Study on the Chemical Components of the Ethyl Acetate Extract from *Herpetospermum Caudigerum*

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INTRODUCTION

*Herpetospermum Caudigerum* Wall (Cucurbitaceae) is distributed in southwest China. The dried ripe seeds of *Herpetospermum Caudigerum* Wall. have been used for the treatment of liver diseases as a Tibetan folk medicine in China.¹-³ The study crushing the dried seeds, then extracting the chemical composition by 95% ethanol. Collecting eluate, evaporating solvent, the concentrated solution dispersed in water, then obtaining different fractions by extracting with petroleum ether, ethyl acetate and n-BuOH.⁴ The sugar and grease in the ethyl acetate extract was removed. Then repeated using the technical means include Silica gel column chromatography, Sephadex chromatography, reversed-phase column chromatography, recrystallization and semi-preparative column chromatography to separate and purificate until obtaining the monomer compound.⁵ Finally, the compound structure was identified by TLC, ¹H-NMR, ¹³C-NMR, etc. We identified the structures of 6 compounds (Figure 1): Herpetin¹, Eicosanoic acid, 2-propenyl ester², Cucurbitacin R³, Cucurbitacin L⁴, 3'-Hydroxydaidzein⁵, Oleic acid⁶, among these compounds, compound 2, 3, 4, 5 were first isolated from *Herpetospermum* seed.

METHODS

General Experimental Procedures

1D and 2D NMR spectra were taken on a BRUKER AVANCE III NMR System-600 NMR spectrometer. ESI-MS and HRESIMS were obtained using an Agilent 6210 TOF LC-MS mass spectrometer. Preparative HPLC was performed on an Agilent 1260, and a reversed-phase C₁₈ column (YMC-Pack ODS-AU 20×250mm, 10μm) was employed. Column chromatography was undertaken over silica gel (200-300m).¹¹ TLC was carried out with glass plate precoated silica gel G. Spots were visualized under UV light and by spraying with 10% H₂SO₄ in 95% EtOH, followed by heating at 100°C. Methanol used in preparative HPLC procedure was in HPLC grade, and other solvents were of analytical grade.

Plant Material

The seeds of *Herpetospermum Caudigerum* Wall. were collected from the kangding area in Sichuan province and authenticated by A/Prof. Liang-Ke Song in School of Life Science and Engineering, Southwest Jiaotong University. Avoucher specimen was deposited in Room3704, 3rd Teaching Building, Southwest Jiaotong University.

Extraction and Isolation

The dried-up and powdered seeds of *Herpetospermum Caudigerum* Wall. (10 kg) were extracted with 95% EtOH at room temperature. After removal of the solvent, the residue after removing EtOH was suspended in water and partitioned with petroleum ether, ethyl acetate, n-BuOH, successively. The ethyl acetate extract (280g) was subjected to column chromatography on silica gel with gradient solvents of DCM-MeOH (100:0-1:1). The collected fractions were combined according to the TLC result to give 9 fractions (BL₁, BL₆). BL₄ (46g) was applies to silica gel column chromatography to give BL₁₁ (10.4g), BL₁₂ was purified by silica gel column chromatography eluted with CHCl₃:MeOH (100:3-100:10)
RESULTS AND DISCUSSION

Compound 1 was obtained as a white amorphous powder, and determined to possess the molecular formula C_{30}H_{34}O_{9} by its pseudo-molecular ion peak at m/z 561.2160 [M+Na]^{+} in the positive HR-ESI-MS experiment. According to the data of 1H-NMR (CD_{3}OD; 400 MHz) δ: 6.94 (1H, d, J = 2.0 Hz, H-2), 6.90 (1H, s, H-1’), 6.90 (1H, s, H-4’), 6.88 (2H, d, J = 8.0 Hz, H-6, 5’), 6.82 (1H, d, H-6), 6.68 (1H, s, H-2’), 6.67 (1H, s, -OH), 5.54 (2H, d, J = 6.4 Hz, -OH), 4.77 (1H, d, J = 7.2 Hz, H-7’), 4.08 (1H, m, -OH), 3.93 (2H, m, H-9), 3.89 (3H, s, -OCH_{3}), 3.88 (3H, s, -OCH_{3}), 3.86 (3H, s, -OCH_{3}), 3.77 (2H, m, H-9’), 3.61 (1H, m, H-8), 2.94 (2H, m, H-9’), 2.74 (2H, m, H-7), 2.58 (2H, m, H-8’, 8”), 2.42 (2H, m, H-7). Its structure was identified as herpetin\textsuperscript{6,7} by comparison of the spectrum of data with those reported in the literatures.

Compound 2 was obtained as a white granular crystal, and determined to possess the molecular formula C_{32}H_{34}O_{2} in the positive HR-ESI-MS experiment. According to the data of 1H-NMR (400 MHz, CDCl_{3}); δ: 85.32-5.17 (2H, m), 4.22 (1H, dd), 4.07 (1H, dd), 2.26-2.22 (3H, m), 1.99-1.91 (4H, m), 1.20 (30H, 3.88 (3H, s, -OCH_{3}), 3.86 (3H, s, -OCH_{3}), 3.77 (2H, m, H-9’), 3.61 (1H, m, H-8), 2.94 (2H, m, H-9’), 2.74 (2H, m, H-7), 2.58 (2H, m, H-8’, 8”), 2.42 (2H, m, H-7). Its structure was identified as herpetin\textsuperscript{6,7} by comparison of the spectrum of data with those reported in the literatures.

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Compound 3 was obtained as a white crystal powder, and determined to possess the molecular formula C$_{30}$H$_{46}$O$_{2}$ in the positive HR-ESI-MS experiment. According to the data of $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 4.38 (1H, s, H-6), 4.30 (1H, t, J = 7.5Hz, H-2), 3.90 (2H, s, J = 4.2Hz, -OH), 3.52 (1H, s, -OH), 3.51 (1H, s, -OH), 3.12 (1H, d, J = 14.8Hz, H-16), 2.92 (1H, dd, J = 8.1Hz,H-23), 2.74 (2H, d, J = 13.5Hz, H-7, 12), 2.64 (2H, m, J = 14.6Hz, H-7, 12), 2.57 (1H, m, J = 6.9Hz, H-10), 2.40 (1H, m, J = 5.2Hz, H-9), 2.23 (1H, m, J = 4.3Hz, H-8), 2.05(2H, s, H-24), 1.82 (4H, dd, J = 13.3, 6.8Hz, H-1, 15), 1.41 (6H, s, -CH$_3$), 1.33 (6H, s, -CH$_3$), 1.27 (3H, s, -CH$_3$), 1.24 (3H, s, -CH$_3$), 1.21 (3H, s, -CH$_3$), 1.18 (3H, s, -CH$_3$). Its structure was identified as Cucurbitacin R$^9$ by comparison of the spectrum of data with those reported in the literatures.

Compound 4 was obtained as a white crystal powder, and determined to possess the molecular formula C$_{30}$H$_{46}$O$_{2}$ in the positive HR-ESI-MS experiment. According to the data of $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 4.14 (1H, s, H-1), 4.13 (1H, s, H-6), 4.10(1H, s, -OH), 4.08 (1H, s, -OH), 3.49 (1H, s, -OH), 3.12 (1H, d, H-16), 2.27 (1H, dd, H-10), 1.81 (1H, d, H-17), 1.78 (1H, m, H-8), 1.62 (3H, s, H-7, 12), 1.57 (1H, m, H-23), 1.30 (1H, m, H-24), 1.28 (6H, m, H-7, 12, 15), 1.27 (3H, s, -CH$_3$), 1.25 (3H, s, -CH$_3$), 1.12 (3H, s, -CH$_3$), 1.10 (3H, s, -CH$_3$), 1.05 (3H, s, -CH$_3$), 0.98 (3H, s, -CH$_3$), 0.94 (3H, m, -CH$_3$), 0.91(3H, s, -CH$_3$). Its structure was identified as Cucurbitacin L$^9$ by comparison of the spectrum of data with compound 3 and those reported in the literatures.

Compound 5 was obtained as a white crystal powder, and determined to possess the molecular formula C$_{18}$H$_{26}$O$_{2}$ in the positive HR-ESI-MS experiment. According to the data of $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.08 (1H, s, H-2), 7.93 (1H, d, Ar-H), 7.51 (1H, d, Ar-H), 7.44 (1H, d, Ar-H), 7.41 (1H, d, Ar-H), 7.31 (1H, s, Ar-H), 7.28 (1H, d, Ar-H), 5.53 (1H, s, H-2'), 5.23 (1H, s, -OH), 4.90 (1H, s, -OH). Its structure was identified as 3'-Hydroxydaidzein$^{10}$ by comparison of the spectrum of data with those reported in the literatures.

Compound 6 was obtained as a white needle crystal, and determined to possess the molecular formula C$_{30}$H$_{46}$O$_{2}$ in the positive HR-ESI-MS experiment. According to the results that the TLC indicated that compound 6 could show the same color pot as the Oleanic acid in the same position by using chloroform-methanol (15:1) or Cyclohexane-acetone-ethyl acetate as developing solvent, and concentrated sulphuric acid: ethanol (10:1) solution as coloration using silica gel G, its structure was identified as Oleanic acid.

**CONCLUSION**

The study crushing the dried seeds, then extracting the chemical composition by 95% ethanol. Collecting eluate, evaporating solvent, the concentrated solution dispersed in water, then obtaining different fractions by extracting with petroleum ether, ethyl acetate and n-BuOH. The ethyl acetate extract was repeated using the technical means include Silica gel column chromatography, Sephadex chromatography, reversed-phase column chromatography, recrystallization and semi-preparative column chromatography to separate and purificate until obtaining the monomer compound. Finally, the compound structure was identified by TLC, $^1$H-NMR, $^{13}$C-NMR, et al. We identified the structures of 6 compounds, they were Herpetin, Eicosanoic acid, 2-propenyl ester, Cucurbitacin R, Cucurbitacin L, 3'-Hydroxydaidzein, and Oleanic acid. What’s more, Eicosanoic acid, 2-propenyl ester, Cucurbitacin R, Cucurbitacin L, and 3'-Hydroxydaidzein were first isolated from *Herpetospermum* seed, it greatly enriched the type of natural products. During the study, the sugar and grease in the ethyl acetate extract was removed to reduce the interference of glycolipids, it also accelerated the speed of monomer purification.

**CONFLICT OF INTEREST**

None.

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