Sesquiterpene lactones isolated from *Carpesium abrotanoides* L. by LC–MS combined with HSCCC inhibit liver cancer through suppression of the JAK2/STAT3 signaling pathway

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Abstract

*Carpesium abrotanoides* L. is rich in sesquiterpene lactones, the experiments of anti-tumor activity in vitro showed that total sesquiterpene lactone extracts exhibited the most cytotoxicity activities against HepG-2 cells (IC₅₀ = 4.2 μg/mL). In order to isolated the active compounds quickly, the strategy of HPLC-ESI-Q-TOF-MS/MS combined with high-speed counter-current chromatography (HSCCC) was implemented. Three sesquiterpene lactone compounds, 2α,5α-dihydroxy-11αH-eudesma-4(15)-en-12,8β-olide (1), telekin (2), oxoeudesm-11(13)-eno-12,8α-lactone (3) were obtained. Their structures were elucidated by detailed analysis of 1D, 2D NMR and HRMS data. In addition, compounds 1–3 were evaluated for their cytotoxic activities against HepG-2 cell line with IC₅₀ value of 9.83, 2.95, and 4.15 μM, respectively. Moreover, all compounds not only can reduce the expression of JAK2 and STAT3 mRNA but also inhibit the p-JAK2 and p-STAT3 protein expression levels in a dose-dependent manner in the HepG-2 cells. In conclusion, three sesquiterpene lactone compounds inhibited the proliferation of HepG-2 cells via suppressing the JAK2/STAT3 signaling pathway.

Keywords  *Carpesium abrotanoides* L. · Sesquiterpene lactones · Cytotoxicity · JAK2/STAT3 signaling pathway

Introduction

*Carpesium* L., a genus of Compositae plants including about 21 species is mainly distributed in southern Europe and Asia. *Carpesium abrotanoides* L., a famous ethnomedicine “Tianmingjing”, which was widely used for treating bronchitis, bruises, hepatitis for a long time in ethnic minority areas of Southwest China, especially for Tujia, and Miao minorities. Phytochemical research had demonstrated that it is enriched in sesquiterpene lactones with the α-methylene-γ-valerolactone skeleton [1–4]. Some of them possessed a wide of pharmacological activities, including anti-cancer [5], anti-inflammatory [6], and anti-bacterial [7, 8].

Liver cancer is the sixth most commonly diagnosed cancer and the third leading cause of cancer death worldwide in 2020, accounts for 906,000 new cases and 830,000 deaths annually [9]. Among them, more than 75% of primary liver cancers are belonging to hepatocellular carcinoma (HCC), which the incidence is increasing year by year [10]. So far, Sorafenib and lenvatinib are the only two first-line agents for treating advanced HCC. However, their median survival times are several months, and had side effects [11]. So it is of great significance to find new safe and effective drugs for the treatment of liver cancer. *Carpesium abrotanoides* L., is rich in sesquiterpene lactones with significant cytotoxicity effects on the HepG-2 cell line. Hence, the present study aimed to find the main active components of this plant against liver cancer and clarify its mechanism of action.

Supplementary information

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The JAK2/STAT3 signaling pathway had drawn increasingly research interests in the development of HCC. Abnormal activation of this pathway is closely related to the occurrence, development, invasion, metastasis, and prognosis of HCC [12]. Modern clinical and pathological studies found evidence that more than 60% of HCC patients have high expression of STAT3 in tissue samples [13]. Besides, phosphorylation of STAT3 is characteristic of STAT3 activation, and its transcription factor relies on the activation of JAK receptors, especially JAK2/STAT3 [14]. The abnormal activation of STAT3 is associated with abnormal regulation of the upstream kinase JAK2, and JAK2 mutations are also involved in the occurrence and development of tumor [15]. On the other hand, whole-genome sequencing revealed that the JAK/STAT signaling pathway is one of the two main oncogenic pathways in HCC [16].

It is well known that the universal-traditional methods of extraction, separation, and analysis spent more time and laborious getting compounds [17, 18]. Therefore, the present work is focus on isolating target sesquiterpenes rapidly via the strategy of high-pressure liquid chromatography coupled with electrospry ionization-quadrupole time-of-flight mass spectrometry (HPLC-ESI-Q-TOF-MS/MS) combined with high-speed counter-current chromatography (HSCCC). Herein, the isolation, structure elucidation, bioassay, and three compounds anti-liver cancer activity through the JAK2/STAT3 signaling pathway were described.

**Results and discussion**

**Anti-tumor bioassay of TEC and SLEC by the CCK-8 method**

The cytotoxicity of TEC and SLEC in vitro was evaluated against seven tumor cell lines, including Hela, HepG-2, SW620, A549, HL-60, SMMC-7721, and PC-3 using the CCK-8 assay, paclitaxel was used as the positive control. The results (Table 1) showed that the total ethanol extracts (TEC) and the total sesquiterpene lactone extracts (SLEC) exhibited potent effect on different tumor cells. In particular, the SLEC extracts showed good cytotoxicity against HepG-2 and SMMC-7721 cells with the IC_{50} values of 4.2 and 23.6 μg/ml, respectively, indicating that the sesquiterpene lactones might be the active constituents of this plant against HCC.

**LC–MS analysis**

According to the results of anti-tumor in vitro, we attempted to analyse the chemical constituents of SLEC fraction. By optimizing the liquid phase and mass spectrometry conditions, mass spectrometry analysis in positive and negative modes respectively, are shown in Fig. 1. The results revealed that sesquiterpene lactones with a higher response in the positive mode, and mainly exhibited in the form of \([M + H]^+\) or \([M + Na]^+\). The retention time, molecular formula, and fragment ions of 18 potential sesquiterpene lactones were summarized in Table 2. Further study of molecular ion peaks and fragmentation pattern of multi-level mass spectrometry revealed that the lactone ring of sesquiterpene lactones will continuously lose one molecule of H2O and CO units which are the characteristic cleavage law in positive mode. However, there are still a large number of isomers that need to be further determined by other methods.

**HSCCC separation**

The selection of an appropriate two-phase solvent system is essential for efficient isolation in HSCCC, which usually required a suitable K-value for the target ingredients by LC–MS tests [19]. Generally, the K-value should be in the range from 0.5 to 2.0 which is suitable for isolation. However, the chemical type we studied is sesquiterpene lactone, which is rarely reported [20, 21]. The K-values of three target compounds were tested by HPLC analysis as shown in Table 3. Through the determination of K-values, the solvent system of n-hexane-ethyl acetate-methanol-water (1:9:9:1, v/v/v/v) and chloroform-methanol-water (4:3:2, v/v/v) were within the scope of 0.5 < K < 2.0, which were applied to further HSCCC separations. Compared to the peak resolution of sesquiterpene, n-hexane-ethyl acetate-methanol-water (1:9:9:1, v/v/v/v) was suitable for the isolation of Peak A and Peak B, but not for Peak C. The combination of chloroform-methanol-water (4:3:2, v/v/v) was effective for Peak C. Thus, n-hexane-ethyl acetate-methanol-water (1:9:9:1, v/v/v/v) and chloroform-methanol-water (4:3:2, v/v/v) were selected for HSCCC analysis of Peaks A, B, and C, respectively.

Besides the solvent system, other factors were also investigated, such as the flow rate of mobile phase, revolution speed, and the loading amount of sample. Different flow rates (1.5, 2.0, 2.5 and 3.0 mL/min), different revolution speed (700, 800, 835 and 850 rpm) and sample loads

| Table 1 Anti-tumor activities data of three different parts of *Carpesium abrotanoides* L. |
|-----------------------------------------------|
| Extracts | Cell lines | IC_{50} (μg/ml) |
|          | Hela       | HepG-2 | SW620 | A549 | HL-60 | SMMC-7721 | PC-3 |
| TEC      | 255.4      | 28.3   | 176.7 | –    | 145.9 | 109.1 | – |
| SLEC     | 58.9       | 4.2    | 92.4  | 281.0 | 23.6  | 272.9 |
| The rest | >300       | >300   | >300  | –    | –     | >300  | – |
(50, 100, 150 and 200 mg) were all examined. The results revealed that when the flow rate, revolution speed, and sample loads were 3.0 mL/min, 835 rpm, and 200 mg respectively, satisfactory separation efficiency and peak resolutions could be achieved, which are shown in Fig. 2.

**Structural elucidation**

Under optimal conditions, three fractions (A, B, C) were separated and their weights were 12.2 mg, 24.3 mg, and 7.6 mg. The purities of these three compounds were 95.8%, 98.2%, and 97.1% by HPLC, respectively (Fig. 3). Three sesquiterpene lactones were determined as 2α,5α-dihy-droxy-11αH-eudesma-4(15)-en-12,8β-olide (1), telekin (2), oxoeudesm-11(13)-eno-12,8α-lactone (3), respectively. Their structures were identified by HRMS, 1H-NMR, 13C-NMR, 2D-NMR, and compared with relevant references.

The molecular formula of compound 1 could be deduced as C_{15}H_{22}O_{4} based on the HRMS spectra. The 1H-NMR spectrum of 1 showed the presence of two methyl groups at
$\delta^H_{\text{H}} 0.89$ (s, 3H) and 1.17 (d, $J = 5.8$ Hz, 3H), an exocyclic CH$_2$ group at $\delta^H_{\text{H}}$ 4.80 (d, $J = 1.5$ Hz) and 4.94 (d, $J = 1.5$ Hz), and an O-bearing CH group at $\delta^H_{\text{H}}$ 4.59 (ddd, $J = 6.0, 4.5, 1.7$ Hz). The $^{13}$C-NMR (DEPT) spectrum of 1 exhibited 15 carbon signals including a $\gamma$-lactone carbonyl resonance at $\delta^C_{\text{C}}$ 180.8 (C-12), two olefinic C-atoms at $\delta^C_{\text{C}}$ 149.7 (C-4) and 108.8 (C-15), and an O-bearing tertiary CH group at $\delta^C_{\text{C}}$ 78.5 (C-8). Based on these findings, compound 1 was assumed to be an eudesmane-type sesquiterpene. The HMBC correlations of H-13 ($\delta^H_{\text{H}}$ 1.17) with C-12 ($\delta^C_{\text{C}}$ 180.8), C-11 ($\delta^C_{\text{C}}$ 40.7), and C-7 ($\delta^C_{\text{C}}$ 37.1), which demonstrated that the methyl attached to C-11. The HMBC correlations of H$_2$-15 with C-15 ($\delta^C_{\text{C}}$ 108.8), C-4 ($\delta^C_{\text{C}}$ 149.7), C-5 ($\delta^C_{\text{C}}$ 72.1), and C-3 ($\delta^C_{\text{C}}$ 40.9), which indicated that the terminal double bond was located at C-4. Compared with relevant literature [22], the compound 1 was determined as 2$\alpha$,5$\alpha$-dihydroxy-11$\alpha$H-eudesma-4(15)-en-12,8$\beta$-olide.

The molecular formula of compound 2 was deduced to be C$_{15}$H$_{20}$O$_3$. The $^1$H-NMR spectra of 2 showed the following clear signals: one methyl groups at $\delta^H_{\text{H}}$ 0.98 (s, 3H), two exocyclic CH$_2$ group at $\delta^H_{\text{H}}$ 4.71 (t) and 4.88 (t), $\delta^H_{\text{H}}$ 5.61 (d, $J = 1.1$ Hz) and 6.16 (d, $J = 1.2$ Hz), and an O-bearing CH group at $\delta^H_{\text{H}}$ 4.58 (td, $J = 5.2, 1.5$ Hz). The $^{13}$C-NMR (DEPT) spectrum of 2 showed the presence of 15 carbon signals, including a $\gamma$-lactone carbonyl resonance at $\delta^C_{\text{C}}$ 170.8 (C-12), four olefinic C-atoms at $\delta^C_{\text{C}}$ 150.1 (C-4), 142.1 (C-11), 120.3 (C-13) and 109.0 (C-15), and an O-bearing tertiary CH group at $\delta^C_{\text{C}}$ 77.0 (C-8). Based on these findings, compound 2 was assumed to be an eudesmane-type sesquiterpene. Compared with relevant literature [23], the data are consistent with those reported. So the compound 2 was determined as telekin.

Combined with the $^1$H- and $^{13}$C-NMR data, the molecular formula of 3 was deduced to be C$_{15}$H$_{20}$O$_3$. The $^1$H-NMR spectrum showed the presence of two methyl groups at $\delta^H_{\text{H}}$ 0.96 (s, 3H) and 1.02 (d, $J = 5.8$ Hz, 3H), an exocyclic CH$_2$ group at $\delta^H_{\text{H}}$ 5.44 (d, $J = 3.2$ Hz) and 6.11 (d, $J = 3.5$ Hz), and an O-bearing CH group at $\delta^H_{\text{H}}$ 4.21 (ddd, $J = 12.0, 9.2, 3.1$ Hz). The $^{13}$C-NMR (DEPT) spectrum of 3 showed that two olefinic C-atoms at $\delta^C_{\text{C}}$ 140.2 (C-11) and 120.1 (C-13), an ester CO group at $\delta^C_{\text{C}}$ 169.8 (C-12), and an O-bearing tertiary CH group at $\delta^C_{\text{C}}$ 80.8 (C-8). Compound 3 was also assumed to be an eudesmane-type sesquiterpene. The HMBC correlations of H-13 ($\delta^H_{\text{H}}$ 6.15) with C-12 ($\delta^C_{\text{C}}$ 169.8), C-11 ($\delta^C_{\text{C}}$ 140.2), and C-7 ($\delta^C_{\text{C}}$ 44.8), of H-13 ($\delta^H_{\text{H}}$ 5.44) with C-12 ($\delta^C_{\text{C}}$ 169.8), C-7 ($\delta^C_{\text{C}}$ 44.8), further confirmed the conjugation of the C=C bond and the lactone C=O group. The data are consistent with those reported previously [24]. Thus, the structure of compound 3 was identified as 1-oxoeudesm-11(13)-eno-12,8$\alpha$-lactone.

Cytotoxicity of isolated compounds

2$\alpha$,5$\alpha$-dihydroxy-11$\alpha$H-eudesma-4(15)-en-12,8$\beta$-olide (1), telekin (2), 1-oxoeudesm-11(13)-eno-12,8$\alpha$-lactone (3) from the SLEC fraction were evaluated for their cytotoxic activities against HepG-2 cell line. The results showed that the IC$_{50}$ values of compound 1 was 9.83 $\mu$M, that of compound 2 was 2.95 $\mu$M, and that of compound 3 was 4.15 $\mu$M.

The effect of compounds 1-3 on the mRNA expression of JAK2 and STAT3 in HepG-2 cells

The HepG-2 cells were treated with different concentrations of compounds 1 (5, 10, and 20 $\mu$M), 2 (1.5, 3, and 6 $\mu$M),
and 3 (2, 4, and 8 μM) for 24 h, respectively (Cell pictures shown in supplementary materials Figure S19). As shown in Fig. 4A, B, the mRNA expression level of JAK2 were significantly decreased by compound 1 and compound 2 at high and medium concentrations, and in a dose-dependent manner compared with the control group (p < 0.01). Moreover, treatment of compound 3 was also significantly decreased the mRNA expression level of JAK2 in a dose-dependent manner compared with the control group (Fig. 4C, p < 0.05). In addition, compounds 1–3 also significantly down-regulated the mRNA expression of STAT3 in a dose-dependent manner compared with the control group (p < 0.01). The results indicated that transcription of the mRNA of JAK2 and STAT3 in JAK2/STAT3 signaling pathway could be more sensitively regulated by compounds 1–3.

Compounds 1-3 inhibited the JAK2/STAT3 signaling pathway

The growing evidence suggests that the JAK2/STAT3 signaling is involved in many types of cancers. Therefore, the protein expression of JAK2, p-JAK2, STAT3, and p-STAT3 in HepG-2 cells was detected by Western blot. The results showed that compounds 1–3 treatment with the high and medium concentrations can significantly reduce the protein expression levels of p-JAK2 and p-STAT3 compared with the control group in a dose-dependent manner (p < 0.05 or p < 0.01). This suggests that compounds 1–3 could significantly inhibit the expression of p-JAK2 and p-STAT3 proteins. In addition, The JAK2 and STAT3 protein expression levels of compounds 1–3 were no statistical significance (Fig. 5).

Discussion

Eucalyptane-type sesquiterpenoids are the predominant constituents within the genus *Carpesium*, especially sesquiterpenoid lactones with an α-methylene-γ-lactone region, which showed significant cytotoxic to human cancer cells [25]. The present study has reported that the total ethanol extracts and total sesquiterpene lactone extracts exhibited the most cytotoxicity activities against HepG-2 cells with IC50 values of 28.3 and 4.2 μg/ml
Fig. 5 Effect of compounds 1–3 on the JAK2/STAT3 pathway in HepG-2 cells. (A–C) The HepG-2 cells were collected after incubation with different concentrations of compounds 1 (0, 5, 10, and 20 μM), 2 (0, 1.5, 3, and 6 μM), and 3 (0, 2, 4, and 8 μM) for 24 hr, the protein expressions of p-JAK2, JAK2, p-STAT3, and STAT3 protein levels were detected by Western blot. All values are expressed as the mean ± SD of three independent experiments, *P < 0.05, **P < 0.01 compared with control.
(Table 1), indicated that the sesquiterpenoid lactones should be the active ingredient. Three active eucalyptane-type sesquiterpenoids (Fig. 3), 2α,5α-dihydroxy-11αH-eudesma-4(15)-en-12,8β-olide (1), telekin (2), oxoeudesma-11(13)-eno-12,8α-lactone (3), rapidly isolated and purified from the whole herb of *Carpesium abrotanoides* L. by the strategy of HPLC-ESI-Q-TOF-MS/MS combined with HSCCC. Compared with traditional separation methods, this strategy will be more quickly and accurately to obtain three target compounds. Among them, compounds 1 and 3 were isolated from this species for the first time, and compounds 2 and 3 possess an α-methylene-γ-lactone group showed more cytotoxicity against HepG-2 better than compound 1, suggesting that an α-methylene-γ-lactone group should be an active group. The present study also was to investigate the mechanism of action of compounds 1–3 in HepG-2 cells.

JAK2/STAT3 signaling pathway is found commonly involved in many types of cancers, which is an important therapeutic target in cancer treatment. The JAK2/STAT3 signaling pathway was activated in the form of phosphorylation [26]. After the phosphorylation of JAK2 caused by cancer-promoting pathological factors, p-JAK2 can cause phosphorylation of STAT3, and p-STAT3 forms a dimer that enters the nucleus and regulates various proliferation and invasion [27]. Our qRT-PCR results (Fig. 4) have showed that compounds 1–3 can significantly down-regulated the mRNA expression of JAK2 and STAT3 in a dose-dependent manner compared with the control group. In addition, the Western blot results (Fig. 5) demonstrated that compounds 1–3 also can significantly decreased the expressions of p-JAK2 and p-STAT3 in HepG-2 cells, suggesting that compounds 1–3 can inhibit the cell proliferation via suppressed the activation of the JAK2/STAT3 pathway.

**Conclusion**

In summary, we investigated the cytotoxic activities of the total extracts and total sesquiterpene lactone extracts against seven tumor cell lines (Hela, HepG-2, SW620, A549, HL-60, PC-3, SMMC-7721), the results demonstrated that these two extracts exhibited the most cytotoxic activities against HepG-2 cell line. After that, 18 sesquiterpene lactones in the total sesquiterpene lactone extracts were determined by HPLC-ESI-Q-TOF-MS/MS method, in addition, three sesquiterpene lactone compounds have been quickly isolated via the HSCCC technology. But more interestingly, these three sesquiterpene lactones exhibited significantly the inhibitory effects on HepG-2 cells by regulating the JAK2/STAT3 signal pathway.

**Materials and methods**

**Apparatus**

HPLC-ESI-Q-TOF-MS/MS was performed on an Agilent 1290 HPLC system (Agilent Technologies, Santa Clara, CA, USA), equipped with an online vacuum degasser, a QuatPump, a manual injection valve with a 20 μL sample loop, a thermostated column compartment, a diode array detector (DAD), and an Agilent ChemStation, has been selected to analysis of samples. Agilent 6530 Accurate-Mass Q-TOF LC/MS system (Agilent Technologies, Santa Clara, CA) equipped with electrospray ionization (ESI) interface was coupled in parallel by splitting the mobile phase 1:3 using an adjustable high-pressure stream splitter (Valco Instrument Company, Houston, TX, USA). MS data were acquired across the range m/z 100–1500 in positive and negative ion modes (dry gas: N2, 325 °C, 5.0 L/min; sheath gas: N2, 350 °C 12 L/min; fragmentor voltage, 135 V; capillary voltage, 3500 V). All operations, acquisition, and analysis of data were monitored by Agilent MassHunter Acquisition Software Version A.01.00 and operated under MassHunter Acquisition Software Version B.05.00.

HSCCC was performed on a model TBE-300C HSCCC instrument (Shanghai Tauto Biotechnique Co.Ltd., China). The instrument was equipped with three multilayered preparation coils (diameter of PTFE tube, 1.6 mm, total volume 300 mL) and a 20 mL sample loop. The revolution speed was adjustable from 0 to 900 rpm. The HSCCC system consists of a TBP-5002 pump, a model of TBD-2000UV detector, a DC-0506 constant temperature circulating implement, and an AV2.2.0 B chromatography workstation. During the separation process, the temperature of separation columns was controlled at 25 °C, and the effluents were monitored at 214 nm. 1H and 13C NMR spectra were measured on Bruker Avance 600 AV (1H at 600 MHz, 13C at 151 MHz) NMR spectrometers. Using tetramethylsilane (TMS) as internal standard, and all chemical shifts were reported in parts per million (ppm, δ).

**Chemicals and plant material**

Ethanol, methanol, n-hexane, ethyl acetate, chloroform, hydrochloric acid, and Sodium hydroxide, which were used for the preparation of the fractions and the separation of HSCCC are purchased from Hunan Huihong Reagent Co., Ltd. (Changsha, China). Ultrapure water was purified and filtered by a MilliQ water (18.2 MΩ) system (Millipore, Bedford, MA, USA). Formic acid and acetonitrile used for LC–MS were of chromatographic grade (Merck, Germany). *Carpesium abrotanoides* L. were obtained from Dao County of Hunan Province in China in 2019. A voucher specimen
(No.2019-TMJ) was deposited in the Department of Pharmaceutical Chemistry, Research Institute of Chinese Medicine.

### Preparation of plant extracts and fractionation

The whole dry crushing plant of *Carpesium abrotanoides* L. (2.0 kg) was extracted by 95% ethanol (20 L) three times and soaked for 24 h. After combining and drying, the total ethanol extracts (TEC) (458.1 g) were obtained. TEC fraction (400.0 g) was dissolved in a solution of 2% NaOH to ethanol extracts (TEC) (458.1 g) were obtained. TEC fraction (400.0 g) was dissolved in a solution of 2% NaOH to pH = 9, and then adjust the pH to 2 with 1% concentrated hydrochloric acid to get precipitate, and finally yielded the total sesquiterpene lactone extracts (SLEC) (42.0 g). pola-

### Anti-tumor activity screening by CCK-8 method

Take the cells (Hela, HepG-2, SW620, A549, HL-60, PC-3, SMMC-7721) in the logarithmic growth phase, the cells were inoculated into 96-well plates at a density of 1 × 10^5/mL well. At 37 °C, 5% CO2 was incubated for 4 h, and the absorbance (OD) value at 450 nm was analyzed with a Bio-Tek microplate reader.

Cell proliferation %

\[
\text{cell proliferation} = \frac{\text{mean OD of treated cells}}{\text{mean OD of untreated cells}} \times 100
\]

### LC–MS analysis

HPLC-ESI-Q-TOF-MS/MS was used to analyze the chemical constituents of SLEC. The final conditions of the instrument are as follows: Agilent HPLC column (Eclipse XDB C18, 4.6 × 250 mm, 5 μm), mobile phase A is 0.1% formic acid water, mobile phase B is acetonitrile, elution procedure (0–8 min, 2% B; 8–10 min, 2–20% B; 10–50 min, 20–52% B; 50–60 min, 52% B; 60–65 min, 52–80% B; 65–68 min, 80–98% B; 68–75 min, 98% B). The column temperature was 25 °C, the flow rate was 1.0 mL/min, and the injection volume was 5 μL. Spectra were recorded from 190 to 400 nm (peak width 0.2 min and data rate 1.25 s⁻¹) while the chromatogram was acquired at 214 nm.

### Measurement of partition coefficients

The selection of solvent system is mainly based on the distribution coefficient of the targets. The specific operation of HPLC method is as follows: the upper and lower phase solvents of a certain volume (20 mL) are prepared according to the proportion of the solvent system, and after the phase separation is balanced, the same volume of upper and lower phase solvents are placed in different containers (10 mL). Add a certain amount of sample (200 mg), shake violently to dissolve, and rest. The same volume of upper and lower phase solvent was injected into HPLC to determine the peak area of the target components in the upper and lower phase (AS and AM), to calculate the partition coefficient (K) (K = AS/AM).

### HSCCC Separation

Five solvent systems consisting of n-hexane-ethyl acetate-methanol-water (1:1:1:1, v/v/v/v), n-hexane-ethyl acetate-methanol-water (1:9:9:1, v/v/v/v), n-hexane-ethyl acetate-methanol-water (3:1:1:1, v/v/v/v), n-hexane-methanol-water (5:4:1, v/v/v), and chloroform-methanol-water (4:3:2, v/v/v) were used for the separation of SLEC. Sample solution was prepared by dissolving 0.1 g of SELC in 20 mL mobile phase of the solvent system for separation.

In each HSCCC separation, the separation column was initiated by filling with the upper phase at 30.0 mL/min, and then the apparatus was rotated 835 rpm. The mobile phase was pumped into the column at 3.0 mL/min. After the equilibration reached, samples were injected into the injection valve. The solvent was continuously monitored at 214 nm and the peak fractions were collected under monitor by with a UV detector.

### The characterization data of compounds 1-3

#### 2a,5a-dihydroxy-11αH-eudesma-4(15)-en-12,8β-olide (1)

White power; M.p. 158–160 °C. HRMS: m/z: 311.1497 [M+COOH]^+ (calcd. for 311.1495, C_{16}H_{23}O_6). 1H NMR (600 MHz, CD_{3}OD). 1H NMR (600 MHz, CD_{3}OD) δ_H: 0.89 (s, 3H, H-14), 1.17 (d, J = 7.2 Hz, 3H, H-13), 1.41 (td, J = 6.2, 5.5, 1.9 Hz, 1H, H-1β), 1.43 (m, 1H, H-6β), 1.69 (dd, J = 13.8, 6.1 Hz, 1H, H-6α), 1.80 (m, 1H, H-1α), 1.83 (m, 1H, H-9β), 2.03 (dd, J = 15.4, 4.7 Hz, 1H, H-9α), 2.40 (ddd, J = 12, 5.4, 1.7 Hz, 1H, H-1β), 2.61 (m, 1H, H-3α), 2.83 (ddd, J = 12.7, 6.4, 4.3 Hz, 1H, H-7), 2.97 (p, J = 7.1 Hz, 1H, H-11), 3.80 (tt, J = 11.3, 5.1 Hz, 1H, H-2), 4.59 (ddd, J = 6.0, 4.5, 1.7 Hz, 1H, H-8), 4.80 (t, J = 1.5 Hz, 1H, H-15), 4.94 (t, J = 1.5 Hz, 1H, H-15). 13C NMR (151 MHz, CD_{3}OD) δ_C: 180.8 (CO, C-12), 149.7 (C, C-4), 108.8 (CH_{2}, C-15), 78.5 (CH, C-8), 72.1 (C, C-5), 66.1 (CH, C-2), 43.7 (CH_{2}, C-1), 40.9 (CH_{2}, C-3), 40.7 (CH, C-11), 37.1 (CH, C-7), 36.5 (C, C-10), 35.4 (CH_{2}, C-9), 26.6 (CH_{2}, C-6), 21.5 (CH_{3}, C-14), 8.2 (CH_{3}, C-13).

#### 1-telekin (2)

White power; M.p. 156-158 °C. HRMS: m/z: 249.1480 [M+H]^+ (calcd. for 249.1491, C_{15}H_{23}O_{5}). 1H NMR (600 MHz, CDCl_{3}). 1H NMR (600 MHz, CDCl_{3}) δ_H: 0.98 (s, 3H, H-14), 3.37 (m, 1H, H-7), 4.58 (td, J = 5.2, 1.5 Hz, 1H, H-8), 4.71(t, 1H,
1-oxoecdus-11(13)-endo-12,8α-lactone (3) white power; M.p. 108-110 °C. HRMS: m/z 249.1480 [M + H]+ (calcd. for 249.1491, C19H23O3). 1H NMR (600 MHz, CDCl3) δH: 9.96 (s, 3H, H-14), 1.02 (d, J = 5.8 Hz, 3H, H-15), 1.35 (q, J = 11.9 Hz, 1H, H-9), 1.45 (dd, J = 14.8, 11.8 Hz, 1H, H-6), 1.55 (m, 1H, H-2), 1.83 (m, 1H, H-5), 1.87 (m, 1H, H-4), 2.04 (m, 1H, H-6), 2.10 (m, 1H, H-2), 2.43 (m, 1H, H-3), 2.39 (dd, J = 9.4, 4.2 Hz, 1H, H-9), 2.70 (m, 1H, H-7), 4.21 (ddd, J = 12.0, 9.2, 3.1 Hz, 1H, H-8), 5.44 (d, J = 3.2 Hz, 1H, H-13), 6.11 (d, J = 3.5 Hz, 1H, H-13). 13C NMR (151 MHz, CDCl3) δC: 222.4(CO, C-1)(ca), 169.8 (CO, C-12), 140.2 (C, C-11), 120.1 (CH2, C-13), 80.8 (CH, C-8), 50.0 (C, C-10), 48.7 (CH, C-5), 44.8 (CH, C-7), 44.1 (CH, C-3), 35.2 (CH2, C-2), 34.5 (CH2, C-9), 29.6 (CH, C-4), 24.1 (CH2, C-6), 22.0 (CH3, C-14), 20.0 (CH3, C-15).

Quantitative real-time PCR analysis

Total RNA was extracted from HepG-2 cells with Trizol reagent (Ambion, Austin, USA). About 500 ng of total RNA for each sample was reverse-transcribed to prepare cDNA using PrimeScript II RTase reverse transcriptase (Takara, Beijing, China). The quantitative real-time polymerase chain reaction (qRT-PCR) was performed using a CFX-Connect 96 (Applied Biosystems, Foster City, CA) with the SYBR FAST qPCR Master Mix (Solarbio, Beijing, China). The primer sequences used for PCR were as follows: JAK2, forward: ACTAAATGCTGTCCCCC, reverse: TTCATGCAGTTGACCGT; STAT3, forward: TGAGGGAGCAGAGATGTG, reverse: TGGGGGCTTGGTAAAA; GAPDH, forward: CCCTGTTGCTGT. The relative expression of each target gene compared with GAPDH was calculated using the 2−ΔΔCt method [28].

Western blotting

The total protein was extracted from HepG-2 cells using RIPA lysis buffer (Solarbio, Beijing, China), and the protein concentration was detected by BCA Protein Assay Kit (Solarbio, Beijing, China). The loading protein (20 µg) was electrophoresed and separated on a 12% SDS-polyacrylamide gel (SDS-PAGE) and then transferred to a polyvinylidene difluoride (PVDF) membrane (0.22 µm, Millipore, USA). The PVDF membrane was blocked in 5% skimmed milk at room temperature for 1 h, the membranes were incubated with primary antibodies (JAK2, p-JAK2, STAT3, p-STAT3, and GAPDH) (Bioswamp) overnight at 4 °C. Then, the membranes followed by secondary antibodies goat anti-rabbit IgG (1:10000) incubation for 1 h at 37 °C and detected by ECL.

Statistical analysis

The results were expressed as means ± standard deviation (SD). One-way analysis of variance (ANOVA) and t-tests were used for comparisons among and between groups, respectively. All statistical analysis was carried out using SPSS 21.0 with statistical significance set at p < 0.05 or p < 0.01.

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