Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

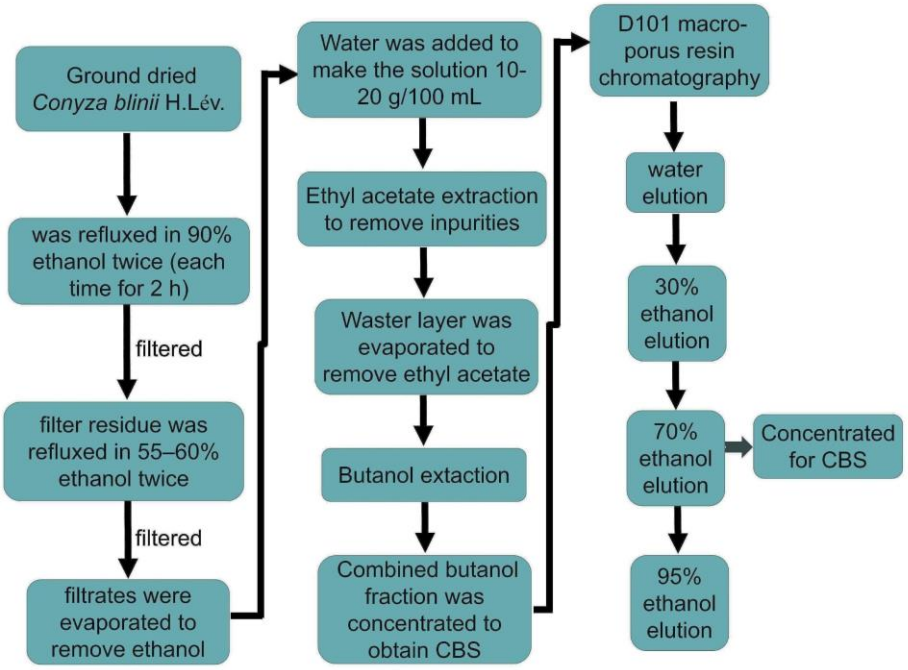

Figure SI-1: Flow chart for preparing CBS.

Materials and Methods

1. Chemicals and antibodies

Chemicals were obtained from from Sigma-Aldrich unless other specified. Cisplatin injection was purchased from Qilu Pharmaceutical Co., LTD (Jinan, China). BD BioCoat Matrigel Invasion Chambers was purchased from BD Biosciences.

2. Preparation and analysis of CBS.

Conyza blinii H.Lév. the accepted name of a species in the genus *Conyza*, family Compositae, which has been checked against websites www.theplantlist.org (The Plant List), and www.ipni.org (The International Plant Names Index). A voucher specimen of *Conyza blinii* H.Lév. was identified by Prof. Tianxiang Li (Tianjin University of Traditional Chinese Medicine, China). Preparation of CBS from dried *Conyza blinii* H.Lév. was strictly conducted as previously publication .[1]

3. HPLC-MS analysis

High resolution HPLC-MS was conducted according to previously publication with slight modifications.[2] Simply, samples were separated on a COSMOSIL Cholester column equipped with a guard column. The mobile phase consisted of water (A) and acetonitrile (B). A gradient program was used for the elution: 0min, 90% A，10%B；5min, 70% A，30%B；30 min, 30% A, 70% B. Flow rate was 1 mL/min with detection wavelength ranges from 190 to 800 nm. The column temperature was set at 30°C. High resolution MS was performed using a Schimadzu LCMS-IT-TOF mass spectrometer.

4. Cell culture

HeLa cells were maintained in DMEM medium. A549 cells were grown in F-12K medium. Each type of medium used was supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin. All cells incubated in a humidified atmosphere with 5% CO_2 at 37 °C.

5. Cell viability assay and IC_{50} calculation

The cell viability was evaluated using MTT assay. Different kinds of cells were seeded in 96-well microtiter plates and left to adhere overnight. The cells were then treated with different concentrations of CBS for 24 or 48 h where necessary. Prepared MTT (0.5 mg/mL, in PBS buffer pH 7.4) was added to each well and the plates were incubated at 37°C for 4 h. Finally, 200 μL dimethylsulphoxide (DMSO) was added to each well to dissolve the formazan dye. Subsequently, the absorbance of each well was measured at 490 nm by a microplate reader. The cell viability was calculated as follows: cell viability (%) = (average A_{490} of the treated group - average A_{490} of the blank group)/(average A_{490} of the control group - average A_{490} of the blank group) × 100%. The cell viability of non-CBS treated group was assigned a value of 100%. The experiments were repeated in triplicate. The IC_{50} values were determined by non-linear regression fitting to a sigmoid curve equation using GraphPad software 5 (San Diego, CA, USA). The cell states were also observed and imaged using a light inverted microscope.

6. Colony formation assay

HeLa cells were plated (1500/well) in triplicate with 2 mL growth medium in 6-well plates. Cells were re-fed with fresh medium containing 3% FBS and treated with different concentrations of CBS. After two weeks, colonies were counted after 5 mg/mL MTT staining.

7. Wound healing assay

HeLa cells were seeded in 6-well plates and allowed to grow until complete confluence. Subsequently, a plastic pipette tip was used to scratch the cell monolayer to create a cleared area. The wounded HeLa cell layer was washed with fresh culture medium to remove loose cells. The cells were then re-fed with fresh medium containing 3% FBS and treated with different concentrations of CBS. The scratched monolayer of HeLa cells were photographed using a light inverted optical microscope.

8. Cell invasion assay

Transwells (BD Biocoat Matrigel 24-well invasion chamber) with filters were coated with an extracellular matrix on the upper surface. The lower chamber was filled with medium containing 10% FBS as chemoattractant agents. The upper chamber was laid over the lower chamber. HeLa cells (8×10^4) were pre-incubated with CBS. for 30 min at room temperature. Then all cell suspension was seeded onto the upper chamber wells in serum-free medium and incubated at 37°C with 5% CO_2 for 24 h. The penetrated cells were then fixed in 4% paraformaldehyde (PFA), stained with hematoxylin–eosin (HE) and counted. Each reported value represents the mean of results obtained in three individual experiments. The cell invasion index was reported as the relative migration of cells across the Matrigel.

9. Cell cycle analysis by flow cytometry

Hela cells (1×10^6/well) were treated with different concentrations of CBS (0, 10 and 20 μg/mL) for 24 h. Then the cells were harvested by trypsinisation, washed in ice-cold phosphate buffered saline, and fixed in 70% ice cold ethanol overnight at -20°C. Subsequently, cells were resuspended in 1 mL of PBS containing 50 U/mL RNase and 50 μg/mL propidium iodide (PI), and then incubated for 40 min in the dark at 4 C. Cell cycle analysis was performed using a FACS (BD Accuri C6), and the population of cells in each phase was calculated using the the Modifit LT software program.

10. Measurement of mitochondrial ΔΨm by flow cytometry

HeLa cells were seeded (2×10^5/well) in 6-well plates and allowed to grow for 24 h. After 24 h of treatment with the test compounds, the cells washed with PBS then stained with 5 μM rhodamine-123 at 37 ^{o}C for 30 min in the dark. Samples were analyzed using a FACS (BD Accuri C6).

11. Western blotting

Cells were lysed in RIPA buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM NaF, 1 mM phenylmethylsulphonyl fluoride, 4 mg/mL leupeptin and 1 mg/mL aprotinin, and 1% Nonidet P-40) supplemented with proteinase inhibitors. Protein samples were extracted and then resolved by 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose filter. The filters were blocked in PBST buffer containing 5% non-fat dry milk for 1 hour at room temperature. Blots were then incubated overnight at 4°C with primary antibodies. Secondary antibody was incubated for 2 hour at room temperature. Finally, Odyssey infrared laser imaging system was used to image the results.

12. Tumour Xenograft animal Experiments.

The animals had free access to food and water in animal cages that were maintained in a pathogen-free environment (24 ± 1^{o}C, humidity of 55 ± 5%) with a 12-h light/dark cycle. Animal welfare and experimental procedures were carried out in accordance with the Ethical Regulations on the Care and Use of Laboratory Animals of Tianjin University of Science and Technology and were approved by the university committee for animal experiments. All experiments were carried out using 4-5 weeks female Kunming mice ranging from 18-22 g. Cancer models were established by injection of 2×10^6 cells/mouse on the right flank. 48 h after inoculation, 24 mice were randomly divided into 3 groups respectively. Mice were treated CBS (i.p.), physiological saline or cisplatin injection for consecutive 10 days, once for a day. 24 h after the last administration, the mice were sacrificed and the tumors and other oragans were excised and weighted. At the end of the experiments, a thorough necropsy was carried out. Collected umors were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned and processed to hematoxylin and eosin (HE) staining.

13. Statistical analysis.

The values obtained in the experiments are expressed as the mean ± standard deviation (SD) and were analysed by Student's t-test or one-way analysis of variance (ANOVA) where

necesssary. All statistical analyses were performed using SPSS 17.0 software and $P < 0.05$ was considered significant.

References:

1 L. Ma and J. Liu, *J. Ethnopharmacol*., 2014, **158**, 358-363.

2 X. Qiao, X. Zhang, M. Ye, Y. f. Su, J. Dong, J. Han, J. Yin and D. a. Guo, *Rapid. Commun. Mass Spectrom*., 2010, **24**, 3340-3350.