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Direct HPLC detection of benzodilactones and quinones in glands of *Lysimachia fordiana*

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ABSTRACT

The gland cells were successfully collected from the stems of *Lysimachia fordiana* Oliver, and the homologous pigments of fordianin A, fordianin B, fordianaquinone A and fordianaquinone B were firstly detected in the glands by HPLC. This indicated that the stem was an ideal material for the preparation of the glands, and the gland was a center for the polycyclic pigments accumulation in this species.

1. Introduction

Plant secondary metabolites, including volatile essential oils and non-volatile alkaloids, quinones, lactones, etc., played an important role in the life of human beings, thus the localization of sites of their biosynthesis and accumulation was of interest for researchers. The volatile essential oils, valued for its medicinal or fragrant properties, can be used in pharmacy, perfume and cosmetics industries. It has been verified that glandular structures were the primary sites of the secretion and storage of essential oils in certain plant species [1–10]. The analytical methods for the qualitative analyses of the essential oils in glandular structures mainly included fluorescence microscopy and histochemical stain which is based on the specific reactions between reagents and the functional groups of chemical compounds in oils [1–9], while the identification of individual chemical compounds in oils was usually performed by gas chromatography–mass spectrometry [10]. Some non-volatile phytoconstituents were also correlated to glands, for example, the antitumor agent camptothecin was proved to be primarily accumulated in secretory canals and glandular trichomes of leaves and stems of *Camptotheca acuminata* (Nyssaceae) by fluorescence microscopy and high performance liquid chromatography (HPLC) analyses [11]; the antidepressant and antiviral agents of hypericin and pseudohypericin were confirmed to exist in glandular structures in the species of Myrsinaceae by histochemical stain, HPLC [12–14], electrospray ionization mass spectrometry [15], light microscopy, scanning electron microscopy and transmission electron microscopy [16,17]. The evidences as the above-mentioned showed that the gland is a kind of center for some secondary metabolites accumulation. Although many plants have been utilized as materials to study the relationship of the gland and its internal phytoconstituents, the direct selective separation of gland cells under natural conditions is even hard to be achieved, because these targets are very small or dense in fresh plant materials. In order to conveniently study the biosynthesis in gland, it is very essential to develop a technical procedure for gland isolation, or to find ideal materials for this purpose.

Lysimachia Linnaeus, with about 191 species of perennial and annual herbs, is traditionally included in the family

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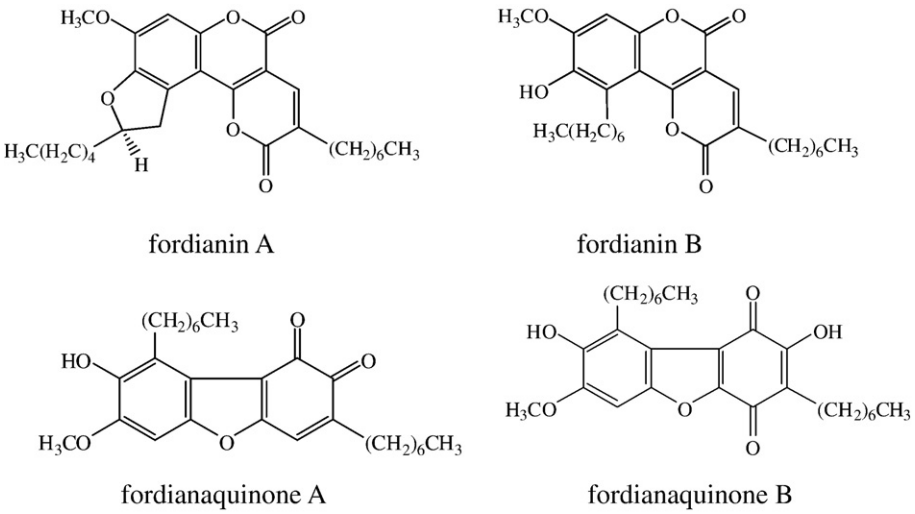

Fig. 1. The chemical structures of the homologous pigments in *L. fordiana*.

Primulaceae but now in Myrsinaceae [18]. The gland, a distinctive feature founded in Myrsinaceae, also occurs in several species of *Lysimachia* L., especially in the leaves, e.g., *Lysimachia fordiana* Oliver [19]. *L. fordiana* is a perennial herb, which is commonly used in folk medicine for the treatment of wounds, scrofula and subcutaneous ulcer in China [20]. Its stems, erect, fleshy, usually simple, are peppered with scattered distinct black gland spots, either on their surfaces or within them, while its leaf blades, bracts and calyx lobes are covered with densely minutely black gland dots [21]. In our previous work, four structurally homologous pigments, i.e., benzodilactones of fordianin A and fordianin B, quinones of fordianaquinone A and fordianaquinone B (Fig. 1) have been isolated and structurally identified from its leaves [22]. Our recent work showed these pigments have antifungal activity, and fordianaquinone B can strongly inhibit the bioactivity of DNA topoisomerase I. However, the contents of these pigments are low in the plant and the plant is rarely distributed, which makes it difficult to collect enough amounts of samples for *in vivo* experiments. The study on the biosynthesis site of these pigments may be helpful for related research in future.

In this study, we firstly attempt to (i) verify the possibility of the separation of gland spots in *L. fordiana*, and (ii) detect the individual constituents in glands by HPLC. It will help to understand whether the gland is the accumulation center for those phytoconstituents.

2. Experimental

2.1. Plant material and sample preparation

L. fordiana was collected in July 2006 from Wuzhishan Mountains in Guangdong province. The voucher specimen (Gang Hao, 387) was deposited at the Herbarium of South China Botanical Garden of Chinese Academy of Sciences.

The epidermises were peeled off from the fresh stems of *L. fordiana*, and the exposed gland spots were cut off under a stereo microscope (Xttxtt-type, China). The glands (20 mg) and surrounding tissues (150 mg) were extracted with 100 mL of acetone for 3 times, respectively. The evaporated extracts were dissolved in100 mL of the solution of ethanol–acetone (2:1, v/v). The extracts were filtered through a 0.2 μm filter prior to use.

2.2. Chemicals

40 μg/mL of ethanol–acetone (2:1, v/v) solutions of the purified fordianin A, fordianin B, fordianaquinone A and fordianaquinone B were prepared according to the reference [22] as the references. After making a single injection of the standard solution for the qualitative identification, these four standard solutions were mixed together in equal volume. For sample preparation and HPLC analyses, Milli-Q (Millipore, America) water and the HPLC grade chemicals were used. All HPLC solvents and sample solutions were filtered through a 0.2 μm filter prior to use.

2.3. Instruments

The Agilent model 1100 HPLC system equipped with autosampler, binary pump, DAD detector and Eclipse SB reversed-phase C_{18} (4.6 mm × 150 mm, 5 μm) column was used.

2.4. HPLC analysis

HPLC parameters: column temperature: 25°C; injection volume: 20 μL; flow rate: 1 mL/min; detector wavelength: 440 nm; run time: 40 min. The 440 nm of detector wavelength was chosen to eliminate the interference of other compounds without long conjugated electron system.

The HPLC analyses were performed using a gradient elution with water (mobile phase A) and methanol (mobile phase B). The gradient elution had the following profile: 0–10 min, 70–85% solvent B; 10–15 min, 85–90% solvent B; 15–30 min, 90–95% solvent B; 30–35 min, 95–100% solvent B; and 35–40 min, 100% solvent B. At the end of each run, flush the system and column for 10 min with 100% methanol. Prior to the next run, the system was equilibrated for 10 min with the