



Regular articles

## Amentoflavone and the extracts from *Selaginella tamariscina* and their anticancer activity

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### Abstract

The extracts and amentoflavone were extracted from the plant of *Selaginella tamariscina*. The extracts mainly contained biflavonoids. The structure of amentoflavone was elucidated by spectral examinations. Amentoflavone and the extracts were screened against five cancer cells, including HeLa (human cervical carcinoma cells), BEL-7402 (human hepatoma carcinoma cells), MCF-7 (human breast cancer cells), PANC-1 (human pancreatic cancer cells) and HL-60 (human leukemia cells). The anticancer activity were determined by means of MTT assay and Trypan Blue cytometry. Assays *in vitro* showed that they were effective to inhibit the proliferation of HL-60, MCF-7, HeLa, BEL-7402, PANC-1 and had reliable activity against HL-60.

**Key words:** amentoflavone; extracts; *Selaginella tamariscina*; anticancer activity

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### Introduction

*Selaginella tamariscina* is a traditional medicine, which belongs to the family Selaginellaceae. It was introduced in Chinese Pharmacopeia (2005 Ed) for the effectiveness in promoting blood circulation<sup>[1]</sup>. Modern pharmacological and clinical studies have shown that *Selaginella tamariscina* has the biological activities of anti-cancer, anti-inflammatory, anti-virus, analgesia, lowering blood pressure, lowering blood glucose and strengthening the role of immune functions<sup>[2-5]</sup>. Flavonoids are

ubiquitous polyphenolic compounds found in vascular plants with a large variety of biological effects. Amentoflavone, a biflavonoid which is a dimer of apigenin, has shown antiviral<sup>[4]</sup>, antioxidant, antidepressant, anti-inflammatory and analgesic activity<sup>[6-9]</sup>.

### Experimental

#### General

NMR spectra were measured on a Bruker ARX-300 NMR spectrometer with tetramethylsilane (TMS) as the internal reference and chemical shifts are expressed in  $\delta$  (ppm). TLC was performed on silica gel GF<sub>254</sub> (10-40  $\mu$ ; Qingdao, China).

#### Plant material

*Selaginella tamariscina* (Beauv.) Spring, the

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whole plant collected in Hebei province of China, were identified by Prof. Jincai Lu. A voucher was deposited in the Traditional Chinese medica of Shenyang Pharmaceutical University.

### *Anticancer materials*

#### **Cells and chemicals**

All of these cells were obtained from the Chinese Academy of Medical Sciences. Fetal bovine serum (FBS) were obtained from Hao Yang Technology Co. Ltd (Tianjin, China). Trypan blue were obtained from GIBCOBRL (Gaithersburg, MD). RPMI 1640 medium, MTT and dimethylsulfoxide (DMSO) were purchased from Sigma Chemical (St. Louis, MO).

#### **Cell culture and methods**

##### **(1) MTT assay**

HeLa, BEL-7402, MCF-7, PANC-1 cells were cultured in RPMI-1640 medium at 37 °C in 5 % CO<sub>2</sub>. The media were supplemented with 10 % (v/v) heatinactivated fetal bovine serum. The cells were routinely cultured in 96-well tissue culture microplate and then inoculated with HeLa, BEL-7402, MCF-7 and PANC-1 cells 100 μl per well. Samples were diluted in the culture medium at different concentrations, each concentration had three parallel wells were cultured at 37 °C in 5 % CO<sub>2</sub> for four days. The cells were evaluated using the MTT assay: after cultivation every well was injected MTT (5 mg/ml) 15 μl and then was maintained for 4 h at 37 °C. Removed clear supernatant, injected DMSO 150 μl to each well, shaken lightly. The concentration of the cells was detected by an enzyme-linked immunosorbent assay (ELISA) kit (Ke Hua Inc, Shanghai, China) following the manufacturer's protocol<sup>[12]</sup>. Inhibition ratio % = (OD<sub>control</sub> - OD<sub>sample</sub>) / OD<sub>control</sub> × 100 %.

##### **(2) Trypan blue cytometry**

HL-60 cells were seeded at 3.0 × 10<sup>4</sup> cells/ml in 24-well plate and incubated with various concentrations of drugs for 96 h. The cells treated with DMSO (0.1 %) were used as a control. The cell number in each group was determined with the aid of hemocytometer. Cell viability was estimated by trypan blue exclusion assay after 96 h of drugs treatment. The concentration of the cells was detected by the following formula: Inhibition ratio % = [living cells (control) - living cells (sample)] / living cells (control) × 100 %.

### **Results and discussion**

The whole plant of *S. tamariscina*. (10.0 kg) was extracted three times with 50 % EtOH (two hours every time), and the extract was obtained under reduced pressure, concentrated to 1:1 (1 g herbs/ml) to get yellow brown suspension. The suspension was centrifuged for 5000 rev/min to obtain the precipitation (100 g) (JB-B). It was isolated by column chromatography on silica gel and gradient with CHCl<sub>3</sub>-MeOH (100:1 to 1:1) to give five fractions. Fraction 4 was subjected to silica gel column chromatography eluted with CHCl<sub>3</sub>-MeOH (10:1) to afford fraction. Fraction was isolated by column chromatography on SephadexLH-20 gel and gradient elution with CHCl<sub>3</sub>-MeOH (1:1) to give amentoflavone (3 g) (JB-A).

JB-A was obtained as a yellow amorphous powder in MeOH. It gave a positive Mg-HCl test revealing a flavonoid compound. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz) δ: 6.18 (1H, d, J = 2.0 Hz, H-6), 6.39 (1H, s, H-6''), 6.45 (1H, d, J = 2.0 Hz, H-8), 6.72 (2H, d, J = 8.7 Hz, H-3''', 5'''), 6.78 (1H, s, H-3''), 6.82 (1H, s, H-3), 7.15 (1H, d, J = 9.3 Hz, H-5'), 7.58 (2H, d, J=8.7 Hz, H-2''', 6'''), 7.99 (1H, d, J = 2.0 Hz, H-2'), 8.01 (1H, d, J = 2.0 Hz, 6.9 Hz, H-6'), 10.28 (1H, s, 7''-OH), 10.55 (2H, brs, 4'-OH and H-4''-OH), 10.82 (1H, s, 7-OH), 12.96 (1H, s, 5''-OH), 13.09 (1H, s, 5-OH). <sup>13</sup>C

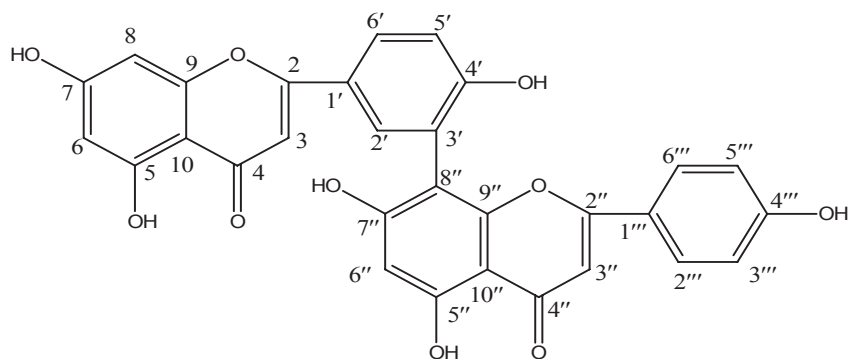


Fig. 1. The structure of amentoflavone (JB-A)

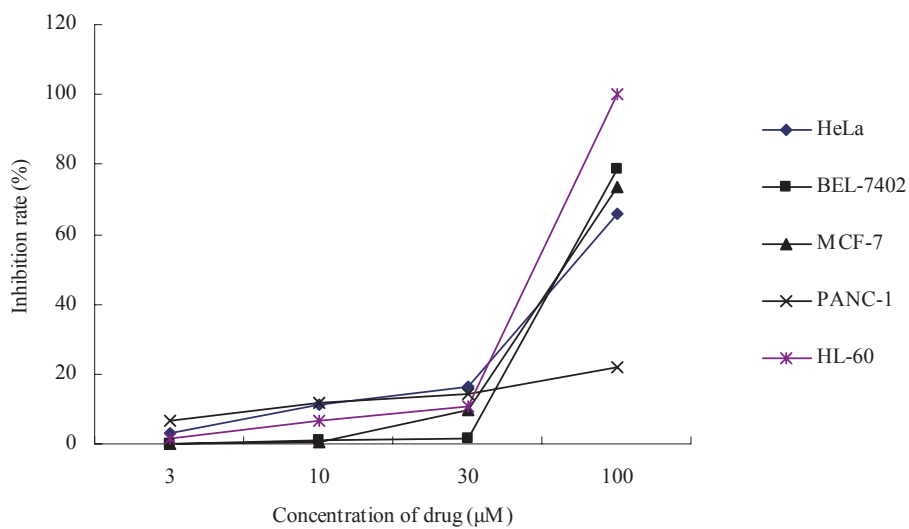


Fig. 2. Effect of JB-A on the inhibition of HeLa, BEL-7402, MCF-7, PANC-1, HL-60.

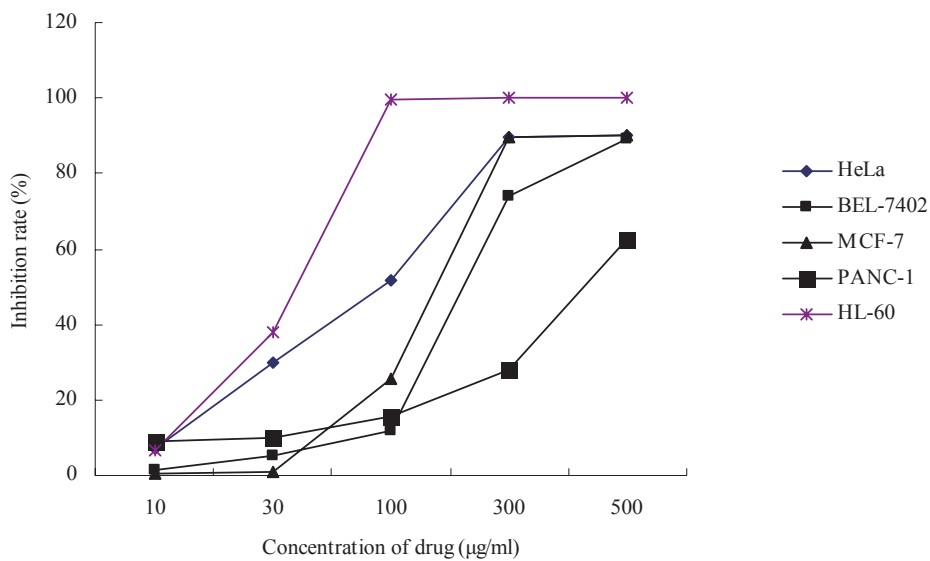


Fig. 3. Effect of JB-A on the inhibition of HeLa, BEL-7402, MCF-7, PANC-1, HL-60.



Table 1. The IC<sub>50</sub> of JB-A and JB-B against HeLa, BEL-7402, MCF-7, PANC-1, HL-60.

| IC <sub>50</sub> | HeLa  | BEL-7402 | MCF-7 | PANC-1 | HL-60 |
|------------------|-------|----------|-------|--------|-------|
| JB-A (μM)        | 76.83 | 72.45    | 67.71 | >100   | 46.97 |
| JB-B (μg/ml)     | 73.39 | 219.6    | 155.9 | 457.1  | 35.68 |

NMR (DMSO-*d*<sub>6</sub>) δ: 94.1 (C-8), 98.7 (C-6), 98.9 (C-6''), 102.7 (C-3''), 103.1 (C-3), 103.6 (C-8''), 103.8 (C-10), 103.8 (C-10''), 115.9 (C-3'''), 115.9 (C-5'''), 116.3 (C-5'), 120.1 (C-1'''), 121.1 (C-1'), 121.5 (C-3'), 127.9 (C-2'), 128.3 (C-2''), 128.3 (C-6'''), 131.5 (C-6'), 154.6 (C-9''), 159.6 (C-9), 160.6 (C-4'), 160.6 (C-5''), 161.1 (C-5), 161.6 (C-4'''), 161.9 (C-7''), 163.9 (C-7), 163.9 (C-2''), 164.2 (C-2), 181.8 (C-4), 182.2 (C-4''). All data were identical to those of amentoflavone<sup>[11]</sup>. The structure of the amentoflavone was shown in Fig. 1.

#### **The activity of JB-A, JB-B against HeLa, BEL-7402, MCF-7, PANC-1, HL-60**

JB-A and JB-B were assayed for their anticancer effect *in vitro*. The results revealed that JB-A and JB-B had no noticeable activity against tested cells PANC-1 and had weak activity against tested cells including HeLa, BEL-7402, MCF-7. But JB-A and JB-B showed conspicuous activity on inhibiting HL-60 cells. The inhibition of the expression of HL-60 cells were exhibited by JB-A and JB-B with 50 % inhibitory concentration (IC<sub>50</sub>) 46.97 μM and 35.68 μg/ml respectively (See Table 1, Fig. 1-2).

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#### **References**

[1] Chinese Pharmacopoeia Commission. Chinese

- pharmacopoeia, 2005, 1: 157.
- [2] Lee HS, Oh WK, Kim BY, Ahn SC, Kang DO, Shin DI, Kim J, Mheen TI, Ahn JS. Inhibition of phospholipase C gamma 1 activity by amentoflavone isolated from *Selaginella tamariscina*. *Planta Medica*, 1996, 62(4): 293.
- [3] Lin LC, Kuo YC, Chou CJ. Cytotoxic biflavonoids from *Selaginella delicatula*. *Journal of Natural Products*, 2000, 63(5): 627-630.
- [4] Lin YM, Flavin MT, Schure R, Chen FC, Sidwell R, Barnard DL, Huffman JH and Kern ER. Antiviral activities of biflavonoids. *Planta Medica*, 1999, 65(2): 120-125.
- [5] Kuo YC, Sun CM, Tsai WJ, Ou JC, Chen WP, Lin CY. Chinese herbs as modulators of human mesangial cell proliferation: Preliminary studies. *Journal of Laboratory and Clinical Medicine*, 1998, 132(1): 76-85.
- [6] Cholbi MR, Paya M, Alcaraz MJ. Inhibitory effects of phenolic compounds on CCl<sub>4</sub>-induced microsomal lipid peroxidation. *Experientia*, 1991, 47(2): 195-199.
- [7] Baureithel KH, Buter KB, Engesser A, Burkard W, Schaffner W. Inhibition of benzodiazepine binding *in vitro* by amentoflavone, a constituent of various species of *Hypericum*. *Pharmaceutica Acta Helveticae*, 1997, 72(3): 153-157.
- [8] Kim HK, Son KH, Chang HW, Kang SS, Kim HP. Amentoflavone, a plant biflavone: A new potential anti-inflammatory agent. *Archives of Pharmacal Research*, 1998, 21(4): 406-410.
- [9] Da Silva KL, Dos Santos AR, Mattos PE, Yunes RA, DelleMonache F, Cechinel-Filho V. Chemical composition and analgesic activity of *Calophyllum brasiliense* leaves. *Therapie*, 2001, 56(4): 431-434.
- [10] Markham KR, Franke A, Molloy BPJ, Webby RF. Flavonoid profiles of New Zealand *Libocedrus* and related genera. *Phytochemistry*, 1990, 29(2): 501-507.
- [11] Zhou Z, Zhang Y, Ding XR, Chen SH, Yang J, Wang XJ, Jia GL, Chen HS, Bo XC, Wang SQ. Protocatechuic aldehyde inhibits hepatitis B virus replication both *in vitro* and *in vivo*. *Antiviral Research*, 2007, 74(1): 59-64.