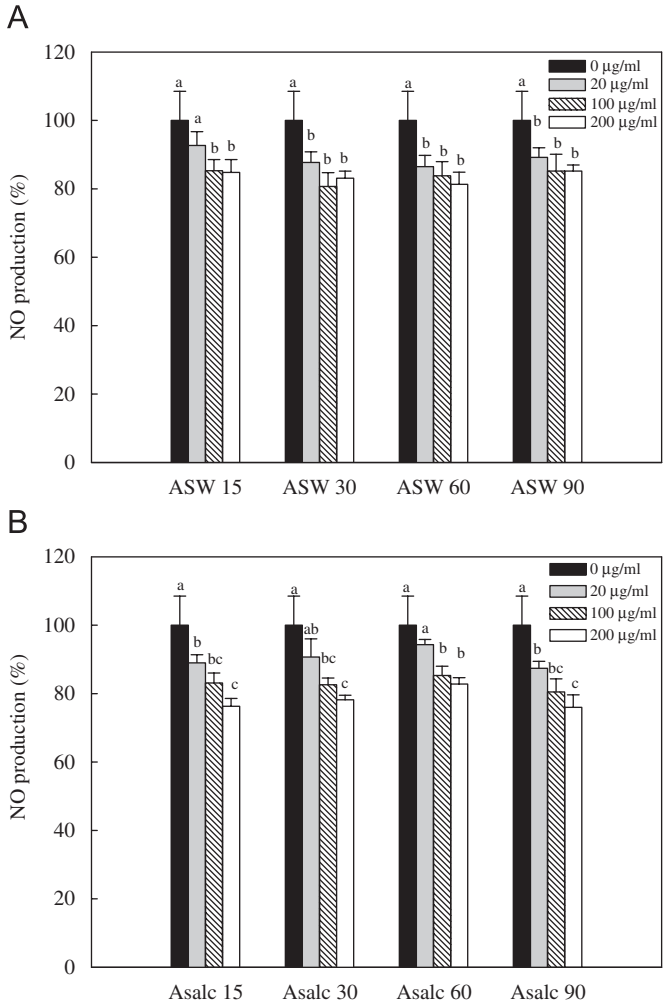


Fig. 4. Effect of AS extract on the NO production of Raw 264.7 cells in the presence of LPS. Raw 264.7 cells were cultured with AS extracts in the presence of 50 ng/mL LPS for 48 h. Water extracts (A), alcohol extracts (B). The accumulation of NO was assayed by the Griess reaction. Data shown are mean ± SD of the results of six cultures.

media were measured with and without AS extracts co-incubated macrophage activated with LPS (50 ng/mL). When LPS was administered to RAW 264.7 macrophages, the NO production increased dramatically. The AS extracts in the concentration range of 20–200 μg/mL showed inhibitory effects on NO production in LPS activated RAW 264.7 macrophage (Fig. 4). In general, the NO production decreased with increased extract concentrations. The results also suggested that a short AS extraction time (15 min) was able to inhibit NO production in a dose-dependent manner at concentrations of 20, 100, and 200 μg/mL without cytotoxic effect (data not shown).

3.4. Relationship between antioxidant activity and bioactive components of AS extracts

The correlation coefficients between antioxidant activity and ferulic acid as well as TPA contents were calculated. It was found that the antioxidant activities (scavenging DPPH, inhibiting lipid peroxidation and NO production) were, in general, positively and linearly correlated with the ferulic acid and TPA for all extracts (Table 3). This result confirms that phenolic compounds in plant

Table 3
Relationship between antioxidant activity and various components in AS extract

Component	Lipid peroxidation, *r*	DPPH scavenging, *r*	Inhibition of NO generation, *r*[a]
Water extract			
Ferulic acid	0.743	0.754	0.984
TPA	0.852	0.789	0.918
Alcohol extract			
Ferulic acid	0.923	0.890	0.898
TPA	0.896	0.921	0.928

TPA: Total peaks area of phenolic acids.
[a] AS extract concentration was 200 μg/mL.

food materials are the major contributors of their antioxidant activity, and *A. sinensis* is no exception.

3.5. Flavor quality of AS extracts

Heating the traditional Chinese medicine for a long time may cause compounds to decompose because some of them were found to be thermal labile (Song et al., 2004; Zhao et al., 2003). Long-term extraction at high temperature might also cause volatile aromatic oil evaporation (Doneanu and Anitescu, 1998), which might result in loss of the attractive special flavor of AS. The amounts of igustilide, butylidene phthalide, and butyl phthalide are the major volatile components of AS extracts (Kim et al., 2006a b), and they were chosen as the indication representatives of flavor property. A typical GC–MS chromatogram for the volatiles in AS is given in Fig. 5, and the changes of the contents of the major volatile components in AS extracts are shown in Fig. 6. The amounts of these compounds were much higher in 20% ethanol extracts than in boiling water. In the water extracts, the amounts of ligustilide (1.28 mg/100 mL, Fig. 6A) and butyl phthalide (0.067 mg/100 mL, Fig. 6C) were the highest in the 60-min extraction while butylidene phthalide (0.082 mg/100 mL, Fig. 6C) was highest in the 15-min extraction. In the 20% ethanol extracts, the amount of ligustilide (4.403 mg/100 mL, Fig. 6B), butylidene phthalide (0.187 mg/100 mL) and butyl phthalide (0.147 mg/100 mL) (Fig. 6D) were higher in the 30-min extraction than in other periods. The results indicate that 20% ethanol is better for extracting highly volatile aromatic oils than water. In the 20% ethanol extraction, more volatile compounds were obtained in 30 min, and volatile compound content decreased once the extraction time exceeded that. This phenomenon was also found in water extraction processing except that the peak amounts of volatile compounds appeared in 60 min.

Matsumoto et al. (1998) reported that ligustilide and butylidene phthalide reversed the decrease in pentobarbital sleep in isolated mice with induced stress, and concluded that these compounds contribute to the sedative action of Japanese angelica root (Angelica acutiloba KITAGAWA). Loss of volatile aromatic oil during processing not only reduced the flavor quality but also may decrease the health benefits of AS extracts.

In conclusion, this study has established a composition/bioactivity change profile for AS under different extraction conditions, and suggests that extracting the AS with water or 20% ethanol for only 15 min can yield a product with the best antioxidant activity. With regard to the flavor quality, extraction of AS with 20% ethanol for 30 min yielded the highest amount of volatile oils. For obtaining a better flavor product with high antioxidant activity, we suggest that 20% ethanol should be used and the extraction time should be less than 30 min.

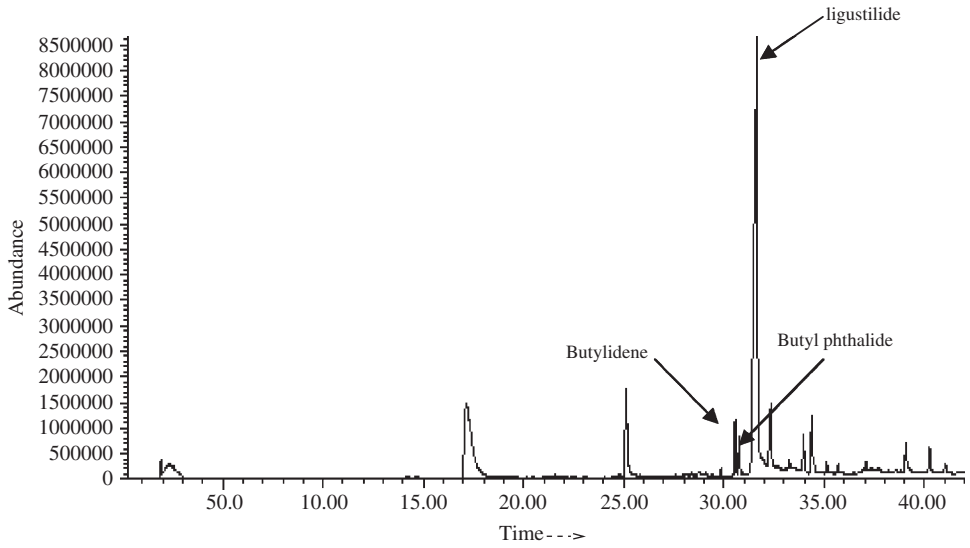


Fig. 5. Gas chromatography of volatile compounds of the *Angelica sinensis* extract. Using a Hewlett-Packard 5890 Series II chromatograph coupled to a Hewlett-Packard 5890A MSD mass spectrometer, and equipped with Carbowax 20 M column (30 m × 0.32 mm i.d., film thickness = 0.25 μM) The injector temperature was 220 °C, detector temperature 260 °C, and the oven programmed from 50 to 220 °C at an increasing rate of 5 °C/min. The carrier gas was helium at a flux of 0.8 mL/min.

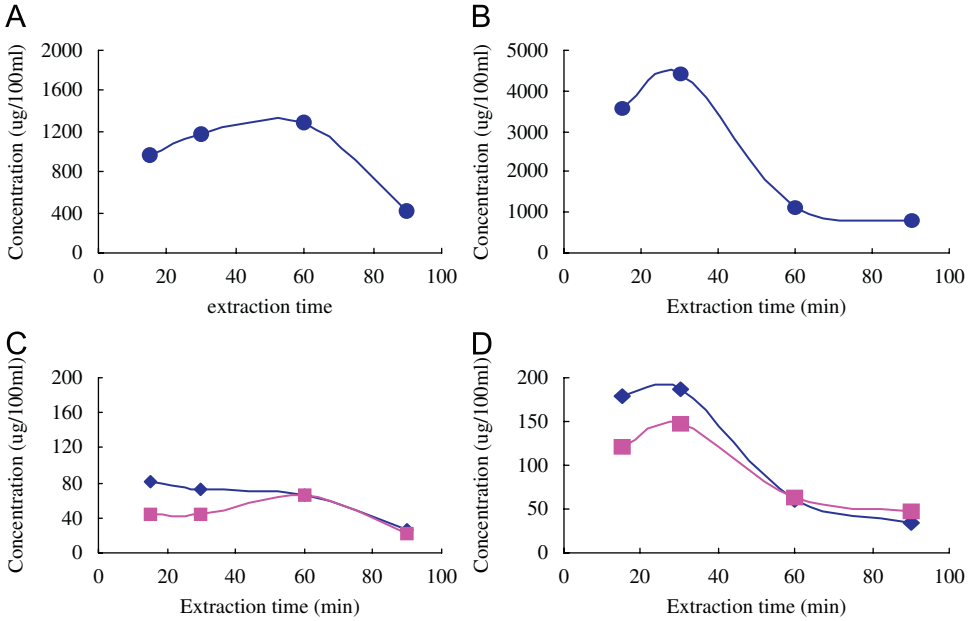


Fig. 6. Quantification of volatile compounds of *A. sinensis* extracts with different extraction conditions. (A,C), *A. sinensis* water extraction; (B,D), *A. sinensis* alcohol extraction.-●-, ligustilide; -◆-, butylidene phthalide; -■-, butyl phthalide.

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