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**Original article**

# Pharmacodynamics of *julibroside* $J_8$ and $J_{12}$ in inhibiting angiogenesis

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***Background:*** *Ablizia julibrissin* could inhibit the proliferation of cancer cells *in vitro* and *in vivo*. However, the effects of the compositions *Julibroside* $J_8$ and $J_{12}$ on the angiogenesis are still poorly understood.
***Objective:*** We compared the pharmacodynamics of *julibroside* $J_8$ and $J_{12}$ in inhibiting angiogenesis in vitro and in vivo.
***Methods:*** *Julibroside* $J_8$ and $J_{12}$ were separated from the crude extract of *Ablizia julibrissin*. The effects of *julibroside* $J_8$ and *julibroside* $J_{12}$ on growth, migration, and matrigel tube formation of human microvascular endothelial cells (HMEC-1) were explored. In addition, the *in vivo* anti-angiogenic effect of *julibroside* $J_8$ and $J_{12}$ was evaluated on a chorioallantoic membrane (CAM).
***Results:*** HMEC-1 cells showed dose-dependent inhibition of growth, migration, and tube formation, when treated with 0.5-4 μg/ml *julibroside* $J_8$ or 0.1-0.5 μg/ml *julibroside* $J_{12}$ respectively. The formation of microvessels on CAM was also inhibited by *julibroside* $J_8$ or *julibroside* $J_{12}$ at concentrations of 10-100 $\mu g.egg^{-1}$ or 5-50 $\mu g.egg^{-1}$ respectively.
***Conclusion:*** *Julibroside* $J_{12}$ confers more potent inhibitory effect on angiogenesis than *julibroside* $J_8$ does.

***Keywords:*** Angiogenesis, chicken chorioallantoic membrane, human microvascular endothelial cells, *julibroside* $J_8$, *julibroside* $J_{12}$

Angiogenesis is a physiological process involving the growth of new blood vessels from pre-existing vessels. Under normal physiological conditions, angiogenesis only occurs in wound during repair and the ovaries and endometrium during the menstrual period. However, continuous and uncontrolled angiogenesis may be found in many diseases including cancers [1-4]. Angiogenesis is a prerequisite for cancer growth and metastasis. Anti-angiogenesis might be an acceptable strategy in suppressing cancer growth and metastasis. Specific inhibition of the proliferation of vascular endothelial cells and the angiogenesis in chicken chorioallantoic membrane (CAM) using angiogenesis inhibitor (AI) may inhibit cancer growth and metastasis. Screening the drugs for anti-angiogenesis has been a hot topic in the anti-neoplastic research. The majority of anti-angiogenic drugs have clinically irreversible side effects, such as bleeding/coagulation disorders, aggravated cardiovascular diseases, disturbance in normal follicular development and menstrual cycle in adult women, etc, which limit its clinical application [5]. In view of the mild side effects of natural medicines, researchers are engaging in identifying effective AIs among natural medicines.

*Ablizia julibrissin* has been widely used in clinical practice as a common traditional Chinese medicine. The Chinese Pharmacopoeia (2000) documents that *Ablizia julibrissin* is the dried stem bark, with the characteristics of tranquilizing, promoting blood circulation, and removing blood stasis. It has been used in the treatment of discomfort, depression, insomnia, lung abscess, and trauma, etc. Studies reported that *Ablizia julibrissin* could inhibit the proliferation of cancer cells *in vitro* and *in vivo* [6-8]. The chemical

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compositions of Albiziae is very complicated. The previous study separated a series of structurally complicated triterpenoid glycosides from *Ablizia julibrissin* by using chromatographic method [9]. *Julibroside* $J_8$ and $J_{12}$ are a kind of triterpenoid saponin containing nine glycons. Zheng et al have demonstrated *julibroside* $J_8$ and $J_{12}$ could exert suppressive effects on the proliferation of cancer cells, but their effects on the angiogenesis are still poorly understood [8]. In the present study, *Julibroside* $J_8$ and $J_{12}$ were isolated from *Ablizia julibrissin* and effects of *julibroside* $J_8$ and $J_{12}$ on angiogenesis were investigated *in vitro* and *in vivo*.

## Materials and methods

The dried stem bark of *Ablizia julibrissin* was collected from Zhejiang Province, China in September 2005, and was identified by Professor Jian-wei Chen, Chief of the Chinese Herbal Pharmacy in Nanjing University of Traditional Chinese Medicine. A voucher specimen (No. 20050909-1) was preserved in the Department of Natural Medicines, Jiangnan University. MCDB-131, epidermal growth factor (EGF), fetal bovine serum (FBS), RPMI-1640 and trypsin were purchased from Sigma (St. Louis, USA). HeLa, Bel-7402, MCF-7, B16F10, and C51 were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences. HMEC-1 and MRC-5 were purchased from the French National Institute for Health and Medicine Research. HMEC-1 cells were grown in MCDB-131 medium supplemented with 10% FBS, 2 mM L-glutamine, 10 ng/ml EGF and 1 μg/mL hydrocortisone. HeLa, Bel-7402, MCF-7, B16F10, C51 and MRC-5 cells were grown in RPMI 1640 containing 10% FBS. All cells were maintained at 37°C in humidified air with 5% $CO_2$.

### *Isolation and characterization of the anti-angiogenic component from Albizia julibrissin Durazz*

The powder of dried stem barks (15 kg) from *Albizia julibrissin* was extracted with 70% ethanol. The ethanol extract was evaporated, dissolved in water, and then sequentially extracted with chloroform, ethyl acetate, and *n*-butanol. The *n*-butanol fraction (214.5 g) with the most cytotoxicity was dissolved in water and fractionated through a Diaion HP-20 resin column with gradient elution (100% water'!100% ethanol). The fraction (65 g) eluted with 75% ethanol was then subjected to silica gel column chromatography using a solvent system of chloroform-methanol-water (9:1:0.1-6:4:1) to collect into 60 fractions (500 mL/fraction). Analysis of individual fractions was performed by thin layer chromatography (TLC) plate with a solvent system: the ratio of acetic acid to butanol to water was 4:1:5, and the separated spots on TLC were visualized under UV and 50% sulfuric acid. The fractions having the same pattern of separated spots on the TLC were collected together, and the cytotoxic activity of each fraction against HMEC-1 was determined by MTT assay. The active fraction (24.5 g) was applied with RP C18 column chromatography (eluted with 55 to 75% methanol), followed by preparative HPLC OBD-$C_{18}$ column chromatography (eluted with 65% methanol, 3.0 ml/min, 215 nm), which produced 45 mg of cytotoxic compound with the $IC_{50}$ of 1.2 μg/mL against HMEC-1. The purity of this cytotoxic compound was over 99%, as determined by HPLC. The chromatographic conditions were as follows: a Nova-pack C18 column (150 mm x 4.6 mm; I.D. 5 μm), mobile phase of methanol/water (65:35, v/v), flow rate of 1.0 ml/min, column temperature at 25°C, and the detection wavelength at 215 nm. The structure of the cytotoxic compound was identified by comparing the chemical and spectral data (m.p., UV, IR, MS, $^1$H- NMR and $^{13}$C-NMR) with those reported in the literature [8] and results showed *julibroside* $J_8$ and $J_{12}$ as shown in **Figure 1A** *Julibroside* $J_8$ and $J_{12}$ were dissolved in water and diluted to the desired concentrations immediately prior to use.

### *SRB test*

HMEC cells in the logarithmic growth phase were seeded in a 96-well plate at a density of 7-8 x $10^3$ cells/well, and then incubated at 37°C in humidified air supplemented with 50 ml/L $CO_2$ overnight. Different concentrations of *julibroside* $J_8$ or $J_{12}$ were independently added into each well, and the well without drug served as the negative control. Treatment was performed for different durations, and then the medium was removed, and fresh medium was added followed by incubation for 72 h. Then, the medium was removed, and fixation was performed with 100 μL of 100 g/L trichloroacetic acid in each well for five minutes, and then at 4°C for one hour. The fixation fluid was removed, and cells were washed with deionized water five times and air-dried. Then, treatment with 100 μL of 4 g/L SRB in each well was carried out at room temperature for 30 min. Cells were washed with 10 ml/L acetic acid solution 5 times. Thereafter, 150 μL

of 10 mmol/L Trisbase (pH 10.5) were added into each well. The absorbance (A) was measured at $A_{570}$ nm with a MK3 microplate reader. The inhibition rate (%) was calculated, and the curves were delineated based on the drug concentration and inhibition rate. The half-inhibitory concentration (IC50) was calculated. Inhibition rate = $[(A_{control} - A_{treatment}) / (A_{control} - A_{blank})] \times 100\%$.

### *Inhibitory effect of julibroside $J_8$ and $J_{12}$ on HMEC-1 cells*

*Julibroside $J_8$ and $J_{12}$* were diluted into a series of concentrations with cell medium. The final concentrations of *julibroside $J_{12}$* were 0.1, 0.2, 0.3, and 0.5 μg/mL, respectively and those of *julibroside $J_8$* were 0.5, 1.0, 1.5, 2.0 and 4.0 μg/mL, respectively. The *julibroside $J_8$* and *$J_{12}$* of different concentrations were added into each well followed by incubation for one to two days. Experiment was performed in quadruplicates for each concentration, and the blank control and negative control were also set. Each well contained 300 □L of medium, which was removed 6, 12, 24, and 48 h after culture. Fresh medium was added into each well for synchronized culture. The inhibitory rate (%) was detected with SRB test, and $IC_{50}$ was calculated.

### *Inhibitory effect of julibroside $J_8$ and $J_{12}$ on cancer cells*

*Julibroside $J_8$* and *$J_{12}$* were diluted into a series of concentrations with cell medium. The final concentrations of *julibroside $J_{12}$* were 0.1, 0.2, 0.3, and 0.5 μg/mL, respectively and those of *julibroside $J_8$* were 1.0, 5.0, 10.0, and 15.0 μg/mL, respectively. The *julibroside $J_8$* and *$J_{12}$* of different concentrations were added into each well followed by incubation for one to two days. Experiment was performed in quadruplicates for each concentration, and the blank control and negative control were also set. Each well contained 300 μL of medium, which was removed 72 h after culture. The inhibitory rate (%) was detected with SRB test, and $IC_{50}$ was calculated.

### *Cell migration assay*

The cell migration was tested as previously described [10]. Briefly, $1\times10^5$ cells were seeded in 24-well plates followed by incubation overnight. When the cells were adherent completely, the cells were then scraped away producing a wound; the width of the wound was about 250 to 270 μm. Then, the medium was refreshed with medium with or without *julibroside $J_8$* (1.2 μg/mL) or *$J_{12}$* (0.2 μg/mL). Cells were incubated for different durations (6 h, 12 h, and 24 h), and randomly selected fields were photographed at a magnification of 100× with a microscope video system (Olympus, IX70, Japan). The widths of each wound were measured at three distinct sites and then averaged.

### *Capillary tube formation assay*

The tube formation assay was performed to determine the effect of *julibroside $J_8$* and *$J_{12}$* on angiogenesis *in vitro*. The capillary tube-like structures of HMEC-1 were assessed in a matrigel angiogenesis model as previously described [11]. Briefly, a 96-well plate coated with 60 μL of matrigel per well was allowed to be dried at 37°C for 1 h. Each well was seeded with $4\times10^4$ HMEC-1 and maintained in MCDB containing various concentrations of *julibroside $J_{12}$* (0.05~0.5 μg/mL) or *$J_8$* (1~4 μg/mL) for 24 h. In addition, negative control group was also set. The enclosed networks of tubes were photographed from five randomly selected fields under a microscope (Olympus, IX70, Japan). The total length of the tubes in each photograph was measured using Adobe Photoshop software [12]. Inhibition of tube formation was calculated as $[1-(\text{tube length}_{treatment}/\text{tube length}_{control})] \times 100\%$.

### *Chicken chorioallantoic membrane (CAM) assay*

Anti-angiogenic activity was measured using CAM assay as previously described [13]. Fertilized, domestic chick embryos were incubated for four days and then windowed with slight modification. Briefly, a small hole (approximately 2 cm in diameter) was made by removing the shell and inner shell membrane from the air space site and then the exposed area was sealed with cellophane tape. The eggs were returned to the incubator at 37.8°C (humidity 55-60%) and incubated with the window upright for three days. On day 9, sterile filter paper disks saturated with *julibroside $J_8$* (10, 50, and 100 μg), *julibroside $J_{12}$* (5, 10, and 50 μg), or saline solution (control) were placed on the surface of the growing CAM vessels. After 48 h exposure, the CAMs were carefully isolated and were fixed in 95% alcohol. CAM vessels in 15 viable embryos of each treatment group were counted under a light microscope and the neovascular zones of CAM under the disks were photographed followed by analysis.

## Results

### *Structural formulas of julibroside $J_8$ and $J_{12}$*

The structural formulas of *julibroside* $J_8$ and $J_{12}$ were shown in **Figure 1**. Except for the group connected to the $R_2$ site, the structures of them were similar. *Julibroside* $J_8$ connects to (-OH), but *julibroside* $J_{12}$ connects to (-NHAC).

### *Effect of julibroside $J_8$ and $J_{12}$ on the proliferation of HMEC-1 cells*

After treatment with *julibroside* $J_8$ or $J_{12}$, the proliferation of HMEC-1 cells was analyzed with SRB test. The results showed that both of them had significantly inhibitory effects on the proliferation of HMEC cells. After treatment with *julibroside* $J_8$ or $J_{12}$ of different concentrations for 6 h, cell proliferation decreased, and the intracellular granules increased in a dose-dependent manner. Under the same concentration, cell growth was significantly inhibited by both *julibroside* $J_8$ and $J_{12}$ in a time-dependent manner. The results are shown in **Figure 2A** and **B**.

### *Effect of julibroside $J_8$ and $J_{12}$ on the proliferation of cancer cells and MRC-5 cells*

*Julibroside* $J_8$ and $J_{12}$ of different concentrations resulted in suppressive effects on the cancer cell growth in a dose-dependent manner. The suppressive effect of *julibroside* $J_{12}$ on the cell proliferation was more evident than that of *julibroside* $J_8$ under the same concentration. The $IC_{50}$ of *julibroside* $J_{12}$ in cancer cells was 0.5 μg/mL, while that of *julibroside* $J_8$ was 10 μg/mL. The differences between them were listed in **Figure 3A** and **B**. The effects of *julibroside* $J_8$ and $J_{12}$ on normal cells (MRC-5) were also listed in the **Figure 3**. Under 0~15.0 μg/mL, *julibroside* $J_8$ did not affect the proliferation of MRC-5, which suggested *julibroside* $J_8$ was not toxic to MRC-5 cells under 0~15.0 μg/mL. However, it could significantly inhibit the growth of cancer cells under these concentrations. Similarly, *julibroside* $J_{12}$ did not inhibit the proliferation of MRC-5 cells under 0~2.0 μg/mL. On the contrary, it could promote the proliferation of MRC-5 cells under certain concentrations.

### *Effect of julibroside $J_8$ and $J_{12}$ on the migration of HMEC-1 cells*

The migration of HMEC-1 cells is shown in **Figure 4**. Under the intervention of *julibroside* $J_8$ or $J_{12}$, the width of the wound in the negative control markedly decreased, which indicated a large number of migrated cells. However, the number of the migrated cells significantly reduced after *julibroside* $J_8$ or $J_{12}$ treatment. The results above suggested both *julibroside* $J_8$ and $J_{12}$ could inhibit the migration of endothelial cells. The characteristic was the same with the effect of the *Ablizia julibrissin* extract (data not shown). Therefore, these compounds may inhibit the angiogenesis through suppressing the migration of vascular endothelial cells.

### *Julibroside $J_8$ and $J_{12}$ inhibited the formation of matrigel tube in vitro*

The formation of HMEC-1 matrigel tube was shown in **Figure 5**. Under the intervention of *julibroside* $J_8$ and $J_{12}$, the formation of HMEC-1 matrigel tube was dramatically suppressed. For example, *julibroside* $J_8$ of 1 μg/mL could affect the formation of HMEC-1 tube, and the number of newly generated tubes decreased. When the concentration of *julibroside* $J_8$ was 2.0 μg/mL, HMEC-1 cells almost did not move after adhesion accompanied by absence of tube formation. When the concentration of *julibroside* $J_8$ was 3.0 μg/mL, the cell adherence was interrupted. After treatment with *julibroside* $J_8$, the lumen areas were significantly changed when compared with the control group. Similar results were also observed after *julibroside* $J_{12}$ treatment. The findings above suggested that *julibroside* $J_8$ and $J_{12}$ exhibited significantly inhibitory effect on the *in vitro* formation of tube.

### *Julibroside $J_8$ and $J_{12}$ inhibited the formation of CAM*

As shown in **Figure 6**, the CAM blood vessels were sparsely distributed in after treatment with *julibroside* $J_8$ *and* $J_{12}$, and an abnormal distribution of blood vessels could be identified at the administration site. The numbers of large, mediate, and small blood vessels significantly reduced (**Table 1**). However, in the control group, blood vessels of CAM showed dendritic and even distribution and abnormal blood vessels decreased or were absent, suggesting that *julibroside* $J_8$ and $J_{12}$ could significantly inhibit the angiogenesis on CAM in a dose-dependent manner.

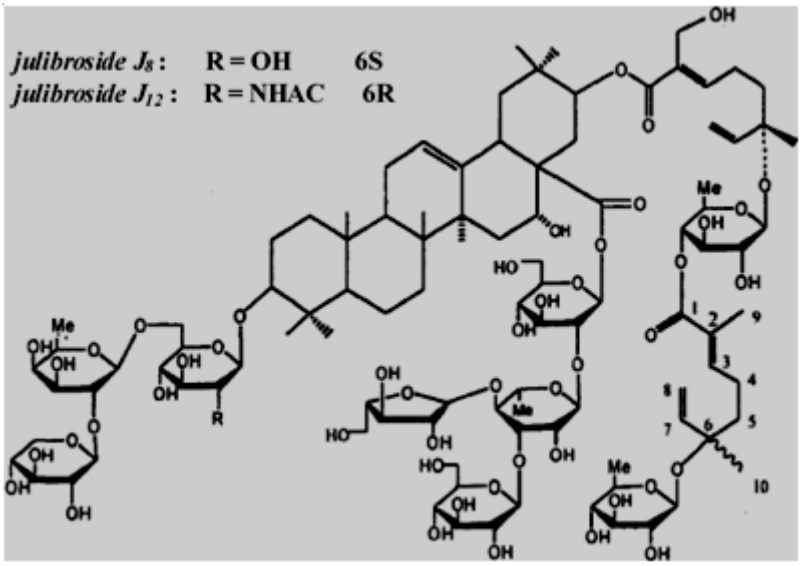


**Figure 1.** Chemical structures of *julibroside* $J_8$ and *julibroside* $J_{12}$

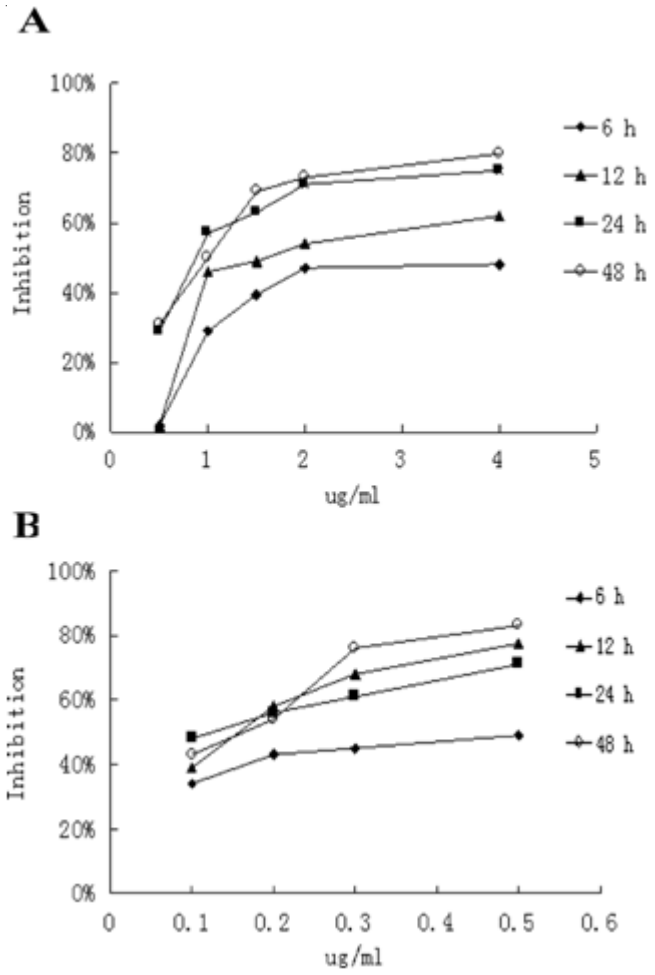


**Figure 2.** Suppressive effect of *julibroside* $J_8$ and *julibroside* $J_{12}$ on the growth of HMEC-1 cells. **A:** Effect of *julibroside* $J_8$ at 0.1, 0.2, 0.3 and 0.5 μg/ml for 6 h, 12 h, 24 h, 48 h on the cell proliferation. **B:** Effect of *julibroside* $J_{12}$ at 0.5, 1.0, 1.5, 2.0 and 4.0 μg/ml for 6 h, 12 h, 24 h and 48 h on the cell proliferation. The number of viable cells was detected using SRB test. Data were expressed as mean±SD and the experiments were repeated five times.

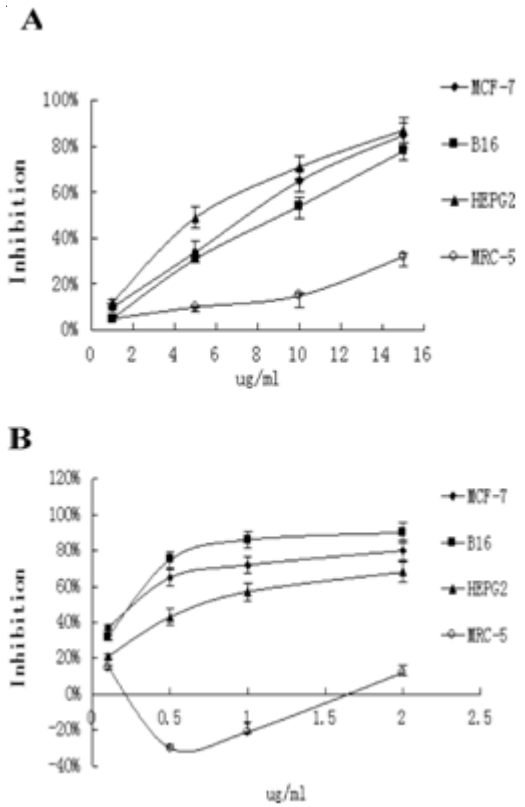


**Figure 3.** Suppressive effects of *julibroside* $J_8$ (**A**) and *julibroside* $J_{12}$ (**B**) on different cell lines. The number of viable cells was detected using SRB test. Data were expressed as mean±SD and the experiments were repeated five times.

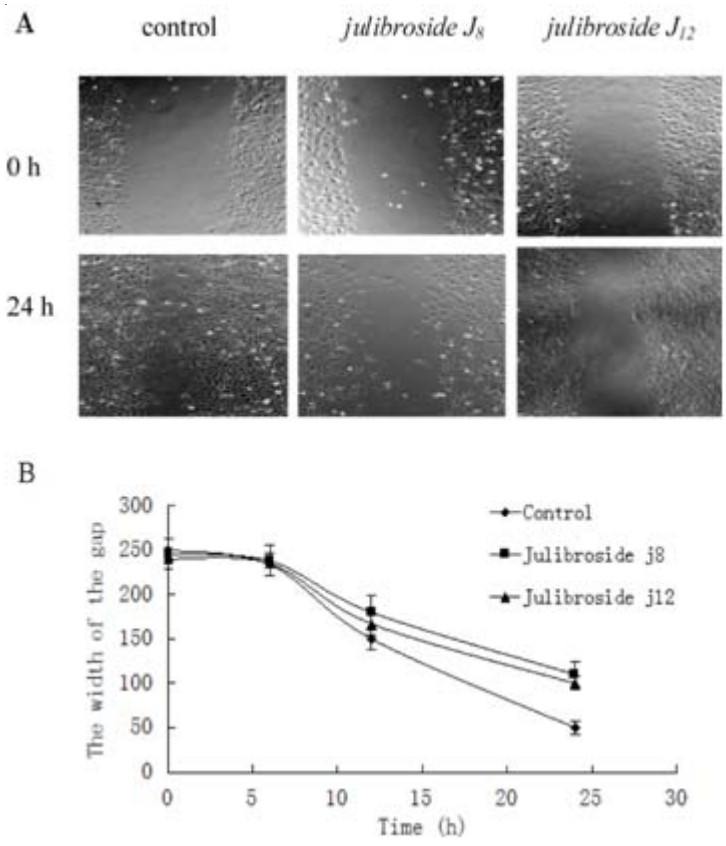


**Figure 4.** Effect of *julibroside* $J_8$ and *julibroside* $J_{12}$ on HMEC-1 cell migration. **A:** The migration of HMEC-1 cells after 24 h of *julibroside* $j_8$ (1.2 μg/ml) and *julibroside* $J_{12}$ (0.2 μg/ml) treatment. **B:** The width was determined at 3 distinct sites from 3 independent experiments. **P <0.01 vs. control at 6 h, 12 h, and 24 h (n=3 per group).

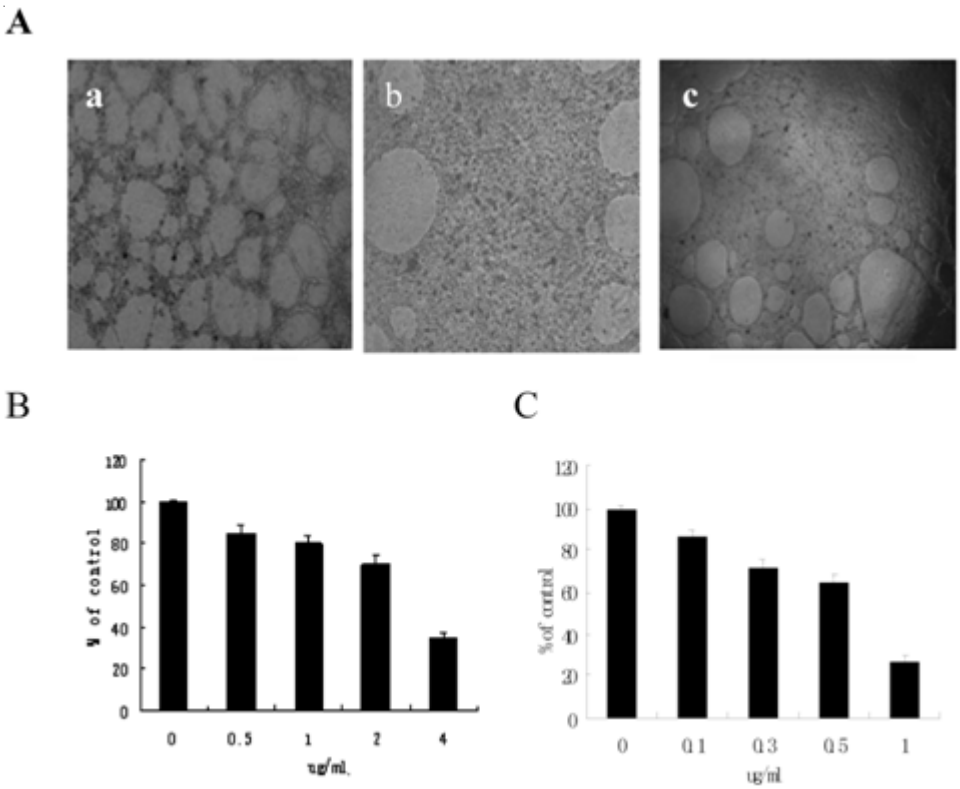


**Figure 5.** Effect of *julibroside* $J_8$ and *julibroside* $J_{12}$ on the matrix-induced tube formation. **A:** HMEC-1 cells were treated with *julibroside* $J_8$ or *julibroside* $J_{12}$ of different concentrations for 24 h. (a) control; (b) *julibroside* $J_8$; (c) *julibroside* $J_{12}$. **B:** Suppressive effects of *julibroside* $J_8$ on the tube formation of HMEC-1 cells was in a dose-dependent fashion. **C:** Suppressive effects of *julibroside* $J_{12}$ on the tube formation of HMEC-1 cells was in a dose-dependent fashion. Each treatment was repeated at least three times. *P <0.05. **P <0.01 vs control.

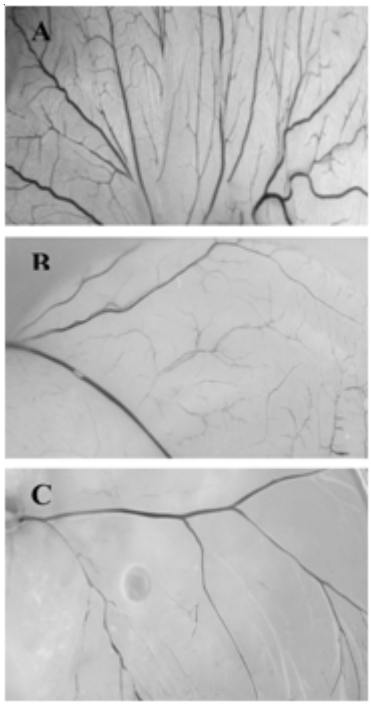


**Figure 6.** Inhibition of *julibroside* $J_8$ and *julibroside* $J_{12}$ on angiogenesis in the chicken chorioallantoic membrane (CAM) model. **A:** control, **B:** *julibroside* $J_8$, **C:** *julibroside* $J_{12}$

**Table 1**. Effects of *julibroside* $J_8$ and $J_{12}$ on neovascularization in CAM (.x±s, n =10)

| | Large vessel | Middle vessel | Small vessel |
|---|---|---|---|
| *julibroside* $J_8$ | | | |
| 0 μg/mL(control) | 2.8±0.20 | 4.5±0.14 | 21.8±1.19 |
| 10 μg/mL | 2.1±0.34 | 3.8±0.16 | 16.6±0.65 |
| 50 μg/mL | 2.6±0.14 | 2.8±0.13 | 9.9±0.30* |
| 100 μg/mL | 2.3±0.17 | 2.5±0.12 | 4.9±0.17** |
| *julibroside* $J_{12}$ | | | |
| 0 μg/mL(control) | 2.8±0.20 | 4.5±0.87 | 21.8±1.19 |
| 5 μg/mL | 2.5±0.46 | 3.7±0.56 | 17.6±0.57 |
| 10 μg/mL | 2.7±0.31 | 2.9±0.27 | 7.9±0.14 * |
| 50 μg/mL | 2.5±0.17 | 2.4±0.19 | 3.8±0.10 ** |

* $p$ <0.05, ** $p$ <0.01 *vs* Control

## Discussions

Applying natural medicines, including those with angiogenic properties, in the treatment of various diseases is a long-standing practice in China. Based on this, some natural medicines may be used for angiogenesis-related diseases. Our department has focused on examining plants widely used in traditional Chinese medicine for the identification of novel anti-angiogenic compounds, which may be candidates in the treatment of angiogenesis-related diseases. In our previous studies, *in vitro* and *in vivo* angiogenesis models have been established in order to screen potentially active compounds from traditional Chinese herbal medicines. Our previous results showed that *Albizia julibrissin* extract had significant anti-angiogenic effects *in vitro* and *in vivo*. Additionally, we also found that the extract of *Albizia julibrissin* had suppressive effect on cancer metastasis.

After confirming the efficacy of *Albizia julibrissin* extract, macroporous resin adsorption, silica gel and reversed-phase C18 column chromatography were used to isolate and purify the potential compounds from *Albizia julibrissin* with suppressive effects on HMEC-1 cell growth. It was found that the active component in *Albizia julibrissin* exhibiting suppressive effects on the proliferation of vascular endothelial cell was the saponins compound. The $IC_{50}$ of this compound in inhibiting HMEC-1 cell proliferation was 1.5 μg/mL (data not shown), and it could interfere with the migration and tube formation of HMEC-1 cells in a dose-dependent manner. Two active monomers A1 and D2 were identified through further isolating this active compound using the reversed-phase C18 column chromatography. Two monomers were white-crystal after being freeze-dried, and they were easy to dissolve in hot water and difficult to dissolve in organic solvents such as methanol and ether. Based on the results from mass spectrum, infrared spectra, and nuclear magnetic resonance of A1 and D2, the components were determined to be *julibroside* $J_8$ and $J_{12}$. Firstly, we compared the anti-angiogenic effects of *julibroside* $J_8$ with ginsenoside $Rg_3$. *Julibroside* $J_8$ significantly inhibited the angiogenesis *in vitro* and *in vivo* at relatively low concentrations and compared favorably with ginsenoside $Rg_3$[14].

In the present study, the effects of *julibroside* $J_8$ and $J_{12}$ on the angiogenesis were evaluated to verify whether the extracted compound from *Albizia julibrissin* had similar inhibitory effects on the angiogenesis. In addition, the the pharmacodynamics of both compounds was also compared. Results showed *julibroside* $J_8$ and $J_{12}$ had inhibitory effects on the proliferation, migration, and angiogenesis of endothelial cells to different extents, and the $IC_{50}$ was decreased from the original 30 μg/mL (*Albizia julibrissin*) to 1.2 μg/mL (*julibroside* $J_8$) and 0.2 μg/mL (*julibroside* $J_{12}$). Furthermore, we found that the growth of endothelial cells was inhibited after *julibroside* $J_8$ and $J_{12}$ treatment for six hours, suggesting that the inhibitory effects of *julibroside* $J_8$ and $J_{12}$ on endothelial cells did not result from the changes in growth environment.

Although there are few differences in the structure between *julibroside* $J_8$ and $J_{12}$, significant differences in pharmacodynamics were noted between both compounds. Firstly, the effects of *julibroside* $J_{12}$ on the proliferation, migration, and angiogenesis of endothelial cells were stronger than those of *julibroside* $J_8$. Secondly, the inhibitory effect

of *julibroside* $J_{12}$ on cancer cells was significantly stronger than *julibroside* $J_8$. When normal human embryonic lung fibroblast MRC-5 cells were treated with *julibroside* $J_8$ or $J_{12}$ at the same concentration, *julibroside* $J_8$ did not show the toxicity, while *julibroside* $J_{12}$ promoted the proliferation of MRC-5 cells at certain concentrations. Those findings suggested the acetylamino sugars in the third site and the cis-trans isomerization in the sixth site of the monoterpenoid acid played important roles in the biological activity of these compounds. The relationship between their structure and their therapeutic effects still need further study.

As reported previously [15], there are about forty kinds of saponins in *Albizia julibrissin* extract, and their structures are similar. Further studies are needed to clarify whether these saponins also affect the angiogenesis.

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The authors have no conflict of interest to report. Hui Hua, Xiaoping Zhang, and Dan Li contributed equally to this article.

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