Original Research

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

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***Herpetospermum* seed, a common folk medicine used by Tibetan medication, is the dried ripe seed of *Herpetospermum Caudigerum* Wall. It is bitter in taste and cold in nature. In Tibet it is popularly known and used in traditional medicine for the treatment of liver diseases, cholic diseases, and dyspepsia. Six compounds, named Herpetin(1), Eicosanoic acid, 2-propenyl ester(2), Cucurbitacin R(3), Cucurbitacin L(4), 3'-Hydroxydaidzein(5), Oleanic acid(6), have been isolated from the ethyl acetate extract of the seeds of *Herpetospermum Caudigerum* Wall, among these compounds, compound 2, 3, 4, 5 were isolated from this plant for the first time.**

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Key Words: *herpetospermum caudigerum wall, cucurbitacin, extraction, isolation*

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 obtainning 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 obtainning the monomer compound.[5] Finally, the compound structure was identified by TLC, ^{1}H-NMR, ^{13}C-NMR, ect. We identified the structures of 6 compounds (**Figure 1**): Herpetin(**1**), Eicosanoic acid, 2-propenyl ester(**2**), Cucurbitacin R(**3**), Cucurbitacin L(**4**), 3'-Hydroxydaidzein(**5**), Oleanic acid(**6**), among this compounds, compound **2-5** 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 C_{18} 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% H_2SO_4 in 95% EtOH, followed by heating at 100ºC. 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 (BL_1-BL_9). BL_4 (46g) was applies to silica gel column chromatography to give $BL_{4\text{-}1}$ (10.4g), $BL_{4\text{-}1}$ was purified by silica gel column chromatography eluted with $CHCl_3$:MeOH (100:3-100:10)

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and the use of Sephadex LH-20 (CH_3OH) to yield compound 1 (18.9mg) and compound 2 (20.1mg). According to the TLC profiles, BL_{4-7} (9.6g) was applies to Silica gel column chromatography eluted with petroleum ether:acetone (50:1~0:1) to give BL_{4-7-1} (2.3g), BL_{4-7-1} was purified by the use of Sephadex LH-20 (CH_3OH) to yield compound 3 (30.2mg). Then BL_{4-7-5} (3.1g) was applies to silica gel column chromatography to give $BL_{4-7-5-1}$ (203mg), $BL_{4-7-5-1}$ was purified by use of silica gel column chromatography, reversed-phase column chromatography and Sephadex LH-20 (CH_3OH) to yield compound 4 (9.8mg). $BL_{4-7-5-4}$ (96mg) was applied to preparative HPLC system [mobile phase: CH_3OH/H_2O (65:35, v/v); flow rate: 5 mL min^{-1}; UV detection at 254 nm] resulting in the isolation of compound 5 (14.8mg). BL_{4-8} (7.8g) was applies to silica gel column chromatography eluted with petroleum ether:ethyl acetate (30:1~0:1) to give BL_{4-8-1}, BL_{4-8-1} was purified by the use of Sephadex LH-20 (CH_3OH) to yield compound **6** (1.02g).

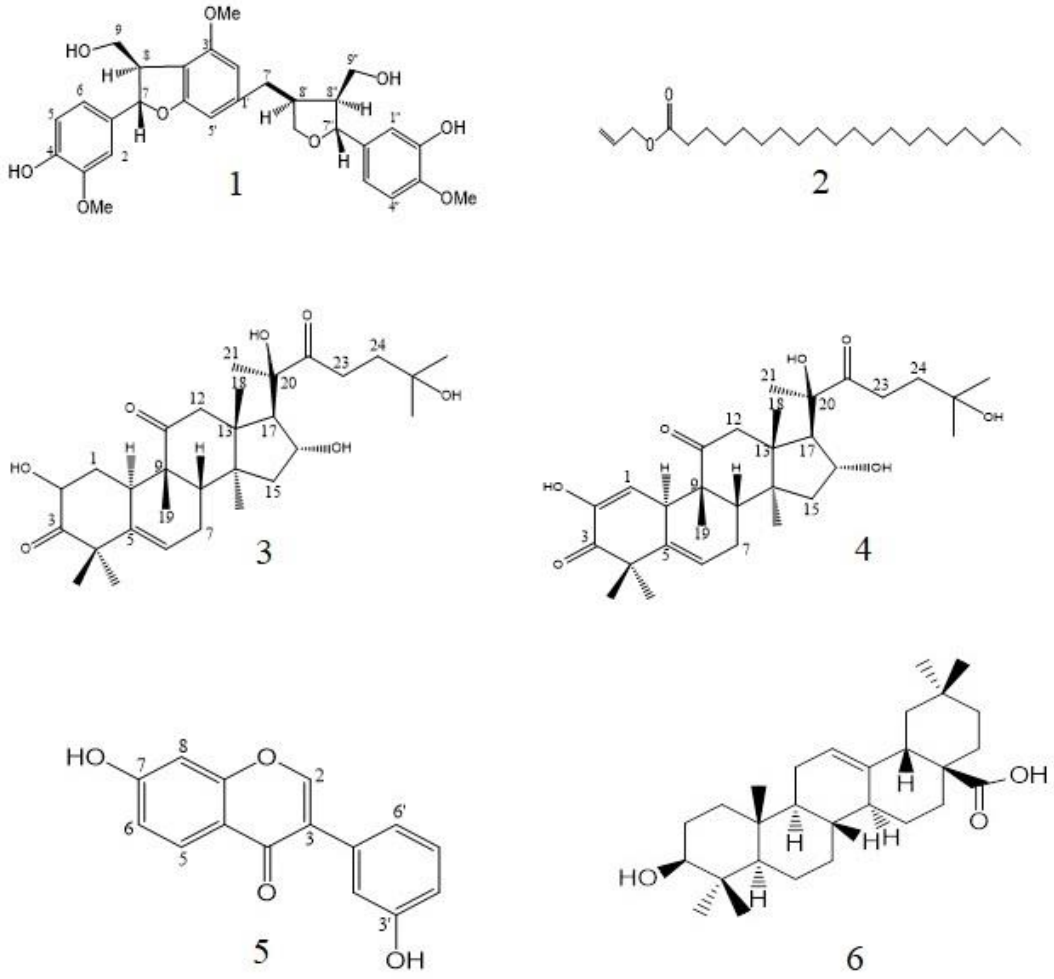

Figure 1. The structures of compounds 1-6.

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 ^{1}H-NMR (CD_3OD, 400MHz) δ: 6.94 (1H, d, J =2.0Hz, H-2), 6.90 (1H, s, H-1"), 6.90 (1H, s, H-4"), 6.88 (2H, d, J = 8.0Hz, 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 = 6.4Hz, -OH), 4.77 (1H, d, J =7.2Hz, 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 [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 $C_{23}H_{44}O_2$ in the positive HR-ESI-MS experiment. According to the data of ^{1}H-NMR (400 MHz, $CDCl_3$): δ5.32-5.17 (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,

q), 0.81 (3H, t). The structures of compound **2** is simple, and its structure was identified as Eicosanoic acid, 2-propenyl ester [8] by comparison of the spectrum of data with those reported in the literatures.

Compound **3** was obtained as a white crystal powder, and determined to possess the molecular formula $C_{30}H_{46}O_7$ in the positive HR-ESI-MS experiment. According to the data of ^{1}H-NMR (400 MHz, $CDCl_3$): δ 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 =16.1,8.1Hz,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 $C_{30}H_{44}O_7$ in the positive HR-ESI-MS experiment. According to the data of ^{1}H NMR (400 MHz, $CDCl_3$): δ 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 $C_{15}H_{10}O_4$ in the positive HR-ESI-MS experiment. According to the data of ^{1}H NMR (400 MHz, CDCl3): δ 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 $C_{30}H_{48}O_3$ 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.

REFERENCES

1. Brent EK. In vitro evaluation of combination therapies against hepatitis B virus replication. J Antiviral Res. 1996;29:49-53.
2. Cheng YM, Flora Xizangica. Science Press (Beijing). 1985;537-539.
3. Chinese Pharmacopoeia Commission, The Drug Standard of Ministry of Health of P.R. China (Tibetan Medicines). Chinese Pharmacopoeia Commission, Ministry of Health of China, Beijing. 1995;9:641-644.
4. Hu S. Study on the chemical constitutions of the ethyl acetate extract from Herpetospermum Caudigerum. M.S. Thesis, Southwest Jiaotong University, Chengdu, China, IN, 2016.
5. Wu WJ. Study on the chemical compounds and monomer active of Herpetospermum seed for the ethyl acetate part. M.S. Thesis, Southwest Jiaotong University, Chengdu, China, IN, 2015.
6. Zhou XS. Systematic Study on the Lignans Compounds of Herpetospermum Caudigerum. M.S. Thesis, Southwest Jiaotong University, Chengdu, China, IN, 2014.
7. Yuan HL, Yang M, Li XY, et al. Hepatitis B Virus Inhibiting Constituents from Herpetospermum Caudigerum. Chem Pharm Bull. 2006;54:1592-1594.
8. Czajkowska D, Morzycki JW. Metathesis reactions of 22-steroids. Tetrahedron Lett. 2009;50:2904-2907.
9. Gan ML, Liu MT, Liu B, et al. Ucurbitane Glucosides from the Root of Machilus yaoshansis. J Nat Prod. 2011:2431-2437.
10. Goto H, Terao Y, Akai S. Synthesis of various kinds of isoflavones,isoflavanes,and biphenyl-ketones and their 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activities. Chem Pharm Bull (Tokyo). 2009;57:346-360.
11. Yu JQ, Hang W, Duan WJ, Wang X, Wang DJ, Qin XM. Two new anti-HBV lignans from Herpetospermum Caudigerum. Phytochem Lett. 2014;10:230-234.