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Analysis of Serum Pharmacochemistry of Hederagenin using UPLC-Q-TOF/MS

(Analisis Farmakokimia Serum Hederagenin menggunakan UPLC-Q-TOF/MS)

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ABSTRACT

This study investigated the changes of plasma absorbed components in rats after oral administration of hederagenin. Serum pharmacochemistry analysis of hederagenin was carried out to understand the changes of its metabolic components in the body. Biological samples were collected and then the migration components of hederagenin-containing serum were established by UPLC/Q-TOF-MS technique. Possible metabolites were obtained for comprehensive analysis through relevant studies and the regulation of broken bonds in the molecular structure of hederagenin. At about 10.262 min, the molecular ion peak of the hederagenin of M/Z 471[M-H]$^-$ was detected in negative ion mode and the prototype product and its associated fragment ions could be detected only at 1, 3, 6, 9, 12, and 24 h after administration. Seventy-one signal peaks of potential metabolites were found in the drug serum. Based on the bond energy characteristics of molecular structure, 47 possible metabolite-related molecular ion peaks through decarboxylation, dehydration, demethylation or methyl shift, deoxygenation, ring opening, and unsaturated biformation were deduced, and signals of 35 metabolite-related molecular ion peaks were identified. Hederagenin can metabolize many products *in vivo*. Important information about the metabolism of hederagenin, which is useful for fully understanding its mechanism of action, was provided in this study.

Keywords: Hederagenin; serum pharmacochemistry; UPLC/Q-TOF-MS

ABSTRAK

Penyelidikan ini mengkaji perubahan komponen yang diserap plasma dalam tikus selepas pemberian oral hederagenin. Analisis farmakokimia serum hederagenin telah dijalankan untuk memahami perubahan komponen metabolik dalam badan. Sampel biologi telah dikumpulkan dan kemudian komponen penghijrahan serum yang mengandungi hederagenin telah dibentuk dengan teknik UPLC/Q-TOF-MS. Metabolit yang mungkin diperoleh menjalani analisis komprehensif melalui kajian yang berkaitan dan pengawalan ikatan pecah dalam struktur molekul hederagenin. Pada 10.262 min, puncak ion molekul hederagenin M/Z 471[MH]- telah dikesan dalam mod ion negatif dan produk prototaip serta ion serpihan yang berkaitan boleh dikesan hanya pada 1, 3, 6, 9, 12 dan 24 jam. Tujuh puluh satu puncak isyarat metabolit yang berpotensi ditemui dalam serum ubat. Berdasarkan ciri tenaga ikatan struktur molekul, 47 daripadanya berkemungkinan ion molekul yang berkaitan dengan metabolit memuncak melalui dekarboksilasi, dehidrasi, demetilasi atau anjakan metil, penyahoksigenan, pembukaan cincin serta biformasi tak tepu dan signal 35 ion molekul berkaitan metabolit puncak dikenal pasti. Hederagenin boleh memetabolismekan banyak produk *in vivo.* Maklumat penting tentang metabolisme hederagenin yang berguna untuk memahami sepenuhnya mekanisme tindakannya telah dikaji dalam kajian ini.

Kata kunci: Farmakokimia serum; hederagenin; UPLC/Q-TOF-MS

INTRODUCTION

The concept of serum pharmacochemistry was first proposed by Hiroko Iwama in 1987. Serum pharmacochemistry refers to the qualitative and quantitative analysis of serum or plasma using modern analytical methods (Iwama et al. 1987) to study the chemical substances and their metabolic regularity in serum. The proposal of this concept has broken the

traditional research method of traditional Chinese medicine and compound prescription. The chemical composition may change after passing through the digestive and intestinal flora. After hydrolysis, oxidation and reduction reactions, the bioactive ingredients are often absorbed into the blood, or loop-cracking, cyclization, deester, dehydrogenation, demethylation, hydrogenation, and other reactions occur under the action of intestinal enzymes, forming metabolites into the blood. Wang et al. (2008) found that there is a close relationship between plasma components, their concentrations and their pharmacological effects. This finding can also be observed in herbal medicines (Xiong et al. 2013; Yan et al. 2013). Therefore, it is necessary to have a comprehensive understanding of drug absorption and pharmacokinetic information in pharmacological studies.

Hederagenin, (3β, 4α)-3,23-dihydroxy olean-12-ene-28-acid, molecular formula $C_{30}H_{48}O_4$, is a pentacyclic triterpenoid compound, widely distributed in a variety of medicinal plants, such as *Hedera helix* L., *Dipsacus asper*, *Clematis*, *Pulsatilla*, *Honeysuckle*, and *Predictor*. Previous studies have shown that hederagenin have anti-tumor (Lee et al. 2000), anti-depression (Zhou et al. 2010), antibacterial, anti-inflammatory (Kim et al. 2017), antiatherosclerosis (Lu et al. 2015), anti-diabetic (Kim et al. 1998) and other pharmacological effects and biological activities (Choi et al. 2005; Wu et al. 2017). Due to the characteristics of poor solubility and low bioavailability of hederagenin found in previous studies, there are relatively few studies on the activity of hederagenin at this stage, and the clinical application is also limited accordingly. However, in the early stage of our laboratory, it was found that hederagenin still has good lipid-lowering activity under the condition of low bioavailability.

For thousands of years, Traditional Chinese medicine (TCM) theories have guided and explained the clinical efficacy of TCM and its extracts. However, the basic application principle and mechanism of action of these TCM cannot be explained by modern science, which is difficult to be accepted internationally. Therefore, the focus of TCM research today is to explore the pharmacological action and mechanism of action (Wang et al. 2018). To solve the problem, hederagenin still has good activity under the condition of low bioavailability, our group aimed to identify the biologically active components absorbed in the plasma of rats after gavage of hederagenin using UPLC/Q-TOF-MS instructed by plasma pharmacochemical ideas. A chemical database of serum pharmacochemistry of hederagenin was established, which will lay a theoretical basis for further reveal the pharmacological effect of hederagenin and its metabolic components.

EXPERIMENTAL DETAILS

EXPERIMENTAL INSTRUMENTS

KD-160 type electronic scale (TANITA Co., Ltd.); KQ-250E Ultrasonic Cleaner (Kunshan Ultrasonic Instrument Co., Ltd.); TGL-16 Desktop High-Speed Refrigerated Centrifuge (Changsha Xiangyi Centrifuge Co., Ltd.); TB-25 type 1/100,000 electronic balance (DENVER, Beijing Denver Instrument Co., Ltd.); Agilent 1290 Series High Performance Liquid Chromatograph (Agilent, USA); Agilent6538 Q-TOF/MS mass spectrometer (Agilent, USA); Agilent 20RBAX SB-C18 (2.7 μm, 3.0*100 mm) (Agilent, USA).

CHEMICALS AND MATERIALS

Hederagenin (Shanghai Chunyou Biotechnology Co., LTD., p0207-201906); Acetonitrile (Merck, Lot: 17025037); Formic acid (Cramal, Lot:ZY161013); Heparin Sodium (Shanghai Xinyi Pharmaceutical Factory Co., Ltd., Lot:151706056A); Methanol (Merck, AR, Lot:168205); Wahaha Pure Water (Hangzhou Wahaha Co., Ltd.).

ANIMALS

Male Sprague-Dawley rats (200±20 g) were purchased from Shanghai Jiesijie Experimental Animal Co., Ltd. (license number: SCXK (Shanghai) 2018-0046). The temperature and humidity in the feeding room were at 24±2 °C and 60±5%, respectively. The animals were given tap water and fed normal food *ad libitum*, who experienced 12 h dark-light cycle for a week acclimation before the experiment. All the animal experiments were carried out according to the Guidelines for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of JiangSu Food & Pharmaceutical Science College. All procedures were in accordance with the National Institute of Health's guidelines regarding the principles of animal care.

METHODS

PREPARATION OF DRUG-CONTAINING SERUM

Hederagenin was orally administered to the rats by gavage at a single dose of 51.21 mg/kg, and the volume

was 10 mL/kg for four consecutive days, once a day. Blood samples were collected at 0, 0.5, 1, 3, 6, 9, 12, 24, and 36 h after the last administration (Huang et al. 2018; Ju et al. 2018). The plasma was obtained by centrifugation at 4000 rpm for 10 min immediately, and the plasma sample was labelled and frozen at -80 °C until analysis.

SAMPLE PRETREATMENT

After the plasma samples were thawed at 4 °C, 0.1 mL of serum was spiked into centrifuge tubes and then add 0.02 mL of internal standard solution, 0.02 mL of formic acid solution, and 0.28 mL of methanol, vortex mixing for 30 s. In order to obtain the upper layer of the mixture, the mixture was centrifuged at 15600 rpm for 5 min, and the upper layer was moved to another tube for evaporation and dried at 37 °C with nitrogen. The residue was reconstituted with 100 μL methanol, and the supernatant was obtained by vortexing for 30 s and centrifuging at 15600 rpm for 5 min. 5 μL supernatant was taken for UPLC-Q-TOF/MS analysis.

ANALYSIS CONDITIONS

The chromatographic separation was performed on a C18 reversed-phase UPLC column (3.0 × 100 mm, 2.7 μm, Agilent) at 35 °C, the flow rate was 0.4 mL /min, and the sample injection volume was 5 μL. The mobile phase consisted of (A) 0.05% formic acid in water and (B) acetonitrile. The optimized gradient program is shown in Table 1.

With an electrospray ionization (ESI) interface operated in negative ionization mode, the mass spectrometric data were collected and scanned within the range of m/z 100 to 1100. The optimized parameters were as follows: capillary voltage, 2.8 kV; sampling cone voltage, 40 V; source temperature, 110 °C; desolvation temperature, 400 °C; desolvation gas, 600 L/h; and cone gas, 50 L/h. Use leucine enkephalin (0.5 μg/mL) as the lock mass. The data corrected during the acquisition process generates reference ions at m/z 554.2615, which are introduced at a flow rate of 5 μL/min through the lock spray.

TABLE 1. Solvent gradient program of UPLC analysis

Time (min)	A (%)	B (%)
0	90%	10%
3	70%	30%
10	20%	80%
23	5%	95%
25	90%	10%

DATA ANALYSIS

The data processing is mainly carried out by the following methods: (1) Profinder B.06.00 software is used for batch data extraction. The extraction standard is limited to an absolute peak height of not less than 300, and its retention time and peak area are extracted. (2) Secondary ion extraction is performed on hederagenin, after obtaining the relevant secondary fragment ions, the fragment ions are subjected to primary extraction to extract their retention time and peak area. (3) According to the structural properties of hederagenin and the law of bond energy breaking, it is speculated that hederagenin may be cleaved, the retention time and peak area of the primary and secondary ions are extracted from the speculated possible compounds in the total ion chromatogram.

RESULTS AND DISCUSSIONS

ION MODE SCREENING

It can be seen from Figure 1 that most of the peaks in the positive ion mode also appear in the negative ion mode, and the peak number and abundance in the negative ion mode are better than the positive ion mode. Therefore, the negative ion mode was selected for further study.

DETERMINATION AND ATTRIBUTION OF PROTOTYPE COMPOUNDS IN DRUG SERUM

As shown in Figure 2, Agilent Mass Hunter Qualitative Analysis software was used to treat hederagenin-9 h sample as an example. Hederagenin (tR=10.262 min) of M/Z 471[M-H] $^-$ was present in ESI$^-$figure. There were m/z 183.7598, 309.8920, 395.4642, 471.3517 and other fragment ions in MS^E figure. The same fragment ions at the same retention time as the hederagenin standard product, is determined as the prototype product of hederagenin.

STUDY ON THE CHARACTERIZATION OF CHEMICAL CONSTITUENT GROUPS OF DRUG SERUM AT DIFFERENT TIME POINTS

The constituents in rat serum were separated and identified by using retention time and mass spectrometry to (Figure 3). Further data extraction and analysis of the total ion chromatogram of serum samples were carried out from different blood collection points. The hederagenin could detect the prototype product and its related fragment ions at 1, 3, 6, 9, 12, and 24 h after administration, while the prototype metabolites could not be detected in the samples at the remaining serum time points. This indicates that the blood component content of the prototype drug in the body increased greatly after hederagenin administration for 1 h, and the prototype product of hederagenin and its primary metabolite related fragment ions were found according to the corresponding MS^E map. The phenomenon of metabolism occurs after the prototype of hederagenin entered the blood, which occurs under various metabolic enzymes and other conditions *in vivo*, and fragment ions are the most at 9 h. The secondary fragments related to hederagenin are organized as shown in Table 2. This indicates that hederagenin was metabolized into more small molecule components and absorbed into blood 9 h after administration, or hederagenin is metabolized into other ion fragments in blood after absorption into blood.

TABLE 2. Prototype products and main fragment ions of hederagenin in drug serum from different blood collection points

Samples	Precursor ion *m/z*	Fragment ion *m/z*
Blank plasma		——
0.5 h		——
1 h	471.3539	318.0787、341.2094、818.1414、1053.6284
3 h	471.3543	241.4987、393.3198、429.2995、588.6519、735.5839
6 h	471.3546	252.2324、262.6855、350.3801、401.1114、426.3417、441.4268、453.3347、497.4651、516.2274、644.9903、747.1644、970.9343、1041.4125
9 h	471.3549	131.5449、138.3020、147.9297、151.8231、176.2130、183.7598、187.7680、196.9713、249.6273、265.3826、285.7966、309.8920、350.2275、395.4642、412.9860、437.5922、474.3717、485.0436、498.2119、503.2071、550.2032、572.4596、620.8917、664.0346、764.3674、782.7546、784.3235、799.7795、805.7684、943.0580、975.0507、985.8233、1058.0665
12 h	471.3555	160.3574、177.7649、205.8066、331.0847、390.3609、429.2995、516.0338、599.0045、621.8683、632.0758、682.0991、894.9666
24 h	471.3545	127.6855、192.6480、235.8388、335.8268、397.6680、435.7197、525.2165、776.9764、917.9047
30 h		——
36 h		——

THE FRAGMENTATION PATHWAYS OF HEDERAGENIN AND RELATED FRAGMENT IONS IN NEGATIVE ION MODE

The secondary fragment ions of hederagenin time-dependent are sorted by the size of m/z, according to the mass spectrum characteristic ions of the prototype compound, possible metabolites, and the bond-breaking law of the prototype compound *in vivo* (Jin et al. 2018; Jun & Tong 2012; Yun yun & Chao 2011; Zhang et al. 2019). Reasonable inferences were made for 71 secondary fragment ions (Table 2) which combined with compounds such as glucuronidation, sulfation, methylation, acetylation, sulfonation and other forms of metabolism, screening and speculation analysis of possible metabolites. The composition of 35 metabolites is shown in Table 3. In addition, according to the chemical structure of hederagenin, the composition of 47 possible metabolites is inferred, and the structure of potential metabolites and the cleavage pathway are drawn (Figure 4).

The m/z 471 $[M-H]^-$ molecular ion peak is generated by hederagenin in negative ion mode conditions. The literature (Jin et al. 2018; Li et al. 2010) shows that pentacyclic triterpenoids are prone to crack the RDA because of the unsaturated double formation in the C ring. Compound 1 (m/z 224) in Table 3 and the compound B-8 (m/z 248) in Figure 4 can be obtained by cracking, respectively. Under certain conditions, the hydroxyl group at the C_3 position of the hederagenin is easy to lose O^{2-} or combine with the H^+ on the adjacent C atom equivalently lose a molecule of H_2O (Jin et al. 2018), namely compound 33 (m/z 455) and compound 32 (m/z 453). The carboxyl group at the C_{28} position of the hederagenin is prone to decarboxylation reaction (Li et al. 2010) under the corresponding conditions, resulting in the formation of compound 28 (m/z 427); or combining with the H^+ on the adjacent C atom to remove a molecule of formic acid (HCOOH) to form unsaturation double construction, getting compound 26 (m/z 425); secondly, it is reported in the literature (Jun & Tong 2012) that compound 27 (m/z 426) was obtained from hederagenin whose parent nucleus structure lost carboxyl group (m/z 45), then E ring cleavage lost m/z 72 under certain conditions, which is compound 13 (m/z 354). In the pentacyclic triterpenes, the corner methyl group between two adjacent rings is easily removed or displaced to form a stable tertiary C^+ ion, especially the corner methyl group at the allyl position, which is easier to be removed and form a robust C^+ ion (Xiong et al. 2005). Hederagenin has corner methyl groups at positions C_{25}, C_{26}, and C_{27}, respectively. Therefore, after losing the corner methyl group, unsaturated carbon double structure is formed on the adjacent ring, and then RDA rearrangement occurs, and compound 5 (m/z 255) and compound 25 (m/z 412) can be obtained.

In the above, the mother nucleus is mainly to produce related molecular ion peaks by decarboxylation, dehydration, demethylation or methyl shift, deoxygenation, ring-opening, and unsaturated double formation. Different order of metabolic forms can produce different molecular ion peaks. The more details analysis is as follows (Figure 4).

The RDA cleavage of the mother nucleus occurs first, and then decarboxylation, dehydration, reduction, and loss of methoxy groups may, respectively, produce compounds B-10 to B-19. Loss of the methoxy group in the mother nucleus may produce compound 31 (m/z 441). In the case of decarboxylation of the nucleus, compounds 26, 27, 28 may be produced, and then compound 10 (m/z 324), compound 20 (m/z 395) and compound 21 (m/z 397) can be obtained by losing the methoxy group, respectively. On the basis of compound 32 (m/z 453), a series of decarboxylation reactions will occur, which may produce compound 11 (m/z 336), compound 22 (m/z 407), and compound 23 (m/z 409) which continue to lose one molecule of H_2O or methoxy group to produce compound 6 (m/z 306), compound 8 (m/z 318), compound 14 (m/z 377), compound 15 (m/z 379), compound 17 (m/z 389), and compound 19 (m/z 391). On the basis of compound 33 (m/z 455) and the same principle as (4), compound 7 (m/z 308), compound 9 (m/z 320) and compound 12 (m/z 338), compound 16 (m/z 381) and compound 24 (m/z 411) can be produced. The molecular ion peak signals of compound 18 (m/z 390) and compound 29 (m/z 435) may be caused by successive removal of two molecules of neutral water from the mother nucleus; and the compound 18 (m/z 390) continue to occur decarboxylation reaction. The molecular ion peak signals of compound 30 (m/z 437) may be caused by loses one molecule of neutral water and then is reduced to lose one molecule of O^{2-}. Considering that the compound may be oxidized or combined with other substances in the related literature, the molecular ion peak of compound 2 (m/z 241), compound 4 (m/z 252), compound 34 (m/z 516) and 35 (m/z 599), respectively, from compound 1+OH, compound 1+CO, $[M-H]^-+HCOO^+$, and $[M-H]^-+SO_3H$+HCOOH is speculated.

TABLE 3. Presumptions of 35 possible metabolites

Peak number	tR (min)	Molecular Ions （*m/z*）	Formula	Strucrure presumed
1	10.914	224.1738	$C_{14}H_{24}O_2$	B-9
2	14.873	241.4987	$C_{14}H_{26}O_3$	B-9+OH
3	11.669	249.6273	$C_{16}H_{24}O_2$	$[M-H]^-$ -(B-9）
4	12.402	252.2324	$C_{15}H_{25}O_3$	B-9+CO
5	16.498	255.2366	$C_{19H2}7$	C-7
6	15.468	306.2521	$C_{23}H_{36}$	A-20
7	17.734	308.2669	$C_{23}H_{32}$	A-10
8	8.191	318.2168	$C_{24}H_{30}$	A-21
9	10.434	320.2302	$C_{24H3}2$	A-9
10	16.544	324.2213	$C_{23}H_{32}$	B-5
11	5.971	336.3368	$C_{24}H_{32}O_2$	A-8
12	24.828	338.3197	$C_{24}H_{34}O$	A-3
13	13.935	354.1415	$C_{24}H_{34O}2$	B-4
14	13.042	377.1464	$C_{28}H_{41}$	A-17
15	14.644	379.1684	$C_{28H4}3$	A-13/A-15
16	17.024	381.1754	$C_{28H4}5$	A-11
17	9.221	389.2692	$C_{29}H_{41}$	A-18
18	10.617	390.3609	$C_{29}H_{42}$	$[M-H]^-$-$2H_2O$-COOH
19	9.06	391.2858	$C_{29H4}3$	A-14/A-16
20	13.729	395.2216	$C_{28}H_{43}O$	B-7
21	15.217	397.2285	$C_{28}H_{45}O$	B-6
22	6.589	407.2734	$C_{29}H_{43}O$	A-7
23	20.755	409.2165	$C_{29}H_{45}O$	A-6/A-5
24	14.942	411.2944	$C_{29}H_{47}O$	A-4
25	13.546	412.986	$C_{27}H_{40}O_3$	C-1
26	15.568	425.1447	$C_{29}H_{45}O_2$	B-2
27	13.615	426.3417	$C_{29}H_{48O}2$	$[M-H]^-$-COOH
28	13.592	427.1566	$C_{29}H_{47O}2$	B-3
29	7.39	435.7197	$C_{30}H_{44}O_2$	$[M-H]^-$-$2H_2O$
30	8.786	437.5922	$C_{30}H_{46}O_2$	$[M-H]^-$-H_2O-O
31	12.722	441.4268	$C_{29}H_{48}O_3$	B-1
32	11.574	453.3347	$C_{30}H_{45}O_3$	A-2
33	10.411	455.3175	$C_{30}H_{47}O_3$	A-1/C-2/C-3/C-4
34	12.262	516.0338	$C_{31}H_{49O}6$	$[M-H]^-$+HCOO
35	9.539	599.3069	$C_{31}H_{51}O_9S$	$[M-H]^-$+SO_3H+HCOOH

EFFECT OF METABOLIC COMPONENTS OF HEDERAGENIN ON PHARMACODYNAMICS

The molecular structures of drugs contribute to their pharmacological actions. The changes of some specific functional groups trigger molecular structure alternation followed by the changes of properties, including the binding force between drugs and receptors, subsequently affecting the efficacy of drugs. Analyzing the effects of specific functional groups on the changes of local structural changes and properties of drugs provides a more comprehensive understanding of the structure-activity relationship. Alkyl and sulfonic acid groups, two groups of structure-related functional groups of drugs are discussed as follows.

Alkyl can increase the lipid solubility of drugs, reduce the degree of dissociation, increase steric hindrance, increase stability and prolong the action time of drugs. For example: Testosterone (not effective orally), but methyltestosterone formed after the introduction of methyl groups at C_{17} is effective orally. Haloids are powerful electron-withdrawing groups, which have effects on charge distribution, lipid solubility and drug action time that can enhance biological activity. For example, fluphenazine has stable effect of 4 ~ 5 times more than that of perphenazine. Hydroxyl groups can form hydrogen bonds, increase water solubility, reduce biological activity, and reduce toxicity. Hydroxyl substitutions on the aliphatic chain often reduce activity and toxicity. Replacement the aromatic ring, binding with alkaline activity and receptor enhance activity and toxicity. Acylation of hydroxyl to ester or hydrocarbon to ether is much less active. Sulfhydryl can increase lipid solubility making the drug easier to absorb and affecting the metabolic process. Ether and sulfide have hydrophilic oxygen atoms and lipophilic carbon atoms which is conducive to drug operation and directional distribution, increase biological activity. Sulfonic acid groups increase water solubility and reduce toxicity, but also reduce biological activity. Carboxyl groups have the properties of increasing water solubility and enhancing biological activity. The ester group can increase lipid solubility and enhance biological activity, which is beneficial to absorption and transport. For example, double hydrogen artemisinin has good antimalarial activity, but low solubility in water, which is not conducive to injection application. Artesunate has good anti-malarial activity and good solubility, because it makes use of succinic acid with double carboxylic acid functional groups to form a carboxyl and double hydrogen artemisinin monoester to increase solubility in water. Amide groups easily form hydrogen bonds with biological macromolecules and easily bind to receptors. Amino can form hydrogen bonds, increase water solubility and biological activity. For example, benzoyl nitrogen salicylic acid has the amino group, which can increase its stability and improve biological activity.

The exact process of metabolism in the body is not clear. In this study, we hypothesized that hederagenin still has good pharmacological efficacy even in the condition of low bioavailability, which may be due to its degradation into small molecules *in vivo* and related to the properties of some functional groups.

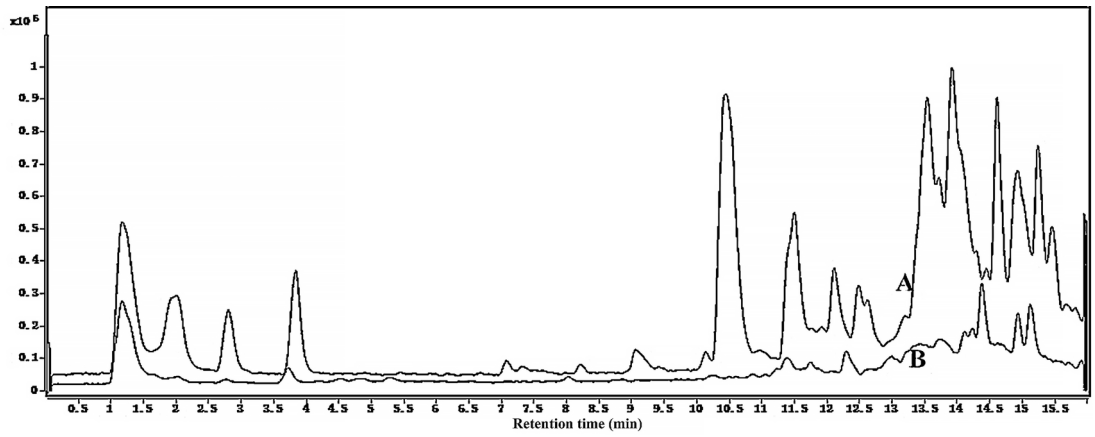

FIGURE 1. Chromatograms of hederagenin (3 h) by UPLC-Q-TOF/MS. A is ESI negative ion mode, B is ESI positive ion mode

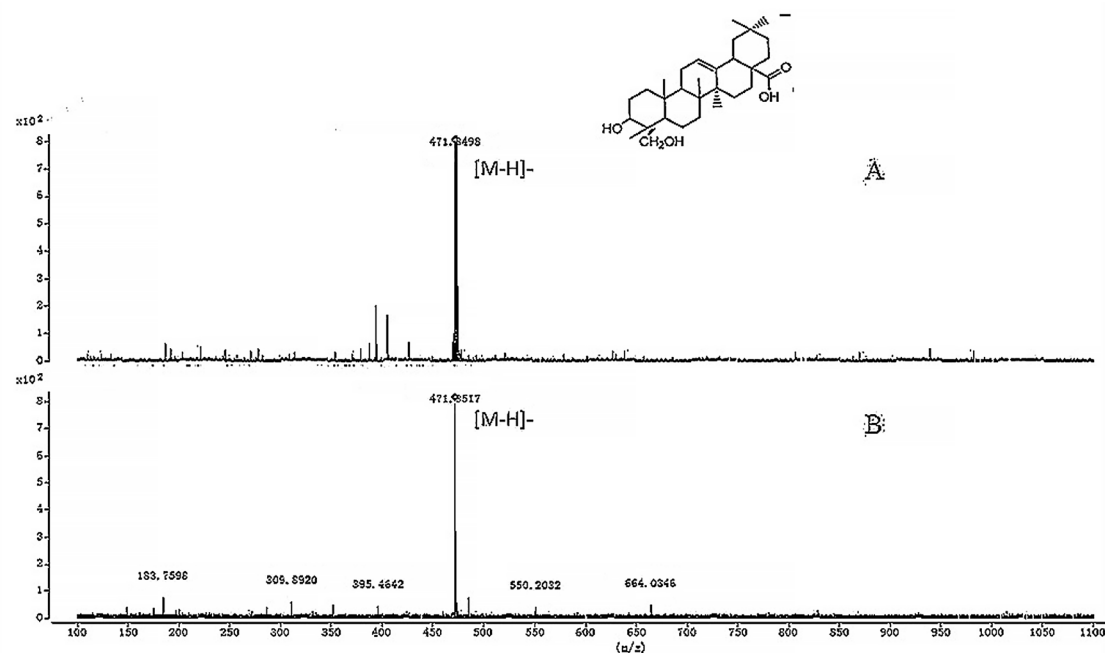

FIGURE 2. Hederagenin prototype attribution (M/Z 471), A: Hederagenin standard product; B: 9 h- drug serum samples

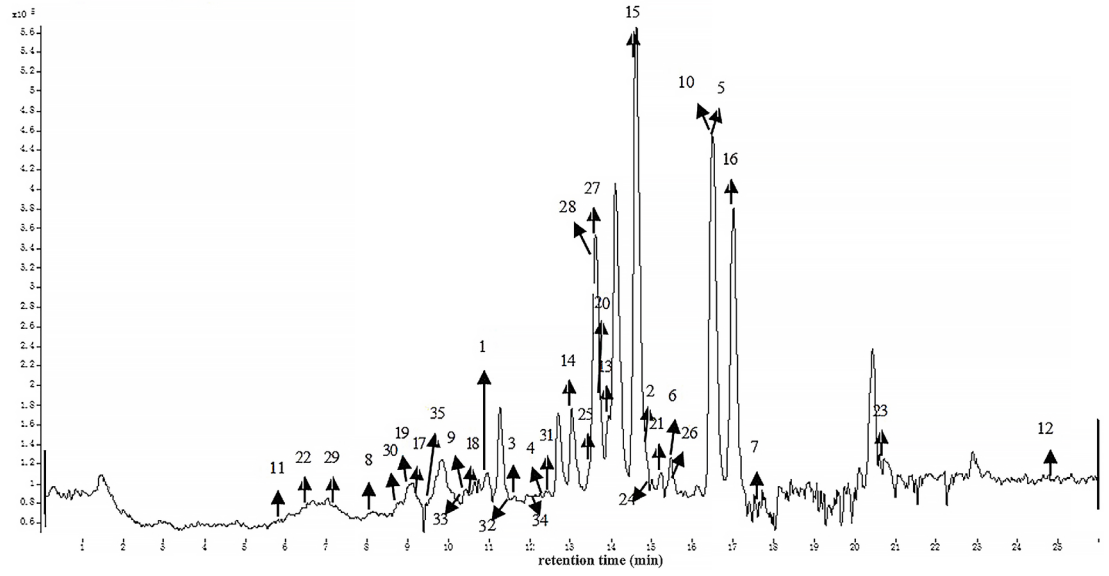

FIGURE 3. Total ion chromatogram of hederagenin-9 h

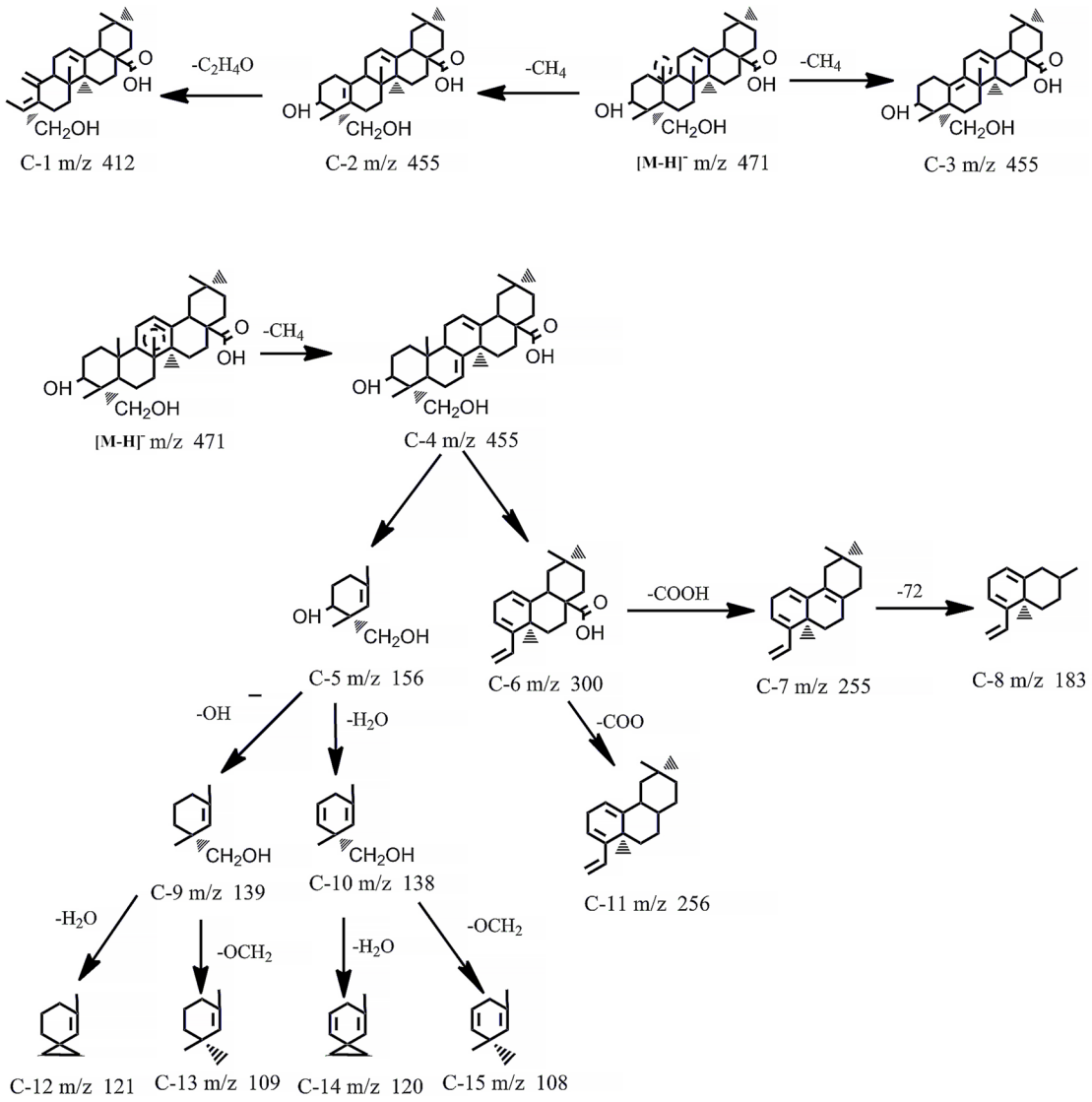

FIGURE 4. Possible metabolite cleavage pathways in hederagenin containing serum

Conclusions

In this study, we obtained data on the migration components of its drug serum through UPLC/Q-TOF-MS, which is the first report *in vivo* serum pharmacochemistry of hederagenin. The serum containing hederagenin mainly contains 71 possible signal peaks of metabolites. Based on the structural characteristics of hederagenin and the metabolism rules in the body, the target components were extracted by using Agilent Mass hunter software, and finally 35 possibilities were inferred. This established a good foundation for further research on the relationship between the efficacy of hederagenin and its chemical composition. Also, this study provides essential information about the metabolism of hederagenin, which is useful for fully understanding its mechanism of action.

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REFERENCES

Choi, J., Jung, H.J., Lee, K.T. & Park, H.J. 2005. Antinociceptive and anti-inflammatory effects of the saponin and sapogenins obtained from the stem of *Akebia quinata*. *J. Med. Food* 8(1): 78-85. doi:10.1089/jmf.2005.8.78

Huang, M., Cheng, Z., Wang, L., Feng, Y., Huang, J., Du, Z. & Jiang, H. 2018. A targeted strategy to identify untargeted metabolites from *in vitro* to *in vivo*: Rapid and sensitive metabolites profiling of licorice in rats using ultra-high performance liquid chromatography coupled with triple quadrupole-linear ion trap mass spectrometry. *J. Chromatogr. B, Analyt. Technol. Biomed. Life Sci.* 1092: 40-50. doi:10.1016/j.jchromb.2018.05.044

Jun, X.H. & Tong, Z.L. 2012. Studies on the pharmacokinetics and quality control methods of triterpenoid saponins in *Pulsatilla chinensis*. Heibei Medical University. Ph.D. Thesis (Unpublished).

Iwama, H., Amagaya, S. & Ogihara, Y. 1987. Effect of shosaikoto, a Japanese and Chinese traditional herbal medicinal mixture, on the mitogenic activity of lipopolysaccharide: A new pharmacological testing method. *J. Ethnopharmacol.* 21(1): 45-53. doi:10.1016/0378-8741(87)90093-6

Jin, M.M., Zhang, W.D., Jiang, H.H., Du, Y.F., Guo, W., Cao, L. & Xu, H.J. 2018. UPLC-Q-TOF-MS/MS-guided dereplication of *Pulsatilla chinensis* to identify triterpenoid saponins. *Phytochem. Anal.* 29(5): 516-527. doi:10.1002/pca.2762

Ju, Z., Li, J., Han, H., Yang, L. & Wang, Z. 2018. Analysis of bioactive components and multi-component pharmacokinetics of saponins from the leaves of Panax notoginseng in rat plasma after oral administration by LC-MS/MS. *J. Sep. Sci.* 41(7): 1512-1523. doi:10.1002/jssc.201701042

Kim, D.H., Yu, K.W., Bae, E.A., Park, H.J. & Choi, J.W. 1998. Metabolism of kalopanaxsaponin B and H by human intestinal bacteria and antidiabetic activity of their metabolites. *Biol. Pharm. Bull.* 21(4): 360-365. doi:10.1248/bpb.21.360

Kim, E.H., Baek, S., Shin, D., Lee, J. & Roh, J.L. 2017. Hederagenin induces apoptosis in cisplatin-resistant head and neck cancer cells by inhibiting the Nrf2-ARE antioxidant pathway. *Oxid. Med. Cell Longev.* 2017: 5498908. doi:10.1155/2017/5498908

Lee, K.T., Sohn, I.C., Park, H.J., Kim, D.W., Jung, G.O. & Park, K.Y. 2000. Essential moiety for antimutagenic and cytotoxic activity of hederagenin monodesmosides and bisdesmosides isolated from the stem bark of *Kalopanax pictus*. *Planta Med.* 66(04): 329-332. doi:10.1055/s-2000-8539

Li, S.L., Lai, S.F., Song, J.Z., Qiao, C.F., Liu, X., Zhou, Y., Cai, H., Cai, B.C. & Xu, H.X. 2010. Decocting-induced chemical transformations and global quality of Du-Shen-Tang, the decoction of ginseng evaluated by UPLC-Q-TOF-MS/MS based chemical profiling approach. *J. Pharm. Biomed. Anal.* 53(4): 946-957. doi:10.1016/j.jpba.2010.07.001

Li, Y.J., Wei, H.L., Qi, L.W., Chen, J., Ren, M.T. & Li, P. 2010. Characterization and identification of saponins in *Achyranthes bidentata* by rapid-resolution liquid chromatography with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 24(20): 2975-2985. doi:10.1002/rcm.4728

Lu, S.H., Guan, J.H., Huang, Y.L., Pan, Y.W., Yang, W., Lan, H., Huang, S., Hu, J. & Zhao, G.P. 2015. Experimental study of antiatherosclerosis effects with hederagenin in rats. *Evid. Based Complement. Alternat. Med.* 2015: 456354. doi:10.1155/2015/456354

Wang, M., Chen, D.Q., Chen, L., Liu, D., Zhao, H., Zhang, Z.H., Vaziri, N.D., Guo, Y., Zhao, Y.Y. & Cao, G. 2018. Novel RAS inhibitors poricoic acid ZG and poricoic acid ZH attenuate renal fibrosis via a Wnt/beta-catenin pathway and targeted phosphorylation of smad3 signaling. *J. Agric. Food Chem.* 66(8): 1828-1842. doi:10.1021/acs.jafc.8b00099

Wang, X., Sun, W., Sun, H., Lv, H., Wu, Z., Wang, P., Liu, L. & Cao, H. 2008. Analysis of the constituents in the rat plasma after oral administration of Yin Chen Hao Tang by UPLC/Q-TOF-MS/MS. *J. Pharm. Biomed. Anal.* 46(3): 477-490. doi:10.1016/j.jpba.2007.11.014

Wu, A.G., Zeng, W., Wong, V.K.W, Zhu, Y.Z., Lo, A.C.Y., Liu, L. & Law, B.Y.K. 2017. Hederagenin and alpha-hederin promote degradation of proteins in neurodegenerative diseases and improve motor deficits in MPTP-mice. *Pharmacol. Res.* 115: 25-44. doi:10.1016/j.phrs.2016.11.002

Xiong, Q., Rocco, F., Wilson, W.K., Xu, R., Ceruti, M. & Matsuda, S.P.T. 2005. Structure and reactivity of the dammarenyl cation: Configurational transmission in triterpene synthesis. *J. Org. Chem.* 70(14): 5362-5375. doi:10.1021/jo050147e

Xiong, Y.K., Lin, X., Liang, S., Hong, Y.L., Shen, L. & Feng, Y. 2013. Identification of senkyunolide I metabolites in rats using ultra performance liquid chromatography/quadrupole-time-of-flight tandem mass spectrometry. *J. Pharm. Biomed. Anal.* 81-82: 178-186. doi:10.1016/j.jpba.2013.04.012

Yan, Y., Chai, C.Z., Wang, D.W., Yue, X.Y., Zhu, D.N. & Yu, B.Y. 2013. HPLC-DAD-Q-TOF-MS/MS analysis and HPLC quantitation of chemical constituents in traditional Chinese medicinal formula Ge-Gen Decoction. *J. Pharm. Biomed. Anal.* 80: 192-202. doi:10.1016/j.jpba.2013.03.008

Yun yun, X. & Chao, Y. 2011. HPLC-UV and UPLC-Q-TOF analysis and metabolism study on celastrol in rats. Shanghai Jiao Tong University. MSc. Thesis (Unpublished).

Zhang, T., Wang, Y.K., Zhao, Q., Xiao, X.R. & Li, F. 2019. UPLC-Q-TOF-MS-based metabolomics study of celastrol. *Zhongguo Zhong Yao Za Zhi* 44(16): 3562-3568. doi:10.19540/j.cnki.cjcmm.20190606.502

Zhou, D., Jin, H., Lin, H.B., Yang, X.M., Cheng, Y.F., Deng, F.J. & Xu, J.P. 2010. Antidepressant effect of the extracts from *Fructus akebiae*. *Pharmacol. Biochem. Behav.* 94(3): 488-495. doi:10.1016/j.pbb.2009.11.003

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