CHAPTER III

EXPERIMENTAL

1. Sources of Plant Materials

The aerial parts of *Ellipeiopsis cherrevensis* (Pierre ex Finet & Gagnep.) R. E. Fr. were collected from Wang Nam Khiao, Nakhon Ratchasima in April and October, 2002. The leaves and stem of *Stelechocarpus cauliflorus* R. E. Fr. were collected from Khao Chong, Trang and Tha-pae Waterfall, Nakhonsrithammarach, Thailand in March, 2003 and March, 2004, respectively. Voucher herbarium specimens of both plants have been deposited at the Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

2. General Techniques

2.1 Solvents

Throughout this work, all organic solvents were of commercial grade and were redistilled prior to use.

2.2 Analytical Thin-Layer Chromatography (TLC)

Technique	:	One dimension, ascending
Adsorbent	:	Silica gel 60 F_{254} (E. Merck) precoated plate
Layer thickness	:	0.2 mm
Distance	:	5.0 cm
Temperature	:	Laboratory temperature (25-30 °C)
Detection	:	1. Ultraviolet light (254 and 365 nm)
		2. 10% Sulfuric acid and heating at 105 °C for 10 min

2.3 Preparative Thin-Layer Chromatography (PTLC)

Technique	:	One dimension, ascending
Adsorbent	:	Silica gel 60 F_{254} (E. Merck) pre-coated plate
Layer thickness	:	1 mm
Distance	:	15 cm
Temperature	:	Laboratory temperature (25-30 °C)
Detection	:	Ultraviolet light (254 and 365 nm)

2.4 Column Chromatography

2.4.1 Vacuum Liquid Column Chromatography

Adsorbent	:	Silica gel 60 (No. 7734) particle size 0.063-0.200 nm (E. Merck)
Packing method	:	Dry Packing
Sample loading	:	The sample was dissolved in a small amount of the organic solvent, mixed with a small quantity of adsorbent, triturated, dried and then placed gently on top of the column.
Detection	:	Fractions were examined by TLC technique in the same manner as described in section 2.2

2.4.2 Flash Column Chromatography

Adsorbent	:	Silica gel 60 (No. 9385) particle size 0.040-0.063 nm (E. Merck)
Packing method	:	Wet packing: The adsorbent was slurried in the eluent, then poured into a column and allowed to settle.
Sample loading	:	The sample was dissolved in a small amount of the eluent, and then applied gently on top of the column.
Detection	:	Fractions were examined by TLC technique in the same manner as described in section 2.2

2.4.3 Gel Filtration Chromatography

Gel Filter	:	Sephadex LH-20 (Pharmacia Biotech AB)
Packing method	:	Gel filter was suspended in the eluent and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tightly.
Sample loading	:	The sample was dissolved in a small amount of eluent and then applied gently on top of the column.
Detection	:	Fractions were examined by TLC technique in the same manner as described in section 2.2

2.4.4 High Pressure Liquid Chromatography (HPLC)

Column (Semi-preparative)	:	Cosmosil 5C18-AR II (20 × 250 mm)

(Analytical)	:	Cosmosil C18 AR II (4.6 × 15 mm)
Flow rate	:	1. 0.7 ml/min (Analytical)
		2. 3 ml/min (Semi-preparative)
Mobile Phase	:	1. MeOH-H_2O gradient (6:4 → 3:7)
		2. MeOH-H_2O gradient (3:7 → 8:2)
Injection volume	:	1. 0.3 µl (Analytical)
		2. 2 ml (Semi-preparative)
Pump	:	Waters 600
Detector	:	996 photodiode-array
Temperature	:	25 °C

2.5 Spectroscopy

2.5.1 Ultraviolet (UV) Spectra

UV spectra were obtained on a Shimadzu UV-160A spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University) and a Hitachi U-3010 spectrophotometer (Kobe Pharmaceutical University).

2.5.2 Infrared (IR) Spectra

IR spectra (KBr disc and film) were recorded on a Perkin Elmer FT-IR 1760X spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University).

2.5.3 Mass Spectra

Electrospray Ionization Time of Flight (ESI-TOF) mass spectra were obtained on a Micromass LCT mass spectrometer (National Center for Genetic Engineering and Biotechnology, BIOTEC, Thailand), and the electron-impact (EI), high resolution electron impact (HR-EI), secondary ion (SI) (with glycerol for a matrix) and high resolution secondary ion (HR-SI) mass spectra were recorded on a Hitachi M-4100 instrument (Kobe Pharmaceutical University, Kobe, Japan).

2.5.4 Proton and Carbon-13 Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR) Spectra

^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were measured on a Bruker DPX-300 FT-NMR spectrometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were measured on a JEOL JMN-A 500 spectrometer, Varian ^{unity}INOVA spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University), Varian VXR-500 spectrometer (Kobe Pharmaceutical University) and Bruker-AV 500 MHz spectrometer (National Center for Genetic Engineering and Biotechnology, BIOTEC, Thailand).

The solvents for NMR spectra were deuterated chloroform ($CDCl_3$), deuterated methanol (CD_3OD), deuterated acetone (acetone-d_6) and deuterated dimethylsulfoxide (DMSO-d_6). The chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal.

2.5.5 Fluorescence Spectrophotometer

Fluorescence spectra were measured on a Hitachi F-2000 Fluorescence Spectrophotometer (Kobe Pharmaceutical University).

2.6 Physical Properties

2.6.1 Melting Points

Melting points were obtained on a Fisher-John Melting Point Apparatus (Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.6.2 Optical Rotations

Optical rotations were measured on a Perkin-Elmer 341 polarimeter using a sodium lamp operating at 589 nm (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University) and a JASCO DIP-370 digital polarimeter (Kobe Pharmaceutical University, Japan).

3. Extraction and Isolation

3.1 Extraction and Isolation of Compounds from the Leaves of *Ellipeiopsis cherrevensis*

3.1.1 Extraction

The dried leaves (500 g) were ground, then macerated with hexane (4 x 3 L), $CHCl_3$ (4 x 3 L) and MeOH (4 x 3 L), respectively. The filtrate was pooled and evaporated under reduced pressure at temperature not exceeding 40 °C to afford the hexane extract (7.2 g, 1.44% based on dried weight of leaves), $CHCl_3$ extract (15.8 g, 3.16% of dried weight) and MeOH extract (37.0 g, 7.40% of dried weight).

3.1.2 Isolation of Compounds from the $CHCl_3$ Extract of *E. cherrevensis* Leaves

The $CHCl_3$ extract (15.8 g) was redissolved in a small amount of $CHCl_3$, triturated with kieselguhr and dried at room temperature. It was then applied on top of a silica gel column (400 g, 13 x 6 cm) for vacuum liquid column chromatography eluting stepwise with a gradient of hexane-EtOAc (1:0 to 0:1). Each collected fraction was 300 ml the eluates were and examined by TLC (solvent system: hexane-EtOAc = 2:1). Forty-two fractions were collected and fractions with similar chromatographic pattern were combined to yield thirteen fractions (A-01 – A-13, Table 6).

Table 6. Combined fractions from $CHCl_3$ extract of *E. cherrevensis* leaves

Fraction Code	Weight (g)
A-1	0.57
A-2	0.93
A-3	0.60
A-4	0.61
A-5	0.38
A-6	0.33
A-7	0.30
A-8	0.31
A-9	1.12
A-10	2.46

Table 6. (continued)

Fraction Code	Weight (g)
A-11	1.54
A-12	3.17
A-13	0.13

3.1.2.1 Isolation of Compound EC-1

Fraction A-4 (0.61 g), which showed one main spot on TLC (solvent system: hexane-EtOAc = 3:1), yielded a white precipitate after partial evaporation of the solvent. It was recrystallized in a mixture of hexane-EtOAc (2:1) to yield compound EC-1 as colorless needles (61.8 mg, 0.012% yield).

3.1.2.3 Isolation of Compound EC-2

Fraction A-6 (0.33 g) was separated on a Sephadex LH-20 column, using $CHCl_3$-MeOH (2:1) as the eluent, to yield six fractions (A-61 - A-66). All fractions were examined on TLC (solvent system: hexane-EtOAc = 1:1), then fractions which showed yellow spot were combined and repeatedly chromatographed on a Sephadex LH-20 column (1.7 x 25 cm) using MeOH as eluent, to give compound EC-2 as orange amorphous powder (12.7 mg, 0.0025% yield).

3.1.2.2 Isolation of Compound EC-3

Fraction A-8 (0.31 g) was subjected to gel filtration chromatography on a Sephadex LH-20 column, using $CHCl_3$-MeOH (2:1) as eluent. Nine fractions (A-81 – A-89, 20 ml each) were collected and evaporated. Fraction A-85 (247.1 mg) was repeatedly chromatographed on a Sephadex LH-20 column, eluted with $CHCl_3$-MeOH (2:1), to yield compound EC-3 as white amorphous powder (72.3 mg, 0.014% yield).

3.1.2.4 Isolation of Compound EC-4

Fraction A-9 (1.12 g) was separated by a Sephadex LH-20 column, using $CHCl_3$-MeOH (2:1) as eluent. Nine fractions (30 ml each) were collected and combined according to their TLC pattern (solvent system: hexane-EtOAc = 2:1) into five fractions (A-91 - A-95). Fraction A-93 (537.6 mg) was further fractionated by a

silica gel column (50 g, 2.4 x 29 cm) eluted with a mixture of hexane-EtOAc (2:1) to give five combined fractions (A-941 - A-945). Compound EC-4 (145.0 mg, 0.029% yield) was obtained as white amorphous powder from fraction A-945.

3.1.3 Isolation of Compounds from the MeOH Extract of *E. cherrevensis* Leaves

The MeOH extract (20 g) was dissolved in a small volume of MeOH, triturated with kieselgur and dried at room temperature. It was separated by vacuum liquid column chromatography using a sintered glass filter column of silica gel (400 g, 13 x 6 cm). Elution was performed in a polarity gradient manner with mixture of $CHCl_3$-MeOH (1:0 to 0:1). Fifty fractions (300 ml each) were collected, then combined according to TLC pattern (solvent system: $CHCl_3$-MeOH = 2:1) to yield twelve fractions (B-1 - B-12, Table 7).

Table 7. Combined fractions from MeOH extract of *E. cherrevensis* leaves

Fraction Code	Weight (g)
B-1	0.24
B-2	0.58
B-3	0.78
B-4	0.45
B-5	0.57
B-6	0.82
B-7	1.32
B-8	3.88
B-9	3.30
B-10	3.37
B-11	1.63
B-12	2.05

3.1.3.1 Isolation of Compound EC-5

From fraction B-4, compound EC-5 (12.3 mg, 0.0025% yield) was obtained as a yellow amorphous powder which showed one spot on TLC (solvent system: $CHCl_3$-MeOH = 2:1).

3.1.3.2 Isolation of Compound EC-6

Fraction B-7 (1.3 g) was subjected to silica gel column chromatography (75 g, 3 x 26 cm) using gradient solvent mixture of $CHCl_3$-MeOH (2:1 to 0:1) as mobile phase, to give thirty-three fractions (150 ml each). These fractions were collected and combined based on their TLC pattern (solvent system: $CHCl_3$-MeOH = 3:1) to afford nine fractions (B-71 - B-79). Fraction B-75 was repeatedly purified on a Sephadex LH-20 column (1.7 x 25 cm) using MeOH as the elutent, to give compound EC-6 as pale yellow amorphous powder (18.6 mg, 0.0037% yield).

Fraction B-9 (3.3 g) was separated on a silica gel column (200 g, 5 x 23 cm) using gradient solvent mixture of $CHCl_3$-MeOH (2:1 to 0:1) as eluent, to afford fifty-five fractions (300 ml each). The eluates were examined on TLC (solvent system: $CHCl_3$-MeOH = 2:1), then combined according to their similarity into nine fractions (B-91 - B-99). Fraction B-93 was repeatedly purified on a Sephadex LH-20 column (1.7 x 25 cm) using MeOH as the elutent, to give compound EC-6 as pale yellow amorphous powder (29.2 mg, 0.0058% yield).

3.2 Extraction and Isolation of Compounds from the Stems of *Ellipeiopsis cherrevensis*

3.2.1 Extraction

The dried stems (370 g) from the first collection of *E. cherrevensis* were chopped into small pieces, then successively macerated with hexane (4 x 3 L), $CHCl_3$ (4 x 3 L) and MeOH (4 x 3 L), respectively. The filtrate was pooled and evaporated under reduced pressure at temperature not exceeding 40 °C to afford the hexane extract (2.2 g, 0.61% of dried weight), $CHCl_3$ extract (4.1 g, 1.11% of dried weight) and MeOH extract (17.5 g, 4.73% of dried weight).

The dried stems (500 g) from the second collection of this plant were treated in the similar manner as above to afford the hexane extract (4.1, 0.83% of dried weight), $CHCl_3$ extract (4.0 g, 0.8% of dried weight) and MeOH extract (18.2 g, 3.64% of dried weight).

3.2.2 Isolation of Compounds from the $CHCl_3$ Extract of *E. cherrevensis* Stems

The $CHCl_3$ extract (8.1 g) was redissolved in a small amount of $CHCl_3$, triturated with kieselguhr and dried at room temperature. It was then applied on top of

a silica gel column (200 g, 5 x 21 cm). Elution was performed in a polarity gradient manner with mixtures of $CHCl_3$ and MeOH (9:1 to 0:1) to give fifty-two fractions (50 ml each). Each fraction was combined according to its TLC pattern (solvent system: $CHCl_3$-MeOH = 20:1) to yield eight fractions (C-1 – C-8, Table 8).

Table 8. Combined fractions from $CHCl_3$ extract of *E. cherrevensis* stems

Fraction Code	Weight (g)
C-1	0.86
C-2	2.12
C-3	2.00
C-4	0.67
C-5	0.03
C-6	0.18
C-7	0.14
C-8	0.36

3.2.2.1 Isolation of Compound EC-7

Fraction C-2 (2.12 g) was further separated on a silica gel column (75 g, 2.5 x 21 cm) using gradient mixture of $CHCl_3$-acetone (20:1 to 1:1) to give thirty fractions. All fractions were examined by TLC (solvent system: $CHCl_3$-acetone = 1:1), then fractions showing a fluorescent orange spot on TLC (R_f = 0.25, solvent system: $CHCl_3$-acetone = 1:1) under UV light at 265 nm were combined and repeatedly separated on a Sephadex LH-20 column using $CHCl_3$-acetone (2:1) as the eluent, and finally on another Sephadex LH-20 column using MeOH as the eluent to afford compound EC-7 as orange amorphous powder (6.5 mg, 0.0007% yield).

3.2.2 Isolation of Compounds from the MeOH Extract of *E. cherrevensis* Stems

The MeOH extract (17 g) was fractionated by a vacuum silica gel column (400 g, 13 x 6 cm) eluted with mixture of $CHCl_3$ and MeOH (1:0 to 0:1) to afford twenty-one fractions (300 ml each). All fractions were examined on TLC (solvent system: $CHCl_3$-MeOH = 10:1), then combined to give ten fractions (D-1 –D-10, Table 9).

Table 9. Combined fractions from MeOH extract of *E. cherrevensis* stems

Fraction Code	Weight (mg)
D-1	27.0
D-2	67.3
D-3	9.5
D-4	825.3
D-5	820.8
D-6	257.6
D-7	345.5
D-8	522.8
D-9	546.3
D-10	562.4

3.2.2.1 Isolation of Compound EC-7

Fraction D-4 (825.3 mg) was subjected to silica gel column chromatography (20 g, 2.1 x 15 cm) using CH_2Cl_2-MeOH (100:1) to give eight combined fractions (D-41 - D-48). Fraction D-43 (75 mg), which showed orange spot with the same R_f value as compound EC-8, was repeatedly purified on a Sephadex-LH-20 column, using $CHCl_3$-MeOH (2:1) as the eluent, to give compound EC-7 (4.1 mg).

3.2.2.1 Isolation of Compound EC-5

Fraction D-5 (820.8 mg) was separated on a silica gel column (40 g, 2.4 x 17 cm) using gradient mixture of EtOAc-MeOH (1:0 to 0:1) to give twenty fractions (50 ml each). All fractions were examined on TLC (solvent system: EtOAc-MeOH = 100:1), then combined to yield four fractions (D-51 - D-54). Fraction D-52 (37.9 mg), when rinsed with $CHCl_3$-MeOH (2:1), gave compound EC-5 (16.8 mg).

3.3 Extraction and Isolation of Compounds from the Leaves of *Stelechocarpus cauliflorus*

3.3.1 Extraction

Dried leaves of the first collection (1.4 kg) were ground, then macerated with hexane (10 x 7 L), EtOAc (10 x 7 L) and MeOH (10 x 7 L), respectively. The filtrates were pooled and evaporated under reduced pressure at temperature not exceeding 40

°C to afford hexane extract (76.6 g, 5.47% of dried weight of leaves), EtOAc extract (65.6 g, 4.69% of dried weight) and MeOH extract (105.2 g, 7.51% of dried weight).

Dried, ground leaves from the second collection (4.3 kg), which were macerated with hexane (12 x 9 L), EtOAc (12 x 9 L) and MeOH (12 x 9 L), respectively, yielded, after removal of the organic solvent, hexane extract (182.5 g, 4.30% of dried weight), EtOAc extract (136.2 g, 3.17% of dried weight) and MeOH extract (242.3 g, 5.63% of dried weight).

3.3.2 Isolation of Compounds from the Hexane Extract of *S. cauliflorus* Leaves

The first batch of hexane extract (20 g) was redissolved with small amount of hexane, triturated with kieselguhr and dried at room temperature. It was applied on top of a sintered glass filter column of silica gel (400 g), then separated by vacuum liquid column chromatography. Elution was performed in polarity gradient manner with mixtures of hexane and ethyl acetate (1:0 to 0:1). Thirty-six fractions (200 ml per fraction) were collected. Fractions with similar TLC pattern (solvent system: hexane-EtOAc = 2:1) were combined to give ten fractions (E-1 – E-10, Table 10).

Table 10. Combined fractions from the first batch of hexane extract of *S. cauliflorus* leaves

Fraction Code	Weight (g)
E-1	3.25
E-2	0.21
E-3	1.55
E-4	2.56
E-5	2.09
E-6	1.53
E-7	1.08
E-8	1.98
E-9	0.80
E-10	1.47

The second batch of hexane extract (30 g) was fractionated on a silica gel column (1 kg, 9.5 x 22 cm) using mixtures of hexane-EtOAc (5:1 to 0:1) as mobile phase. All ninety-seven fractions (400 ml each) were combined according to their TLC pattern (solvent system: hexane-EtOAc = 2:1) to yield fourteen fractions (F-1 – F-14, Table 11).

Table 11. Combined fractions from the second batch of hexane extract of *S. cauliflorus* leaves

Fraction Code	Weight (g)
F-1	2.19
F-2	1.36
F-3	0.95
F-4	3.36
F-5	1.40
F-6	4.27
F-7	1.30
F-8	1.39
F-9	0.94
F-10	0.98
F-11	1.37
F-12	0.74
F-13	2.10
F-14	0.75

3.3.2.1 Isolation of Compound SC-1

Fraction E-7 (1.08 g) was subjected to silica gel column chromatography (40 g, 2.3 x 18 cm). A mixture of hexane-EtOAc (2:1) was used as the mobile phase. All twenty-six fractions (100 ml each) were combined based on their similar TLC pattern (solvent system: hexane-EtOAc = 2:1) into seven fractions (E-71 – E-77). Fraction E-71 (895.1 mg) was crystallized in a mixture of hexane-EtOAc (2:1) to yield compound SC-1 (282 mg, 0.02% yield) as white amorphous powder.

3.3.2.2 Isolation of Compound SC-2

Fraction F-4 (3.36 g) was re-chromatographed on a silica gel column (75 g, 2.9 x 24 cm) eluting with a mixture of hexane-acetone (20:1) to give thirty-six fractions (50 ml each). Similar fractions were combined, after being examined by TLC (solvent system: hexane-acetone = 10:1), into five fractions (F-41 – F-45). Fraction F-43 (954.8 mg) was further purified on a Sephadex LH-20 column using $CHCl_3$-MeOH (2:1) as the eluent. Ten fractions (15 ml per fraction) were collected, then combined according to their TLC pattern (solvent system: hexane-acetone = 10:1) into five fractions (F-431 – F-435). Compound SC-2 (20.7 mg, 0.0015% yield) was obtained as light brown oil from fraction F-434.

3.3.2.3 Isolation of Compound SC-3

White amorphous powder (489.8 mg) which precipitated from fraction F-6 displayed one major (R_f = 0.20) and one minor spot (R_f = 0.33) on TLC (solvent system: hexane-acetone = 4:1). Both spots fluoresced under UV light at 254 nm and turned pink after spraying with 10% sulfuric acid and heated. This white precipitate was divided into two portions: F-61 (240.3 mg) and F-62 (249.5 mg).

Portion F-61 was fractionated by silica gel column chromatography (15 g, 2 x 19 cm), eluted with a mixture of hexane-EtOAc (100:1 to 10:1). The eluates (62 fractions, 50 ml each) were collected and combined according to their TLC pattern (solvent system: hexane-acetone = 4:1) into five fractions (F-611 – F-615). Fraction F-615, which exhibited a pink spot when detected with 10% sulfuric acid reagent, gave compound SC-3 (33.0 mg) as colorless crystals.

Portion F-62 (249.5 mg) was subjected to silica gel column chromatography (40 g, 2.3 x 17 cm) using mixtures of hexane-acetone (4:1 to 1:1) as the mobile phase. Fourteen fractions (50 ml each) were collected and combined according to their behavior on TLC (solvent system: hexane-acetone = 4:1) into 3 fractions (F-621 – F-623). After removal of the solvent, fraction F-622 yield an additional quantity of compound SC-3 (182.8 mg, 0.015% total yield).

3.3.2.4 Isolation of Compound SC-4

Fraction F-612 (3.6 mg) was further separated by preparative TLC, using a mixture of hexane-acetone (4:1) as the mobile phase (double development). Compounds SC-3 (1.2 mg, R_f = 0.41) and SC-4 (2.1 mg, R_f = 0.23) were obtained.

Fraction F-621 (9.4 mg) was also subjected to preparative TLC, developing twice with a mixture of hexane-acetone (4:1) as the mobile phase. Additional quantities of compounds SC-3 (5.5 mg, R_f = 0.43) and SC-4 (3.3 mg, R_f = 0.29) were purified.

3.3.3 Isolation of Compounds from the EtOAc Extract of *S. cauliflorus* Leaves

The first batch of EtOAc extract (30 g) was redissolved in a small amount of hexane, triturated with kieselguhr and dried at room temperature. It was applied on top of a silica gel column (400 g), then fractionated by quick column chromatography. Elution was performed in a polarity gradient manner with the mixtures of hexane and EtOAc (1:0 to 0:1). A total of sixty-six fractions (200 ml each) were collected. Fractions with similar TLC pattern (solvent system: hexane-EtOAc = 3:1) were combined into eleven fractions (G-1 – G-11, Table 12).

Table 12. Combined fractions from the first batch of EtOAc extract of *S. cauliflorus* leaves

Fraction Code	Weight (g)
G-01	1.34
G-02	0.38
G-03	4.29
G-04	1.11
G-05	2.56
G-06	1.42
G-07	0.95
G-08	1.38
G-09	4.22
G-10	3.04
G-11	9.22

The second batch of EtOAc extract (25 g) was dissolved in $CHCl_3$ and partitioned with water (5 x 100 ml) to give an aqueous extract. The aqueous extract was partitioned with EtOAc (8 x 50 ml) to yield EtOAc extract (3.0 g), which was

fractionated on a silica gel column (2.4 x 30 cm), eluted with gradient mixture of $CHCl_3$ and MeOH (1:0 to 4:1). Two hundred and seven fractions (50 ml each) were collected and subsequently combined according to their TLC behavior (solvent system: $CHCl_3$-MeOH = 4:1) to yield seventeen fractions (H-1 – H-17, Table 13).

Table 13. Combined fractions from the second batch of EtOAc extract of *S. cauliflorus* leaves

Fraction Code	Weight (mg)
H-1	12.0
H-2	267.1
H-3	103.1
H-4	128.7
H-5	78.6
H-6	171.9
H-7	66.0
H-8	72.0
H-9	86.2
H-10	110.6
H-11	29.0
H-12	501.2
H-13	43.1
H-14	67.2
H-15	52.4
H-16	110.4
H-17	420.3

3.3.3.1 Isolation of Compound SC-5

Fraction G-10 (1.35 g) was subjected to silica gel column chromatography (2.2 x 30 cm) eluted with gradient mixtures of $CHCl_3$ and MeOH (1:0 to 1:1). Seventy-eight fractions (40 ml each) were collected and combined based on their TLC pattern (solvent system: $CHCl_3$-MeOH = 5:1) to give fifteen fractions (G-1001 – G-1015). Fraction G-1006 (207.1 mg) was further fractionated on another silica gel column (15 g, 1.7 x 16 cm) eluting with gradient mixtures of EtOAc and MeOH from

1:0 to 49:1. Thirty-one fractions (15 ml per fraction) were collected and then combined according to their TLC behavior (solvent system: EtOAc-MeOH = 99:1) to yield seven fractions: G-10061 – G-10067.

Fraction G-10063 (115.7 mg) was repeatedly purified by preparative HPLC using an ODS column (Cosmosil 5C18-AR II, 20 × 250 mm, flow rate 4 ml/min, detected at 280 nm) with water-MeOH (3:2 to 3:7) as the eluent to give compound SC-5 as light red amorphous powder (29.7 mg, 0.0021% yield).

3.3.3.2 Isolation of Compound SC-6

Fraction H-12 (501.2 mg) was fractionated by preparative HPLC using an ODS column (Cosmosil 5C18-AR II, 20 × 250 mm, flow rate 3 ml/min, detected at 280 nm) with water-MeOH (7:3 to 1:4) as the eluent to give sixteen fractions. Recrystallization of fraction H-1207 in MeOH gave compound SC-6 as light red crystals (88.8 mg, 0.0021% yield).

3.4 Extraction and Isolation of Compounds from the Stems of *Stelechocarpus cauliflorus*

3.4.1 Extraction

Dried, chopped stems of *S. cauliflorus* from the first collection (5.2 kg) was successively macerated with hexane (7 x 11 L), EtOAc (7 x 11 L) and MeOH (7 x 11 L). The filtrates were pooled and evaporated under reduced pressure at temperature not exceeding 40 °C to afford the hexane extract (72.2 g, 1.39% based on dried weight of stems), EtOAc extract (100.3 g, 1.93% of dried weight) and MeOH extract (149.2 g, 2.87% of dried weight).

Similar treatment of the second batch of *S. cauliflorus* stems (1.8 kg) yielded the hexane extract (57.1 g, 3.18% of dried weight), EtOAc extract (27.2 g, 1.51% of dried weight) and methanol extract (80.3 g, 4.46% of dried weight).

3.4.2 Isolation of Compounds from the Hexane Extract of *S. cauliflorus* Stems

The second batch of hexane extract (30 g) was dissolved in a small amount of hexane, triturated with kieselguhr and dried at room temperature. It was then separated by vacuum liquid column chromatography (silica gel 500 g, 13 x 8 cm),

eluted stepwise with mixtures of hexane and EtOAc (1:0 to 0:1). Forty-two fractions (200 ml each) were collected, then combined according to their TLC patterns (solvent system: hexane-EtOAc = 3:1) to yield ten fractions (I-1 – I-10, Table 14).

Table 14. Combined fractions from the second batch of hexane extract of *S. cauliflorus* stems

Fraction Code	Weight (g)
I-1	13.0
I-2	1.50
I-3	1.65
I-4	0.48
I-5	1.06
I-6	1.68
I-7	1.74
I-8	2.60
I-9	5.40
I-10	1.05

3.4.2.1 Isolation of Compound SC-4

Fraction I-6 (335.8 mg) was further separated on a silica gel column (75 g, 3.2 x 20 cm) eluted with mixtures of hexane and EtOAc (9:1 to 1:1). The eluates (40 ml each) were examined by TLC (solvent system: hexane-EtOAc = 4:1) and then combined to yield seven fractions: I-61 – I-67. Fraction I-64 (211.8 mg) was fractionated on a silica gel column (10 g, 2 x 12 cm). Elution was performed in a polarity gradient manner with hexane-EtOAc (90:1 to 85:15) to give six fractions (I-641 – I-646). Further purification of fraction I-642 (79.8 mg) by silica gel column chromatography (10 g, 1.5 x 17 cm), eluted with hexane-acetone (5:1) yield compound SC-4 (12.1 mg).

The same compound was also obtained when fraction I-7 (1.33 g) was subjected to silica gel column chromatography (75 g, 2.9 x 20 cm) using gradient mixtures of hexane-acetone (90:1 to 3:7) as eluent, to afford eighty-one fractions (50 ml each). Similar fractions, after being examined by TLC (solvent system: CH_2Cl_2-acetone = 4:1), were combined into eleven fractions: I-701 – I-711. Fraction I-707

(70.5 mg) was subjected to another silica gel column (10 g, 2 x 12 cm) using hexane-acetone (19:1) as the mobile phase. Sixteen fractions (20 ml each) were combined based on TCL pattern (solvent system: hexane-acetone = 4:1) into three main fractions (I-7071 – I-7073). Fraction I-7072 (65 mg), which showed a major pink spot upon detection with 10% sulfuric acid, was further purified by preparative TLC using hexane-acetone (4:1) as the mobile phase (double developing) to give compound SC-4 (63.3 mg, 0.0042% total yield).

3.4.2.2 Isolation of Compound SC-7

White amorphous powder (92.4 mg), which precipitated from fraction I-9, appeared as a single spot on TLC (solvent system: hexane-EtOAc = 4:1) when visualized under UV wavelength of 254 nm and turned pink upon 10% sulfuric acid detection.

3.4.3 Isolation of Compounds from the EtOAc Extract of *S. cauliflorus* Stems

The first batch of EtOAc extract (20 g) was re-dissolved with a small amount of hexane, triturated with kieselguhr and dried at room temperature. It was then fractionated by vacuum liquid column chromatography using a sintered glass filter column of silica gel (350 g, 13 x 6 cm). Eluation was performed in a polarity gradient manner with mixtures of $CHCl_3$ and MeOH (1:0 to 0:1). Thirty-two fractions (200 ml each) were collected and examined by TLC (solvent system: $CHCl_3$-MeOH = 20:1). Fractions with similar chromatographic pattern were combined to yield twelve fractions (J-1 – J-12, Table 15).

Table 15. Combined fractions from the first batch of EtOAc extract of *S. cauliflorus* stems

Fraction Code	Weight (g)
J-1	1.66
J-2	0.78
J-3	0.53
J-4	0.29
J-5	0.27
J-6	0.24
J-7	1.18
J-8	8.10
J-9	0.80
J-10	1.66
J-11	1.23
J-12	1.59

The second batch of EtOAc extract (20 g) was similarly fractionated on a silica gel column (600 g, 9.5 x 17 cm) eluted with gradient mixtures of hexane and EtOAc (5:1 to 1:1). Combination of similar fractions after TLC examination (solvent system: hexane-EtOAc = 4:1) yielded nineteen major fractions (K-01 – K-19, Table 16).

Table 16. Combined fractions from the second batch of EtOAc extract of *S. cauliflorus* stems

Fraction Code	Weight (g)
K-01	2.40
K-02	0.81
K-03	0.75
K-04	0.28
K-05	1.28
K-06	0.28
K-07	0.34

Table 16. (continued)

Fraction Code	Weight (g)
K-08	0.90
K-09	0,04
K-10	0.29
K-11	0.88
K-12	1.18
K-13	0.14
K-14	0.25
K-15	0.56
K-16	0.27
K-17	0.89
K-18	0.21
K-19	0.68

3.4.3.1 Isolation of Compound SC-8

Fraction J-8 (8.10 g) was fractionated on a silica gel column (180 g, 5 x 22 cm), eluted with gradient mixtures of CH_2Cl_2 and MeOH (100:1 to 60:40). Ninety-one fractions (50 ml each) were collected, then combined according to their TLC pattern (solvent system: CH_2Cl_2-MeOH = 5:1) into eleven fractions: J-81 - J-811. Fraction J-89 (441.4 mg) was further separated on another silica gel column (40 g, 2.5 x 20 cm), using hexane-acetone (10:3) as the mobile phase to yield fifty-six fractions (20 ml each). TLC examination (solvent system: CH_2Cl_2-MeOH = 100:1) led to combination of these into twelve fractions. Gel filtration chromatography of fraction J-8910 (12.8 mg) on a Sephadex LH-20 column washed down with $CHCl_3$-MeOH (2:1) gave compound SC-8 as pale yellow amorphous powder (5.1 mg, 0.0001% yield).

3.4.3.2 Isolation of Compound SC-9

Fraction J-9 (798.9 mg) was separated on a silica gel column (40 g, 2.5 x 20 cm) eluted with gradient mixtures of CH_2Cl_2 and MeOH (20:1 to 1:1) to give thirty-five fractions (20 ml each). Similarity of TLC pattern (solvent system: CH_2Cl_2-

MeOH = 10:1) led to nine combined fractions (J-91 – J-99). Fraction J-95 was repeatedly purified on a Sephadex LH-20 column (2.8 x 86 cm) using $CHCl_3$-MeOH (2:1) as the eluent to give compound SC-9 as yellow amorphous powder (11.0 mg, 0.0002% yield).

Compound SC-9 was also found precipitated as a yellow amorphous powder (39.4 mg, 0.0022% yield) from fraction K-08.

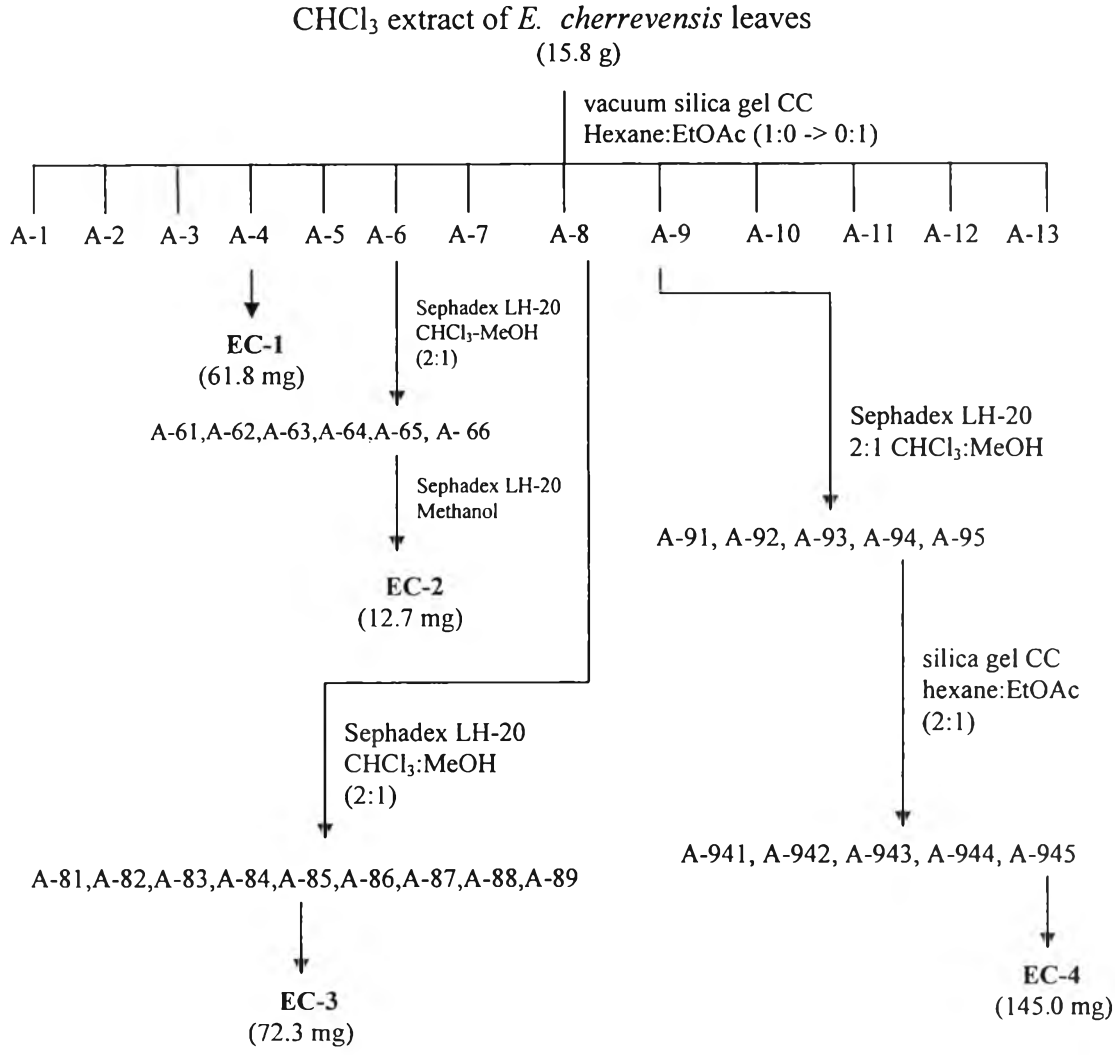
3.4.3.3 Isolation of Compound SC-10

Fraction K-05 (1.28 g) was subjected to silica gel column chromatography (40 g, 2.4 x 20 cm) using gradient mixtures of 5-15% acetone in CH_2Cl_2 to yield forty-eight fractions (50 ml each). These fractions were combined according to their TLC pattern (solvent system: CH_2Cl_2-acetone = 10:1) into eight fractions: K-051 – K-058.

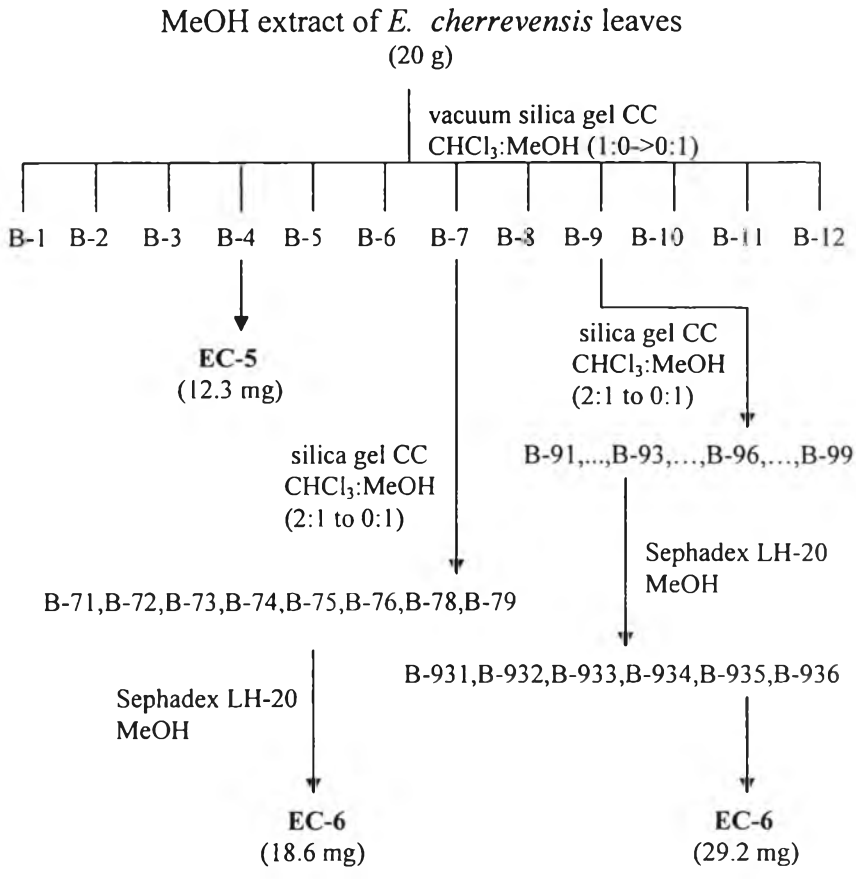
Fraction K-055 (69.6 mg) was further fractionated by a Sephadex LH-20 column eluting with $CHCl_3$-MeOH (2:1). Seventeen 15-ml fractions were collected and combined into three main fractions: K-0551, K-0552 and K-0553. Compound SC-10 (21.7 mg, 0.0012% yield) was obtained from fraction K-0553 as yellow amorphous powder.

3.4.3.4 Isolation of Compound SC-11

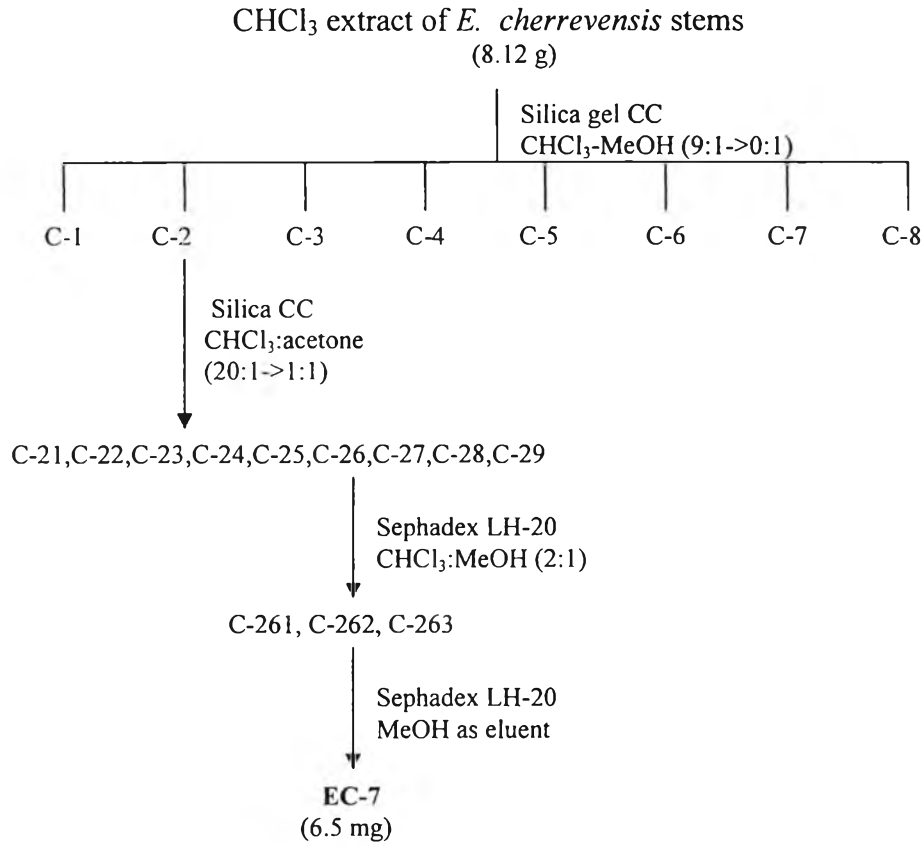
Fraction K-11 (876.3 mg) was fractionated by silica gel column chromatography (75 g, 2.9 x 18 cm) using gradient mobile phase of 5-30% acetone in dichloromethane to afford fifty fractions (100 ml each). After each fraction was examined by TLC (solvent system: CH_2Cl_2-MeOH = 20:1), they were combined into eight fractions: K-111 – K-118. Fraction K-115 (160.9 mg) was repeatedly purified on a Sephadex LH-20 column with $CHCl_3$-MeOH (2:1) as the eluent to give compound SC-11 as orange amorphous powder (4.1 mg, 0.0002% yield).



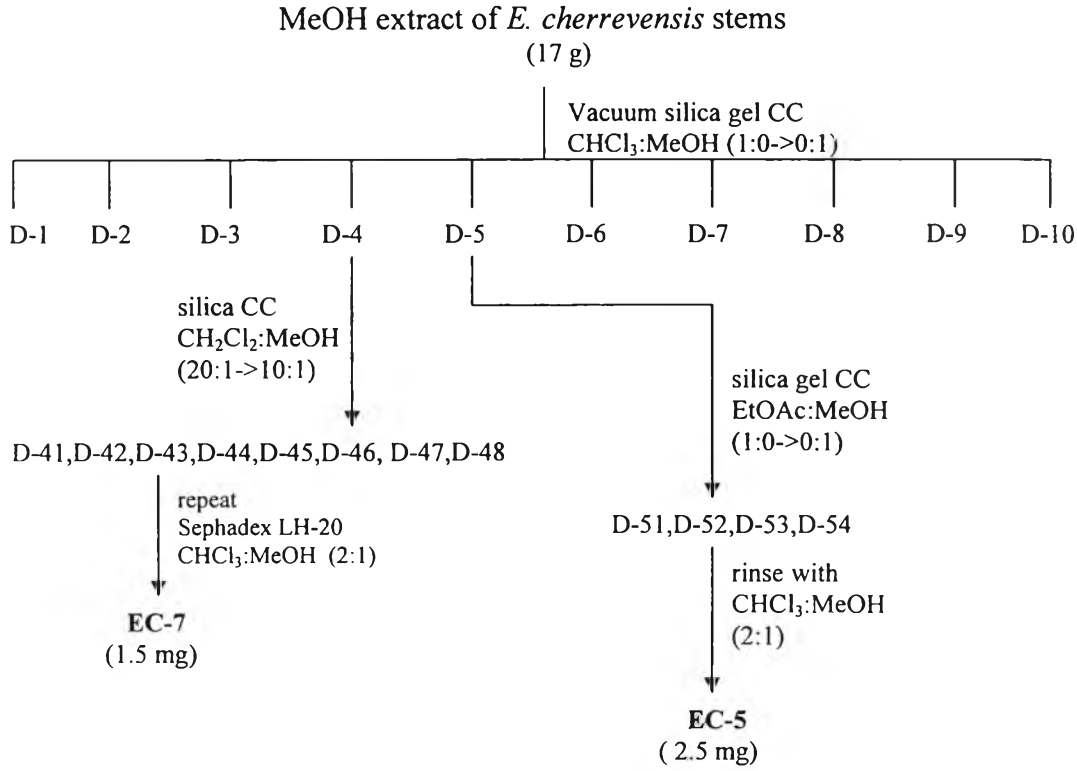
Scheme 1. Separation of $CHCl_3$ extract from the leaves of *Ellipeiopsis cherrevensis*



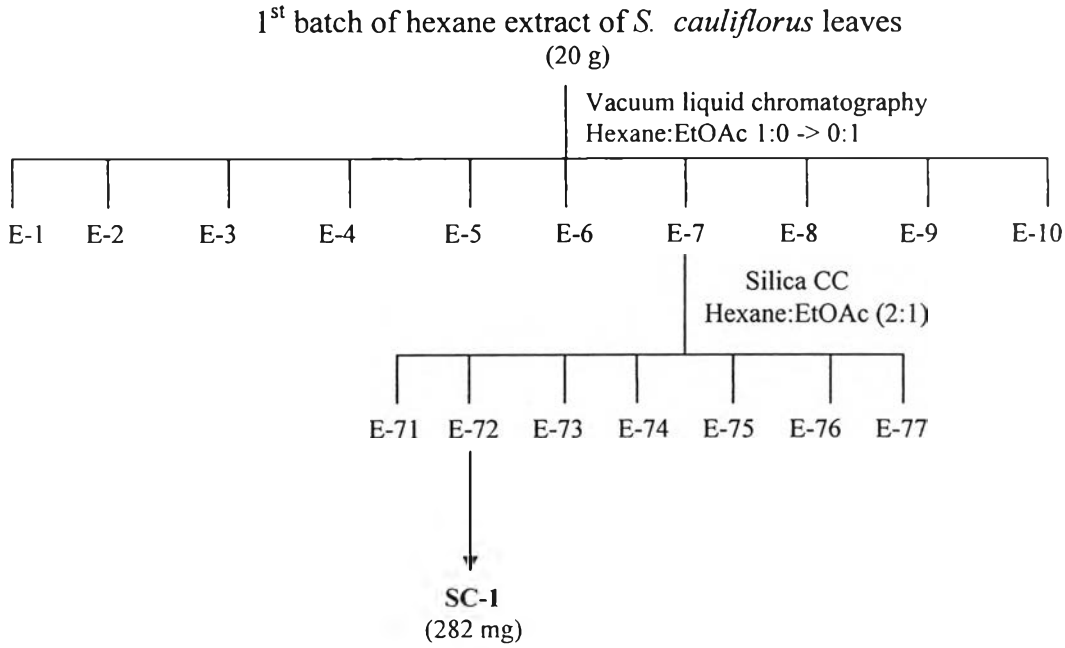
Scheme 2. Separation of MeOH extract from the leaves of *E. cherrevensis*



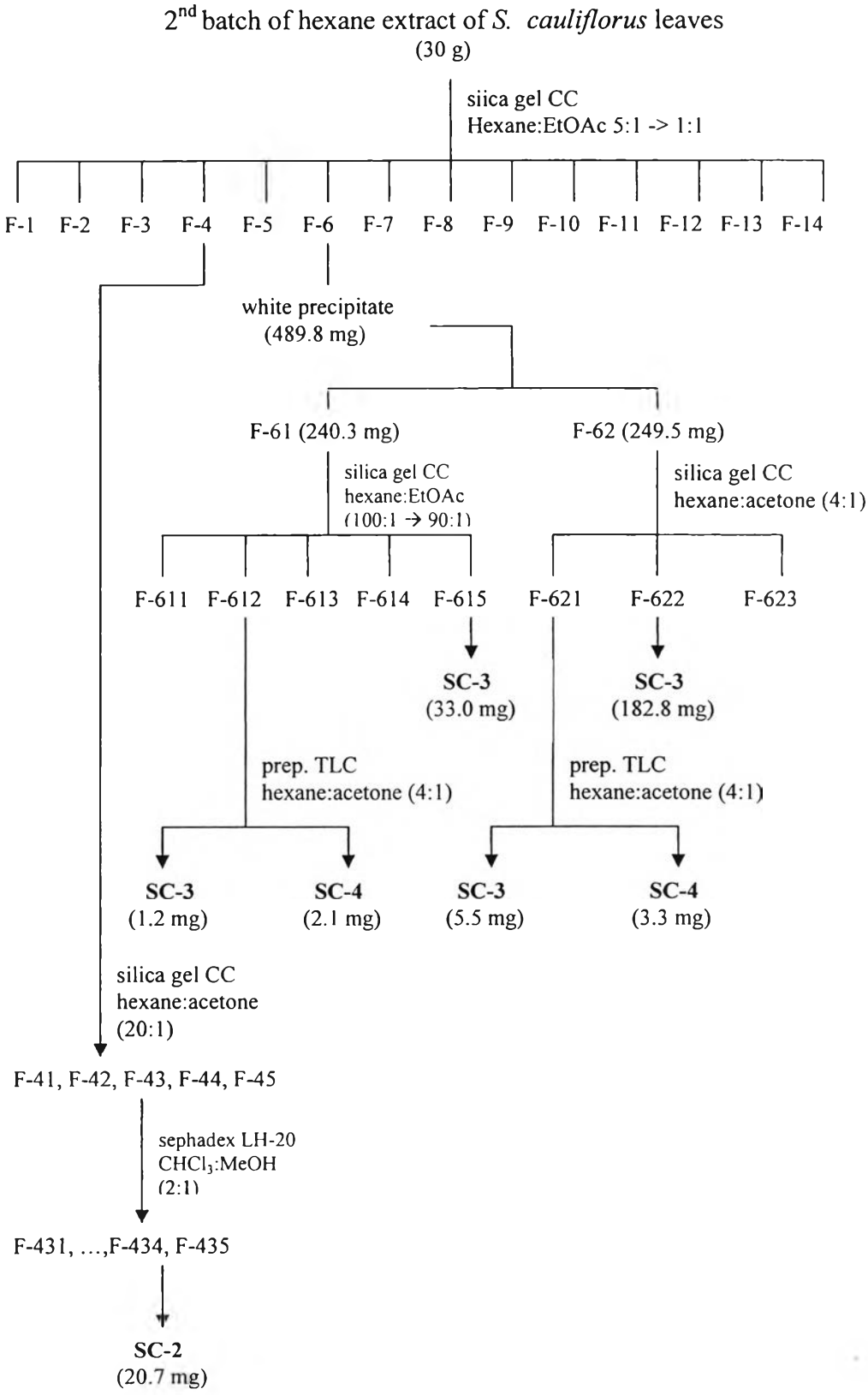
Scheme 3. Isolation of compound EC-8 from the $CHCl_3$ extract of the stems of *E. cherrevensis*



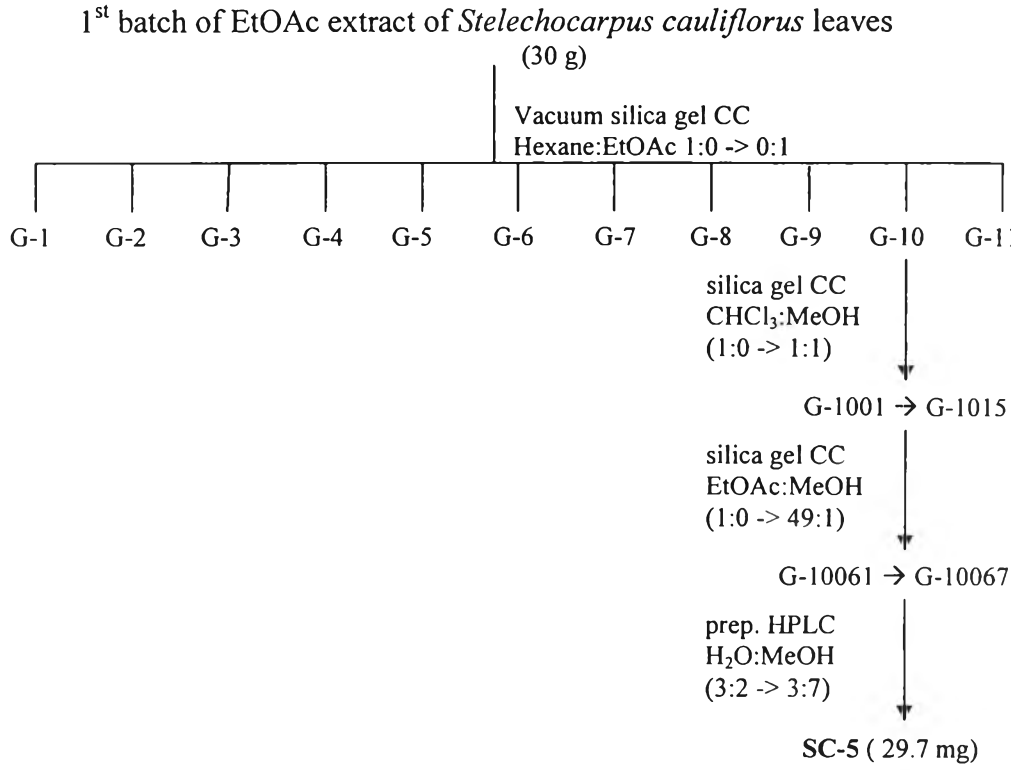
Scheme 4. Separation of MeOH extract from the stems of *Ellipeiopsis cherrevensis*



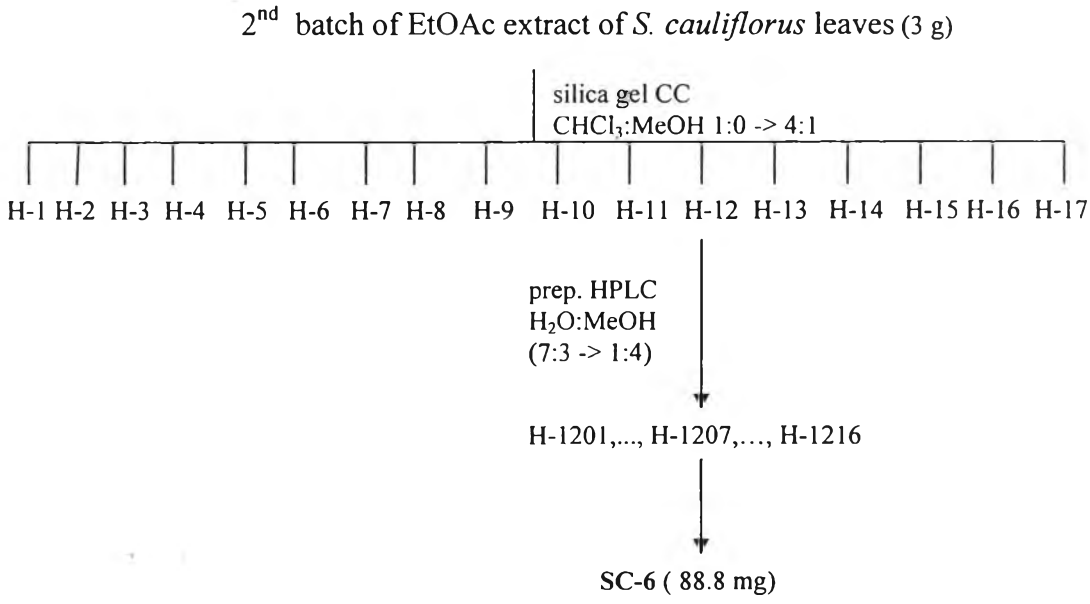
Scheme 5. Separation of the first batch of hexane extract from the leaves of *S. cauliflorus*



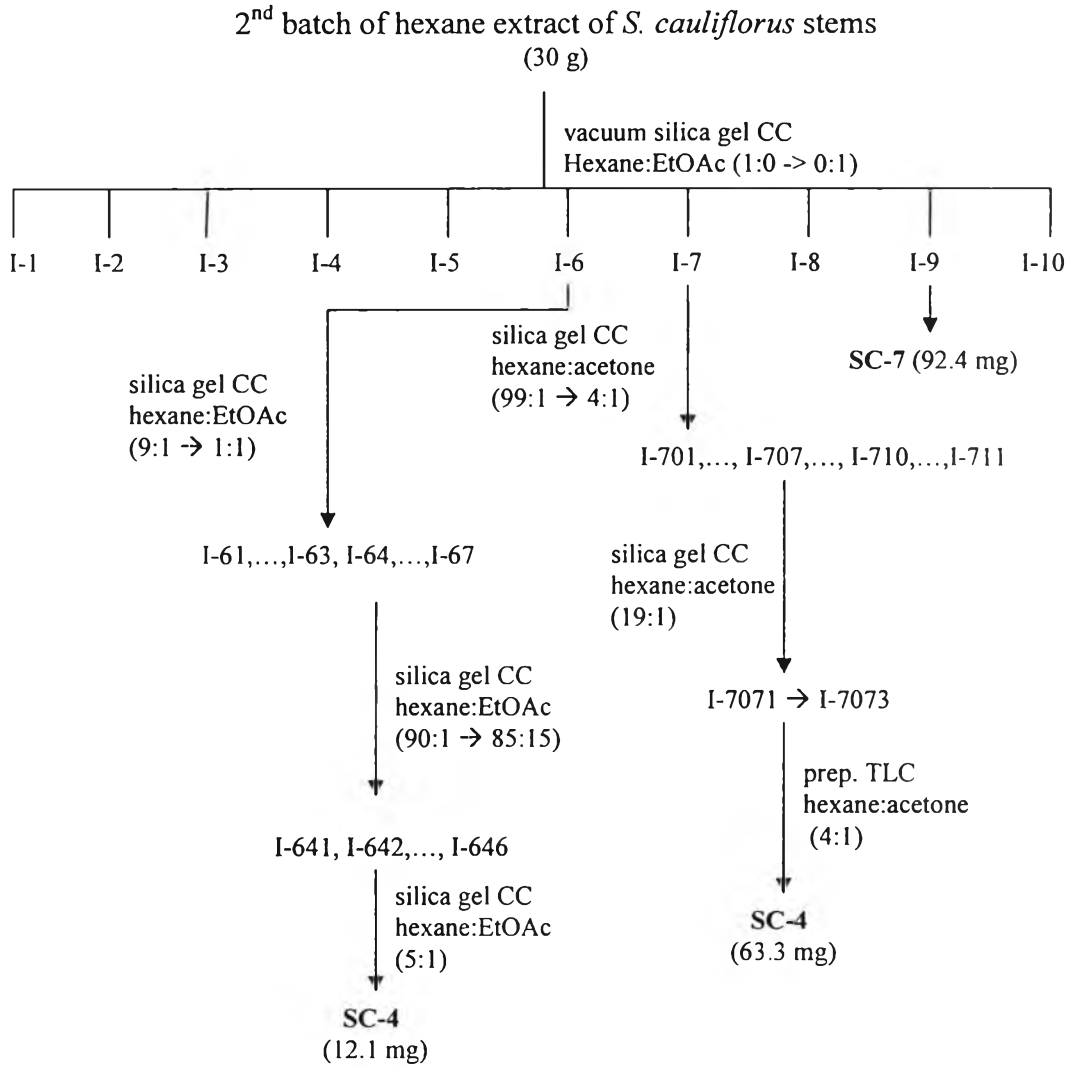
Scheme 6. Separation of the second batch of hexane extract from the leaves of *S. cauliflorus*



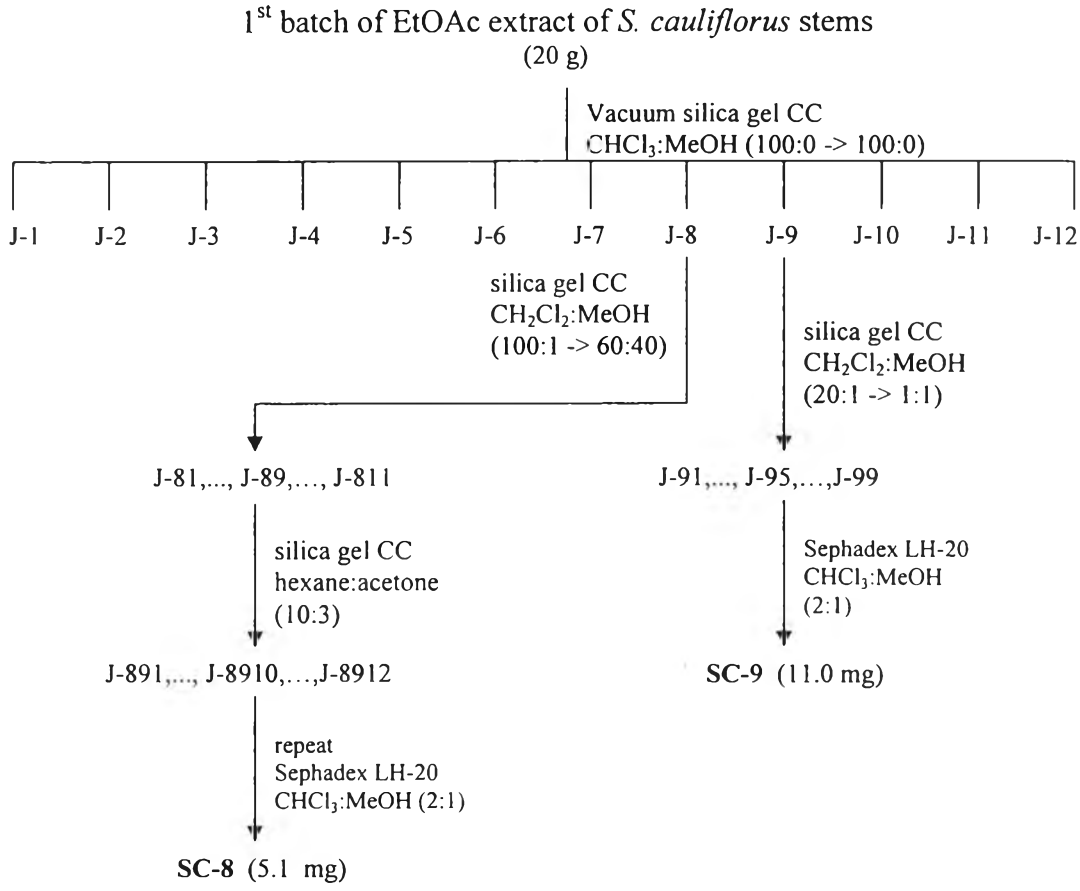
Scheme 7. Separation of the first batch of EtOAc extract from the leaves of *S. cauliflorus*



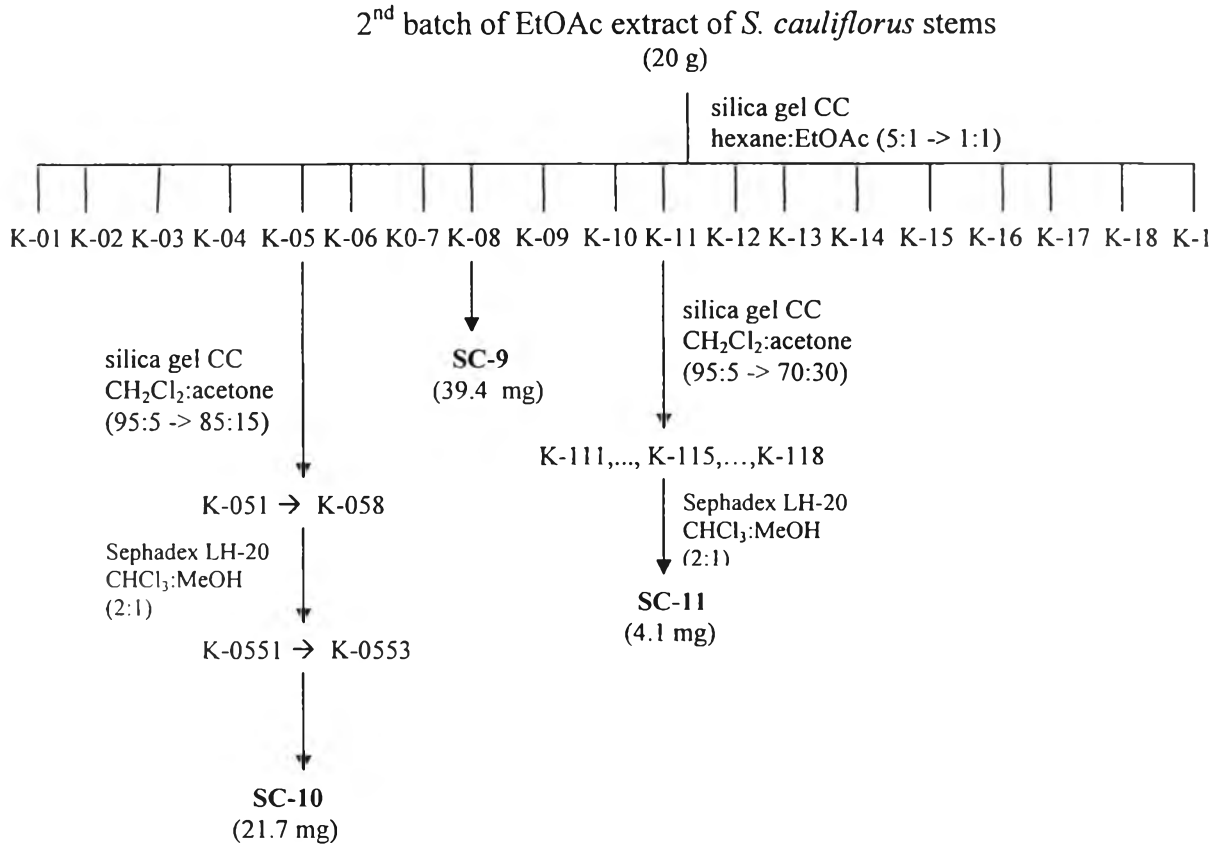
Scheme 8. Separation of the second batch of EtOAc extract from the leaves of *S. cauliflorus*



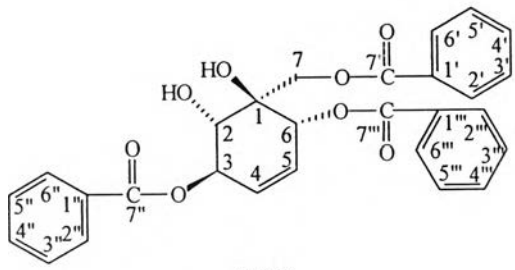
Scheme 9. Separation of the second batch of hexane extract from the stems of *S. cauliflorus*



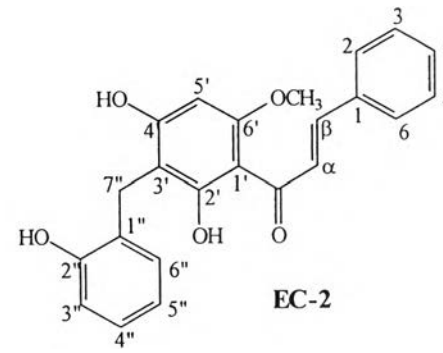
Scheme 10. Separation of the first batch of EtOAc extract from the leaves of *S. cauliflorus*



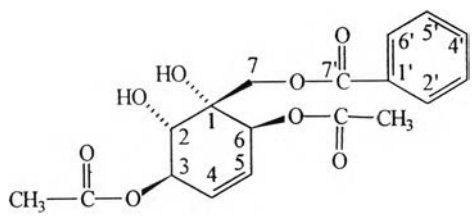
Scheme 11. Separation of the second batch of EtOAc extract from the stems of *S. cauliflorus*



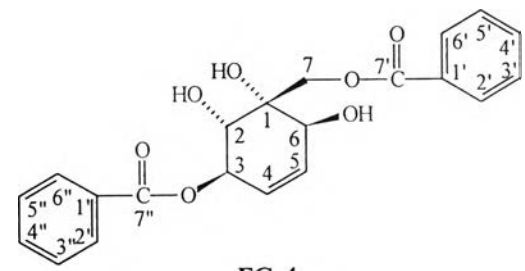
EC-1



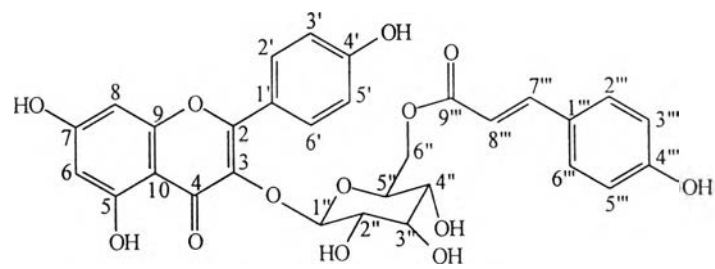
EC-2



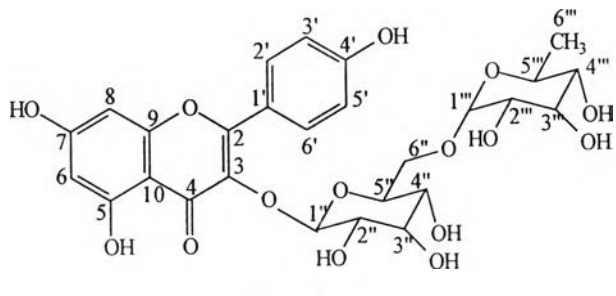
EC-3



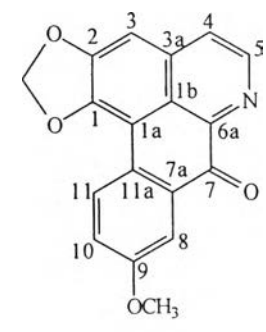
EC-4



EC-5

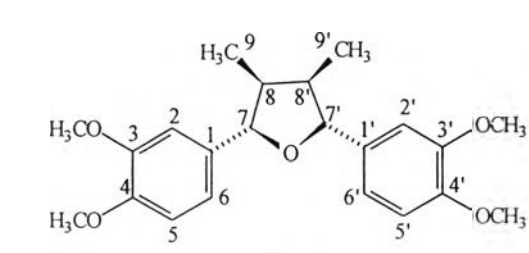


EC-6

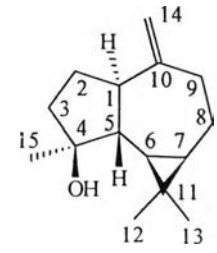


EC-7

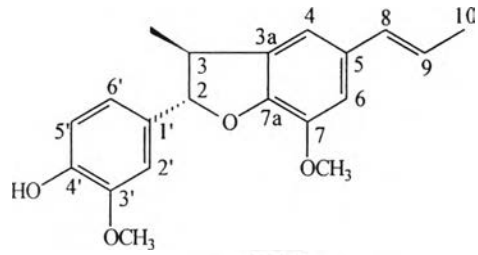
Figure 8. Structure of compounds isolated from aerial parts of *E. cherrevensis*



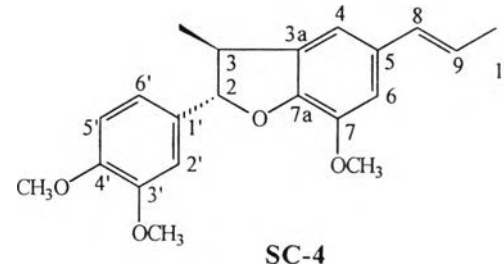
SC-1



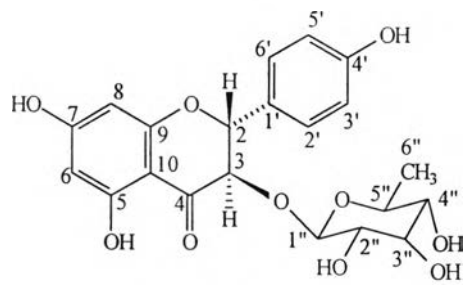
SC-2



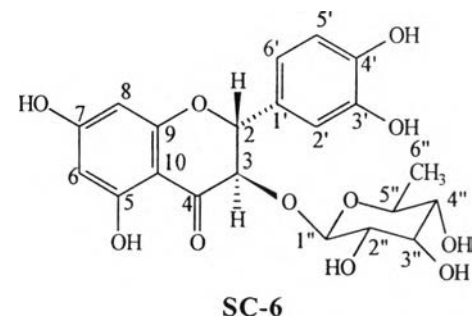
SC-3



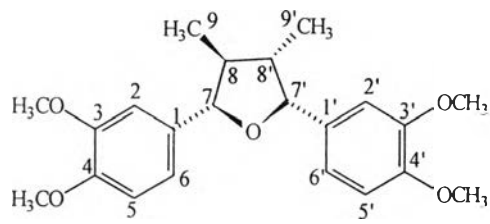
SC-4

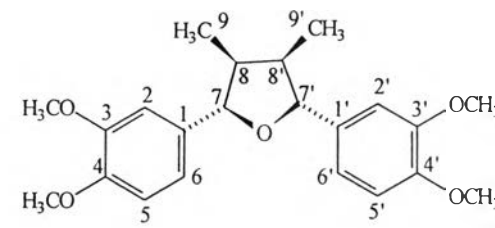


SC-5

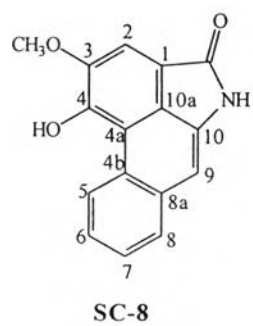


SC-6

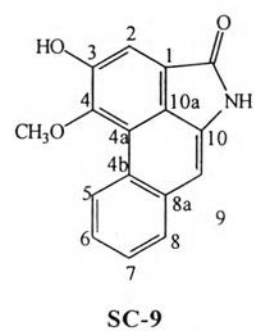




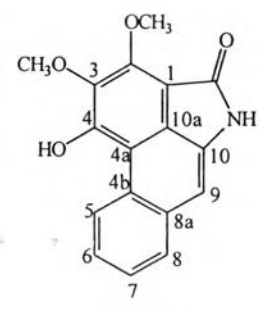
SC-7



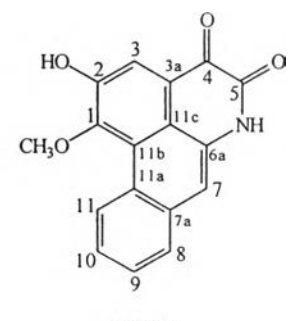
SC-8



SC-9



SC-10



SC-11

Figure 9. Structure of compounds isolated from aerial parts of *S. cauliflorus*

4. Physical and Spectral Data of Isolated Compounds

4.1 Compound EC-1

Compound EC-1 was obtained as white needle crystals (61.8 mg, 0.012 % based on dried weight of leaves).

CIMS : *m/z*; 488 $[M]^+$; see **Figure 12**

mp : 182 – 183 °C

$[\alpha]^{25}_D$: -189.5° (*c* 0.22, $CHCl_3$)

UV : λ_{max} nm (log ε), in $CHCl_3$; 243 (4.35), 276 (3.65); see **Figure 10**

IR : ν_{max} cm^{-1}, KBr; 3449, 1716, 1272, 1112, 712; see **Figure 11**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$;
4.29 (1H, *d*, *J* = 8.5 Hz), 4.74 (1H, *d*, *J* = 12.0 Hz), 4.80 (1H, *d*, *J* = 12.0 Hz), 5.80-5.84 (2H, *m*), 5.86 (1H, *dt*, *J* = 10.3, 2.0 Hz), 5.98 (1H, *dt*, *J* = 10.3, 2.0 Hz), 7.30 (2H, *tt*, *J* = 8.0, 1.0 Hz), 7.32 (2H, *tt*, *J* = 8.0, 1.5 Hz), 7.44 (2H, *tt*, *J* = 7.8, 1.2 Hz), 7.47 (1H, *tt*, *J* = 8.0, 1.0 Hz), 7.49 (1H, *tt*, *J* = 8.0, 1.0 Hz), 7.57 (1H, *tt*, *J* = 7.8, 1.2 Hz), 7.90 (2H, *dd*, *J* = 8.0, 1.0 Hz), 7.97 (2H, *dd*, *J* = 7.8, 1.2 Hz), 8.07 (2H, *dd*, *J* = 8.0, 1.5 Hz).

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$;
62.7, 72.9, 75.5, 76.1, 76.7, 127.9, 128.3, 128.4, 128.5, 129.0, 129.4, 129.6, 129.7, 129.9, 130.0, 133.1, 133.4, 133.6, 166.5, 166.6, 167.2

4.2 Compound EC-2

Compound EC-2 was obtained as orange needle crystal (12.7 mg, 2.54 x 10^{-3} % based on dried weight of leaves).

HRESIMS : *m/z*; 376.1311 (calcd. for $C_{23}H_{20}O_5$: 376.1305); see **Figure 22**

mp : 179 – 181 °C

UV : λ_{max} nm (log ε), in MeOH; 243 (3.92), 347 (4.31); see **Figure 20**

IR : ν_{max} cm^{-1}, KBr; 3272, 1627, 1449, 1338, 1235, 1115, 756, 568; see **Figure 21**

^{1}H NMR : δ ppm, 500 MHz, in Acetone-d_6;
3.90 (2H, *s*), 3.95 (3H, *s*), 6.19 (1H, *s*), 6.73 (1H, *ddd*, *J* = 7.6, 7.6, 1.2 Hz), 6.82 (1H, *dd*, *J* = 7.6, 1.2 Hz), 6.99 (1H, *ddd*, *J* = 7.6, 7.6, 1.2 Hz),

7.25 (1H, *dd*, *J* = 7.6, 1.2 Hz), 7.45 (3H, *m*), 7.72 (2H, *dd*, *J* = 8.2, 1.8 Hz), 7.83 (1H, *d*, *J* = 15.6 Hz), 8.04 (1H, *d*, *J* = 15.6 Hz), 15.04 (1H, *s*)

^{13}C NMR : δ ppm, 125 MHz, in Acetone-d_6;
22.9, 56.3, 92.4, 106.1, 108.1, 116.0, 120.6, 127.8, 127.9, 128.5, 129.2, 129.8, 130.9, 131.2, 136.5, 142.7, 155.2, 162.6, 164.2, 166.4, 193.2

4.2 Compound EC-3

Compound EC-3 was obtained as white amorphous powder (64.3 mg, 0.92 % based on dried weight of leaves).

HRESIMS : *m/z*; 364.1159 (calcd. for $C_{18}H_{20}O_8$: 364.1152); see **Figure 32**

mp : 138 – 140 °C

$[\alpha]^{25}_D$: -107.9° (*c* 0.24, $CHCl_3$)

UV : λ_{max} nm (log ε), in $CHCl_3$; 243 (3.96), 275 (3.31); see **Figure 30**

IR : ν_{max} cm^{-1}, KBr; 3464, 1722, 1275, 1116, 714; see **Figure 31**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$;
2.03 (3H, *s*), 2.05 (3H, *s*), 4.04 (1H, *d*, *J* = 7.0 Hz), 4.46 (1H, *d*, *J* = 12.0 Hz), 4.76 (1H, *d*, *J* = 12.0 Hz), 5.44 (1H, *d*, *J* = 4.3 Hz), 5.49 (1H, *m*), 5.79 (1H, *dd*, *J* = 10.0, 2.0 Hz), 5.86 (1H, *ddd*, *J* = 10.0, 4.3, 2.0 Hz), 7.42 (2H, *dd*, *J* = 7.8, 1.3 Hz), 7.55 (1H, *m*), 7.98 (2H, *dd*, *J* = 7.8, 1.3 Hz)

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$;
21.1, 66.5, 70.2, 71.3, 73.1, 74.7, 125.9, 128.5, 128.8, 129.7, 129.8, 133.4, 167.0, 171.7, 179.0

4.4 Compound EC-4

Compound EC-4 was obtained as an white amorphous powder, soluble in $CHCl_3$ (145.4 mg, 0.029 % based on dried weight of leaves).

ESIMS : *m/z*; 384 $[M]^+$; see **Figure 42**

mp : 104 – 105 °C

$[\alpha]^{25}_D$: -113.2° (*c* 0.25, $CHCl_3$)

UV : λ_{max} nm (log ε), in $CHCl_3$; 243 (4.19), 276 (3.54); see **Figure 40**

IR : ν_{max} cm^{-1}, KBr; 3462, 1679, 1281, 1119, 712; see **Figure 41**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$;

4.22 (1H, *d*, *J* = 6.0 Hz), 4.32 (1H, *d*, *J* = 4.0 Hz), 4.72 (1H, *d*, *J* = 12.0 Hz), 4.86 (1H, *d*, *J* = 12.0 Hz), 5.69 (1H, *m*), 5.84 (1H, *ddd*, *J* = 10.3, 2.5, 0.5 Hz), 5.98 (1H, *ddd*, *J* = 10.3, 4.0, 2.0 Hz), 7.36 (4H, *m*), 7.51 (2H, *m*), 7.94 (2H, *dd*, *J* = 8.3, 1.5 Hz), 7.98 (2H, *dd*, *J* = 8.3, 1.5 Hz)

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$;
66.7, 68.6, 70.8, 74.2, 75.9, 126.8, 128.4, 129.2, 129.4, 129.7, 129.8, 133.4, 133.5, 167.1, 167.8

4.5 Compound EC-5

Compound EC-5 was obtained as pale yellow amorphous powder, soluble in MeOH (12.3 mg, 2.46 x 10^{-3} based on dried weight of stems).

ESIMS : *m/z*; 617.1263 $[M+Na]^+$; see **Figure 52**

mp : 182 – 184 °C

UV : λ_{max} nm (log ε), in MeOH; 208 (4.51), 267 (4.33), 348 (4.25); see **Figure 50**

IR : ν_{max} cm^{-1}, KBr; 3422, 1659, 1608, 1510, 1364, 1182, 1064; see **Figure 51**

^{1}H NMR : δ ppm, 300 MHz, in DMSO-d_6;
0.97 (3H, *d*, *J* = 6.0 Hz), 3.03-3.69 (10H, *m*), 5.09 (1H, *d*, *J* = 8.0 Hz), 5.29 (1H, *d*, *J* = 7.2 Hz), 6.18 (1H, *br s*), 6.39 (1H, *br s*), 6.86 (2H, *d*, *J* = 8.4 Hz), 7.97 (2H, *d*, *J* = 8.4 Hz), 12.54 (1H, *br s*)

^{13}C NMR : δ ppm, 75 MHz, in DMSO-d_6;
18.0, 67.0, 68.4, 70.1, 70.5, 70.7, 71.9, 74.3, 75.9, 76.5, 93.9, 98.9, 100.8, 101.4, 103.9, 115.1, 120.9, 130.8, 133.2, 156.4, 156.7, 159.8, 161.1, 164.3, 177.2

4.6 Compound EC-6

Compound EC-6 was obtained as pale yellow amorphous powder, soluble in MeOH (18.6 mg, 3.72 x 10^{-3} % based on dried weight of stems).

ESIMS : *m/z*; 617.1472 $[M+Na]^+$; see **Figure 61**

mp : 218 – 220 °C

UV : λ_{max} nm (log ε), in MeOH; 207 (4.87), 267 (4.67), 313 (4.78); see **Figure 59**

IR : ν_{max} cm^{-1}, KBr; 3460, 1684, 1608, 1502, 1356, 1183, 1068; see **Figure 60**

^{1}H NMR : δ ppm, 300 MHz, in DMSO-d_6; 3.16-3.47 (4H, *m*), 4.08 (1H, *dd*, *J* = 11.1, 5.7 Hz), 4.27 (1H, *d*, *J* = 11.1 Hz), 5.44 (1H, *d*, *J* = 6.6 Hz), 6.10 (1H, *d*, *J* = 16.9 Hz), 6.14 (1H, *d*, *J* = 1.8 Hz), 6.37 (1H, *d*, *J* = 1.8 Hz), 6.78 (2H, *d*, *J* = 7.8 Hz), 6.85 (2H, *d*, *J* = 8.7 Hz), 7.34 (1H, *d*, *J* = 16.9 Hz), 7.35 (2H, *d*, *J* = 7.8 Hz), 7.98 (2H, *d*, *J* = 8.7 Hz), 10.12 (1H, *br s*), 12.56 (1H, *br s*)

^{13}C NMR : δ ppm, 75 MHz, in DMSO-d_6; 63.1, 70.1, 74.2, 76.3, 93.7, 98.8, 101.0, 103.8, 113.6, 115.1, 115.7, 120.7, 124.9, 130.1, 130.7, 133.0, 144.5, 156.2, 159.6, 159.8, 161.0, 164.2, 166.0, 177.2

4.7 Compound EC-7

Compound EC-7 was obtained as orange amorphous powder, soluble in MeOH

ESIMS : *m/z*; 306.0772 $[M+H]^+$; see **Figure 70**

mp : > 300 °C

UV : λ_{max} nm (log ε), in MeOH; 246 (3.06), 263 (2.92), 273 (2.95), 438 (2.30); see **Figure 68**

IR : ν_{max} cm^{-1}, KBr; 2920, 1712, 1604, 1462, 1378, 1341, 1304, 1265, 1229, 1035, 962; see **Figure 69**

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; 3.97 (3H, *s*), 6.33 (2H, *s*), 7.12 (1H, *s*), 7.28 (1H, *d*, *J* = 9.3 Hz), 7.75 (1H, *d*, *J* = 5.1 Hz), 7.98 (1H, *br s*), 8.54 (1H, *d*, *J* = 9.3 Hz), 8.86 (1H, *d*, *J* = 5.1 Hz)

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; 55.8, 102.3, 102.4, 108.2, 110.3, 122.6, 122.7, 124.3, 129.1, 132.9, 135.9, 144.8, 145.3, 148.0, 151.8, 159.8, 182.3

4.8 Compound SC-1

Compound SC-1 was obtained as white amorphous powder, soluble in $CHCl_3$

EIMS : *m/z* (% relative intensity); 372 (M^+, 47.2), 206 (100.0), 165 (47.4),

95 (15.7), 77 (13.5); see **Figure 78**

mp : 115 – 117 °C

$[\alpha]^{25}_{D}$: 0° (*c* 0.25, $CHCl_3$)

UV : λ_{max} nm (log ε), in $CHCl_3$; 232 (3.16), 243 (3.91), 258 (3.32), 281 (3.81); see **Figure 76**

IR : ν_{max} cm^{-1}, KBr; 2955, 2868, 2835, 1595, 1517, 1462, 1412, 1265, 1236, 1142, 1027, 1000, 859, 810, 752; see **Figure 77**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$;
1.04 (6H, *d*, *J* = 6.5 Hz), 2.33 (2H, *m*), 3.88 (12H, *s*), 4.52 (2H, *d*, *J* = 6.5 Hz), 6.85 (2H, *d*, *J* = 8 Hz), 6.97 (4H, *dd*, *J* = 8.5, 2.0 Hz), 6.99 (2H, *d*, *J* = 2.0 Hz)

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$;
12.9, 44.4, 55.8, 87.2, 109.7, 110.9, 118.6, 134.8, 149.0

4.10 Compound SC-2

Compound SC-2 was obtained as light brown oil, soluble in $CHCl_3$

ESIMS : *m/z* ; 243.1723 $[M+Na]^+$; see **Figure 87**

UV : λ_{max} nm (log ε), in $CHCl_3$; 245 (2.95), 232 (2.14); see **Figure 85**

IR : ν_{max} cm^{-1}, KBr; 3375, 2928, 2868, 1673, 1462, 1377, 1097, 1029, 888, 735; see **Figure 86**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$;
0.45 (1H, *t*, *J* = 10.2 Hz), 0.69 (1H, *dd*, *J* = 10.2, 6.9 Hz), 0.94 (1H, *m*), 1.01 (3H, *s*), 1.03 (3H, *s*), 1.26 (3H, *s*), 1.29 (1H, *m*), 1.47 (1H, *m*), 1.61 (1H, *m*), 1.73 (1H, *m*), 1.79 (1H, *m*), 2.02 (1H, *m*), 2.10 (1H, *m*), 2.17 (1H, *m*), 2.40 (1H, *m*), 4.65 (2H, *d*, *J* = 7.8 Hz)

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$;
16.3, 20.2, 24.8, 26.1, 26.6, 26.7, 27.4, 29.9, 38.8, 41.7, 53.4, 54.3, 80.9, 106.2, 153.4

4.9 Compound SC-3

Compound SC-3 was obtained as white crystal (215.8 mg, 0.015 % based on dried weight of the leaves).

ESIMS : *m/z*; 327.1596 $[M+Na]^+$; see **Figure 93**

mp : 128 - 130 °C

$[\alpha]^{25}_D$: -43.6° (*c* 0.012, $CHCl_3$)

UV : λ_{max} nm (log ε), in $CHCl_3$; 234 (3.53), 244 (4.12), 250 (4.05), 279 (4.37); see **Figure 91**

IR : ν_{max} cm^{-1}, film; 3456, 2962, 2933, 1606, 1517, 1479, 1454, 1434, 1336, 1271, 1208, 1144, 1125, 1033, 963, 858, 820, 757, 636; see **Figure 92**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$;
1.36 (3H, *d*, *J* = 6.72 Hz), 1.85 (3H, *d*, *J* = 6.71 Hz), 3.43 (1H, *dq*, *J* = 9.46, 6.71 Hz), 3.85 (3H, *s*), 3.88 (3H, *s*), 5.08 (1H, *d*, 9.46 Hz), 5.66 (1H, *s*), 6.09 (1H, *dq*, *J* = 15.86, 6.71 Hz), 6.35 (1H, *dd*, *J* = 15.86, 1.53 Hz), 6.75 (1H, *br s*), 6.77 (1H, *br s*), 6.78 (1H, *d*, *J* = 7.93 Hz), 6.89 (1H, *dd*, *J* = 8.24, 1.53 Hz), 6.96 (1H, *d*, *J* = 1.22 Hz)

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$;
17.6, 18.4, 93.7, 45.6, 56.0, 56.0, 109.0, 109.2, 113.3, 114.1, 120.0, 123.5, 131.0, 132.1, 132.2, 133.3, 144.1, 145.8, 146.7, 146.7

4.12 Compound SC-4

Compound SC-4 was obtained as pale yellow gum (75.4 mg, 4.18 x 10^{-3} % based on dried weight of the leaves).

ESIMS : *m/z*; 363.1572 $[M+Na]^+$; see **Figure 103**

$[\alpha]^{25}_D$: -23.9° (*c* 0.20, $CHCl_3$)

UV : λ_{max} nm (log ε), in CHCl3; 233 (2.31), 244 (3.09), 254 (2.96), 280 (3.19); see Figure 101

IR : ν_{max} cm^{-1}, KBr; 3000, 2928, 2853, 1734, 1600, 1517, 1497, 1456, 1422, 1379, 1336, 1266, 1143, 1029, 963, 857, 813, 761; see **Figure 102**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$;
1.36 (1H, *d*, *J* = 7.0 Hz), 1.84 (1H, *dd*, *J* = 6.7, 1.7 Hz), 3.44 (1H, *dq*, *J* = 9.5, 6.7 Hz), 3.85 (3H, *s*), 3.85 (3H, *s*), 3.87 (3H, *s*) 3.87 (3H, *s*), 5.09 (1H, *d*, 9.5 Hz), 6.09 (1H, *dq*, *J* = 15.6, 6.7 Hz), 6.34 (1H, *dd*, *J* = 15.6, 1.7 Hz), 6.75 (1H, *br s*), 6.77 (1H, *br s*), 6.82 (1H, *d*, *J* = 8.1 Hz), 6.93 (1H, *dd*, *J* = 8.1, 2.0 Hz), 6.97 (1H, *d*, *J* = 2.0 Hz),

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$;
17.5, 18.3, 45.5, 55.8, 55.8, 55.9, 93.6, 109.1, 109.2, 110.7, 113.2, 119.1, 144.1, 123.4, 130.8, 132.1, 132.6, 133.2, 146.5, 149.0, 149.0

4.17 Compound SC-5

Compound SC-5 was obtained as light brown amorphous powder (29.7 mg, 2.12 x 10^{-3} % based on dried weight of leaves).

EIMS : *m/z* (% relative intensity); 434 (M^+, 0.7), 380 (0.5), 317 (2.9), 270 (100.0), 153 (55.6), 107 (64.5); see **Figure 113**

mp : 177 – 178 °C

$[\alpha]^{25}_D$: -17.2° (*c* = 0.5, MeOH)

UV : λ_{max} nm (log ε), in MeOH; 235 (4.29), 293 (4.20), 335 nm (3.49); see **Figure 111**

IR : ν_{max} cm^{-1}, KBr; 3413, 2934, 1643, 1518, 1469, 1381, 1292, 1262, 1168, 1086, 1064, 1035, 830, 596, 559; see **Figure 112**

^{1}H NMR : δ ppm, 500 MHz, in CD_3OD;
1.18 (3H, *d*, *J* = 6.5 Hz), 3.31 (1H, *dd*, *J* = 5.0, 1.5 Hz), 3.50 (2H, *dd*, *J* = 3.0 and 1.5 Hz), 3.65 (1H, *dd*, *J* = 10 and 3.5 Hz), 4.01 (1H, *d*, *J* = 1.5 Hz), 4.25 (1H, *m*), 4.62 (1H, *d*, *J* = 11.0 Hz), 5.14 (1H, *d*, *J* = 11.0 Hz), 5.89 (1H, *d*, *J* = 2.0 Hz), 5.92 (1H, *d*, *J* = 2.5 Hz), 6.84 (2H, *d*, *J* = 9 Hz), 7.35 (2H, *d*, *J* = 9 Hz)

^{13}C NMR : δ ppm, 125 MHz, in CD_3OD;
17.9, 70.5, 71.8, 72.2, 73.8, 83.9 (C-2), 78.7 (C-3), 96.3, 102.2, 97.4, 102.6, 116.4, 116.4, 128.6, 130.1, 130.1, 159.5, 164.2, 165.6, 168.6, 196.1

4.18 Compound SC-6

Compound SC-6 was obtained as light brown crystal (88.8 mg, 2.07 x 10^{-3} % based on dried weight of leaves).

SIMS : *m/z* (% relative intensity); 449 (M^-, 100.0), 286 (38.6), 179 (6.6), 151 (19.5), 91 (9.2); see **Figure 123**

mp : 185 – 188 °C

$[\alpha]^{25}_D$: -7.2° (*c* = 0.5, MeOH)

UV : λ_{max} nm (log ε), in MeOH; 235 (4.69), 291 (4.41), 335 nm (3.57); see **Figure 121**

IR : ν_{max} cm^{-1}, KBr; 3419, 2935, 1643, 1523, 1472, 1368, 1293, 1262, 1154, 1116, 1089, 1066, 1038, 978, 822, 779, 647, 592; see **Figure 122**

^{1}H NMR : δ ppm, 500 MHz, in CD_3OD;
1.18 (3H, *d*, *J* = 6.5 Hz), 3.31 (1H, *dd*, 5.0, 2.0 Hz), 3.54 (2H, *dd*, *J* = 3.5 and 1.5 Hz), 3.66 (1H, *dd*, *J* = 9.5 and 3.5 Hz), 4.05 (1H, *d*, *J* = 1.5 Hz), 4.25 (1H, *m*), 4.57 (1H, *d*, *J* = 11.0 Hz), 5.07 (1H, *d*, *J* = 11.0 Hz), 5.89 (1H, *d*, *J* = 2.5 Hz), 5.92 (1H, *d*, *J* = 2.5 Hz), 6.81 (1H, *d*, 8 Hz), 6.84 (1H, *d*, *J* = 8, 1.5 Hz), 6.96 (1H, *d*, *J* = 2 Hz, 2H)

^{13}C NMR : δ ppm, 125 MHz, in CD_3OD;
17.9, 70.5, 73.8, 72.2, 71.8, 78.6, 84.0, 96.3, 97.4, 102.2, 102.5, 115.5, 116.3, 120.2, 129.2, 146.4, 147.4, 164.1, 165.5, 168.6, 196.0

4.11 Compound SC-7

Compound SC-7 was obtained as white amorphous powder, soluble in $CHCl_3$

mp : 105 - 107 °C

UV : λ_{max} nm, in $CHCl_3$; 233, 243, 259, 281; see **Figure 131**

IR : ν_{max} cm^{-1}, film; 2957, 2934, 2836, 1593, 1516, 1462, 1419, 1384, 1264, 1236, 1161, 1138, 1029, 860, 808, 763,629; see **Figure 132**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$;
0.61 (*d*, 7.02), 0.97 (2H, *d*, 6.71), 1.02 (3H, *d*, 6.41), 1.74 (1H, *m*), 2.20 (1H, *m*), 2.28 (2H, *m*), 3.81 (3H, *s*), 3.82 (3H, *s*), 3.82 (3H, *s*), 3.83 (3H, *s*), 3.83 (3H, *s*), 3.86 (3H, *s*), 4.37 (1H, *d*, 9.46), 4.47 (2H, *d*, 6.41), 5.08 (1H, *d*, 8.54), 5.81 (1H, 8.09), 6.79 (2H, *d*, 7.52), 6.82 (1H, *d*, 7.32), 6.84 (1H, *dd*, 8.09, 1.38), 6.85 (1H, *d*, 1.38), 6.93 (2H, *dd*, 7.52, 1.83), 6.96 (2H, *d*, 1.83), 7.00 (1H, *dd*, 7.32, 1.98), 7.04 (1H, *d*, 1.98),

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$;
12.7, 14.8, 14.8, 44.1, 45.8, 47.7, 55.6, 55.6, 55.7, 82.8, 87.0, 87.0, 109.5, 109.7, 110.2, 110.5, 110.8, 110.9, 118.4, 118.5, 119.0, 133.3, 133.6, 133.7, 147.8, 147.8, 148.2, 148.2, 148.4, 148.7

4.14 Compound SC-8

Compound SC-8 was obtained as pale yellow amorphous powder (5.1 mg, 9.81 x 10^{-5} % based on dried weight of stems).

EIMS : *m/z* (% relative intensity); 265 (M^+, 100.0), 250 (32.4), 222 (15.5), 166 (12.7), 139 (6.6), 71 (6.0), 57 (10.5), 43 (33.2), 43 (100.0), 28 (6.5); see **Figure 141**

UV : λ_{max} nm (log ε), in MeOH; 276 (4.22), 342 (3.67), 356 (3.51), 365 (3.55), 383 (3.53); see **Figure 139**

IR : ν_{max} cm^{-1}, KBr; 3408, 3188, 1698, 1657, 1501, 1459, 1413, 1377, 1325, 1271, 1232, 1184, 1131, 1055, 1034, 845, 742, 683; see **Figure 140**

^{1}H NMR : δ ppm, 300 MHz, in DMSO-d_6; 4.04 (3H, *s*), 7.12 (1H, *s*), 7.53 (2H, *m*), 7.76 (1H, *s*), 7.94 (1H, *s*), 9.25 (1H, *s*), 10.65 (1H, *s*)

^{13}C NMR : δ ppm, 75 MHz, in DMSO-d_6; 58.07, 105.25, 109.43, 115.28, 116.80, 125.19, 125.83, 127.48, 127.54, 128.34, 129.56, 135.04, 136.03, 149.11, 150.25, 169.77

4.13 Compound SC-9

Compound SC-9 was obtained as yellow amorphous power (2.76 mg, 2.11 x 10^{-4} % based on dried weight of stems).

EIMS : *m/z* (% relative intensity); 265 (M^+, 3.5), 250 (1.6), 222 (0.7), 166 (0.7), 43 (100.0); see **Figure 149**

UV : λ_{max} nm (log ε), in MeOH; 248 (4.40), 272 (4.18), 281 (4.26), 370 (3.79), 388 (3.87); see **Figure 147**

IR : ν_{max} cm^{-1}, KBr; 3294, 3200, 2949, 1702, 1626, 1501, 1422, 1357, 1329, 1293, 1238, 1182, 1032, 985, 875, 849, 742, 615; see **Figure 148**

^{1}H NMR : δ ppm, 300 MHz, in DMSO-d_6; 4.00 (3H, *s*), 7.09 (1H, *s*), 7.55 (2H, *m*), 7.61 (1H, *s*), 9.10 (1H ,*d*, J = 7.5 Hz), 9.92 (1H, *d*, J = 7.8 Hz), 10.78 (1H, *br s*)

^{13}C NMR : δ ppm, 75 MHz, in DMSO-d_6; 59.56, 103.99, 113.50, 120.45, 121.89, 122.42, 125.38, 126.09, 126.88, 127.37, 129.05, 134.94, 135.40, 148.96, 152.33, 168.59

4.15 Compound SC-10

Compound SC-10 was obtained as pale yellow amorphous powder (21.7 mg, 1.21 x 10^{-3} % based on dried weight of stems).

ESIMS : *m/z* ; 318.0750 $[M+Na]^+$; see **Figure 158**

mp : 218 – 220 °C

UV : λ_{max} nm (log ε), in MeOH; 247 (4.54), 273 (4.26), 281 (4.36), 348 (3.80), 356 (3.77), 377 (3.89), 389 (4.04); see **Figure 156**

IR : ν_{max} cm^{-1}, film; 3248, 2926, 2852, 2684, 1610, 1457, 1419, 1274, 1230, 1154, 1073, 977, 743; see **Figure 157**

^{1}H NMR : δ ppm, 300 MHz, in CD_3OD; 4.09 (3H, *s*), 4.43 (3H, *s*), 7.12 (1H, *s*), 7.50 (2H, *dd, J* = 6.00, 3.30), 7.82 (1H, *dd, J* = 6.00, 3.30), 9.15 (1H, *dd, J* = 6.00, 3.30)

^{13}C NMR : δ ppm, 75 MHz, in CD_3OD; 60.4, 63.3, 106.1, 110.3, 117.6, 123.4, 126.3, 127.4, 127.5, 127.8, 129.8, 135.3, 135.7, 145.2, 150.0, 151.4, 169.4

4.16 Compound SC-11

Compound SC-11 was obtained as orange amorphous powder (4.10 mg, 2.28 x 10^{-4} % based on dried weight of stems).

ESIMS : *m/z*; 316.0586 $[M+Na]^+$; see **Figure 168**

UV : λ_{max} nm (log ε), in MeOH; 244 (4.02), 284 (3.62), 315 (3.72), 444 (3.49); see **Figure 166**

IR : ν_{max} cm^{-1}, film; 3367, 2925, 2791, 2212, 1679, 1658, 1615, 1564, 1516, 1384, 1278, 1114, 1025, 952, 831, 741; see **Figure 167**

^{1}H NMR : δ ppm, 500 MHz, in DMSO-d_6; 4.04 (3H,*s*), 7.48 (2H, *m*), 7.63 (2H, *m*), 7.91 (1H, *m*), 8.07 (1H, *s*), 9.44(1H, *m*), 12.01 (1H, *br s*)

^{13}C NMR : δ ppm, 125 MHz, in DMSO-d_6; 59.7, 112.1, 117.2, 117.3, 124.2, 124.9, 126.2, 126.8, 126.8, 128.0, 128.5, 130.5, 132.6, 151.3, 153.0, 155.8, 177.1

5. Evaluation of Biological Activities

5.1 Determination of antimycobacterial activity

Antimycobacterial activity was assessed against *Mycobacterium tuberculosis* $H_{37}Ra$ using the Microplate Alamar Blue Assay (MABA) (Collins and Franzblau, 1997). The mycobacteria was grown in 100 ml of 7H9GC broth containing 0.005 % Tween 80. Culture was incubated in 500 ml plastic flask on a rotary shaker at 200 rpm and 37 °C until they reached an optical density of 0.4-0.5 at 550 nm. Bacteria were washed and suspended in 20 ml of phosphate-buffered saline and passed through a filter. The filtrates were aliquoted and stored at -80°C.

The susceptibility testing was performed in 96-well microplates. Samples were initially diluted with either dimethyl sulfoxide or distilled deionized water, then diluted by Middlebrook 7H9 media containing 0.2 % v/v glycerol and 1.0 gm/L broth 7H9GC, and subsequent two-fold dilutions were performed in 0.1 ml of 7H9CG broth in microplates. Frozen inocula were diluted 1:100 in 7H9GC broth and adding of 0.1 ml to the well resulted in final bacterial titers of about 5×10^4 CFU/ml. Wells containing sample only were used to determine whether the tested-samples themselves can reduce the dye or not. Additional control wells consisted of bacteria only (B) and medium only (M). Plates were incubated at 37 °C. Starting at day 6 of incubation, 20 µl of Alamar Blue solution and 12.5 µl of 20% Tween 80 were added to one B well and one M well, and plates were reincubated at 37°C. The B wells were observed for a color change from blue to pink, at which time reagents were added to all remaining wells. Plates were then incubated at 37 °C, and results were recorded at 24 h post-reagent addition. Visual MIC values were defined as the lowest concentration of sample that prevented a color change. Standard drugs: rifampicin, isoniazid and kanamycin sulfate, were used as the reference compounds.

5.2 Determination of antimalarial activity

Plasmodium faiclparum (K1, multi-drug resistant strain) was cultivated *in vitro* using the method of Trager and Jensen (Trager and Jensen, 1976) in RPMI 1640 medium containing 20 mM HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid), 32 mM $NaHCO_3$ and 10% heat-inactivated human serum with 3% erythrocytes and incubated at 37 °C in an incubator with 3 % CO_2. Cultures were diluted with fresh medium and erythrocytes every day according to cell growth. Quantitative

assessment of antimalarial activity *in vitro* was determined by microculture radioisotope techniques based upon the method of Desjardins *et al.* (1979). Briefly, a mixture of 200 µl of 1.5 % erythrocytes with 1 % parasitemia at the early ring stage was pre-exposed to 25 µl of the medium containing a test sample dissolved in 1 % DMSO (0.1% final concentration) for 24 h employing the incubation condition described above. Subsequently, 25 µl of [^{3}H]-hypoxanthine (Amersham, USA) in culture medium (0.5 µCi) were added to each well and plates were incubated for an additional 24 h. Levels of incorporated labeled hypoxanthine indicating parasite growth were determined using the TopCount microplate scintillation counter (Packard, USA). IC_{50} value represents the concentration which indicates 50 % reduction of parasite growth. The standard sample was dihydroartemisinin (DHA).

5.3 Determination of cytotoxic activity

5.3.1 Human small cell lung carcinoma (NCI-H187)

Cytotoxicity to NCI-H187 cells (Human small cell lung carcinoma, ATCC CRL-5804) was determined by MTT assay (Plumb *et al.*, 1989). Briefly, cells were diluted to 10^5 cells/ml. Test compounds were diluted in distilled water and added to microplates in a total volume of 200 µl. Plates were incubated at 37 °C, 5 % CO_2 for 5 days. Then, 50 µl of 2 mg/ml MTT solution (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromine; Thiazolyl blue) was added to each well of the plate. Plates were wrapped with aluminium foil and incubated for 4 h. After incubation period, the microplates were spinned at 200 × g for 5 min. MTT was then removed from the wells and the formazan crystals were dissolved in 200 µl of DMSO and 25 µl of Sorensen'glycine buffer. Absorbance was read in microplate reader at the wavelength of 510 nm. The reference substance was ellipticine. The activity was expressed as 50 % inhibitory concentration (IC_{50}), the concentration which inhibits cell growth by 50 % compared with untreated cells.

5.3.2 Human epidermoid carcinoma (KB) and breast cancer (BC)

Cytotoxicity to KB (Human epidermoid carcinoma of cavity, ATTCC CCL-17) and BC (Breast cancer) cell lines were determined by a colorimetric assay that measured cell growth from cellular protein content (Skehan *et al.*, 1990). Ellipticine and doxorubicin were used as positive control. DMSO was used as

negative control. Briefly, cells at a logarithmic growth phase were harvested and diluted to 10^5 cells/ml with fresh medium and gently mixed. Extracts or test compounds were diluted in distilled water and put into microplates in a total volume of 200 µl. Plates were incubated at 37 °C, 5 % CO_2 for 72 h. After incubation period, cells were fixed by 50 % trichloroacetic acid. The plates were incubated at 4 °C for 30 min, washed with tap water and air-dried at room temperature. The plates were then stained with 0.05 % sulforhodamine B (SRB), dissolved in 1 % acetic acid for 30 min. After staining period, SRB was removed with 1 % acetic acid. Plates were air-dried before bound dye was solubilized with 10 mM Tris-base for 5 min on shaker. Absorbance was read in microplate reader at the wavelength of 510 nm. The criteria of cytotoxic potency of the compound testing in this system are as follows:

IC_{50} (µg/ml)	Activity
> 20	Inactive
> 10 – 20	Weakly active
5 – 10	Moderately active
< 5	Strongly active

5.3.3 Vero cell

Compounds were tested for their cytotoxicity against Vero cells (African green monkey kidney fibroblast) in 96-well tissue culture plates. Vero cell suspension (190 µl) containing 1×10^5 cells/ml and 10 µl of tested compound solution were added to each well in triplicate. Ellipticine and 10 % DMSO were used as positive and negative control, respectively. The cells were incubated at 37 °C for 72 h in 5% CO_2. After incubation, the cytotoxicity was determined as in 5.3.2. If % cell viability ≥ 50% reported IC_{50} > 50 µg/ml and if % cell viability < 50% reported IC_{50} from two-fold serial dilution.

5.4 Determination of anti-herpes simplex activity

Anti-herpes simplex virus type 1 (HSV-1) activity of pure compounds was tested against HSV-1 strain ATCC VR 260, using colorimetric microplate assay as in 5.3.2. The growth of host cells (vero cell line ATCC CCL-81) infected with virus and treated with the extract was compared with control cells, which were infected with virus only. Acyclovir and DMSO were used as positive and negative control,

respectively. The extracts were tested at non-cytotoxic concentrations (inhibition of cell growth < 50 %). The criteria of potency of activity are as follows:

% inhibition	potency of activity
25-35 %	weakly active
35-50 %	moderately active
> 50 %	active

Extracts that inhibited virus more than 50 % were further tested to determine the concentrations that inhibit viral activity by 50 % (IC_{50}).

5.5 Determination of lymphocyte proliferation stimulation

The in vitro lymphocyte proliferation and cytotoxicity to splenic lymphocytes were determined by using Alamar Blue method (Ahmed, Gogal and Walsh, 1994; Nakayama *et al.*, 1997; Zhi-Jun *et al.*, 1997).

The splenic lymphocyte suspension obtained from Wistar rat was adjusted to 2.5 x 10^6 cells/ml in complete RPMI 1640 medium. One hundred microlitres of the suspension were placed in 96-well sterile culture plate containing 10 µl of two-fold dilution of 1.6 – 200 µg/ml of plant extract or compound or 0.5 % DMSO as vehicle control or 5 µg/ml of Concanavalin A or 50 µg/ml of LPS as positive control. Then, 90 µl of complete RPMI 1640 medium were added. The plate was incubated in 5 % CO_2 under humidified conditions at 37 °C for 48 h. After that, 20 µl/well of Alamar Blue were added and the plate was re-incubated for 24 h. Since Alamar Blue contained an oxidation-reduction (Redox) indicator, cellular proliferation induced chemical reduction of the media which resulted in a change in Redox color from blue to red. The intensity of red color reflected the extent of cellular proliferation. The plates was then measured the absorbance at 570 nm (reduced form) and 600 nm (oxidized form) using microplate reader. Specific absorbance (specific OD), obtained by subtracting the absorbance at 600 nm from that of 570 nm, was used in the calculation of the stimulation index (SI) of lymphocyte proliferation.

$$SI = \text{specific OD (sample)} / \text{specific OD (solvent control)}$$

$$\% \text{ cytotoxicity} = \left[\frac{\text{specific OD (control)} - \text{specific OD (sample)}}{\text{specific OD (control)}}\right] \times 100$$

5.5 Determination of aldose reductase inhibitory activity

The activities of recombinant human aldose reductase (AR) enzyme were measured according to the procedure of Nishimura (Nishimura *et al.*, 1991). The plant extract or pure compound was dissolved in DMSO. The reaction mixtures, containing 10 mM DL-glyceraldehyde, 0.15 mM β-NADPH, 5 µl of the recombinant human AR and 5 µl of test sample solution or DMSO in a total volume of 1 ml of 100 mM sodium phosphate buffer (pH 6.2), were incubated in advance at 25 °C for 3 min. Then, the reaction was started by addition of the enzyme and the decrease of absorbance was measured at 340 nm for 1 min using a Hitachi U-3010 spectrophotometer. The % inhibition of test sample was calculated as:

$$[1 - (\Delta A \text{ sample/min} - \Delta A \text{ blank/min})/(\Delta A \text{ control/min} - \Delta A \text{ blank/min})] \times 100$$

whereas ΔA sample/min represents a decrease of absorbance for 1 min with a test sample, ΔA blank/min with DMSO and water instead of a sample and a substrate, respectively, and ΔA control/min with DMSO in place of a sample.

5.6 Determination of advance glycation endproducts (AGEs) formation inhibitory activity

Assay of this inhibitory activity employed the measurement of fluorescent material based on AGEs in order to detect the inhibitory effect of test samples on the Maillard reaction (Matsuura *et al.*, 2002). The plant extract or pure compound was dissolved in DMSO. The reaction mixtures, containing 400 µg bovine serum albumin (BSA), 200 mM glucose and 10 µl of test sample solution or DMSO in a total volume of 500 µl of 50 mM phosphate buffer, pH 7.4, were incubated at 60 °C for 30 hr. The blank sample, which contained no plant extract or pure compound, was kept at 4 °C until measurement. After cooling, aliquots of 250 µl were transferred to 1.5 ml plastic tubes, then, 25 µl of trichloroacetic acid (TCA) was added to each tube and stirred. The supernatant was removed after centrifugation (15,000 rpm) at 4 °C for 4 min and the AGEs-BSA precipitate was dissolved with 1 ml of alkaline phosphate buffer saline (PBS). These solutions were monitored by using spectrofluorometer to compare their fluorescence spectra (ex. 360 nm) and the changes in fluorescence intensity (ex. 360 nm, em. 460 nm) based on AGEs. The % inhibition was calculated similar to the above method for AR inhibitory activity.