Research Article

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Rapid identification of direct-acting pancreatic protectants from *Cyclocarya paliurus* leaves tea by the method of serum pharmacochemistry combined with target cell extraction

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Abstract: Extracts of *Cyclocarya paliurus* (CP) leaves, a popular sweet tea, inhibit pancreatic β cell apoptosis and have potent hypoglycemic effects, but the identities of the anti-apoptotic bioactive components are still unknown. In the present study, a method using UPLC-Q-TOF/MS based on serum pharmacochemistry combined with target cell extraction was established to rapidly identify direct-acting pancreatic protectants from CP. After orally administering a set amount of CP extract to rats, blood samples were collected to characterize the components that can be absorbed into the blood using UPLC-Q-TOF/MS. Also, target cells (pancreatic β NIT-1 cells) were incubated with CP extract for 24 hours, and cells were collected to identify the components that can bind to the cells using UPLC-Q-TOF/MS. Finally, to evaluate the protective effect of the bioactive components of CP, MTT and TUNEL assays were performed on treated NIT-1 cell induced by streptozotocin (STZ). Three potential direct-acting pancreatic protectants -- kaempferol, quercetin, quadranoside IV -- were

identified, and anti-apoptotic effects of kaempferol and quercetin were confirmed in STZ-induced NIT-1 cells. The findings indicate that this combined approach is a feasible, rapid, and expedient tool for capturing potential direct-acting components from natural products such as those from CP leaves.

Keywords: *Cyclocarya paliurus*; pancreatic protectants; serum pharmacochemistry; target cell extraction; UPLC-Q-TOF/MS.

List of Abbreviations

CP: The leaves of *Cyclocarya paliurus*

DMSO: dimethylsulfoxide

DMEM: Dulbecco's modified Eagle's medium

FBS: fetal bovine serum

U.S. FDA: United States Food and Drug Administration

MTT: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide

UPLC-Q-TOF/MS: ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry

STZ: streptozotocin

TCMs: Traditional Chinese medicines

TUNEL: terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling assay

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1 Introduction

Cyclocarya paliurus (Batal.) Ijinskaja (Juglandaceae) is a type of tall tree distributed in the highlands of the south and southeast of China [1]. *C. paliurus* (CP) leaves possess natural sweetness, and local people have long

been consuming it as a nutraceutical tea. In addition, CP leaves are also an herbal medicine of Traditional Chinese medicine (TCM). The properties of “clearing heat and removing toxicity” are recorded in the ancient Chinese pharmacopeia *Zhong-hua-ben-cao*, and extracts have been widely used as an obesity and diabetes treatment [2, 3]. Based on their dual properties as food and drug, CP herbal teas have been approved as a dietary supplement product by the United States Food and Drug Administration (U.S.FDA) since 1991 [2]. Nowadays, dietary supplements derived from CP are in great demand among Chinese diabetes patients.

In the past decade, pharmacological studies have revealed that CP has versatile biological properties such as antioxidant, antihyperglycemic, antihyperlipidemic, and anticancer activities, in addition to many other positive effects [2, 4-7]. Beyond that, phytochemical studies have indicated that CP contains various bioactive constituents, including carbohydrates, flavonoids, triterpenoids, phenolic acids, and sterols [8-11]. However, few direct correlations between the chemical components and the pharmacological effects have been reported. In our previous study, we reported that CP exhibited a potent hypoglycemic effect in diabetic mice by inhibiting pancreatic β cell apoptosis via regulating MAPK and Akt signaling pathways [2]; therefore, we have continued to uncover the association of CP bioactive components with their biological functions in the present study.

According to the intracorporal course of orally administered drugs, the active components presented in oral drugs are initially absorbed into the bloodstream, afterwards distributed to the tissues and organs of the body. In the tissues and organs, the active components bind to receptors on cell membranes or move into the inner cellular systems to elicit their biological functions [12]. Therefore, there are two required conditions for oral drugs to act on tissues and organs: one is to be absorbed into the bloodstream, and another is to be able to interact with receptors on the cell membrane or within cells. This provides us a productive direction in the identification of direct-acting bioactive components from natural products.

Conventional procedures for screening potential active components from CP are to first isolate and purify the constituents from CP extracts and then perform in vivo and in vitro pharmacological evaluation approaches. Since CP contains hundreds of components [13], the traditional isolation and purification methods are not an ideal way to screen active components from CP due to their complex procedures and intensive labor. In recent years, the approach of serum pharmacochimistry coupled with liquid chromatography-mass spectrometry has been

widely applied to track bioactive components of TCMs that can be absorbed into the bloodstream [14-16]. Moreover, target cell extraction coupled with liquid chromatography-mass spectrometry was also successfully applied to characterize the potential bioactive components from TCMs [17-19]. These make it possible to rapidly identify direct-acting active components from natural products like CP.

In this study, we aimed to establish an analytical platform using UPLC-Q-TOF/MS based on serum pharmacochimistry combined with target cell extraction approaches, in order to rapidly identify potential direct-acting pancreatic protectants from CP against pancreatic β cell apoptosis. Accordingly, we aimed to evaluate protective effects of identified compounds on STZ-induced pancreatic β cell apoptosis in vitro to confirm the feasibility of this approach.

2 Experimental procedure

2.1 Herbs and chemicals

C. paliurus (Batal.) Iljinskaja leaves were obtained from the garden of Nanjing Forestry University, China (Nanjing, Jiangsu, China) in March 2015. The authentication of leaves was performed by Prof. Hubiao Chen (School of Chinese Medicine, Hong Kong Baptist University), and voucher specimens (No. CP20151201) were deposited in Research Laboratory, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong. Formic acid and streptozotocin (STZ), dimethylsulfoxide (DMSO), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide (MTT) were from Sigma (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM) powder, fetal bovine serum (FBS), penicillin-streptomycin (PS), and trypsin-EDTA were from Gibco (Grand Island, NY, USA). Standard chemicals quercetin (98% purity) and kaempferol (98% purity) were provided by Baoji Herbest Bio-Tech Co., Ltd. (Baoji, Shanxi, China). LC-MS grade acetonitrile was obtained from Merck (EMD Millipore, MA, USA). Deionized water was produced by a milli-Q system (Millipore, MA, USA).

2.2 Preparation of aqueous extract of CP

CP was first extracted by water and then concentrated to yield the crude extract (yield 16.6%) as previously described [2].

2.3 Study of serum pharmacchemistry

2.3.1 Animals

Three Male Sprague-Dawley rats weighing 200–250 g were acquired from the Laboratory Animal Services Center, The Chinese University of Hong Kong, Hong Kong. The rats were provided with standard rodent diet ad libitum and free access to water and were housed in environment controlled rooms at $22 \pm 1^\circ\text{C}$ with a 12 h light/dark cycle (lights on 6:00-18:00). The Animal Ethics Committees of Hong Kong Baptist University, approved all experimental protocols, in accordance with “Institutional Guidelines and Animal Ordinance” from Department of Health, Hong Kong Special Administrative Region (Registration No. LIUYE/15-16/01-CLNC).

2.3.2 Serum pharmacchemistry analysis

For serum pharmacchemistry analysis, rats were firstly subjected to fasting for 18 hours. Subsequently, approximately 0.5 mL blood samples were collected from the ophthalmic veins under anesthesia in heparinized centrifuge tubes via sterile capillary tubes at 0, 10, and 30 minutes, 1, 2, 4, 8, and 12 hours after the oral administration of CP extract at 10.5 g/kg body weight. The blood serum was obtained by centrifugation at 3500 rpm for 30 minutes at room temperature. A total of 90 μL serum was added to 200 μL MeOH and vortexed vigorously. Two phases were formed after centrifugation at 14000 rpm for 10 minutes at 4°C . The supernatant was collected for UPLC-Q-TOF/MS analysis, and the injection volume was 2 μL .

2.4 Assay of cell extraction

2.4.1 Cell culture

Mouse pancreatic β NIT-1 cells were obtained from American Type Culture Collection (ATCC, 40–60 passages) and cultured as a monolayer in RPMI 1640 medium (Gibco Chemical, USA) supplemented with 10% heat-inactivated FBS, glucose (11.1 mM), penicillin (100 U/mL), and streptomycin (0.1 mg/mL). The cells were preserved under standard cell culture conditions at 37°C in a humidified environment of 5% CO_2 , and they were routinely sub-cultured every 2–3 days.

2.4.2 Cell extraction

NIT-1 cells grown in complete RPMI medium were seeded into 100-mm culture dishes with densities of 1.0×10^6 cells/mL and were maintained at 37°C in a 5% CO_2 environment for 24 hours. After discarding the culture medium, serum-free RPMI medium and CP extract (100 $\mu\text{g/mL}$ final concentration) were added into the dishes followed by continuous incubation for 24 hours. The cells were harvested and then centrifuged at 3000 rpm for 5 minutes, and deposited cells were washed five times with PBS. Afterwards, deposited cells were extracted with 1 mL of 70% acetonitrile by ultrasonic extraction for 1 hour. Centrifugation was performed at 13,000 rpm at 4°C for 10 minutes, and the supernatant was filtered through 0.45 μm nylon membranes for analysis.

2.5 UPLC-Q-TOF/MS analysis

An Agilent 1290 Infinity UPLC system equipped with a standard auto-sampler and binary solvent delivery system (Santa Clara, CA, USA) was used for chromatographic separation. A 100 mm $\times$ 2.1 mm Acquity UPLC HSS T3 1.8 μm column (Waters Corp., Milford, MA, USA) was used. The mobile phase consisted of A (0.1% formic acid) and B (0.1% formic acid in acetonitrile). The optimized chromatography strategy, with a flow rate of 0.3 mL/min was as follows: (1) from 0–5 minutes, a linear gradient from 2–5% B; (2) from 5–25 minutes a linear gradient from 5–40% B; and from 25–30 minutes, a linear gradient from 40–98% B. The injection volume was 2 μL and the column temperature was maintained at 35°C for each run.

MS analysis was performed in a quadrupole time-of-flight (Q-TOF) tandem mass spectrometer (Agilent Technologies, USA) equipped with electrospray ionization (ESI) with negative ionization mode. The operating parameters were as follows: capillary voltage 3.5 KV; nebulizer 45 psi; drying gas flow 7 L/minutes; drying gas temperature 300°C ; sheath gas flow, 10 L/minutes; sheath gas temperature 350°C . To ensure reproducibility and mass accuracy, the mass spectrometer was calibrated over a range of 50–1500 Da. The operations were controlled by Mass Hunter software version 6.00 (Agilent Technologies Inc., USA).

2.6 Cell viability

The NIT-1 β cells were cultured in 96-well microplates at densities of 1×10^4 cells/well and incubated with 0.2, 2, or 10 μ M kaempferol or quercetin, with or without exposure to 32 mM STZ. Following a 24 h incubation, the culture medium was withdrawn and cell viability was measured using the MTT method as described previously [2].

2.7 TUNEL assay

Cells were plated on coverslips after exposure to 10 μ M kaempferol or quercetin for 24 h and were fixed with 4% paraformaldehyde for 30 minutes. Nonspecific chromogen reaction induced by endogenous peroxidase was inhibited with 3% H_2O_2 in methanol for 10 minutes at room temperature, and cells were further rinsed in distilled PBS and incubated with equilibration buffer for 1 minute. A TUNEL kit ApopTag* Peroxidas In Situ Apoptosis Detection Kit (Merck Millipore, Temecula, CA) was used for TUNEL assay in accordance with instructions of manufacturer. TUNEL-positive cells were counted in 5 randomly selected fields (200 $\times$ magnification), and the percentage was calculated against total cells.

2.8 Statistical analysis

All data were investigated with GraphPad PRISM 5.0 (GraphPad Software Inc., San Diego, CA, USA) software using one-way ANOVA, monitored by Duncan's multiple range tests. All quantitative values were expressed as the mean $\pm$ SEM. Differences were determined to be significant when $P < 0.05$.

3 Results and discussion

3.1 Serum pharmacochimistry for tracking bioactive components from *C. paliurus* leaf extracts

For rapid screening and identification of bioactive components of CP extracts absorbed in the serum of rats, a simple three-step approach was developed. First, an in-house library that covers the known components of CP as described in the literature was established after searching databases including Pubmed, Chinese National

Knowledge Infrastructure (CNKI) and Web of Science (**Supplementary Table 1**). Subsequently, the information of 108 known compounds, including name and molecular formula, was edited using PCDL software and MassHunter software to establish a library similar to the Standard Reference Database of the National Institute of Standards and Technology (NIST).

After the establishment of this molecular library, the total ion chromatograms (TICs) of CP extract samples, blank serum samples, and serum samples of CP extract-treated rats were obtained by using UPLC-Q-TOF-MS under individually positive and negative chemical ionization modes. These data were subjected to the mass extractor function of MassHunter Workstations Software Qualitative Analysis Version B.06.00 (Agilent Technologies, Inc.2012), and the potentially active molecules with matches in the library described above were automatically identified from TICs of CP extract samples, blank serum samples, and serum samples of CP extract-treated rats according to their precise molecular weights. Compared to obtaining in positive chemical ionization mode, TICs obtained in negative chemical ionization mode exhibited greater responsiveness and richer mass spectrum information for the potential active molecules. Therefore, these TICs were adopted to analyze the peaks of potential active molecules.

Among the TICs obtained from serum samples of CP extract-treated rats at different checkpoint times, those obtained at 30 minutes after oral administration of CP extract showed the most candidate peaks, but multiple candidate peaks were also present at other checkpoint times. Taken together, a total of 13 potential active molecules were detected by comparing TICs of CP extract, blank serum samples, and serum samples of rats after CP extract oral administration for 30 minutes (**Figure 1**). The structures of these 13 potential active molecules were identified by their MS and MS/MS information of ions (**Supplementary Figure 1**). For example, the parent $[M-H]^-$ of the candidate peak of 6 is at m/z 477.0705, with MS^2 fragmentations at m/z 301.0355, formed by neutral loss of 176 Da (glucuronide). On basis of MS and MS/MS data obtained, the molecular formula of this ion was surmised as $C_{21}H_{18}O_{13}$; hence, it was inferred to be quercetin-3-O-glucuronide. Likewise, a total of 13 absorbed components were tentatively recognized as quinic acid, gallic acid, p-hydroxybenzoic acid, neochlorogenic acid, chlorogenic acid, quercetin-3-O-glucuronide, kaempferol-3-O-glucopyranoside, kaempferol-3-O-rhamnoside, quercetin, kaempferol, quadranoside IV, asiatic acid and loganin-7-O-pentoside, respectively (**Figure 1B** and **Table 1**).

Figure 1: The total ion chromatograms of the chemical constituents in rat serum followed by oral administration of CP extract.

Total ion chromatograms (TICs) in negative mode of CP extract (a, blue line), serum samples of rats following oral administration of CP extract for 30 minutes (b, green line), and blank plasma samples (c, purple line). (A) The overall profile of ion chromatograms; (B) Enlarged ion chromatograms of potential active absorbed components.

(1) quinic acid, (2) gallic acid, (3) p-hydroxybenzoic acid, (4) neochlorogenic acid, (5) chlorogenic acid, (6) quercetin-3-O-glucuronide, (7) kaempferol-3-O-glucopyranoside, (8) kaempferol-3-O-rhamnoside, (9) quercetin, (10) kaempferol, (11) quadranside IV, (12) asiatic acid and (13) loganin-7-O-pentoside,

Table 1: LC-MS/MS data for the compounds of CP absorbed into blood.

No.	Rt (min)	Molecular formula	Ion information (m/z)	Identification
1	1.051	$C_7H_{12}O_6$	$191.0575(M-H)^- \rightarrow 173.0446(M-18-H)^-$	Quinic acid
2	2.090	$C_7H_6O_5$	$169.0157(M-H)^-$	Gallic acid
3	6.563	$C_7H_6O_3$	$137.0249(M-H)^-$	p-hydroxybenzoic acid
4	8.948	$C_{16}H_{18}O_9$	$353.0885(M-H)^- \rightarrow 191.0566(M-162-H)^-, 173.0476(M-162-18-H)^-$	Neochlorogenic acid
5	9.633	$C_{16}H_{18}O_9$	$353.0884(M-H)^- \rightarrow 191.0565(M-162-H)^-, 173.0462(M-162-18-H)^-$	Chlorogenic acid
6	13.852	$C_{21}H_{18}O_{13}$	$477.0705(M-H)^- \rightarrow 301.0355(M-176-H)^-$	Quercetin-3-O-D-glucuronide
7	15.352	$C_{21}H_{20}O_{11}$	$447.0942(M-H)^- \rightarrow 285.0405(M-162-H)^-$	Kaempferol-3-O-D-glucopyranoside
8	16.724	$C_{21}H_{20}O_{10}$	$431.1004(M-H)^- \rightarrow 285.0402(M-146-H)^-$	Kaempferol-3-O-L-rhamnoside
9	18.728	$C_{15}H_{10}O_7$	$301.0354(M-H)^-$	Quercetin
10	21.110	$C_{15}H_{10}O_6$	$285.0407(M-H)^-$	Kaempferol
11	22.722	$C_{36}H_{58}O_{10}$	$695.4020(M+HCOO)^- \rightarrow 649.3958(M-H)^- \rightarrow 487.3425(M-162-H)^-$	Quadranside IV
12	27.871	$C_{30}H_{48}O_5$	$487.3430(M-H)^-$	Asiatic acid
13	28.516	$C_{22}H_{34}O_{14}$	$521.3137(M-H)^- \rightarrow 389.2714(M-132-H)^-, 371.2588(M-132-18-H)^-$	Loganin-7-O-pentoside

3.2 Target cell extraction for tracking bioactive components from *C. paliurus* leaf extracts

Our previous study showed that an aqueous extract of CP could alleviate STZ-induced apoptosis of NIT-1 (pancreatic β) cells [2]. Therefore, pancreatic β NIT-1 cells were used as an in vitro model for developing an affinity capturing system for pancreatic protectants from CP extract. After obtaining TICs of desorption eluate of NIT-1 β cells treated with or without CP extract, the potential active molecules with matches in the library were also automatically screened out via the aforementioned method. As seen in **Figure 2**, in comparison with a control incubation lacking CP extract, 7 potential active molecules were identified in the desorption eluate of pancreatic β NIT-1 cells incubated with CP extract for 24 h. In addition, 5 of 7 isolated components were tentatively identified as quercetin, kaempferol, quadranside IV, cyclocarioside K and cyclocarioside K isomer, respectively (**Supplementary Figure 1**). However, the other two components have not yet been identified. Interestingly, some relatively abundant components in CP extract have no measurable binding affinity with the target cell in the assay, while only several low content components are detected in CP extract-treated cell extraction. These data indicated that components with low content have significant interactions with the target cells; in other words, the interactions between cells and components are selective.

3.3 Direct-acting pancreatic protectants identification and activity evaluation

Direct-acting pancreatic protectants of CP must have two qualities: (1) they must be able to be absorbed into the bloodstream, and (2) they must interact with specific receptors, enzymes and/or channels on the cell membrane or within the cells. In our study, we have found three components, namely kaempferol, quercetin, quadranside IV, that are detected both in the serum after oral administration and desorption eluate of target cell extraction. Hence, they were identified as potential direct-acting pancreatic protectants (**Figure 3**). Subsequently, the available standards of kaempferol and quercetin were obtained for the evaluation of their protective effects against STZ-induced pancreatic β cell apoptosis. As shown in **Figure 4**, both kaempferol and quercetin exhibit potent protective effects against STZ-induced pancreatic β cell death. The TUNEL staining assay also demonstrated that both kaempferol and quercetin at a concentration of 10 mM could significantly reduce STZ-induced cell apoptosis. Unfortunately, the protective activity of quadranside IV has not been studied because no standard is commercially available. It is worth mentioning that STZ-induced NIT-1 pancreatic β cell apoptosis is associated with increased oxidative and inflammatory status [20]. Interestingly, quadranside IV has already been confirmed as an anti-inflammatory agent to effectively suppress nitric oxide

Figure 2: The total ion chromatograms of the chemical constituents in desorption eluate of pancreatic β NIT-1 cells incubated with CP extract. Total ion chromatograms (TICs) in negative mode of CP extract (a, blue line), desorption eluate of pancreatic β NIT-1 cells incubated with CP extract for 24 h (b, green line), desorption eluate of pancreatic β NIT-1 cells incubated without CP extract for 24 h (c, purple line). (A) The overall profile of ion chromatograms; (B) Enlarged ion chromatograms of potential active absorbed components.

Figure 3: Direct-acting pancreatic protectants identification. (A) Summary of identified potential active components by using serum pharmacology and cell extraction; (B) The structures of identified potential direct acting pancreatic protectants.

Figure 4: The effects of kaempferol and quercetin on STZ-induced apoptosis on NIT-1 cells. (A) NIT-1 cells were preincubated with kaempferol and quercetin (0.2, 2, 10 μM) for 2 h formerly 32 mM STZ treated for 24 h. Cell viability was measured by using MTT method; (B) NIT-1 cells were preincubated with kaempferol and quercetin (10 μM) for 2 h formerly 32 mM STZ treated for 24 h, After staining with 50 μL TUNEL reaction mixture, TUNEL-positive cells were analyzed. Data are expressed as the mean $\pm$ SEM ($n = 3$). ### $P < 0.001$, compared with the control group; $P < 0.05$, compared with the STZ-treated group.

production and TNF- α in activated RAW 264.7 cells [21], which is suggestive of its potential protective effect against STZ-induced apoptosis in pancreatic β cells.

4 Conclusions

In this study, based on the method of serum pharmacology combined with target cell extraction, three direct-acting pancreatic protectants, kaempferol, quercetin and quadranoside IV, were successfully identified from CP extract. Compared to conventional methods, this method is simpler and faster for screening out and identifying potential active components from a complex matrix (e.g. an extract of a TCM), and this method therefore shows great significance for elucidating the efficacies of natural medicines, such as pharmaceutical plant and mineral medicines.

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References

- [1] Wu Z, Gao T, Zhong R, Lin Z, Jiang C, Ouyang S, et al. Antihyperlipidaemic effect of triterpenic acid-enriched fraction from *Cyclocarya paliurus* leaves in hyperlipidaemic rats. *Pharm Biol.* 2017;55:712-21.
- [2] Xiao HT, Wen B, Ning ZW, Zhai LX, Liao CH, Lin CY, et al. *Cyclocarya paliurus* tea leaves enhances pancreatic beta cell preservation through inhibition of apoptosis. *Sci Rep.* 2017;7:9155.
- [3] Jiang C, Yao N, Wang Q, Zhang J, Sun Y, Xiao N, et al. *Cyclocarya paliurus* extract modulates adipokine expression and improves insulin sensitivity by inhibition of inflammation in mice. *J Ethnopharmacol.* 2014;153:344-51.
- [4] Ma Y, Jiang C, Yao N, Li Y, Wang Q, Fang S, et al. Antihyperlipidemic effect of *Cyclocarya paliurus* (Batal.) Iljinskaja extract and inhibition of apolipoprotein B48 overproduction in hyperlipidemic mice. *J Ethnopharmacol.* 2015;166:286-96.
- [5] Wang Q, Jiang C, Fang S, Wang J, Ji Y, Shang X, et al. Antihyperglycemic, antihyperlipidemic and antioxidant effects of ethanol and aqueous extracts of *Cyclocarya paliurus* leaves in type 2 diabetic rats. *J Ethnopharmacol.* 2013;150:1119-27.
- [6] Xie JH, Shen MY, Xie MY, Nie SP, Chen Y, Li C, et al. Ultrasonic-assisted extraction, antimicrobial and antioxidant activities of *Cyclocarya paliurus* (Batal.) Iljinskaja polysaccharides. *Carbohydr Polym.* 2012;89:177-84.
- [7] Xie JH, Liu X, Shen MY, Nie SP, Zhang H, Li C, et al. Purification, physicochemical characterisation and anticancer activity of a polysaccharide from *Cyclocarya paliurus* leaves. *Food Chem.* 2013;136:1453-60.
- [8] Gao Y, He C, Bi W, Wu G, Altman E. Bioassay guided fractionation identified hederagenin as a major cytotoxic agent from *Cyclocarya paliurus* leaves. *Planta Med.* 2016;82:171-9.
- [9] Yang HM, Yin ZQ, Zhao MG, Jiang CH, Zhang J, Pan K. Pentacyclic triterpenoids from *Cyclocarya paliurus* and their antioxidant activities in FFA-induced HepG2 steatosis cells. *Phytochemistry.* 2018;151:119-27.
- [10] Li J, Lu YY, Li F, Zhang YJ, Huang XS, Su XJ. Study on chemical constituents of *Cyclocarya paliurus*. *J Asian Nat Prod Res.* 2006;16:441-2.
- [11] Li S, Cui B, Liu Q, Tang L, Yang Y, Jin X, et al. New triterpenoids from the leaves of *Cyclocarya paliurus*. *Planta Med.* 2012;78:290.
- [12] Yang Z, Kulkarni K, Zhu W, Hu M. Bioavailability and pharmacokinetics of genistein: mechanistic studies on its ADME. *Anticancer Agents Med Chem.* 2012;12:1264-80.
- [13] Cao Y, Fang S, Yin Z, Fu X, Shang X, Yang W, et al. Chemical Fingerprint and Multicomponent Quantitative Analysis for the Quality Evaluation of *Cyclocarya paliurus* Leaves by HPLC-Q-TOF-MS. *Molecules.* 2017;22:pii: E1927.
- [14] Hu Y, Jiang P, Wang S, Yan S, Xiang L, Zhang W, et al. Plasma pharmacokinetics based approach to screening potential bioactive components in Huang-Lian-Jie-Du-Tang using high performance liquid chromatography coupled with mass spectrometric detection. *J Ethnopharmacol.* 2012;141:728-35.
- [15] Wang X, Sun W, Sun H, Lv H, Wu Z, Wang P, et al. 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.* 2008;46:477-90.
- [16] Wang XJ, Zhang N, Sun WJ, Sun H. Preliminary study on serum pharmacokinetics of Liu Wei Di Huang Wan. *Chin J Nat Med.* 2004.
- [17] Hong M, Wang XZ, Wang L, Hua YQ, Wen HM, Duan JA. Screening of immunomodulatory components in Yu-ping-feng-san using splenocyte binding and HPLC. *J Pharm Biomed Anal.* 2011;54:87-93.
- [18] Li X, Sun H, Zhang A, Liu Z, Zou D, Song Y, et al. High-throughput LC-MS method for the rapid characterization of multiple chemical constituents and metabolites of Da-Bu-Yin-Wan. *J Sep Sci.* 2017;40:4102-12.
- [19] Sheng LH. Live cell extraction and HPLC-MS analysis for predicting bioactive components of traditional Chinese medicines. *J Pharm Biomed Anal.* 2006;41:576-81.
- [20] Hong L, Qin W, Shuzhen G, Juncheng H, Hanju S, Xiaoxiang Z, et al. The protective effect of MT- α -glucan against streptozotocin (STZ)-induced NIT-1 pancreatic β -cell damage. *Carbohydr Polym.* 2013;92:1211-17.

- [21] Nhiem NX, Tai BH, Quang TH, Kiem PV, Minh CV, Nam NH, et al. A new ursane-type triterpenoid glycoside from *Centella asiatica* leaves modulates the production of nitric oxide and secretion of TNF- α in activated RAW 264.7 cells. *Bioorg Med Chem Lett*. 2011;21:1777-81.

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