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# Three coumarins from seed of *Cnidium monnieri* and their multidrug resistance reversal effects

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**Abstract** **Object** To isolate the active compounds on reversing multidrug resistance (MDR) of tumor cell from the ethanol extract in the seeds of *Cnidium monnieri* (L.) Cuss. **Methods** The fractionation directed by bioactivity was carried out with silica gel chromatography and RP-HPLC. **Results** Three active coumarins were obtained: imperatorin (Ⅰ), edultin (Ⅱ) and 3′-isobutyryloxy-*O*-acetyl columbionetin (Ⅲ). Their sturctures were identified by spectroscopic analysis. **Conclusion** These three compounds have a medium reversing MDR of KBV200 *in vitro*.

**Key words** *Cnidium monnieri* (L.) Cuss.; coumarin; multidrug resistance (MDR); KBV 200

# 蛇床子中 3种逆转肿瘤细胞多药耐药活性香豆素

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摘 要:目的 从蛇床子 *Cnidium monnier* 中分离逆转肿瘤细胞多药耐药活性成分。方法 用生物活性跟踪法,经硅胶柱层析、RP-HPLC等。结果 分离得到 3种活性成分,分别为欧芹属素乙(imperatorin),爱得尔庭(edultin),9-异丁酰氧基-*O*-乙酰基哥伦比亚苷元(3′-isobutyryloxy-*O*-acetyl columbionetin)。结论 体外实验表明 3种化合物对耐药的肿瘤细胞 KBV 200具有明显的逆转作用。

关键词:蛇床子;香豆素;多药耐药;KBV200

中图分类号:R283.3 文献标识码:A 文章编号:0253-2670(2003)02-0104-03

## 1 Introduction

One of the major problem of cancer chemotherapy is intrinsic or acquired multidrug resistance (MDR). Many kinds of compounds, such as calci-

⑳收稿日期:2002-10-12

um channel bolckers, calmodulin inhibitors and indole alkaloids are known to reverse MDR, however, they all have not been recommended to routine clinical use due to toxicity[1]. For example, Verapamil, the most extensively studies MDR reversing agent, induces severe toxicity at the doses required. Thus we need to develop new classes of MDR reversing agents with less toxicity to the host.

Many traditional Chinese drugs, alone or combined with chemotherapic agents, are used in clinical cancer treatment. They are proven to increase curative effects and decrease the toxicities of the chemotherapic agents. Some compounds isolated from traditional Chinese herbs were reported to have MDR reversing activites[2]. We believed we might find the new classes of MDR reversing agents from traditional Chinese drugs. In this work we found the ethanolic extracts of *Cnidium monnieri* (L.) Cuss. exhibited reversing MDR activities. Activity-bioguided fractionation using chromatography was conducted and led to isolate three active compounds.

## 2 Materials and methods

2.1 General experimental procedures. The $^{1}$HNMR and $^{13}$CNMR spectra ($CDCl_3$) were obtained with JNM-GX 400 or INOVA-600 instruments. FAB-MS was performed on a Zabspec spectrometer. Preparative HPLC was carried out with a water system equipped with a 600E pump, a 996 PDA detector at 254 nm and a Delta-Pak $C_{18}$ prepacked radial compression column (40 mm × 200 mm, 15 μm), elution with MeOH-$H_2O$ at various mixtures at flow rate 15 mL/min.

2.2 Plant material. The seeds of *Cnidium monnieri* (L.) Cuss. were collected in Jiangsu Province. Identification of specimens was carried out by Professor Zhang Ming-qing from Nanjing University of TCM.

2.3 Extraction and activity-directed fractionation. The dry seeds (5 kg) were macerated with 95% EtOH for three times. The EtOH extract was concentrated to dryness *in vacuo* and submitted to liquid-liquid partition with $CHCl_3$-$H_2O$. The $CHCl_3$ phase was partitioned again with hexane/90% MeOH. The active aqueous MeOH phase (29.8 g) was mixed with silica gel (150 g) and evaporated to dry *in vacuo* and washed with petroleum ether, petroleum ether-$CH_2Cl_2$ mixtures (100∶1 to 1∶1), $CH_2Cl_2$ and MeOH. The bioassay results indicated petroleum ether fraction (12 g) and petroleum ether-$CH_2Cl_2$ (50∶1) fraction (1.5 g) were active. The petroleum ether fraction (6 g) was subjected to flash chromatography on silica gel, eluting with petroleum ether-EtOAc (10∶1 to 1∶1). The active fractions combined and further purified with preparative RP-HPLC to give edultin Ⅱ, (28 mg) and 3′-isobutyryolxy-*O*-acetylcolumbianetin Ⅲ, (31 mg), eluting with $CH_3OH$-$H_2O$ (55∶45). The petroleum ether-$CH_2Cl_2$ (50∶1) fraction was chromatographed with preparative $C_{18}$ HPLC to yield imperatorin Ⅰ (35 mg), eluting with $CH_3OH$-$H_2O$ (55∶45).

2.4 Cell culture and bioassay. Resistant human oral epidermoid carcinoma cell line, KBV200 was derived from the parent sensitive KBS cell Line by stepwise exposure to vincristine (VCR), they were kindly gifted by Professor Wang Yu-zi from Beijing Institute of Radiation Medicine. KBV200 cells were maintained in the presence of 1 μg/mL VCR. KBV200 and KBS were cultured in PRMI-1640 supplement with 10% fetal calf serum and were grown at 37℃ in humidified atomsphere with 5% $CO_2$. MTT method was used for cytotoxicity assays[3]. In 96 well plates $2\times10^4$ cells were seeded and treated with graded concentrations of the extracts or compounds (dissolved in DMSO). The plates were incubated for 72 hours at 37℃ (100% humidity with a 5% $CO_2$ atmosphere in air). MTT was added and the plates were incubated for four hours, then 120 μL DMSO were added to dissolve formazam, the absorbance was measured at 570 nm, using a microplate reader. Each concentration was assayed in triplicate.

2.5 Reversal factor[4]. The $ED_{50}$ values of VCR alone and with reversal compounds against KBV200 were obtained and the reversal factor was calculated as following: Reversal factor= $ED_{50}$ of

VCR alone/$ED_{50}$ of VCR in the presence of a given concentration of reversal compound.

## 3 Results and discussion

From screening the common Chinese drug for reversing MDR of tumor cells, we found the EtOH extract of *C. monnieri* showed cytotoxicity of the MDR of KBV200 cell line ($ED_{50}$ 22.5 μg/mL) in the presence of VCR, while exhibited no significant cytotoxicity to KBV200 and the parental KBS cells in the absence of VCR. Fractionation guided by cytotoxicity of KBV200 cells in the presence of VCR led to isolate three active coumarins, imperatorin (Ⅰ), edultin (Ⅱ) and 3′-isobutyryloxy-*O*-acetyl columbionetin (Ⅲ). The structures of the three compounds were identified by comparison of their physical and spectroscopic data[5].

To examine the MDR reversing activity of Ⅰ-Ⅲ, KBV200 and KBS cells were treated with graded concentrations of VCR in the presence and absence of Ⅰ-Ⅲ. All three compounds exhibited weak cytotoxic activity against both KBV200 and KBS cells in the absence of VCR. KBV200 cells became more sensitive to VCR in the presence of Ⅰ-Ⅲ, $ED_{50}$ values of VCR decreased 1.2-8.2 fold depending on the concentration of Ⅰ-Ⅲ (shown in Table 1). While KBS cells showed no mediated cytotoxic response, but in the presence of Ⅰ, KBS cells showed slightly augmented cytotoxic response, the reason is unknown. These results clearly showed that Ⅰ-Ⅲ reversed the MDR of KBV200. The reversal factors of Ⅰ-Ⅲ are shown in Table 1.

**Table 1 Reversal factors of Ⅰ-Ⅲ against KBV200**

| | Compounds /(μg·mL$^{-1}$) | $ED_{50}$ / (μg·mL$^{-1}$) of VCR with compound | Reversal factor |
|---|---|---|---|
| Ⅰ | 0 μg/mL | 1.82 | |
| | 2.5 μg/mL | 1.48 | 1.23 |
| | 5.0 μg/mL | 1.10 | 1.65 |
| | 10.0 μg/mL | 0.66 | 2.78 |
| Ⅱ | 0 μg/mL | 1.20 | |
| | 2.5 μg/mL | 0.71 | 1.69 |
| | 5.0 μg/mL | 0.53 | 2.27 |
| | 10.0 μg/mL | 0.15 | 8.27 |
| Ⅲ | 0 μg/mL | 1.20 | |
| | 2.5 μg/mL | 0.64 | 1.89 |
| | 5.0 μg/mL | 0.37 | 3.22 |
| | 10.0 μg/mL | 0.22 | 5.45 |

The seeds of *C. monnieri* are used as a topical agent for eczenia and pruritus in China, some anti-allergic principles were isolated from this species. We isolated three coumarins with reversing MDR activity, the interactions of these compounds with Pgp and reversal activities *in vivo* will be tested.

**Acknowledgement** The authors wish to thank the financial support of National Natural Science Foundation of China (39970892)

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需要多方关注、共同参与的目标和任务。

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