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ScienceDirect
Chinese Journal of Natural Medicines 2017, **15**(10): 0766–0774
Chinese Journal of Natural Medicines

Anti-proliferation activity of terpenoids isolated from *Euphorbia kansui* in human cancer cells and their structure-activity relationship

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Available online 20 Oct., 2017

[ABSTRACT] *Euphorbia kansui* is a commonly used traditional Chinese medicine for the treatment of edema, pleural effusion, and asthma, etc. According to the previous researches, terpenoids in *E. kansui* possess various biological activities, e.g., anti-virus, anti-allergy, antitumor effects. In this work, twenty five terpenoids were isolated from *E. kansui*, including thirteen ingenane- and eight jatrophane-type diterpenoids (with two new compounds, kansuinin P and Q) and four triterpenoids. Eighteen of them were analyzed by MTS assay for *in vitro* anticancer activity in five human cancer cell lines. Structure-activity relationship for 12 ingenane-type diterpenoids in colorectal cancer Colo205 cells were preliminary studied. Significant anti-proliferation activities were observed in human melanoma cells breast cancer MDA-MB-435 cells and Colo205 cells. More than half of the isolated ingenane-type diterpenoids showed inhibitory activities in MDA-MB-435 cells. Eight ingenane- and one jatrophane-type diterpenoids possessed much lower IC_{50} values in MDA-MB-435 cells than positive control staurosporine. Preliminary structure-activity relationship analysis showed that substituent on position 20 was important for the activity of ingenane-type diterpenoids in Colo205 cells and substituent on position 3 contributed more significant biological activity of the compounds than that on position 5 in both MDA-MB-435 and Colo205 cells.

[KEY WORDS] Terpenoids; Anti-proliferation activity; Human cancer cells; Structure-activity relationship

[CLC Number] R965 **[Document code]** A **[Article ID]** 2095-6975(2017)10-0766-09

Introduction

Euphorbia kansui T.N. Liou ex T.P. Wang is a plant mainly distributed in the northwest of China. The dried roots of *E. kansui* is a common used toxic traditional Chinese medicine (TCM) recorded in Chinese Pharmacopoeia for the treatment of edema, pleural effusion, ascites, and asthma [1]. More clinical applications of *E. kansui* for pancreatitis [2-3] and intestinal obstruction [4] have also been studied.

Previous researches have demonstrated that terpenoids in *E. kansui* (ingenane-, tigliane- and daphane-type diterpenoids) possess anti-virus [5], anti-allergy [6], anti-nematodal [7], anti-fertility [8] and anti-tumor [9] activities. Among them, the anti-tumor activity has attracted more attention and the compounds have been screened in multiple cultured cancer cell lines. Wu *et al.* [9] have reported the antileukemia and anti-tumor activities of two diterpene esters, kansuiphorins A and B from *E. kansui*. A series of human cancer cell lines have been screened with those two compounds; kansuiphorin A shows potential anti-proliferation activity in many cell lines, including MOLT-4, HL-60TB, and K-562 (leukemia) H-322 and HOP-62 (non-small cell lung cancer); SW-620 (colon cancer); SK-MEL-5, RPMI-7951, and Maime-3M (melanoma); and A-498, A-704, and SN-12K1 (renal cancer). Wang *et al.* [10, 11] have further investigated cell cleavage inhibition activity of diterpenes from *E. kansui*. Nine ingenol derivatives and one jatrophane diterpenes (kansuinin B) show significant cleavage arrest activity on division of cells from early *Xenopus laevis*

[Received on] 30-Dec.-2016
[Research funding] This work was supported by the National Natural Science Foundation of China (No. 81073026).
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These authors have no conflict of interest to declare.

embryo. Yasukawa *et al.* [12] have found that a triterpene alcohol (euphol) from *E. kansui* possesses the inhibitory activity of 12-*O*-tetradecanolyphorbol-13-acetate induced tumor in mouse skin. Besides terpenoids, Yu *et al.* [13-14] have demonstrated that six methyl esters and derivatives isolated from *E. kansui* exhibit obvious apoptosis and proliferation inhibition activity in human gastric cancer cells (SGC- 7901).

In this work, 13 ingenane- and eight jatrophane-type diterpenoids (including two new compounds) and four triterpenoids were isolated from *E. kansui* and identified by NMR. Anti-proliferation activities of each compound were evaluated *in vitro* against five human cancer cell lines which haven`t been screened before. In addition, structure-activity relationships of jatrophane-type diterpenoids were preliminarily studied.

Materials and Methods

Chemical and reagents

MTS testing kit (cat#G3581，Promega Corporation，Madison,USA), 0.25% Trypsin-EDTA (GIBCO, cat#25200), RPMI-1640 (GIBCO, cat#A10491-01), Fetal bovine serum (FBS, GIBCO, cat#16000-044), Penicillin-Streptomycin, liquid (GIBCO, cat#15140 -122) were all obtained from Invitrogen Co., Ltd., Carlsbad, CA, USA. Dimethyl sulfoxide (DMSO) (Sigma, cat#D2650, Sigma-Aldrich LLC.,USA), 96-well cell culture cluster (Corning, cat#3599, Corning, NY, USA). All other reagents were analytical grade obtained from Sinopharm chemical reagent Co. Ltd. (Shanghai, China).

Plant Material

The *Euphorbia kansui* radix was collected from Shannxi Province of China in 2010. They were identified by Professor Guo De-An (Shanghai Institute of Materia Medica, Shanghai, China). The Voucher specimens for *E. kansui* radix were deposited at National Engineering Laboratory for TCM Standardization Technology in Shanghai Institute of Materia Medica, Shanghai, China.

Instrumentation

The UV Spectra were obtained in methanol on a Shimadzu UV -2550 spectrophotometer (SHIMADZU Corporation, Kyoto, Japan). The NMR spectra were recorded on a Varian Mercury plus 400 M spectrometer (Agilent corporation, Santa Clara, CA, USA). The mass spectra (MS) were performed on a Bruker HCT APCI-MS and an Agilent 6410B ESI-MS spectrometer (Bruker Corporation, Billerica, MA, USA). Polarimetry analysis was obtained on PerkinElmer polarimeter 341 (PekinElmer Corporation, Boston, MA). Column chromatography was carried out with silica gel (200–300 sieve), Sephadex LH-20 (25–100 μm, GM) and Chromatorex C18, 20–45 μm, FUJI SiLYSIA chemical Ltd. (Kozoji-cho, Kasugai Aichi, Japan). HPLC were performed on Waters 2996 HPLC System (Waters Corporation, Milford, MA, USA) comprised of a quaternary solvent delivery system, an on-line degasser, an auto-sampler, a photodiode array detector (PDA), and an analytical workstation (Empower 2 software) (Waters Corp, Milford, MA, USA). An Allsphere Silica (10 mm × 250 mm i.d., 5 μm) column (Grace Corporation, Columbia, MD, USA) an Agilent XDB C_{18}(9.4 mm × 250 mm i.d., 5 μm) and an Agilent Rx C_8 ODS (10 mm × 250 mm i.d., 5 μm) column were used for preparative purpose. Agilent Poroshell 120 EC-C_8 (3 mm × 100 mm, 2.7 μm) (Agilent corporation, Santa Clara, CA, USA), with an online filter was used. A Branson B3500S-DTH ultrasonic bath (140 W, 42 kHz) (Branson Ultrasonic, Shanghai, China) was used for sample preparation.

Extraction and isolation of compounds from Euphorbia kansui

The dried powder of *E. kansui* roots (16 kg) were stirred and extracted twice with ethanol (128 L, 90 L) under reflux. Evaporation of the solvent under reduced pressure from the combined extracts gave 672 g of ethanol extract.

The ethanol extract (640 g) was subjected to silica gel column chromatography (eluted with petroleum ether and ethyl acetate in increasing polarity). The column chromatographic fractions were combined according to TLC monitoring into five portions. Fraction 3 (60 g), eluted with petroleum ether-dichloromethane-methanol, was isolated and further purified by column chromatography and HPLC. **CI-13** (12 mg) was isolated by HPLC (Agilent Rx C_8, 9.4 mm × 250 mm, 5 μm, MeCN–MeOH–H_2O = 49 : 49 : 2, 4 mL·min^{-1}, 220 nm, t_R = 25 min); **CI-4** (46 mg) was isolated by HPLC (Agilent XDB C_{18}, 21.1 mm × 150 mm, 5 μm, MeOH: 0.1% TFA (95 : 5), 10 mL·min^{-1}); **CI-10** (29 mg), **CI-3** (6 mg) and **CJ-16** (47 mg) were isolated by HPLC (XDB C_{18}, MeOH: 0.1% TFA, 20 mL·min^{-1}); **CT-22** (6.5 mg), **CT-25** (16 mg) and **CJ-15** (72 mg) were isolated by HPLC (XDB C_{18}, MeCN, d = 0.785, 8 mL·min^{-1}); **CI-2** (40 mg), **CI-5** (7.5 mg), **CI-8** (40 mg), **CI-9** (21 mg), **CJ-16** (21 mg), **CI-6** (10.8 mg), **CI-7** (5.3 mg, XDB C_{18} MeCN, d = 0.872, 4 mL·min^{-1}), **CI-11** (3.3 mg), **CJ-17** (4.8 mg, XDB C_{18} MeCN, d = 0.831, 4 mL·min^{-1}) and **CI-12** (36 mg, XDB C_{18} MeCN, d = 0.815, 4 mL·min^{-1}) were isolated by HPLC (XDB C_{18}, 85% MeCN, d = 0.830). The mixture of **CT-23** and **CT-24** were obtained abundantly in the form of precipitate during elution, and was furtherly purified by RP-HPLC (XDB C18, 9.4 mm × 250 mm, 5 μm, MeCN–MeOH–H_2O = 49 : 49 : 2, 4 mL·min^{-1}) to give **CT-23** (18 mg) and **CT-24** (176 mg).

Fraction 4 (40 g) was also isolated and further purified by column chromatography and HPLC (XDB C_{18}, 0.96 cm × 25 cm, 5 μm, MeCN, d = 0.890, 4 mL·min^{-1}) to give **CI-1** (7.1 mg), **CI-20** (12.9 mg), **CI-21** (9.8 mg), **CJ-14** (23 mg), **CJ-19** (124 mg, MeCN, d = 0.898) and **CJ-18** (39 mg, MeCN, d = 0.870).

Cell lines and Cell culture

Five cancer cell lines, including human colorectal cancer Colo205 cells, human melanoma cells breast cancer MDA-MB-435 cells, human leukemia DAUDI cells, human prostate cancer PC3 cells, and human ovary cancer SKOV-3 cells, were obtained from ATCC (American Type Culture Collection) or CAS (Chinese Academy of Sciences cellbank). All cell lines were cultured in RPMI1640 supplemented with 10% fetal bovine serum.