**Molecular mechanisms of a traditional Chinese medicine in the treatment of dysfunctional uterine bleeding**

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**Experiments**

**1. Quality inspection of three types of medicinal materials**

**1.1 Pretreatment of medicinal materials**

*Saururus chinensis*, *Celosia cristata* and *Spatholobus suberectus* were purchased from Hunan Gaoqiao Market (Changsha, China). Each medicinal material is made into coarse powder by ordinary pulverizer, and then pulverized by ultrafine pulverizer (300 g medicinal material, 25 min / time, 0~15°C) after the oven-dried (60°C, 12 h). The obtained medicinal ultrafine pieces are stored in a ventilated and dry place for future use.

**1.2 Identification of medicinal materials by TLC**

***Saururus chinensis*:**After solution preparation, the test solution (10 µL), control medicinal solution (10 µL), and the reference solution (5 µL) were respectively added into the silica gel plate. Then placed it in 10 mL petroleum ether ( 60～90°C) -acetone (5:2) chromatographic cylinder. After it dried, sprayed 10% sulfuric acid ethanol coloring solution and put it in the oven at 105 °C until the spots appear clearly.

***Celosia cristata*:** After solution preparation, pipette 10 µL each of the test solution and control medicinal solution into the silica gel plate. Then placed it in a 10 mL cyclohexane-acetone (5:1) chromatographic tank. After 5% vanillin sulfuric acid coloring solution, put it in 105 °C oven until spots appear clearly.

***Spatholobus suberectus*:** After solution preparation, the test solution (10 µL) and reference solution (5 µL) were respectively added into the silica gel plate, and then put it in a 10 mL chloroform-methanol (20:1) chromatography tank to unfold. photographed at 254 nm and recorded at an UV gel imaging system.

The results of TLC images of three herbs see additional file 6: Figure S3.

**1.3 Identification of medicinal materials by HPLC**

According to the *Chinese Pharmacopoeia 2010*, saccharone is the index component to identify the quality of *S. chinensis*, and formononetin is the index component to identify the quality of *S. suberectus.*

***S. chinensis*:**Weigh 0.5 g of *S. chinensis* in 25 mL methanol, and let stand for 30 min. Ultrasound (500 W, 25 kHz) was conducted for 40 min, followed by methanol to make up the reduced weight. The control sample sauchinone was taken and made into a 40 ug/mL solution with methanol. The samples were then detected by liquid chromatography. The results of HPLC images of *S. chinensis* see additional file 7: Figure S4.

***S. suberectus*:** The previous treatment was consistent with *S. chinensis*. The control sample formononetin was taken and made into a 40 ug/mL solution with methanol. The samples were then detected by liquid chromatography. The results of HPLC images of *S. suberectus* see additional file 8: Figure S5.

**2. Preparation of XBR solution**

According to the conversion reaction (Table S1), the herbs were added 1 L of 100% ethanol and boiled for 30 min. After cooling, filtered the residue and added 1 L of distilled water boiling for 30 min, and then removed the residue again. After the double frying, the alcohol extract filtrate was dissolved and placed in a vacuum freeze dryer. Finally, the powder was stored in a dry state. Before use, dissolve the powder in proportion with saline and set aside at 4℃.

**3. Scanning electron microscopy of endometrium in rats**

Endometrial samples were fixed with 2% pentanediol and 1% osmic acid, followed by dehydration with a gradient of ethanol. The samples were then embedded, and semi-thin sections and ultra-thin sections were yielded by the microtome. The images were photographed under SEM (model Carl Zeiss - EVO-40) with accelerated voltage of 10 kV at a magnification of ×2000.

**4. Organ histologic sections of rats**

The clean organ samples were immersed in neutral formalin for one week, and then dehydrated with a gradient of ethanol. Then, the xylene solution was used for transparent treatment, and then the samples were embedded with paraffin. Paraffin sections were sectioned at 5 μm thickness and mounted on superfrost plus slides. Every tenth section was stained with hematoxylin and eosin and examined by light microscopy.

**5. Detection of TIMP-1 mRNA and MMP-1 mRNA expression in rat endometrium**

The ovaries and uterus were immediately taken out of the rats after dissection, and 1 mL of Trizol was added to the pipette. Add 0.2 times volume of chloroform and let stand at room temperature for 3~5 min. After gradient centrifugation, the concentration of substrate was measured using an UV spectrophotometer. The mRNA was then used as a template to produce cDNA by reverse transcription. The primer sequence design of qRT-PCR is shown in Table S2