Asian Journal of Research in Medical and Pharmaceutical Sciences

Volume 12, Issue 4, Page 21-26, 2023; Article no.AJRIMPS.104908
ISSN: 2457-0745

Study on Chemical Constituents of *Styrax dasyanthus* Perk

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRIMPS/2023/v12i4228

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/104908

Original Research Article

Received: 17/06/2023
Accepted: 22/08/2023
Published: 05/09/2023

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 (-)-secoisolariciresinol-4-O- β -D-glucopyranoside (6).
Conclusion: This study represents the first isolation of these six compounds from *Styrax dasyanthus*.

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Keywords: Styrax dasyanthus; (-)-secoisolarciresinol; dihydromyricetin; kaeMpferol-3-O-β-D-glucopyranoside.

1. INTRODUCTION

Styrax dasyanthus Perk is a tree of Styrax family. 《Gui Zhou Min Jian Yao Wu》[1]《Zhong Hua Ben Cao》[2]《Zhong Yao Da Ci Dian》[3]《Qiang Zu Yi Yao》[4] 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 basis for clinical applications and quality control, we will investigate its chemical composition in this experiment.

2. MATERIALS AND METHODS

2.1 Instruments

BrukerAVANCEIII500MHz Nuclear Magnetic Resonance Spectrometer (Bruker Company, Germany); RE-52AA Rotary Evaporator (Shanghai Yarong Biochemical Instrument Factory); LC-16HPLC Chromatography (Shimadzu Corporation, Japan); Inertsustain C18 Column (250mm×4.6mm,5μm); 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.

2.2 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.

3. EXTRACTION AND SEPERATION

3.1 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).

3.2 Composition Separation

Compound 1 (76 mg) was isolated using a rapid liquid phase 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).

4. STRUCTURAL IDENTIFICATION

4.1 Compound 1

This compound contains 10 carbon atoms, of which δC145.45, δC143.82, δC132.45, δC121.69, δC114.12, and δC111.40 are aromatic carbon atoms (phenyl carbon) and δC60.93 and δC55.84 are two carbons linked to oxygen atoms. 1H-NMR : δH6.61 (1H, dd, *J*=7.9, 1.8 Hz), δH6.70 (1H, d, *J*=7.9 Hz), δH6.72 (1H, d, *J*=1.8 Hz). These three hydrogen signals are

consistent with the carbon spectrum, indicating the presence of a benzene ring. δH3.77 (3H, s) is the signal of the methoxy group on the benzene, δH1.90 (2H, m), δH2.65 (2H, dd, *J*=13.7, 6.7 Hz), δH2.70 (2H, dd, *J*=13.7, 7.6 Hz), δH3.57 (2H, m), and δH3.67 (2H, m) represent the five aliphatic hydrogen signals. The signal δH5.54 (2H, s) represents a hydroxyl hydrogen on the benzene, and δH2.95 (2H, s) represents a fatty hydroxyl signal. After comprehensive analysis of the ^{1}H 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: ^{1}H-NMR (600MHz, $CDCl_3$) δ: 6.80 (2H, d, *J*=8.0Hz, H-5,5'), 6.63 (2H, dd, H-2,2'), 6.59 (2H, d, *J*=8.0Hz, H-6, 6'), 3.82 (6H, s, 3, 3'-OCH3), 3.82 (2H, m, H-9,9'), 3.65 (2H, dd, *J*=13.7,6.8Hz, 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, CD3OD) 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 agree with those of (-)-secoisolariciresinol [5].

4.2 Compound 2

This compound contains 14 carbon signals, with δC167.74 as the base carbon. δC132.33, δC130.92, and δC128.85 are three phenyl carbon atoms. δC65.59 represents a carbon adjacent to oxygen. δC30.58, δC19.19, and δC13.73 are three aliphatic carbon atoms. However, the phenyl carbon signal only shows three carbons, and the δH 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: δH [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}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 [6]. The compound was identified as dibutyl phthalate.

4.3 Compound 3

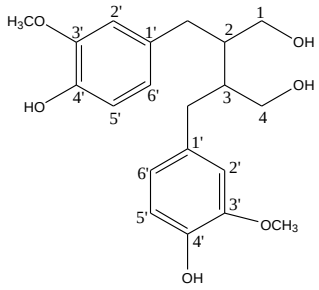
This compound contains 15 carbon signals, with δC198.05 possibly being the 4th carbon of a flavonoid. The signals at δ83.70 and δ72.09 may represent the 2nd and 3rd carbons of the flavonoid, respectively. Furthermore, there are 12 carbon signals with values greater than δ90. Based on these observations, 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}C-NMR (100MHz, DMSO-d6, 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 [7]. The compound was identified as Dihydromyricetin.

4.4 Compound 4

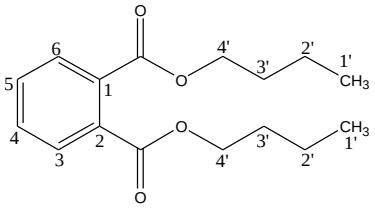
The compound exhibits 21 carbon signals and a terminal carbon signal at δ101.38 in the carbon spectrum, suggesting the presence of glycosides. The hydrogen spectrum reveals a group of AA'BB' aromatic hydrogen signals, including δ8.03, δ6.88 (2H each, d, *J*=8.8 Hz, H-2', 6' and 3', 5'), as well as a set of intercoupled aromatic hydrogen signals at δ6.40, δ6.18 (1H each, d, *J*=2.0 Hz, H-6, 8). In addition, there is an end-based hydrogen signal for a sugar at δ5.29 (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}H-NMR (400MHz, DMSO-d6) δ: 8.03 (2H, d, *J*= 8.8Hz, H-2′,6′), 6.88 (2H, d, *J*=8.8Hz, H-3′, 5′), 6.40 (1H, d, *J*= 2.0Hz, H-8), 6.18 (1H, d, *J*= 2.0Hz, H-6), 5.46 (1H, d, *J*=7.2Hz, H-1″); ^{13}C-NMR (100MHz, DMSO-d6) 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 consistent with the report [8]. The compound was identified as kaeMpferol-3-*O*-β-*D*-glucopyranoside.

4.5 Compound 5

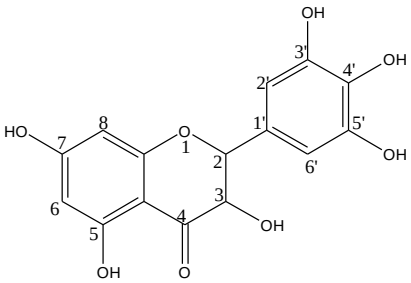
The compound exhibits 27 carbon signals, including two terminal carbon signals at δ101.40 and δ100.80, suggesting the presence of glycosides. The hydrogen spectrum reveals a group of AA'BB' aromatic hydrogen signals, including δ7.98, δ6.88 (2H each, d, *J*=8.8 Hz, H-2', 6' and 3', 5'), as well as a coupled aromatic



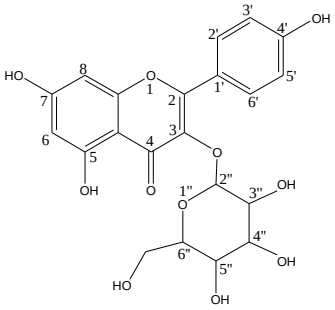
Compound 1



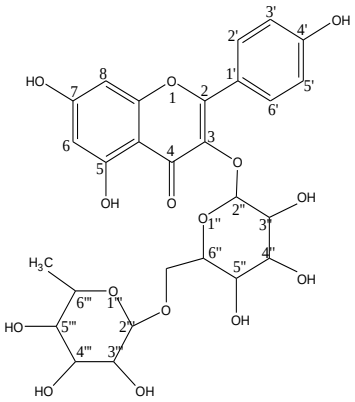
Compound 2



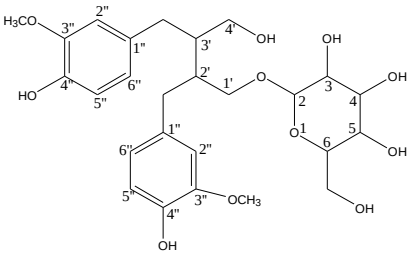
Compound 3



Compound 4



Compound 5



Compound 6

Fig. 1. Structures of compounds 1-6

hydrogen signal at δ6.40, δ6.19 (1H each, d, *J*=2.0 Hz, H-6, 8). In addition, there is an end-based hydrogen signal for a sugar at δ5.29 (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}H-NMR (DMSO-d6) 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-I" Rha-1), 0.98 (3H, d, *J*=6Hz, Rha-6); ^{13}C-NMR (DMSO-d6) 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-I '), 115.56 (C-3', C-5'). 104.35 (C-b). 101.83 (C-1-glc). 101.23 (C-I-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 [9]. The compound was identified as kaeMpferol-3-rutinoside.

4.6 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}H-NMR (400 MHz, DMSO-d6) 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-OCH_3), 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′-OCH_3), 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}C-NMR (100MHz, DMSO-d6) 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-OCH_3), 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′-OCH_3), 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 [10]. The compound was identified as (-)-secoisolariciresinol-4-*O*-β-*D*-glucopyranoside.

5. 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 [11]. 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.

6. CONCLUSION

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.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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