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Comparative study on hemostatic, cytotoxic and hemolytic activities of different species of *Paris* L.

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ABSTRACT

Ethnopharmacological relevance: The rhizoma of *Paris polyphylla* var. *yannanensis* or *P. polyphylla* var. *chinensis* called *Rhizoma Paridis* as a traditional Chinese medicine has an effect of heat-clearing and detoxicating, detumescence and acesodyne in folk for a long time. The increasing application of *Rhizoma Paridis* resulted in the shortage of wildlife resources. Here, we compared the major activities of other species of genus *Paris* to find the replacement plants.

Materials and methods: Six species (*P. polyphylla* var. *yunnanensis*, *P. delavayi* var. *delavayi*, *P. fargesii* var. *Fargesii*, *P. bashanensis* Wang et Tang, *P. polyphyllar* var. *minora*, and *P. polyphylla* var. *pseudothibetical*) were collected from three Provinces in China, and compared the hemostatic, cytotoxic and hemolytic activities by different assays.

Results: For the hemostatic activity, all the plants except *Paris fargesii* var. *Fargesii* could significantly shorten the tail bleeding time and blood clotting time ($P < 0.05$). For further mechanism study, they reduced the prothrombin time (PT) and activated partial thromboplastin time (APTT), but they had no significant effect on thrombin time (TT). *P. fargesii* var. *Fargesii* showed the similar cytotoxicity to *P. polyphylla* var. *yunnanensis* (IC_{50}: 18.21 and 15.73 μg/mL, respectively). HD_{50} was used as the index of hemolytic activity. *P. delavayi* var. *delavayi* and *P. bashanensis* Wang et Tang were the last to have this activity as the values were 3.027 and 1.222 mg/mL.

Conclusions: The different species of genus *Paris* have different activities. *Paris delavayi* var. *delavayi* and *Paris bashanensis* Wang et Tang could be used as the resources of hemostatic drugs and *P. fargesii* var. *Fargesii* as the antitumor medicine.

1. Introduction

Paris L. belonging to the family of Liliaceae grows primarily in tropical to temperate regions of Eurasia, and has 24 species in the earth, of which 19 distribute in China (Li, 1998). The rhizome of *Paris polyphylla* var. *yannanensis* and *P. polyphylla* var. *chinensis* called *Rhizoma Paridis* has been used as a traditional Chinese folk medicine for a long time. It was recorded in "Shengnong Herb" and Li Shizhen's "Compendium of Materia" and now in Chinese Pharmacopoeia (Committee of National Pharmacopoeia, 2005, 2010). *Rhizoma Paridis* frequently plays an important role in clinics for treating of snake bite, fractures, parotitis, tumors, analgesia and traumatic bleeding. With the development of research, a lot of saponins have been isolated and identified from *Rhizoma Paridis* (Zhang et al., 2010), and they act as the major effective constituents for significantly biological activities. According to the findings of therapy of traumatic bleeding, a drug for treatment of abnormal uterine bleeding called Gongxuening Capsule was developed from the extract of *Rhizoma Paridis* in China market (Zhao and Shi, 2005; Guo et al., 2008). While *Rhizoma Paridis* is one of the major components of the well-known prepared Chinese medicines like Yunnan Baiyao Powder which is famous for the hemostatic activity and Jidesheng Sheyao Tablet which is used to treat of snake bite. In our laboratory, we have reported many papers about the chemical components and anti-tumor activity of *Rhizoma Paridis* (Yan et al., 2009; Man et al., 2009a, 2009b, 2010, 2011a, 2011b). Now the endangered status is more and more severe with the increasing application of *Rhizoma Paridis*. So, it is extremely urgent to search for alternative resources or new resources for *Rhizoma Paridis*. Expanding the sources of *Rhizoma Paridis* from other species of *Paris* L. is a useful method.

Abbreviations: PY, *P. polyphylla* var. *yunnanensis*; PD, *P. delavayi* var. *Delavayi*; PF, *P. fargesii* var. *fargesii*; PB, *P. bashanensis* Wang et Tang; PM, *P. polyphyllar* var. *minora*; PP, *P. polyphylla* var. *pseudothibetical*; PT, prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time

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Other species of the genus *Paris* are also using in folk (Traditional Chinese Medicine Dictionary group, 1996) and a lot of saponins have also been isolated and identified from them (Li, 1998), such as *P. polyphylla* var. *pubescens* (Huang et al., 2009a), *Paris verticillata* M. Beib. (Huang et al., 2009b), *Paris axialis* H. Li (Huang et al., 2010a), *P. polyphylla* var. *pseudothibetical* (Zhao et al., 2011a), and *Paris fargesii* Franch (Zhao et al., 2011b). However, not all the species of genus *Paris* have the same biological activities, while some species possessed antitumor activities, others are considered to have hemostatic activities. Six species of *Paris* L. were collected from different Provinces of China that are *Paris delavayi* var. *delavayi* (PD), *P. fargesii* var. *Fargesii* (PF), *Paris bashanensis* Wang et Tang (PB), *P. polyphyllar* var. *minora* (PM), and *P. polyphylla* var. *pseudothibetical* (PP). Therefore, this study was conducted to investigate and compare of them for hemostatic, cytotoxic and hemolytic activities in order to provide a basis for the application in folk use and founding the substitute resources of *Rhizoma Paridis*.

2. Materials and methods

2.1. Plant material and preparation

Different species of *Paris* L. were collected from 5 Provinces of China (Table 1). They had been identified by Dr. Gao, and voucher specimens were deposited at the School of Pharmaceutical Science and Technology in Tianjin University.

Every kind of dried roots was powdered to a homogeneous size by a mill, sieved through a No. 40 mesh, and further dried at 40 °C in the oven for 2 h. The powder samples accurately weighed (10.0 g) were added to a round-bottomed flask containing 40 mL of 70% ethanol and the mixture was heated under reflux for 3 times, 2 h for each time. The ethanol solution was filtered and evaporated with a rotary evaporator (Shenzhen Co., Shanghai, China), and then made up to powder in vacuum drying oven (Tanya Co., Tianjin, China).

2.2. Animals and cells

Kunming mice, weighing about 18–20 g, of SPF degree, were purchased from Tianjin Experimental Animal Center (License No. SCXK (Jin) 2009-0002) and involved in this trial. New Zealand White rabbits (2.0–2.5 kg) were obtained from Laboratory Animal Center of Health Science, Peking University, Beijing, China. The animals were given free access to food and drinking water, under controlled temperature, humidity and photoperiod. Mice were allowed to be acclimated for one week. This animal study was approved by the Institutional Animal Care and Use Committee of China, and institutional guidelines for animal welfare and experimental conduct were followed.

Mouse B16 melanoma cell line (B16) obtained from Peking Union Medical College (Beijing, China), was maintained in RPMI-1640 Medium (Gibson, BRL) supplemented with 10% heat-inactivated (56 °C, 30 min) fetal calf serum (FCS) (Gibson, BRL), penicillin (100 U/mL) and streptomycin (100 μg/mL). The cell culture was maintained at 37 °C in a humidified atmosphere of 5% CO_2. Cells were passaged each 3–4 day.

Table 1
Plant materials used in this study.

No.	Plant name	Voucher specimen	Growing area
1	*P. polyphylla* var. *yunnanensis*	PY201110	Gansu
2	*P. delavayi* var. *delavayi*	PD201110	Chongqing
3	*P. fargesii* var. *fargesii*	PF201110	Chongqing
4	*P. bashanensis* Wang et Tang	PB201110	Chongqing
5	*P. polyphyllar* var. *minora*	PM201110	Chongqing
6	*P. polyphylla* var. *pseudothibetical*	PP201110	Guizhou

2.3. Drugs and drug administration

Yunnan Baiyao Powder was produced by Baiyao Group Co., Ltd. (Yunnan, China). Activated partial thromboplastin time (APTT) reagent, prothrombin time (PT) reagent, and thrombin time (TT) reagent were purchased from Shanghai Sun Biotechnology Co., Ltd. (Shanghai, China).

All the drugs were dissolved in water for oral administration in mice.

2.4. Tail bleeding times

Forty minutes after the final oral administration of the extracts (total of 5 times, once per day), the tail transection bleeding time was determined according to the method of Kim and Lee, 2006 with slightly modification. The mouse tail was transected at 5 mm from the tip and then the tail lesion was blotted with a filter paper every 30 s. The interval, from the time of the tail incision until the time that blood is no longer apparently transferred to the filter paper, was recorded as the bleeding time. Bleeding time beyond 600 s was considered as cut off time for the purpose of statistical analysis.

2.5. Blood clotting assays

Forty minutes after the final oral administration of the extracts (total of 5 times, once per day), the blood clotting assays were determined by two methods. One method was capillary method. The mouse blood samples (0.5 mL) were collected from the eye socket using a glass capillary, and the time interval required for blood coagulation inside the capillary was measured (Wang et al., 2012).

In addition, the mouse blood samples (collected before) were spread onto the glass slides (slide method). Briefly, two drips of blood was dripped on the two sides of a clean glass slide (one drip was used for repeat test), starting a stopwatch immediately. At intervals of 30 s, the blood drip was gently teased with a clean 9# needle from boundary to middle, to check for blood clot formation. This was continued until the fibrin filament could be observed, and the stopwatch was immediately stopped and the blood clotting time was recorded (Li et al., 2010). The experiments of all the tests were controlled at 27 °C with a humidity of 60%.

2.6. In vitro anticoagulation assays

Measurements of plasma activated partial APTT, PT, and TT were performed as the recorded method in the APTT kit, PT kit, and TT kit (Shanghai sun biotechnology Co., Shanghai, China). 40 min after the final oral administration of the extracts (total of 5 times, once per day), blood were collected from inner canthus and added 3.8% sodium citrate (1:9, v/v). The mixture were centrifuged at 3000 rpm for 10 min and obtained the mice plasma. For PT assay, 0.1 mL citrated mice plasma was incubated for 3 min at 37 °C. Clotting was initiated by addition of 0.2 Ml pre-warming PT reagent. Similarly, the TT was measured by addition of 0.2 mL thrombin reagent to a mixture of mice plasma 0.2 mL. The APTT was measured by incubating mice plasma (0.1 mL) with the APT reagent (0.2 mL) for 5 min at 37 °C. Clotting was initiated by adding 25 mmol/L $CaCl_2$ (37 °C, 0.1 mL). Clotting times are based on the average of three separate determinations. At intervals of 5 s, the solutions were slightly shaken. This was continued until the solution immovability could be observed, and the stopwatch was immediately stopped and the PT, TT, and APTT were recorded.