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Comparison of the chemical profiles and anti-platelet aggregation effects of two "Dragon's Blood" drugs used in traditional Chinese medicine

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ABSTRACT

Ethnopharmacological relevance: "Dragon's Blood" has been used as a medicine since ancient times by many cultures. In traditional Chinese medicine, the resin obtained from *Daemonorops draco* (RDD) and the resin from *Dracaena cochinchinensis* (RDC) are equally prescribed as "Dragon's Blood" for facilitating blood circulation.

Aim of the study: To verify the traditional efficacy and elucidate the mechanism, the present study compared the chemical profiles and the pharmacological effects of two species of "Dragon's Blood" mainly used in China.

Materials and methods: A UPLC-MS fingerprinting method was developed to compare the chemical profiles of the two medicines. The anti-platelet aggregation effects of the two medicines induced by arachidonic acid (AA) were investigated.

Results: The chemical profiles of these two species of "Dragon's Blood" were significantly different. The characteristic constituents were found to be: flavanes in RDD and stilbenes in RDC. In the *in vivo* platelet inhibition test, performed with the dose of 200 mg/kg on rats, the peak inhibitory effects of RDD and RDC were 35.8% and 27.6%, respectively, compared with the control group. With the *in vitro* concentrations of 0.2, 0.4 and 0.8 mg/ml, RDD exerted significant inhibition of aggregation by 18.7%, 20.0%, and 61.6%, respectively, and RDC exerted significant inhibition of aggregation by 13.3%, 20.2%, and 31.6%, respectively.

Conclusion: The fingerprinting method used here is suitable for distinguishing them. All pharmacological tests indicated that RDD was more potent than RDC against platelet aggregation.

1. Introduction

"Dragon's Blood" is a deep red resin obtained from species of *Dracaena* (Dracaenaceae), *Daemonorops* (Palmaceae), *Croton* (Euphorbiaceae) and *Pterocarpus* genera (Fabaceae) (Pearson and Prendergast, 2001). It has been used as a famous ethnomedicine since ancient times by many cultures (Gupta et al., 2008). Having a reputation for facilitating blood circulation and dispersing blood stasis, in traditional Chinese medicine, this resinous medicine is commonly prescribed to invigorate blood circulation for the treatment of traumatic injuries, blood stasis and pain (Commission of Chinese Materia Medica, 1999; Chinese Pharmacopoeia Commission, 2010).

The historical uses of "Dragon's Blood" can be traced back to ancient Greece and ancient Arabia (Angiosperm Phylogeny Group, 1974). In A.D. 77–78, "Dragon's Blood" was firstly listed in *De Materia Medica* by the Greek doctor Dioscorides (A.D. 40–90); it is believed that the botanical source of the drug at that time was several species of the *Dracaena* genus, such as *Dracaena draco* and *Dracaena cinnabari*, distributed in the Soktra Island of Yemen (Milburn, 1984; Mabberley, 1998). Later, "Dragon's Blood" was not only very famous in Europe, but also in China, reaching the Far East via the "Silk Road" during the Sui and Tang dynasties (A.D. 581–907). With the development of maritime trade between China and Southeast Asia from Ming dynasty (A.D. 1368–1644), the resin secreted from the fruit of *Daemonorops draco* (Willd.) Blume, a plant indigenous to Indonesia and Malaysia, was shipped to China and used as "Dragon's Blood" (Xie, 1989). Due to the higher price of resin from *Daemonorops draco*, the search for alternative sources has been ongoing. Until 1972, a new plant source of "Dragon's Blood", *Dracaena cochinchinensis* (Lour.) S.C. Chen, was found in Yunnan province of China. Since then, the resin extracted from stems of *Dracaena cochinchinensis* with ethanol has been used as "Dragon's Blood" (Cai and Xu, 1979). Subsequently, *D. cambodiana* Pierre ex Gagnep., another species of the *Dracaena* genus distributed in Hainan province of China, was also studied for obtaining "Dragon's Blood"; however, rarity blocked industrial-scale production (Zheng et al., 2003). In summary, two species are currently the primary

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Fig. 1. Photos of the resin obtained from *Daemonorops draco* (RDD, the upper row) and the resin from *Dracaena cochinchinensis* (RDC, the lower row).

sources for the widely used ethnomedicine "Dragon's Blood" in China; these are the resin obtained from *Daemonorops draco* (RDD) and the resin from *Dracaena cochinchinensis* (RDC) (Fig. 1).

There are two problems in the current use of "Dragon's Blood" resins from these two species: (1) distinguishing one from the other; and (2) determining whether they are in fact equally effective in clinical use. Distinguishing the two is important because, while the resins derived from the two look similar, they differ significantly in price. RDD is much more expensive. Hence, there are many attempts to make RDC appear to be RDD, and sell it at higher prices (Chen, 2005). Unfortunately, attempts to distinguish between the two medicines using empirical methods have met with little success (Wang, 2005; Ren et al., 2006), and the identification carried out by spectrophotometry and thin layer chromatography cannot provide the exact information of characteristic compounds (Song and Hu, 2009). Recently, a HPLC method based on flavylium chromophores as species markers has been reported to identify three species of "Dragon's Blood" commonly traded in Europe; however, RDC was not one of the research objectives (Sousa et al., 2008), and RDC may be unsuitable for this method due to absence of flavylium chromophores (Gupta et al., 2008). To solve this problem, it is desirable to develop a novel method based on chemical identification to distinguish the two resinous medicines used in China. At the same time, we do not actually know whether the two species are equally effective as drugs. Comparisons of their pharmacological potencies based on the clinical indications are needed. Laboratory studies suggest that "Dragon's Blood" species exert their clinical effects by inhibiting blood platelet aggregation (Commission of Chinese Materia Medica, 1999; Lu et al., 2003); thus measuring anti-platelet aggregation is an accepted test for evaluating their clinical effects (Jackson, 2007).

Aware of these two fundamental problems, in recent years, our research group has focused on the research on "Dragon's Blood". In our previous study, we reported the microscopic features and major constituents of *Dracaena* plants, one genus of the original plants for obtaining "Dragon's Blood" (Fan et al., 2008, 2009). Thus, in the present follow-up study, we further differentiated two "Dragon's Blood" medicines using chemical fingerprinting method, and compared their inhibitory effects on rat platelet aggregation induced by arachidonic acid. The results revealed that the developed protocol could unambiguously authenticate the two medicines, and that the characteristic constituents are flavanes in RDD and stilbenes in RDC. Anti-platelet aggregation tests showed that the inhibitory effects of RDD were more potent than those of RDC. These results suggest that the two drugs should be distinguished when sold and used.

2. Materials and methods

2.1. Materials

The sources of the RDD and RDC samples are listed in Tables 2 and 3. Identity of these samples was confirmed by Dr. Hu-Biao Chen, and voucher specimens were deposited in the School of Chinese, Hong Kong Baptist University (JK-01 for RDD and GC-05 for RDC).

2.2. Chemicals and reagents

The standard compounds of loureirin A, loureirin B and resveratrol were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Other standard compounds were isolated by our laboratory with a purity of more than 98%, and their chemical structures were elucidated by comparing with literature data of ^{1}H and ^{13}C NMR (Tsai, 1993; Mu et al., 1999; Tu et al., 2003; Shen et al., 2007). Their chemical structures are shown in Fig. 2. Acetonitrile and methanol of chromatography grade were purchased from Lab-scan (Bangkok, Thailand). Formic acid and ethanol of analytical grade were purchased from Merck (Darmstadt, Germany).

2.3. Sample extraction

The sample powder (0.1 kg) was extracted with 95% ethanol by means of sonication at room temperature for 30 min. The operations were repeated until the extract became colorless.