
Original Research Article

Study on Chemical Constituents of *Styrax Dasyanthus* Perk.

Abstract: objective This study aimed to investigate the chemical components present in the ethyl acetate extract of *Styrax dasyanthus* leaves.. **Methods** Chemical components were isolated and purified using organic solvent decolorization, extraction, preparation of the liquid phase, silica gel column chromatography, and semi-preparative high-performance liquid chromatography. The structure identification of the isolated compounds was based on nuclear magnetic resonance (NMR) data and carbon spectrum matching analysis. **Results** A total of six compounds were isolated from *Styrax dasyanthus*, which were identified as (-)-secoisolarciresinol (1), dibutylphthalate (2), dihydromyricetin (3), kaempferol-3-O- β -D-glucopyranoside (4), kaempferol-3-rutinoside (3,4',5,7-tetrahydroxyflavone-3-rutinoside) (5), and (-)-secoisolarciresinol-4-O- β -D-glucopyranoside (6). **Conclusion** This study represents the first isolation of these six compounds from *Styrax dasyanthus*.

Key words: *Styrax dasyanthus*; (-)-secoisolarciresinol ; Dihydromyricetin ; kaempferol-3-O- β -D-glucopyranoside.

Introduction

Styrax dasyanthus Perk is a tree of *Styrax* family. 《Gui Zhou Min Jian Yao Wu》《Zhong Hua Ben Cao》《Zhong Yao Da Ci Dian》《Qiang Zu Yi Yao》 have its medicinal records. In the application of traditional Chinese medicine of Qiang, the leaves of *Styrax dasyanthus* are used as a remedy, which has medicinal properties of alleviating coughs, moistening the lungs, clearing heat, detoxifying, reducing swelling, and relieving pain, as well as promoting blood circulation and cooling the blood. It can be used to treat conditions such as cough, dry lungs, edema, injuries, stasis, and pain. Currently, research on *Styrax dasyanthus* primarily focuses on reproduction technology, while its medicinal value has not been thoroughly studied, and its chemical composition has not been reported yet.

To further elucidate the chemical composition of *Styrax dasyanthus* and provide a b

asis for clinical applications and quality control, we will investigate its chemical composition in this experiment.

1. MATERIALS AND INSTRUMENTS

1.1 Instruments: Bruker AVANCE III 500 MHz Nuclear Magnetic Resonance Spectrometer (Bruker Company, Germany); RE-52AA Rotary Evaporator (Shanghai Yarong Biochemical Instrument Factory); LC-16 HPLC Chromatography (Shimadzu Corporation, Japan); Inertsustain C18 Column (250 mm × 4.6 mm, 5 μm); ATY124 Electronic Balance (Shimadzu Company, Japan); SBm-T Rapid Liquid Chromatography Instrument (Changzhou Santai Technology Co., LTD); FV64 Nitrogen Blower (Guangzhou Demei International Biotechnology Co., LTD.); Column Chromatographic Silica Gel (100~200 mesh, 300~400 mesh, Qingdao Marine Chemical Co, LTD.); The liquid phase was pure with methanol and acetonitrile. All the other reagents were analytically pure.

1.2 Plant material: *Styrax dasyanthus* leaves were collected from Zhangjiaying Village, Pengkou Town, Liancheng County, Fujian Province in October of 2021, with the latitude and longitude of 25°32'24"N, 116°40'27"E. They were identified as *Styrax dasyanthus* Perk by Associate Professor Wang Huadong. The plants were planted in the Pharmaceutical Botanical Garden of Sichuan College of Traditional Chinese Medicine.

2. EXTRACTION AND SEPERATION

Extraction: Take 1.1 kg of dried leaves and process them through ultra-micro crushing. Soak the crushed leaves with 10 times their volume of mineral ether for 24 hours, then discard the supernatant. Add 10 times their volume of ethyl acetate to the remaining material and soak for another 24 hours. After soaking, combine all the extracts and concentrate them using a rotary evaporator to obtain 13 g of ethyl acetate extracts. Mix the extracts with 50 g of crude silica gel and successively elute them with petroleum ether, petroleum ether-ethyl acetate (2:1, 1:1, 1:2), ethyl acetate, and methanol to obtain 6 compounds (A→E).

Composition separation: Compound 1 (76 mg) was isolated using a rapid liquid ph

ase preparation system with a fraction C flow rate of 25.0 ml/min and a solvent system of ethyl acetate-methanol (methanol 40%-100%, 40 cv). Compound 2 (12 mg), compound 3 (26 mg), and compound 4 (65 mg) were isolated using a rapid liquid-phase preparation system with a fraction E flow rate of 25.0 ml/min and a solvent system of methanol-water (methanol 30% to 50%, 20 cv). Finally, compound 5 (23 mg) and compound 6 (10.5 mg) were isolated using a rapid liquid-phase preparation system with a fraction F flow rate of 25.0 ml/min and a solvent system of methanol-water (methanol 30%-50%, 20 cv).

3. STRUCTURAL IDENTIFICATION

Compound 1: This compound contains 10 carbon atoms, of which $\delta C145.45$, $\delta C143.82$, $\delta C132.45$, $\delta C121.69$, $\delta C114.12$, and $\delta C111.40$ are aromatic carbon atoms (phenyl carbon) and $\delta C60.93$ and $\delta C55.84$ are two carbons linked to oxygen atoms. 1H -NMR : $\delta H6.61$ (1H, dd, $J=7.9$, 1.8 Hz), $\delta H6.70$ (1H, d, $J=7.9$ Hz), $\delta H6.72$ (1H, d, $J=1.8$ Hz). These three hydrogen signals are consistent with the carbon spectrum, indicating the presence of a benzene ring. $\delta H3.77$ (3H, s) is the signal of the methoxy group on the benzene, $\delta H1.90$ (2H, m), $\delta H2.65$ (2H, dd, $J=13.7$, 6.7 Hz), $\delta H2.70$ (2H, dd, $J=13.7$, 7.6 Hz), $\delta H3.57$ (2H, m), and $\delta H3.67$ (2H, m) represent the five aliphatic hydrogen signals. The signal $\delta H5.54$ (2H, s) represents a hydroxyl hydrogen on the benzene, and $\delta H2.95$ (2H, s) represents a fatty hydroxyl signal. After comprehensive analysis of the 1H NMR and ^{13}C -NMR signals, it was concluded that this compound is a simple phenylpropanoid with structural symmetry. The hydrocarbon signal attribution is as follows: 1H -NMR (600MHz, $CDCl_3$) δ : 6.80 (2H, d, $J=8.0$ Hz, H-5,5'), 6.63 (2H, dd, H-2,2'), 6.59 (2H, d, $J=8.0$ Hz, H-6, 6'), 3.82 (6H, s, 3, 3'-OCH₃), 3.82 (2H, m, H-9,9'), 3.65 (2H, dd, $J=13.7, 6.8$ Hz, H-7, 7'), 2.74 (2H, dd, $J=13.7$, 6.8Hz, H-7, 7'), 2.65 (2H, dd, $J=13.7$, 7.8Hz, H-7, 7'), 1.86 (2H, tdd, H-8, 8'); ^{13}C -NMR (150MHz, CD_3OD) δ : 145.45 (C-3, 3'), 143.82 (C-4, 4'), 132.45 (C-1, 1'), 121.69 (C-6, 6'), 114.12 (C-5, 5'), 111.40 (C-2, 2'), 60.93 (C-9, 9'), 55.84 (3, 3'-OCH₃), 43.84 (C-8, 8'), 35.94 (C-7, 7'). These NMR data are in agreement with those of (-)-secoisolariciresinol [1].

Compound 2: This compound contains 14 carbon signals, with $\delta C167.74$ as the base carbon. $\delta C132.33$, $\delta C130.92$, and $\delta C128.85$ are three phenyl carbon atoms. $\delta C65.59$ represents a carbon adjacent to oxygen. $\delta C30.58$, $\delta C19.19$, and $\delta C13.73$ are three aliphatic

carbon atoms. However, the phenyl carbon signal only shows three carbons, and the δ H shows only two sets of identical hydrogen signals at 7.53 (2H, dd, $J=7.0, 3.7$, H-3, 6) and 7.71 (2H, dd, $J=7.0, 3.7$, H-4, 5), as well as three sets of aliphatic hydrogen signals. Based on these observations, it is inferred that this compound has a symmetrical structure. The hydrocarbon signal attribution is as follows: δ H[7.70 (2H, dd, $J=7.0, 3.7$, H-3, 6), 7.50 (2H, dd, $J=7.0, 3.7$, H-4, 5)], [4.29 (4H, t, $J=6.5$, H-1'), 1.70 (2H, m, H-2'), 1.42 (4H, sextet, $J=7.5$, H-3), 0.96 (6H, t, $J=7.5$, H-4')]. $^{13}\text{C-NMR}$ (CDCl_3 , δ , ppm), 167.7 (COO-), 132.3 (C-1, 2), 130.9 (C-4, 5), 128.8 (C-3, 6), 65.6 (C-1'), 30.6 (C-2'), 19.2 (C-3'), 13.7 (C-4'). The above data are basically consistent with the report [2]. The compound was identified as dibutyl phthalate.

Compound 3: This compound contains 15 carbon signals, with δ C198.05 possibly being the 4th carbon of a flavonoid. The signals at δ 83.70 and δ 72.09 may represent the 2nd and 3rd carbons of the flavonoid, respectively. Furthermore, there are 12 carbon signals with values greater than δ 90. Based on these observations, it is inferred that this compound is a flavonoid. The hydrocarbon signal attribution is as follows: 6.40 (2H, s, H-2', 6'), 5.90 (1H, d, $J=2.0$, H-8), 5.86 (1H, $J=2.0$, H-6), 4.90 (1H, d, $J=10.8$, H-2), 4.43 (1H, dd, $J=10.8, 4.0$, H-3), 11.89 (1H, s, 5-OH); $^{13}\text{C-NMR}$ (100MHz, DMSO- d_6 , ppm): 83.70 (C-2), 72.09 (C-3), 198.05 (C-4), 163.79 (C-5), 96.42 (C-6), 167.31 (C-7), 95.43 (C-8), 162.97 (C-9), 100.92 (C-10), 127.58 (C-1'), 107.41 (C-2',6'), 146.16 (C-3',5'), 133.91 (C-4'). The above data are basically consistent with the report [3]. The compound was identified as Dihydromyricetin.

Compound 4: The compound exhibits 21 carbon signals and a terminal carbon signal at δ 101.38 in the carbon spectrum, suggesting the presence of glycosides. The hydrogen spectrum reveals a group of AA'BB' aromatic hydrogen signals, including δ 8.03, δ 6.88 (2H each, d, $J=8.8$ Hz, H-2', 6' and 3', 5'), as well as a set of intercoupled aromatic hydrogen signals at δ 6.40, δ 6.18 (1H each, d, $J=2.0$ Hz, H-6, 8). In addition, there is an end-based hydrogen signal for a sugar at δ 5.29 (1H, d, $J=1.2$ Hz, H-1''). These observations indicate that the inferred compound is a flavonoid glycoside. The hydrocarbon signal attribution is as follows: $^1\text{H-NMR}$ (400MHz, DMSO- d_6) δ : 8.03 (2H, d, $J=8.8$ Hz, H-2',6'), 6.88 (2H, d, $J=8.8$ Hz, H-3', 5'), 6.40 (1H, d, $J=2.0$ Hz, H-8), 6.18 (1H, d, $J=2.0$ Hz, H-6), 5.46 (1H, d, $J=7.2$ Hz, H-1''); $^{13}\text{C-NMR}$ (100MHz, DMSO- d_6) δ : 177.85 (C-4), 165.2 (C-7), 161.6 (C-5), 160.4 (C-4'), 156.9 (C-2), 156.6 (C-9), 133.6 (C-3), 131.3 (C-2', 6'), 121.3 (C-1'), 115.5 (C-3', 5'), 104.2 (C-10), 101.3 (C-1''), 98.7 (C-6), 94.1 (C-8), 77.9 (C-3''), 76.9 (C-5''), 74.6 (C-2''), 70.3 (C-4''), 61.3 (C-6''). The above data are basically con

sistent with the report [4]. The compound was identified as kaempferol-3-*O*- β -*D*-glucopyranoside.

Compound 5: The compound exhibits 27 carbon signals, including two terminal carbon signals at δ 101.40 and δ 100.80, suggesting the presence of glycosides. The hydrogen spectrum reveals a group of AA'BB' aromatic hydrogen signals, including δ 7.98, δ 6.88 (2H each, d, J =8.8 Hz, H-2', 6' and 3', 5'), as well as a coupled aromatic hydrogen signal at δ 6.40, δ 6.19 (1H each, d, J =2.0 Hz, H-6, 8). In addition, there is an end-based hydrogen signal for a sugar at δ 5.29 (1H, d, J =7.6 Hz, H-1"). These observations suggest that the compound is a flavonoid glycoside with two saccharides. The hydrocarbon signals attribution is as follows: $^1\text{H-NMR}$ (DMSO- d_6) ppm 7.98 (2H, d, J =7Hz, H-2', H-6'), 6.88 (2H, d, J =7Hz, H-3', -5'), 6.40 (1H, d, J = 2Hz, H-8), 6.19 (1H, d, J =2Hz, H-6), 5.30 (1H, d, H-1", Glu-1), 5.07 (1H, m, H-1" Rha-1), 0.98 (3H, d, J =6Hz, Rha-6); $^{13}\text{C-NMR}$ (DMSO- d_6) δ : ppm: 177.81 (C-4), 164.91 (C-7), 161.65 (C-5), 160.36 (C-4'), 157.26 (C-9), 156.99 (C-2), 133.68 (C-3), 131.34 (C-2',C-6'). 121.36 (C-1'), 115.56 (C-3', C-5'). 104.35 (C-b), 101.83 (C-1-glc), 101.23 (C-1-rha), 99.27 (C-6), 94.25 (C-8), 76.83 (C-3-glc), 76.21 (C-5-glc), 74.64 (C-2-glc), 72.29 (C-4-rha), 71.07 (C-3-rha), 70.81 (C-2-rha), 70.39 (C-4-glc), 68.71 (C-5-rha), 67.36 (C-6-glc), 18.19 (C-6-rha). The above data are basically consistent with the report [5]. The compound was identified as kaempferol-3-rutinoside.

Compound 6: The carbon spectrum signals revealed 26 carbons, including 12 aromatic carbons and one terminal carbon. The hydrogen spectrum signals displayed two sets of phenolic hydrogen, two benzene methoxy groups, terminal group hydrogen, and a series of aliphatic hydrocarbon hydrogen. Based on a comprehensive analysis, the compound was identified as a lignanoid glycoside. The hydrocarbon signals attribution is as follows: $^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ : 6.68 (1H, d, J = 2.0 Hz, H-2), 6.95 (1H, d, J = 8.8Hz, H-5), 6.62 (1H, dd, J =2.0, 8.8Hz, H-6), 2.47~2.57 (2H, overlap, H-7), 1.82~1.85 (1H, m, H-8), 3.30~3.40 (2H, overlap, H-9), 3.69 (3H, s, 3-OCH₃), 6.62 (1H, d, J = 1.8 Hz, H-2'), 6.63 (1H, d, J = 8.0Hz, H-5'), 6.50 (1H, dd, J = 1.8, 8.0Hz, H-6'), 2.47~2.57 (2H, overlap, H-7'), 1.82~1.85 (1H, m, H-8'), 3.30~3.40 (2H, overlap, H-9'), 3.69 (3H, s, 3'-OCH₃), 4.83 (1H, d, J = 7.2 Hz, H-1"), 3.20~3.25 (3H, overlap, H-2", 3", 5"), 3.12~3.18 (1H, m, H-4"), 3.62~3.64 (1H, m, H-6"), 3.41~3.47 (1H, m, H-6"); $^{13}\text{C-NMR}$ (100MHz, DMSO- d_6) δ : 135.75 (C-1), 113.70 (C-2), 149.13 (C-3), 145.10 (C-4), 115.58 (C-5), 121.61 (C-6), 34.36 (C-7), 43.01 (C-8), 60.71 (C-9), 56.02 (3-OCH₃), 132.66 (C-1'), 113.41 (C-2'), 147.73 (C-3'), 144.77 (C-4'), 115.56 (C-5'), 121.48 (C-6'), 34.35 (C-7'), 42.86 (C-8'), 60.68 (C-9'), 55.98 (3'-OCH₃), 100.86 (C-1"), 73.73 (C-2"), 77.35 (C-3"), 70.18 (C-

4"), 77.45 (C-5"), 61.16 (C-6"). The above data are basically consistent with the literature reports [6]. The compound was identified as (-)-secoisolariciresinol-4-*O*- β -D-glucopyranoside.

4. RESULTS AND DISCUSSION

In this experiment, six chemical compositions of *Styrax dasyanthus* were isolated and identified, including three flavonoids, one lignin, and one phenylpropanoid. According to the literature reports, compound 6 was previously isolated from *Styrax perkinsiae* Rehd [7], while the remaining compounds were isolated for the first time from this genus. All compounds were extracted from the plants for the first time. As a traditional Chinese medicine with proven medicinal efficacy, few studies have focused on its chemical composition and pharmacological effects. This study was the first to conduct a chemical composition analysis of *Styrax dasyanthus*, thereby filling a research gap, enriching the compound library of *Styrax dasyanthus*, and providing a material basis for further research and development of this medicinal plant.

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UNDER PEER REVIEW

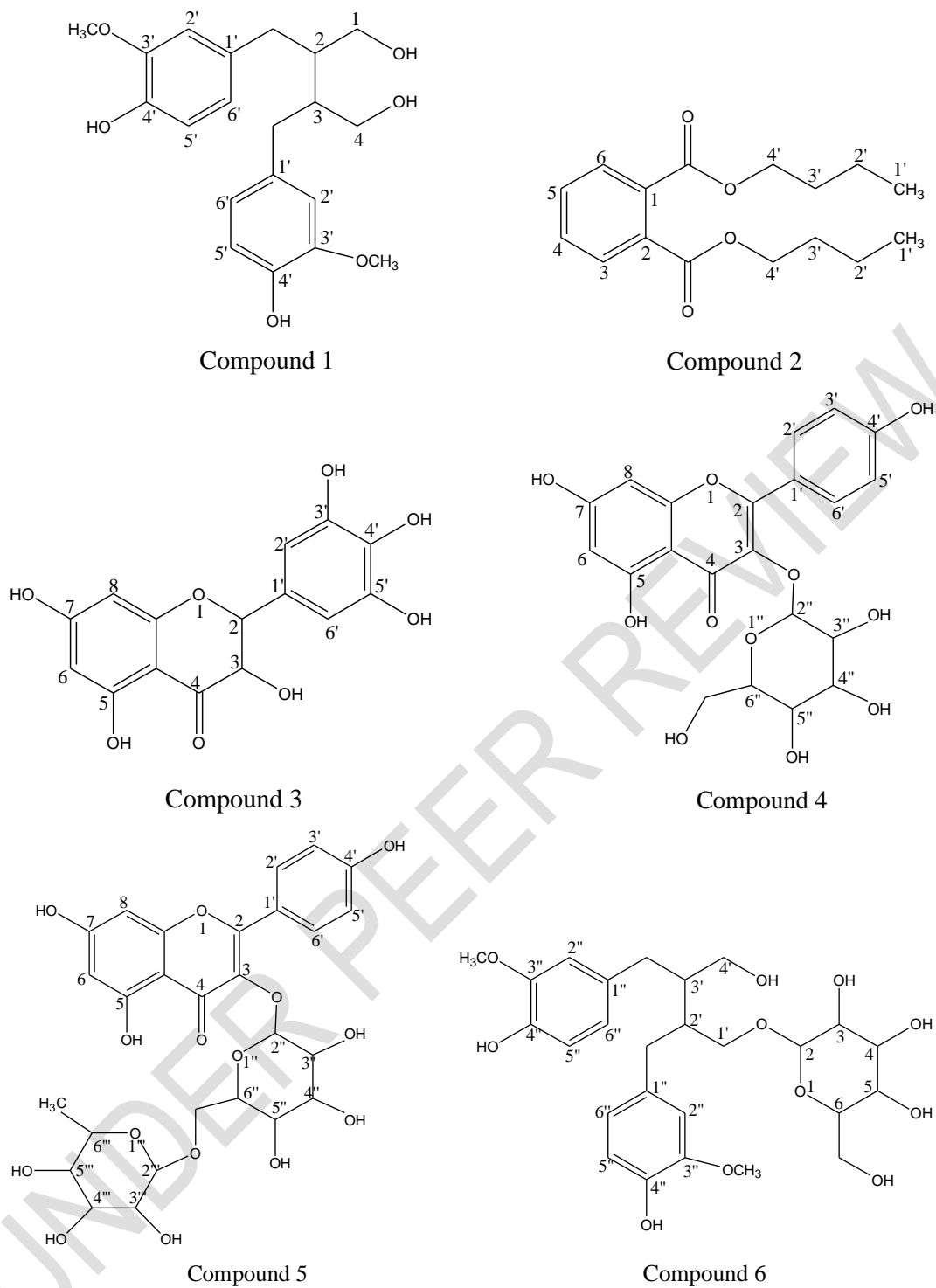


Figure 1 Structures of compounds 1-6