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Metabolic study of *Angelica dahurica* extracts using a reusable liver microsomal nanobioreactor by liquid chromatography-mass spectrometry

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Abstract

Highly active and recoverable nanobioreactors prepared by immobilizing rat liver microsomes on magnetic nanoparticles (LMMNPs) were utilized in metabolic study of *Angelica dahurica* extracts. Five metabolites were detected in the incubation solution of the extracts and LMMNPs, which were identified by means of HPLC-MS as trans-imperatorin hydroxylate (M1), cis-imperatorin hydroxylate (M2), imperatorin epoxide (M3), trans-isoimperatorin hydroxylate (M1'), and cis-isoimperatorin hydroxylate (speculated M2'), respectively. Comparing to the metabolisms of imperatorin and isoimperatorin, it was found that those five metabolites were all transformed from these two major compounds presented in the plant. Since no study on isoimperatorin metabolism by liver microsomal enzyme system has been reported so far, its metabolites (M1' and M3') were isolated by preparative HPLC for structure elucidation by ¹H-NMR and MS² analysis. M3' was identified as isoimperatorin epoxide, which is a new compound as far as its chemical structure is concerned. But interestingly, M3' was not detected in the metabolism of the whole plant extract. In addition, study with known chemical inhibitors on individual isozymes of the microsomal enzyme family revealed that CYP1A2 was involved in metabolisms of both isoimperatorin and imperatorin, while CYP3A4 only in that of isoimperatorin.

Keywords

Angelica dahurica; furocoumarins; metabolites; immobilized liver microsomes; magnetic nanoparticles; HPLC-MS

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Introduction

Metabolization of the active ingredients of herbal medicines as well as phytochemical research is important to understand the herbs' therapeutic effects and the action mechanisms. However, to identify the metabolites and elucidate the metabolic pathways of herbal extracts remain a challenge because of their complicated chemical compositions (Li *et al.*, 2013; Xin *et al.*, 2011). Over the past years, metabolic study of herbal extracts has been carried out mainly *in vivo*. In these studies, metabolite profiles in sample matrices such as urine, plasma, and tissues were determined after giving oral administrations to experimental animals (Liang, *et al.*, 2013; Liu *et al.*, 2013). As is well known, the profiles obtained can be incomplete because metabolites may be produced at tiny amounts and not detectable in these complex matrices. *In vitro* studies, liver microsomes or microbials have been reported as enzymatic systems (Fan *et al.*, 2009). These studies mimicking only a part of the body simplify the tests and data interpretation, and normally produce more repeatable and comprehensive results because the experimental conditions are controllable. However, in most *in vitro* metabolic studies of herbal medicines reported so far, only one or several active ingredients instead of the whole herbal extract were investigated (Li *et al.*, 2006; Jahn *et al.*, 2011; Hackenmueller *et al.*, 2012). This has been due to the difficulties with metabolite identification in complex sample matrices and a lack of enzymatic systems for an effective metabolism of whole herbal extracts.

Various types of enzyme functionalized bioreactors have been developed for *in vitro* metabolic study of drugs (Ginai *et al.*, 2013; Nicoli *et al.*, 2008). Recently, magnetic nanoparticles (MNPs) have attracted increasing interest as solid phase support for enzymes to confer them with improved activity and stability since they provide large enzyme loading capacity and increase the collision frequency between immobilized enzyme molecules and substrate molecules due to Brownian motion of magnetic nanoparticles (Gupta *et al.*, 2011). Their special nanostructures and magnetic properties also offer practical advantages including easy magnetic solid-liquid separation and good reusability which make them very usable for biomedical analysis as well as food analysis (Liu *et al.*, 2014; Qing *et al.*, 2010; Safarikova *et al.*, 1999). Biological macromolecules such as proteins, enzymes, and DNA immobilized on MNPs can serve as excellent solid phase extraction absorbents to screen and isolate active ingredients from herbal medicines. We previously reported the use of α -glucosidase-MNP and protein tyrosine phosphatase 1B-MNPs to screen for enzyme inhibitors from the extract of *Granati pericarpium* (Qing *et al.*, 2012). Furthermore, metabolic enzymes immobilized onto the surface of MNPs have been proved to be a powerful tool for *in vitro* metabolism study of drugs (Xue *et al.*, 2013).

Dried rhizomes of *A. dahurica* have been used as an herbal medicine for treatments of acne, ulcer, carbuncle, rheumatism, headache and toothache (Zhang *et al.*, 2009; Kang *et al.*, 2008). The major bioactive components present in this herb are coumarins, which exhibit various biological activities including anti-cancer, anti-inflammation, antibacterial, and anti-dermatosis (Luo *et al.*, 2011; Kang *et al.*, 2009; Kleiner *et al.*, 2008). Two linear furocoumarins, i.e. imperatorin and isoimperatorin, are predominant components in this plant. They have been found to show potent anti-inflammatory, anti-tumor and β -secretase inhibitory activities (Ban *et al.*, 2003; Kawaii *et al.*, 2001; Marumoto *et al.*, 2010).

Pharmacokinetic profiles of imperatorin and isoimperatorin obtained from a study with rats indicated a rapid absorption and an extensive metabolic elimination after administration (Yang *et al.*, 2010). It was reported that less than 0.01% of imperatorin and less than 1% of isoimperatorin given as parent drugs were recovered from rat bile and urine (Liu *et al.*, 2010), indicating that they were apt to be metabolized *in vivo* and excreted mainly as metabolites. Two oxidation metabolites of imperatorin were reported in a previous study (Wang *et al.*, 2012), and the metabolic pathways were recently proposed (Qiao *et al.*, 2013). Metabolisms of isoimperatorin in rat and in *cunninghamella blakesleana* AS 3.970, a strain of fungi, were investigated (Shi *et al.*, 2013). To the best of our knowledge, no *in vitro* metabolic study of imperatorin or isoimperatorin with liver microsomal enzymes has been reported so far, not to say that of the whole extract of *A. dahurica*.

In this work, metabolism of imperatorin, isoimperatorin, and *A. dahurica* extract were comparatively studied using highly active and recoverable nanobioreactors in combination with HPLC/MS and MNR techniques. The nanobioreactors were prepared by immobilizing rat liver microsomes onto magnetic nanoparticles (LMMNPs). Biological activity of LMMNPs was assessed using isoimperatorin as the test compound. All the metabolites produced from incubating the samples with LMMNPs were characterized by HPLC-UV-MS. Since isoimperatorin metabolism has not been studied *in vitro* by liver microsomal enzymes before, semi-preparative HPLC was used to prepare enough amounts of the metabolites for structure elucidation by MS/MS and ¹H-NMR. Finally, the microsomal isozymes responsible for the metabolism of imperatorin and isoimperatorin were also investigated.

Experimental

Chemicals and reagents

A. dahurica was obtained from local markets in Chengdu, Sichuan province, China. Imperatorin and isoimperatorin were isolated from *A. dahurica* in our lab. Their chemical structures (Fig.1) were elucidated by MS and ¹H-NMR. β -Naphthoflavone, polydiallyldimethylammonium chloride (PDDA), β -nicotinamide adenine dinucleotide phosphate hydrate (NADP), glucose-6-phosphate and yeast glucose-6-phosphate dehydrogenase were purchased from Sigma (MO, USA). Acetonitrile (Fisher, Fair Lawn, USA) was HPLC grade and the water was from a Milli-Q water system (Millipore Corp, Bedford, MA, USA). Tetraethyl orthosilicate (TEOS), α -naphthoflavone, quinidine and ketoconazole were purchased from TCI (Tokyo, Japan). Other chemicals and solvents were of analytical reagent grade.

Preparation and characterization of rat liver microsomes

Rat liver microsomes were prepared as previously reported (Xue *et al.*, 2013). Briefly, male Sprague Dawley rats were fed normally and acclimatized at 12 h light/dark cycle for 7 days before experiment. β -naphthoflavone, dissolved in vegetable oil at 8 mg/mL was given to the rats by intraperitoneal injection for three days to induce hepatic cytochrome P450 enzymes from the eighth day. The rats were fasted for 12 h before being used. Then the rats were sacrificed and fresh livers were removed immediately, weighted and homogenized

with 3× volumes of potassium phosphate buffer (PH 7.4). The homogenate was centrifuged at 10,000 g for 20 min. The supernatant was mixed with 88 mM CaCl₂. The mixture was centrifuged at 27,000 g for 30 min. Then the precipitate was washed in Tris-HCl buffer (PH 7.4). Finally, the precipitate was resuspended in 0.1 M potassium phosphate buffer (PH 7.4) containing 0.25 M glycerol and aliquoted and stored at −80°C before use. The temperature was kept at 4°C during the whole period of preparation, and all solutions were stored at 4°C before using. Protein concentrations of the prepared microsomes were calculated using the methods of Bradford assay with the bovine serum albumin (BSA) as standard (Bradford, 1976).

The animal study was approved by the Animal Ethics Committee of Chengdu Institute of Biology, Chinese Academy of Sciences.

Immobilization of liver microsomes on magnetic nanoparticles

Firstly, Fe₃O₄ magnetic nanoparticles (MNPs) were prepared via co-precipitation of Fe²⁺ and Fe³⁺ with ammonia water under vigorous stirring, and the resultant MNPs were then coated with SiO₂ with TEOS (Qing *et al.*, 2010). Immobilization of rat liver microsomes onto MNPs by the electrostatic layer-by-layer self-assembling technique was carried out following a procedure reported previously (Bajrami *et al.*, 2008). Briefly, the SiO₂-coated Fe₃O₄ were dispersed in a 2 mg/mL PDDA solution and incubated for 20 min. PDDA-MNPs were then separated by an external magnet and washed with water for three times before being dispersed in a microsomal suspension. The mixture of PDDA-MNPs and microsomes was incubated at room temperature for 30 min. Then LMMNPs were separated and washed twice with potassium phosphate buffer (0.1 M, PH 7.4). The amount of immobilized microsomes was calculated by comparing the UV absorption of the supernatant before and after immobilization at the wavelength of 595 nm by Bradford assay.

Preparation of *A. dahurica* extract

Dried rhizomes of *A. dahurica* were powdered, and an aliquot of 5 g powder was ultrasonic extracted with 60 mL of 95% ethanol for 40 min and then filtered. The filtrate was then dried using a rotary evaporator and the residue was resolved in 4 mL of methanol and stored at 4 °C until HPLC-MS analysis and metabolization.

Metabolism of imperatorin, isoimperatorin and *A. dahurica* extract

One hundred µL of LMMNP suspension, 10 µL imperatorin solution (1 mg imperatorin in 500 µL methanol) and 150 µL incubation buffer (0.1 M potassium phosphate buffer, PH 7.4) were transferred to a 1.5 mL Eppendorf tube. The final incubation solution contained 10 mM magnesium chloride, 1.3 mM NADP, 3.3 mM glucose-6-phosphate, 1 U/mL yeast glucose-6-phosphate dehydrogenase, and 74 µM imperatorin. The mixture was incubated at 37 °C for 30 min. The reaction was terminated by magnetic separation of LMMNPs from the incubation solution. LMMNPs were then washed with 0.1 M potassium phosphate buffer three times before being reused.

The procedure for treating isoimperatorin or *A. dahurica* extract was the same as described above. Isoimperatorin or *A. dahurica* extract (10 μ L) was added to the incubation system. The supernatant was then filtrated through a 0.45 μ m filter prior to HPLC analysis.

Assessment of the activity and the reusability of LMMNPs

Isoimperatorin was used as the test compound to assess the activity and the reusability of LMMNPs. LMMNPs were washed with 0.1 M potassium phosphate buffer three times before being reused. And the equivalent reaction condition was adopted during each metabolic test. Six times of metabolism of isoimperatorin were conducted using the same LMMNPs.

Instrumentation

HPLC analysis—HPLC analysis was performed using a liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a binary HPLC pump (LC-20AD), a dual wavelength detector (SPD-20A), a manual injector (7725i-049) and a LC Solution Station for data collection and handling. Chromatographic separation was achieved on an Agilent zorbax SB C18 column (4.6 mm $\times$ 250 mm, 5 μ m) with a similar guard column (4.6 mm $\times$ 10 mm). The column compartment was set at 35 $^{\circ}$ C. The mobile phase consisted of water (A) and acetonitrile (B). The flow rate was 0.8 mL/min. The gradient elution was as follows: 20% to 40% B at 0 – 3 min, 40% to 55% B at 3 -15 min, 55% to 95% B at 15-20 min and 95% B at 30 min. The wavelength was set at 300 nm.

HPLC–MS/MS analysis—An Agilent 1100 system equipped with a microTOF-QII mass spectrometer (Bruker, Billerica, MA, USA) was used. Data were acquired and processed by using Bruker Compass Data Analysis 4.0 software. The mobile phase, column and other conditions were the same as the HPLC analysis described above, and 20 μ L of each sample was injected for analysis. The mass spectrometer was operated in the positive ion mode by using ESI under the following general conditions: the capillary voltage was 4.5 kV, the collision cell RF was 150 Vpp. The source and desolvation temperature were set at 100 $^{\circ}$ C and 180 $^{\circ}$ C. Nitrogen was used as the desolvation gas at a flow rate of 8.0 L/min. The argon was used as collision gas at a flow rate of 0.15 mL/min. The collision energy was set at 10 eV.

Semi-preparative HPLC isolation of isoimperatorin metabolites—Metabolization of isoimperatorin was scaled up by the same process as described above. After the incubation with and removing of LMMNPs, the metabolic solution was extracted with ethyl acetate in a 100 mL separating funnel for three times. The organic phase was dried using a rotary evaporator at 50 $^{\circ}$ C. The residue was re-dissolved in 6 mL of methanol and stored at 4 $^{\circ}$ C until metabolite isolation.

The isolation of metabolites of isoimperatorin was performed using a semi-preparative HPLC system (Waters 2545). Separation was performed on a Kromasil C18 column(10 mm $\times$ 250 mm, 5 μ m) with a mobile phase consisting of methanol and H₂O (60:40, v/v) at 35 $^{\circ}$ C and a constant flow rate of 4 mL/min. The injection volume was 500 μ L, and the UV detector was set at a wavelength of 254 nm. The fraction of effluent between 8.75-9.50 min

was collected as M1' and 10.80-11.50 min collected as M3'. They were dried by rotary evaporator at 50 °C and stored at 4 °C before analysis.

¹H-NMR measurement of the isolated metabolites—¹H-NMR spectra were measured on a Bruker AV 600 MHz spectrometer, and chemical shifts were reported in ppm downfield relative to tetramethylsilane. The isolated compound M1' was dissolved in methanol-*d*₄ and M3' were dissolved in chloroform-*d*₁.

Results and discussion

Preparation of LMMNP nanobioreactors

The nanobioreactors were prepared by using the layer-by-layer (LBL) technique previously reported. The loading capability of SiO₂-coated magnetic nanoparticles for microsomes was determined by measuring protein contents in the microsomal suspension before and after immobilization. The microsomes immobilized on the nanoparticles were calculated about 65 µg/mg nanoparticle. It was noted that the suspendability of LMMNPs was much better as compared with that of the precursor MNPs. A diluted suspension of LMMNPs was stable for several days in terms of its appearance. These nanoparticles showed no significant changes in their metabolic bioactivity after being kept in a refrigerator for one month at 4 °C.

HPLC-MS/MS identification of imperatorin and isoimperatorin metabolites with LMMNPs

The two major active ingredients in *A. dahurica* extract, imperatorin and isoimperatorin, were individually incubated with LMMNPs in order to simplify the metabolite identification. The metabolic solutions were analyzed by HPLC-UV-MS. From HPLC-UV chromatograms obtained (Fig.2), incubation of imperatorin or isoimperatorin with LMMNPs for 30 min produced three metabolites respectively. The six metabolites were well separated under the HPLC conditions established in this work. Surprisingly, MS detection of these metabolite peaks revealed that they were from the same two ions i.e. *m/z* 325 and 287. It was noted that the mass difference suggested a pair of [M+K]⁺ and [M+H]⁺. In the MS² spectra of these metabolites, certain common product ions, including *m/z* 203 and *m/z* 123 were observed. Based on these *m/z* values and other characteristic product ions detected (see table 1 for details), the six metabolites were identified as the oxidation products of the parent drugs. A previous *in vivo* study of imperatorin identified two metabolites, i.e. cis-imperatorin hydroxylate and imperatorin epoxide (Wang *et al.*, 2012). The MS² data of M2 and M3 were found to be in consistent with those two metabolites (shown in Fig. 3) and therefore, identified as cis-imperatorin hydroxylate and imperatorin epoxide. Since the HPLC retention time of M1 was very close to that of M2, and both compounds possess very similar mass spectrometric properties, M1 was deduced to be trans-imperatorin hydroxylate. Similarly, the three metabolites produced from isoimperatorin were identified as trans-isoimperatorin hydroxylate (M1'), cis-isoimperatorin hydroxylate (M2') and isoimperatorin epoxide (M3'), respectively. Their MS² spectrometric properties are showed in Fig 4. To our knowledge, there has been no report on the *in vitro* phase I metabolites of isoimperatorin in a liver microsomal system. Furthermore, we isolated M1' and M3' from the isoimperatorin-LMMNPs incubation solution using a semi-preparative HPLC system, and their structures were confirmed by ¹H-NMR. The amount of M2' in the incubation solution was so small

that it was hard to collect enough for an NMR analysis. However, it could be speculated as cis-isoimperatorin hydroxylate from its HPLC chromatographic behavior and the biogenesis point of view.

Reusability of LMMNP nanoparticle bioreactors

A significant advantage of using the proposed liver microsomal nanobioreactors in a metabolic study is that they can be easily recovered from the incubation solution and can be reused for many times. In addition, the subsequent chemical analysis is greatly simplified due to the absence of proteins in the solution. To evaluate the reusability of the LMMNPs, six consecutive incubation experiments were performed using a same sample of LMMNPs. As can be seen from Fig. 5, the LMMNPs were reusable for at least six times. No obvious changes in the metabolite concentrations were observed after the sixth time.

NMR analysis of the two metabolites of isoimperatorin

The ^1H -NMR spectrum (600 MHz, methanol- d_4) data of M1' was as follows: δ 8.28 (d, 1H, $J = 12$ Hz, H-9), 7.80 (d, 1H, $J = 3.0$ Hz, H-4), 7.21 (s, 1H, H-8), 7.19 (d, 1H, $J = 1.5$ Hz, H-3), 6.29 (d, 1H, $J = 12$ Hz, H-10), and 5.84 (t, 1H, $J = 6$ Hz, H-2'), 5.10 (d, 2H, $J = 6$ Hz, H-1'), 3.98 (s, 2H, H-5'), 1.70 (s, 3H, H-4'), 4.54 (s, 1H, OH-4'). Based on the MS data described above, NMR analysis results, and the information in literature (Shi *et al.*, 2013; Bajrami *et al.*, 2008), M1' was identified as trans-isoimperatorin hydroxylate.

The ^1H NMR (600 MHz, chloroform- d_1) data of M3' was as follows: δ 8.21 (d, 1H, $J = 10.2$ Hz, H-9), 7.61 (d, 1H, $J = 2.4$ Hz, H-4), 7.20 (s, 1H, H-8), 6.95 (d, 1H, $J = 1.2$ Hz, H-3), 6.32 (d, 1H, $J = 9.6$ Hz, H-10), 4.58 (dd, 1H, $J = 10.8$ and 4.2 Hz, H-1'), 4.43 (dd, 1H, $J = 10.8$ and 6 Hz, H-1'), 3.23 (dd, 1H, $J = 6.6$ and 4.8 Hz, H-2'), 1.41 (s, 3H, H-5'), 1.33 (s, 3H, H-4'). Based on the MS data described above and NMR analysis results, and M3' was identified as isoimperatorin epoxide metabolite. It's worth mentioning that M3' was identified as a metabolites of isoimperatorin for the first time in this work.

Identification of the components in *A. dahurica* extract

The *A. dahurica* extract was subjected to HPLC-MS/MS analysis. The structures of the eight major compounds appeared in the HPLC chromatogram were identified by comparison of their retention times, molecular weights and MS² data with the authentic samples as well as those reported in literatures (Franke *et al.*, 2001; Kang *et al.*, 2008; Liu *et al.* 2004).

The HPLC chromatogram of *A. dahurica* extract is shown in Figure 6 (trace d). The chromatographic and MS data of the main coumarins are listed in Table 2. Imperatorin and isoimperatorin were the main compounds present in the extract, and were quantitatively determined using the calibration curves. The calibration curve of imperatorin and isoimperatorin showed a good linearity over the concentration range of 0.5-10 $\mu\text{g/mL}$. The regression equation of imperatorin was $y = 0.3138x + 0.0278$ and the correlation coefficient (r^2) was 0.9993. The regression equation of isoimperatorin was $y = 0.3971x + 0.0428$ and the correlation coefficient (r^2) was 0.9994. The concentration of imperatorin and isoimperatorin in *A. dahurica* extract was calculated as 6.99 $\mu\text{g/mL}$ and 4.75 $\mu\text{g/mL}$, respectively.

Metabolism of *A. dahurica* extract

Metabolism of *A. dahurica* extract by LMMNPs was subsequently performed and the incubation solution was analyzed by HPLC-UV-MS. The HPLC-UV chromatogram obtained was very complex with many peaks, which made the identification of metabolites difficult. However, by comparing the HPLC-UV chromatograms obtained from the incubation solution of *A. dahurica* extract and those of imperatorin and isoimperatorin (Fig. 6), the metabolites produced from *A. dahurica* extract were identified. Five of the six metabolites described above were detected in the incubation solution, among which three were from imperatorin and two from isoimperatorin. Isoimperatorin epoxide was not detected.

Determination of CYP isozymes involved in the metabolism

Since immobilized liver microsomes were used, the CYP isozymes responsible for the metabolism of imperatorin and isoimperatorin were investigated to examine if any changes in metabolic mechanisms were involved. In this work, we determined the acting CYP isozymes by using isozyme inhibitors. It has been reported that α -naphthoflavone, quinidine and ketoconazole are the inhibitors of CYP1A2, CYP2D6 and CYP3A4, respectively (Dasgupta *et al.*, 2010). Therefore, these isozyme inhibitors were used. Table 3 shows the test results. In these tests, incubation solutions were all prepared in parallel except that different isozyme inhibitors were added in the respective experiment groups. From comparing the relative amount of metabolites of imperatorin and isoimperatorin between experiment groups and blank groups obtained from HPLC-UV analysis, the metabolic CYP isozymes were determined. The results suggested that CYP1A2 was the metabolic isozyme for both isoimperatorin and imperatorin, but CYP3A4 metabolized only isoimperatorin effectively.

Conclusions

By using a highly effective and reusable liver microsomal nanobioreactor and HPLC-UV-MS/MS in combination with NMR analysis, *in vitro* microsomal metabolism of *A. dahurica* extracts was investigated for the first time. The nanobioreactors were prepared by immobilizing rat liver microsomes on SiO₂-coated Fe₃O₄ magnetic nanoparticles. They were easily recovered from the incubation solution by means of an external magnet, making the incubation solution enzyme free for a convenient HPLC analysis, which turned out to be a powerful tool for *in vitro* metabolic study for plant extracts. By means of HPLC-MS and/or NMR, five metabolites were identified as trans-imperatorin hydroxylate, cis-imperatorin hydroxylate, imperatorin epoxide, trans-isoimperatorin hydroxylate, and cis-isoimperatorin hydroxylate. Comparing the respective metabolites of imperatorin and isoimperatorin, all of them were found to be the oxidation products transformed from these two major compounds present in the plant extracts. In addition, metabolic study of isoimperatorin revealed a new compound, i.e. isoimperatorin epoxide, which is interestingly not detected in the metabolites of the plant extracts. Furthermore, CYP1A2 was proved to be the metabolic isozyme responsible for both isoimperatorin and imperatorin, while only CYP3A4 for isoimperatorin.

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Fig 1.

Chemical structures of imperatorin, isoimperatorin, and the proposed metabolites. M1, trans-imperatorin hydroxylate; M2, cis-imperatorin hydroxylate; M3, isoimperatorin epoxide; M1', trans-isoimperatorin hydroxylate; M2', cis-isoimperatorin hydroxylate; and M3', isoimperatorin epoxide.

Fig 2.
HPLC-UV chromatograms obtained from the metabolic solutions of imperatorin (A) and isoimperatorin (B) at incubation times of 0 and 30 min, respectively.

Fig 3. MS² properties of the emperorin metabolites detected in the metabolic solution with LMMNPs for 30 min.

Fig 4.
MS² spectrometric properties of the isoimperatorin metabolites detected in the metabolic solution with LMMNPs for 30 min.

Fig 5.
The reusability of LMMNPs using isoimperatorin as the test compound.

Fig 6. Overlaid HPLC-UV chromatograms obtained from imperatorin-LMMNP (trace a), isoimperatorin-LMMNP (trace b), *A. dahurica* extract-LMMNP (trace c) incubation solutions, and *A. dahurica* extract (trace d).

Table 1

MS properties of imperatorin, isoimperatorin and their metabolites

Compound	[M+K] ⁺	[M+H] ⁺	Major fragment ions
imperatorin	309	271	107, 203
M1	325	287	123, 203, 241, 269
M2	325	287	123, 203
M3	325	287	123, 203, 269
Isoimperatorin	309	271	107, 176, 203
M1'	325	287	122, 203
M2'	325	287	123, 203
M3'	325	287	123, 203

Table 2The analysis results of the chemical constituents in the extracts of *Angelica dahurica* by HPLC-MS/MS

t_R(min)	Identification	Formula	Mass	MS	MS/MS
9.02	Xanthotoxol	C ₁₁ H ₆ O ₄	202	201	173, 156
9.75	Oxypeucedanin hydrate	C ₁₆ H ₁₆ O ₆	304	305	203
9.99	Byakangelicin	C ₁₇ H ₁₈ O ₇	334	335	317, 233
15.48	Bergapten	C ₁₂ H ₈ O ₄	216	217	202
15.80	Pabulenol	C ₁₆ H ₁₄ O ₅	286	287	203, 147
24.02	Imperatorin	C ₁₆ H ₁₄ O ₄	270	271	203, 146
25.98	Phelloptorin	C ₁₇ H ₁₆ O ₅	300	301	233, 217
28.45	Isoimperatorin	C ₁₆ H ₁₄ O ₄	270	271	203, 146

Table 3

Results of inhibition tests on CYP isozymes in the metabolism of imperatorin and isoimperatorin

<u>The compound</u>	Imperatorin	Isoimperatorin
Inhibitor		
Blank control	/	/
α -naphthoflavone	+	+
quinidine	-	-
ketoconazole	-	+

Note : “+” represents that the inhibitor has an inhibitory effect on the metabolism, while “-” indicates no effects.