CHAPTER III

EXPERIMENTAL

1. Sources of Plant Materials

The leaves, wood and stem bark of *Chisocheton penduliflorus* Planch. ex Hiern were collected from Na Yong, Trang in April, 2002. The leaves, fruits and seeds of cf. *Aglaia erythrosperma* C.M. Pannell were collected from Ai Khiao Waterfall, Nakhon Sri Thammarat, Thailand in March, 2004. Voucher specimens of both plants have been deposited at the herbarium of 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 at wavelengths of 254 and 365 nm 2. 10% Sulfuric acid in ethanol and heated at 105 °C for 10 min

2.3 Column Chromatography

2.3.1 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.

Vacuum system : Vacuum was created by suction pump at the outlet of the column.

Detection : Fractions were examined by TLC technique in the same manner as described in section 2.2

2.3.2 Column Chromatography (CC)

Column : Flat bottom glass column (various diameter)

Stationary phase : Silica gel 60 (No. 9385, E. Merck) particle size 0.040-0.063 mm (230-400 mesh ASTM)

Packing method : Dry and wet packing

Sample loading : 1) Dry packing: The sample was dissolved in a small amount of suitable organic solvent, mixed with a small quantity of the adsorbent, triturated, dried and then loaded on top of the column.

2) Wet packing: The sample was dissolved in a small amount of the eluent, then loaded on top of the column.

Solvent system : Various solvent systems depending on materials.

Detection : Fractions were examined by TLC technique in the same manner as described in section 2.2

2.3.3 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 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 Spectroscopy

2.4.1 Ultraviolet (UV) Spectra

UV spectra (in MeOH) were obtained on a Shimadzu UV-160A spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand).

2.4.2 Infrared (IR) Spectra

IR spectra (KBr disc and film) were recorded on a Perkin Elmer FT-IR1760X spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University) and a JASCO FT/IR-300E spectrophotometer (Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan).

2.4.3 Mass Spectra

Electrospray Ionization Time of Flight (ESI-TOF) mass spectra, high resolution Electrospray Ionization Time of Flight (HRESI-TOF) mass spectra, and MS 1200L Quadrupole MS/MS Varian (Positive mode) were obtained on a Micromass LCT mass spectrometer (National Center for Genetic Engineering and Biotechnology, Thailand). Electron-impact (EI) mass spectra were recorded on a JEOL JMS-AM20 mass spectrometer (Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan).

2.4.4 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 (400 MHz) and ^{13}C (100 MHz) NMR spectra were obtained on a JEOL JNM-ECP400 spectrometer (Chiba University).

^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were measured on a JEOL JMN-A 500 spectrometer, a Varian unity INOVA spectrometer (Scientific andTechnological Research Equipment Center, Chulalongkorn University), a Bruker-AV 500 MHz spectrometer (National Center for Genetic Engineering and Biotechnology, Thailand), or a JEOL JNM-GSX500A spectrometer (Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan).

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 Physical Properties

2.5.1 Melting Points

Melting points (uncorrected) were measured on a micro melting point hot-stage apparatus (Yanagimoto) (Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan) or a Fisher-John melting point apparatus (Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.5.2 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).

3. Extraction and Isolation

3.1 Extraction and Isolation of compounds from the leaves of *Chisocheton penduliflorus*

3.1.1 Extraction

The dried, powdered leaves (650 g) were macerated with 95% ethanol. After evaporation under reduced pressure, the residue was pre-adsorbed on kieselguhr and successively washed down with hexane (5 × 3 L), $CHCl_3$ (5 × 3 L) and MeOH (5 × 3 L), respectively. Each extract was evaporated under reduced pressure at temperature not exceeding 45°C to afford the hexane extract (25.8 g, 3.97% based on dried weight of leaves), $CHCl_3$ extract (15.9 g, 2.45% of dried weight), and MeOH extract (20.2 g, 3.11% of dried weight).

3.1.2 Isolation of Compounds from the Hexane Extract of *C. penduliflorus* Leaves

The hexane extract (25.8 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 × 6 cm) and eluted stepwise with gradient of hexane-EtOAc (100:1 to 10:1). The eluates were examined by TLC (solvent system: hexane-EtOAc = 5:4). Fifty-three fractions (300 ml each) were collected and fractions with similar chromatographic pattern were combined to yield ten fractions (A-1 - A-10, Table 6).

Table 6. Combined fractions from hexane extract of *C. penduliflorus* leaves

Fraction Code	Weight (g)	Fraction Code	Weight (g)
A-1	2.65	A-6	1.54
A-2	2.37	A-7	2.45
A-3	1.56	A-8	2.38
A-4	1.63	A-9	3.59
A-5	1.52	A-10	4.06

3.1.2.1 Isolation of Compound CP-1 (Hollongdione)

Fraction A-7 (2.45 g) was subjected to silica gel column chromatography (100 g, 3 × 26 cm) using gradient mixture of hexane-EtOAc (5:1 to 1:1) as mobile phase, to give thirty-two fractions (100 ml each). These fractions were collected and combined based on their TLC pattern (solvent system: hexane-EtOAc = 6:1) to afford eight fractions (A-71 - A-78). Fraction A-76 (458.5 mg) was further fractionated by a silica gel column (50 g, 2.4 × 29 cm) eluted hexane-EtOAc (5:1) to give five combined fractions (A-761 - A-765). Compound CP-1 (167.8 mg, 0.026% yield) was obtained as white amorphous powder from fracion A-763.

3.1.2.2 Isolation of Compound CP-2 (Dammaradienone)

Fraction A-9 (3.59 g) was subjected to silica gel column chromatography (50 g, 2.4 × 29 cm) eluted with hexane-EtOAc (4:1) to give ten fractions (A-901 - A-910). Fraction A-905 (358.4 mg) was further fractionated by a silica gel column (50 g, 2.4 × 29 cm) eluted with hexane-EtOAc (5:1) to give five combined fractions (A-9051-A-9055). Compound CP-2 (67.8 mg, 0.010% yield) was obtained as white amorphous powder from fraction A-9055.

3.1.3 Isolation of Compound CP-3 from the $CHCl_3$ Extract of *C. penduliflorus* Leaves

The $CHCl_3$ extract (18.5 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 × 6 cm), eluted stepwise with gradient of hexane-acetone (5:1 to 0:1). The eluates were examined by TLC (solvent system: hexane-

acetone = 4:1). Forty-two fractions (300 ml each) were collected and fractions with similar chromatographic pattern were combined to yield nine fractions (B-1- B-9).

From fraction B-3, compound CP-3 (15.8 mg, 0.0024% yield) was obtained as a white amorphous powder which showed one spot on TLC (solvent system: hexane-acetone = 4:1).

3.2 Extraction and Isolation of compounds from the wood of *Chisocheton penduliflorus*

3.2.1 Extraction

The dried powdered wood (500 g) was macerated with 95% ethanol. After evaporation under reduced pressure, the residue was pre-adsorbed on kieselguhr and successively washed down with hexane (5 × 3 L), $CHCl_3$ (5 × 3 L) and MeOH (5 × 3 L), respectively. Each extract was evaporated under reduced pressure at temperature not exceeding 45°C to afford the hexane extract (20.0 g, 4.0% based on dried weight of wood), $CHCl_3$ extract (24.0 g, 4.82% of dried weight), and MeOH extract (10 g, 2.0% of dried weight).

3.2.2 Isolation of Compounds from the Hexane Extract of *C. penduliflorus* Wood

The hexane extract (20.0 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 × 6 cm) and eluted stepwise with gradient of hexane-acetone (5:1 to 0:1). The eluates were examined by TLC (solvent system: hexane-acetone = 5:1). Fifty-five fractions (300 ml each) were collected and fractions with similar chromatographic pattern were combined to yield twelve fractions (C-1-C-12, Table 8).

Table 7. Combined fractions from hexane extract of *C. penduliflorus* wood

Fraction Code	Weight (g)	Fraction Code	Weight (g)
C-1	1.22	C-7	1.48
C-2	1.12	C-8	1.02
C-3	0.78	C-9	0.56
C-4	1.65	C-10	1.29
C-5	2.22	C-11	1.13
C-6	1.53	C-12	1.45

3.2.2.1 Isolation of Compound CP-3 (Cabraleadiol)

Fraction C-5 (2.22 g) was further separated on a silica gel column (75 g, 2.5× 21 cm). Hexane-acetone (5:1) was used as the mobile phase. Twenty fractions (100 ml each) were collected and combined based on their similar TLC pattern (solvent system: hexane-acetone = 5:1) into seven fractions (C-51 - C-57). Fraction C-53 (295.1 mg) was recrystallized in hexane-acetone (5:1) to yield compound CP-3 (53 mg, 0.011% yield).

3.2.2.2 Isolation of Compound CP-4 (14-Hydroxyviridiflorol)

Fraction C-7 (1.48 g) was re-chromatographed on a silica gel column (50g, 2.5 × 21 cm). A mixture of hexane-acetone (4:1) was used as the mobile phase. Twenty-six fractions (30 ml each) were combined based on their similar TLC pattern (solvent system: hexane-acetone = 4:1) into six fractions (C-71 - C-76). Compound CP-4 (72.5 mg, 0.0145% yield) was obtained as colorless needle crystals from fraction C-74.

3.2.3 Isolation of Compounds from the $CHCl_3$ Extract of *C. penduliflorus* Wood

The $CHCl_3$ extract (24.0 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 × 6 cm) for vacuum liquid column chromatography, eluting stepwise with gradient of hexane-acetone (2:1 to 1:2). The eluates were examined by TLC (solvent system: hexane-acetone = 2:1). Fifty-five fractions (300 ml each) were collected and combined according to similar chromatographic pattern were combined to yield ten fractions (D-1-D-10, Table 9).

Table 8. Combined fractions from $CHCl_3$ extract of *C. penduliflorus* wood

Fraction Code	Weight (g)	Fraction Code	Weight (g)
D-1	1.24	D-6	1.45
D-2	1.67	D-7	2.56
D-3	1.15	D-8	2.34
D-4	2.45	D-9	1.23
D-5	1.76	D-10	2.65

3.2.3.1 Isolation of Compound CP-4 (14-Hydroxyviridiflorol)

Fraction D-4 (2.45 g), which showed one main spot on TLC (solvent system: hexane-acetone = 3:1), yielded a white precipitate after partial evaporation of the solvent. The precipitate was recrystallized in hexane-acetone (2:1) to yield compound CP-4 (300.7 mg, 0.060% yield).

3.2.3.2 Isolation of Compound CP-5 (14-Hydroxyepiviridiflorol)

Fraction D-5 (1.76 g) was subjected to silica gel column chromatography (50 g, 2.3 × 18 cm) eluting with hexane-acetone (2:1) to give twenty-four fractions (30 ml each). Similar fractions were combined, after being examined by TLC (solvent system: hexane-acetone = 2:1), into five fractions (D-51 – D-55). Fraction D-52 was recrystallized in hexane-acetone (2:1) to yield compound CP-5 as colorless needle crystals (30.7 mg, 0.006% yield).

3.2.3.3 Isolation of Compound CP-3 (Cabraleadiol)

Fraction D-6, which showed one main spot on TLC (solvent system: hexane-acetone = 3:1), yielded white precipitate after partial evaporation of the solvent. It was recrystallization of this precipitate in hexane-acetone (4:1) further yielded compound CP-3 (10.3 mg, 0.0021% yield).

3.2.3.4 Isolation of Compound CP-6 (Eichlerialactone)

Fraction D-8 (2.34 g) was further separated on a silica gel column (75 g, 2.5 × 21 cm), eluted with hexane - acetone (4:1). The eluates (40 ml each) were examined (TLC solvent system: hexane-acetone = 4:1) and then combined to yield six fractions:

fraction D-81 – D-86. Fraction D-83 (187.1 mg) was recrystallized in hexane-acetone (4:1) to yield compound CP-6 (20.3 mg, 0.0041% yield) as white amorphous powder.

3.2.3.5 Isolation of Compound CP-7 (Cabralealactone) and CP-8 (Cabraleahydroxylactone)

Fraction D-9 (1.23 g) was separated on a Sephadex LH-20 column, using $CHCl_3$-MeOH (2:1) as the eluent. Thirty-seven fractions (20 ml each) were collected and combined according to their TLC pattern (solvent system: hexane-acetone = 4:1) into five fractions (D-91-D-95). Compound CP-7 (18.7 mg, 0.0037% yield) was obtained as white amorphous powder from fraction D-92 and compound CP-8 (15.8 mg, 0.0032% yield) was obtained as white amorphous powder from fraction D-93.

3.2.3.6 Isolation of Compound CP-9 (Scoparone), CP-10 (Scopoletin), CP-11 (Vanillic Acid), CP-12 (*β*-Sitosterol glucoside) and CP-13 [(-)-10*β*,13,14-Trihydroxy-*allo*- aromadendrane]

Fraction D-10 (2.65 g) was separated on a silica gel column (75 g, 2.5× 21 cm) eluting with $CHCl_3$-MeOH (30:1) to give thirty-five fractions (25 ml each). Similarity of TLC pattern (solvent system: hexane-acetone = 2:1) led to combination of these into five combined fractions (D-101 – D-105). Fraction D-104 (300.6 mg) was further separated on another silica gel column (40 g, 2.3 × 17 cm). Hexane-acetone (3:1) was used as the mobile phase. Thirty-six fractions (15 ml each) were combined based on their similar TLC pattern (solvent system: hexane-acetone= 2:1) into five fractions (D-1041 – D-1045). Compound CP-9 was obtained as yellow needle crystals (28.9 mg, 0.006% yield) from fraction D-1043, whereas compound CP-10 was obtained as yellow needle crystals (27.2 mg, 0.005% yield) from fraction D-1044 and compound CP-11 was obtained as colorless needle crystals (25.8 mg, 0.005% yield) from fraction D-1045.

Fraction D-105 (576.8 mg) was fractionated by silica gel column chromatography (75 g, 3.2 × 20 cm), eluted with hexane and acetone (2:1) to give thirty-six fractions (15 ml each). After each fraction was examined by TLC (solvent system: hexane-acetone = 2:1), they were combined into six fractions: D-1051 - D-1056. Compound CP-12 was obtained as white amorphous powder (27.6 mg, 0.006% yield) from fraction D-1054.

Fraction D-1055 (347.9 mg) was further separated on a Sephadex LH-20 column, using $CHCl_3$-MeOH (2:1) as the eluent. Twenty fractions (15 ml each) were collected and combined according to their TLC pattern (solvent system: hexane-acetone = 2:1) into six fractions (D-10551 – D-10556). Compound CP-13 was obtained as yellow needle crystals (23.6 mg, 0.005% yield) from fraction D-10553.

3.3 Extraction and Isolation of Compounds from the Stem Bark of *Chisocheton penduliflorus*

3.3.1 Extraction

The dried, powdered stem bark (640 g) was macerated with 95% ethanol. After evaporation under reduced pressure, the residue was pre-adsorbed on kieselguhr and successively washed down with hexane (5 × 3 L), $CHCl_3$ (5 × 3 L) and MeOH (5 × 3 L), respectively. Each extract was evaporated under reduced pressure to afford the hexane extract (30.0 g, 4.69% based on dried weight of stem bark), $CHCl_3$ extract (26.9 g, 4.20% of dried weight), and MeOH extract (27.0 g, 4.22% of dried weight).

3.3.2 Isolation of Compounds from the Hexane Extract of *C. penduliflorus* Stem Bark

The hexane extract (30.0 g) was redissolved in a small amount of $CHCl_3$, triturated with kieselguhr and left to dry. It was then applied on top of a silica gel column (400 g, 13 × 6 cm), eluted stepwise with hexane-acetone gradient (6:1 to 0:1). Fifty-two fractions (200 ml each) were collected and fractions with similar chromatographic pattern (TLC solvent system: hexane-acetone = 5:1) were combined to yield fourteen fractions (E-1-E-14, Table 10).

Table 9. Combined fractions from hexane extract of *C. penduliflorus* stem bark

Fraction Code	Weight (g)	Fraction Code	Weight (g)
E-1	1.85	E-6	1.79
E-2	2.71	E-7	2.24
E-3	1.89	E-8	1.87
E-4	1.54	E-9	1.17
E-5	2.04	E-10	1.96

Table 9. Combined fractions from hexane extract of *C. penduliflorus* stem bark (continued)

Fraction Code	Weight (g)	Fraction Code	Weight (g)
E-11	2.01	E-13	2.49
E-12	1.11	E-14	2.76

3.3.2.1 Isolation of Compound CP-14 (5-Hydroxy-7-methoxy-2-pentyl chromone)

Fraction E-4 (1.54 g) was separated on a silica gel column (75 g, 3 × 26 cm) using hexane-acetone (10:1) as the mobile phase, to give thirty-nine fractions (100 ml each). These fractions were collected and combined based on their TLC pattern (solvent system: hexane-acetone = 6:1) to afford seven fractions (E-41 - E-47). Fraction E-43 (544.1 mg) was fractionated a silica gel column (50 g, 2.4 × 29 cm) eluted with hexane-acetone (15:1) to give six combined fractions (E-431 – E-436). Fraction E-433 (304.1 mg) was further separated on another silica gel column (50 g, 2.4 × 29 cm) eluted with hexane-acetone (15:1) to give five combined fractions (E-4331- E-4335). Compound CP-14 was obtained as colorless oil (15.7 mg, 0.0024% yield) from fraction E-4334.

3.3.2.2 Isolation of Compound CP-4 (14-Hydroxyviridiflorol)

Fraction E-12 (1.11 g) was fractionated on a silica gel column (40 g, 2.4 × 20 cm) eluted with hexane-acetone (5:1). Forty-two fractions (20 ml each) were collected and combined based on their TLC pattern (solvent system: hexane-acetone = 3:1) to give five fractions (E-121 – E-125). Fraction E-124 (675.8 mg) was repeatedly purified on a Sephadex LH-20 column (1.7 × 25 cm), using $CHCl_3$-MeOH (1:1) as the eluent, to give compound CP-4 (45.7 mg, 0.0071% yield).

3.3.3 Isolation of Compounds from the $CHCl_3$ Extract of *C. penduliflorus* Stem Bark

The $CHCl_3$ extract (26.9 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 × 6 cm) eluted with hexane-acetone gradient (2:1 to 1:2). The eluates were examined by TLC (solvent system: hexane-acetone = 3:1).

Fifty-seven fractions (300 ml each) were collected and combined to yield eight fractions (F-1-F-8, Table 11).

Table 10. Combined fractions from $CHCl_3$ extract of *C. penduliflorus* stem bark

Fraction Code	Weight (g)
F-1	2.79
F-2	2.65
F-3	3.97
F-4	2.03
F-5	2.98
F-6	3.76
F-7	2.48
F-8	2.69

3.3.3.1 Isolation of Compound CP-15 (Ethyl Orsellinate), CP-3 (Cabraleadiol) and CP-8 (Cabraleahydroxylactone)

Fraction F-2 (2.65 g) was separated on a silica gel column (75 g, 2.5 × 21 cm). Using hexane-acetone (5:1) as the mobile phase. Thirty-two fractions (50 ml each) were combined based on their TLC pattern (solvent system: hexane-acetone = 3:1) into five fractions (F-21-F-25). Fraction F-22 (896.5 mg) was further fractionated on another silica gel column (40 g, 2.4 × 17 cm) eluting with hexane-acetone (5:1). Twenty-five fractions (20 ml each) were collected and then combined according to their TLC behaviour (solvent system: hexane-acetone = 4:1) to yield five fractions: (F-221 - F-225). Fraction F-222, which exhibited an orange spot when detected with 10% sulphuric acid reagent, was separated on a Sephadex LH-20 column, using $CHCl_3$-MeOH (1:1) as the eluent, to yield compound CP-15 as colorless needles (78.9 mg, 0.012% yield).

Fraction F-24 (850.7 mg) was separated on a silica gel column (40 g, 2.5 × 20 cm) eluting with hexane-acetone (4:1) to give thirty-five fractions (20 ml each), which were then combined according to their TLC pattern (solvent system: hexane-acetone = 4:1) into five fractions (F-241 – F-245). Fraction F-242 (467.9 mg) was further purified on a silica gel column (40 g, 2.5 × 20 cm) eluting with hexane-acetone (6:1). Thirty fractions (15 ml each) were collected and then combined according to their

TLC behaviour (solvent system: hexane-acetone = 4:1) to yield four fractions: (F-2421 - F-2424). Again, compound CP-3 (86.5mg, 0.013% yield) was obtained from fraction F-2422 and compound CP-8 (50.8 mg, 0.008% yield) also precipitated from fraction F-2423.

3.3.3.2 Isolation of Compound CP-9 (Scoparone)

Fraction F-3 (3.97 g) was fractionated on a silica gel column (100 g, 5 × 22 cm) eluted with hexane - acetone (3:1) to give thirty-six fractions (20 ml each). After examined by TLC (solvent system: hexane-acetone = 2:1), they were combined into six fractions: (F-31 - F-36). Fraction F-35 (478.3 mg) was separated on another silica gel column (40 g, 2.3 × 17 cm) eluting with hexane - acetone (3:1). Thirty-seven fractions (15 ml each) were collected and combined (TLC solvent system: hexane:acetone = 3:1) to yield fractions F-351 - F-354. Fraction F-353 (256 mg) was further fractionated on another silica gel column (40 g, 2.4 × 17 cm) eluting with hexane-acetone (4:1) into twenty-five fractions (15 ml each), then combined (TLC solvent system: hexane-acetone = 2:1) into three fractions: (F-3531- F-3533). Another quantity of compound CP-9 (95.5 mg, 0.015% yield) was obtained from fraction F-3531

3.3.3.3 Isolation of Compound CP-12 (*β*-sitosterol glucoside)

Fraction F-6 (3.76 g) was fractionated on a silica gel column (100 g, 5 × 22 cm) eluted with hexane - acetone (2:1). Forty-two fractions (30 ml each) were collected, then combined according to their TLC pattern (solvent system: hexane-acetone = 2:1) into seven fractions: (F-61- F-67). Compound CP-12 (58.9 mg, 0.219% yield) was obtained from fraction F-66.

3.4 Extraction and Isolation of Compounds from the Leaves of cf. *Aglaia erythrosperma*

3.4.1 Extraction

The dried, powdered leaves (400 g) were macerated with 95% ethanol. After evaporation under reduced pressure, the residue was pre-adsorbed on kieselguhr,loaded onto a silica gel column and successively washed down with hexane (5 × 3 L), $CHCl_3$ (5 × 3 L) and MeOH (5 × 3 L), respectively. Each extract was evaporated under reduced pressure to afford the hexane extract (32 g, 8% based

on dried weight of leaves), $CHCl_3$ extract (25 g, 6.25% of dried weight), and MeOH extract (23 g, 5.75% of dried weight).

3.4.2 Isolation of Compounds from the Hexane Extract of cf. *Aglaia erythrosperma* Leaves

The hexane extract (32.0 g) was dissolved in a small amount of $CHCl_3$, triturated with kieselguhr and dried at room temperature, then applied on top of a silica gel column (400 g, 13 × 6 cm) and eluted stepwise with hexane-acetone (6:1). The eluates were examined by TLC (solvent system: hexane-acetone = 5:1). Fifty-eight fractions (300 ml each) were collected and combined into eleven fractions (G-1-G-11, Table 12).

Table 11. Combined fractions from hexane extract of cf. *Aglaia erythrosperma* leaves

Fraction Code	Weight (g)
G-1	2.78
G-2	1.57
G-3	3.67
G-4	2.18
G-5	1.98
G-6	3.75
G-7	2.59
G-8	3.96
G-9	1.43
G-10	1.99
G-11	3.93

3.4.2.1 Isolation of Compound AE-1(Cabraleadiol)

Fraction G-6 (3.75 g) was separated on a silica gel column (75 g, 2.5 × 20 cm) eluting with hexane-acetone (4:1) to give thirty-five fractions (30 ml each). Similar TLC pattern (solvent system: hexane-acetone = 4:1)of these fractions led to five combined fractions (G-61 – G-65). Fraction G-62 (867.9 mg) was further purified on a silica gel column (40 g, 2.5 × 20 cm) eluting with hexane-acetone (6:1). Thirty

fractions (15 ml each) were collected and then combined according to their TLC behaviour (solvent system: hexane-acetone = 4:1) to yield fractions G-621 - G-624. Compound AE-1 (76.5 mg, 0.019% yield) was obtained as white amorphous powder from fraction F-622. Comparison of the TLC pattern and ^{1}H-NMR spectra later showed compounds AE-1 and CP-3 to be identical.

3.4.2.2 Isolation of Compound AE-2 (Cabraleahydroxylactone)

Fraction G-7 (2.59 g) was separated on a Sephadex LH-20 column, using $CHCl_3$-MeOH (2:1) as the eluent. Thirty-seven fractions (20 ml each) were collected and combined (TLC solvent system: hexane-acetone = 4:1) into five fractions (G-71-G-75). Compound AE-2 (18.7 mg, 0.0005% yield) was obtained as white amorphous powder from fraction G-72. Comparison of this compound was later shown, through TLC pattern and NMR spectra, to be the same as compound CP-8.

3.4.3 Isolation of Compounds from the $CHCl_3$ Extract of cf. *Aglaia erythrosperma* Leaves

The $CHCl_3$ extract (25 g) was redissolved in a small amount of $CHCl_3$, triturated with kieselguhr and dried. It was then applied on top of a silica gel column (400 g, 13 × 6 cm) eluting with $CHCl_3$. Fifty-seven fractions (300 ml each) were collected and fractions with similar TLC pattern in $CHCl_3$ were combined to yield eight fractions (H-1-H-8, Table 13).

Table 12. Combined fractions from $CHCl_3$ extract of cf. *Aglaia erythrosperma* leaves

Fraction Code	Weight (g)	Fraction Code	Weight (g)
H-1	2.45	H-5	1.44
H-2	1.24	H-6	2.76
H-3	2.07	H-7	2.59
H-4	2.45	H-8	2.76

3.4.3.1 Isolation of Compound AE-3 (Scoparone)

Separation of fraction H-5 (1.44 g) on a Sephadex LH-20 column, using $CHCl_3$-MeOH (1:1) as the eluent, yielded four fractions (H-51 –H-54). Then, fraction

H-52 (479.5 mg) were repeatedly chromatographed on a Sephadex LH-20 column (1.7 × 25 cm) using the same eluent, to give compound AE-3 as yellow needle crystals (79.8 mg, 0.02% yield). This compound was later shown to be the same as compound CP-9.

3.4.3.2 Isolation of Compound AE-4 (Aglaialactone)

Fraction H-6 (2.76 g) was separated on a Sephadex LH-20 column, with $CHCl_3$-MeOH (1:1) as the eluent. Ten fractions (30 ml each) were collected and combined (TLC solvent system: $CHCl_3$-MeOH = 30:1) into five fractions (H-61 – H-65). Fraction H-62 (657.5 mg) was further fractionated on a silica gel column (50 g, 2.4 × 29 cm) eluted with $CHCl_3$-MeOH (30:1) to give five combined fractions (H-621 –H-625). Compound AE-4 was obtained as white amorphous powder (102.4 mg, 0.0256% yield) from fraction H-623.

3.5 Extraction and Isolation of compounds from the pericarp of cf. *Aglaia erythrosperma*

3.5.1 Extraction

The dried, powdered fruits (1kg) were macerated with 95% ethanol. The dried residue was pre-adsorbed on kieselguhr and successively washed down with hexane (5 × 3 L), $CHCl_3$ (5 × 3 L) and MeOH (5 × 3 L), respectively. Each extract was evaporated under reduced pressure to afford the hexane extract (60.0 g, 6.00% base on dried weight of fruits), $CHCl_3$ extract (40.0 g, 4.00% of dried weight), and MeOH extract (31.2 g, 3.12% of dried weight).

3.5.2 Isolation of Compounds from the Hexane Extract of cf. *Aglaia erythrosperma* Pericarp

The hexane extract (60.0 g) was dissolved in a small amount of $CHCl_3$, triturated with kieselguhr and dry-packed onto a silica gel column (500 g, 13 × 8 cm). Vacuum liquid column chromatography, eluting stepwise with hexane – acetone gradient (10:1 to 0:1) and examined by TLC (solvent system: hexane-acetone = 5:1) yielded sixty fractions (300 ml each), which were then combined into nine major fractions (I-1-I-9, Table 14).

Table 13. Combined fractions from hexane extract of cf. *Aglaia erythrosperma* pericarp

Fraction Code	Weight (g)
I-1	3.18
I-2	2.56
I-3	2.14
I-4	2.17
I-5	4.56
I-6	4.18
I-7	3.13
I-8	3.34
I-9	3.43

3.5.2.1 Isolation of Compound AE-1 (Cabraleadiol)

Fraction I-5 (4.56 g) was fractionated on a silica gel column (100 g, 5 × 8 cm), eluted with hexane - acetone (5:1) to give thirty-five fractions (20 ml each). Similarity of TLC pattern (solvent system: hexane-acetone = 4:1) into six fractions (I-51 - I-56). Fraction I-53 (875.9 mg) was separated on a silica gel column (40 g, 2.5 × 20 cm) using hexane-acetone (4:1) as the eluent and TLC system, to afford thirty-two fractions (20 ml each). Six combined fractions: (I-531 - I-536) were obtained. Further purification of fraction I-533 (275.3 mg) on a silica gel column (50 g, 3.2 × 20 cm) eluted with hexane-acetone (4:1) again yielded compound AE-1 (194.4 mg, 0.019% yield).

3.5.2.2 Isolation of Compound (Cabraleahydroxylactone)

Fraction I-6 (4.18 g) was chromatographed on a silica gel column (75 g, 2.9 × 24 cm) eluting with hexane-acetone (4:1) to give thirty-six fractions (30 ml each). Similar fractions TLC (solvent system: hexane-acetone = 4:1) were pooled into seven fractions (I-61 - I-67). Compound AE-2 (278.5 mg, 0.028% yield) was obtained from fraction I-65.

3.5.2.3 Isolation of Compound AE-5 (Ethyl eichlerianoate)

Fraction I-7 (3.13 g) was subjected to silica gel column chromatography (75 g, 2.9 × 24 cm) using hexane-acetone (2:1) as the solvent system to yield forty-three

fractions (30 ml each). These fractions were combined according to their TLC pattern (solvent system: hexane-acetone = 3:1) into five fractions: (I-71 - I-75). Fraction I-73 (845.2 mg) was fractionated on another silica gel column (20 g, 2.1 × 15 cm) eluted with hexane-acetone (3:1) to give nine fractions (I-731 - I-739). Further purification of fraction I-734 (438.7 mg) on another silica gel column (75 g, 2.9 × 18 cm) eluted with hexane-acetone (5:1) gave compound AE-5 as white amorphous powder (5.4 mg, 0.0005% yield).

3.5.2.4 Isolation of Compound AE-6 (Eichlerialactone)

Fraction I-8 (3.34 g) was separated on a silica gel column (75 g, 2.9 × 24 cm) eluted and mornitored with hexane-acetone (4:1) to give thirty-five fractions (20 ml each), later combined into fractions I-81 - I-87. Compound AE-6 precipitated as white amorphous powder (86.3 mg, 0.008% yield) from fraction I-85. It was subsequently shown to be the same as compound CP-6.

3.5.3 Isolation of Compounds from the $CHCl_3$ Extract of cf. *Aglaia erythrosperma* Pericarp

The $CHCl_3$ extract (40.0 g) was dry-packed onto a silica gel column (400 g, 13 × 6 cm) eluted with hexane-acetone gradient (3:1 to 0:1). Fifty-seven fractions (300 ml each) were collected and fractions with similar chromatographic pattern were combined after TLC inspection (solvent system: hexane – acetone = 3:1) to yield eight fractions (J-1-J-8, Table 15).

Table 14. Combined fractions from $CHCl_3$ extract of cf. *Aglaia erythrosperma* pericarp

Fraction Code	Weight (g)	Fraction Code	Weight (g)
J-1	3.43	J-3	3.01
J-2	3.76	J-4	3.56

Table 14. Combined fractions from $CHCl_3$ extract of cf. *Aglaia erythrosperma* pericarp (continued)

Fraction Code	Weight (g)	Fraction Code	Weight (g)
J-5	3.18	J-7	3.76
J-6	4.97	J-8	3.65

3.5.3.1 Isolation of Compound AE-7 (Aglinin A)

Fraction J-4 (3.56 g) was fractionated on a silica gel column chromatography (75 g, 2.9 × 24 cm), eluted with hexane-acetone (4:1). The eluates (55 fractions, 30 ml each) were collected and combined according to their TLC pattern (solvent system: hexane-acetone = 4:1) into 5 fractions (J-41 – J-45). After removal of the solvent, fraction J-44 yielded compound AE-7 as white amorphous powder (150.7 mg, 0.015% yield).

3.5.3.2 Isolation of Compound AE-1 (Cabraleadiol) and Compound AE-2 (Cabraleahydroxylactone)

Fraction J-5 (3.18 g) was subjected to a silica gel column (75 g, 2.9 × 24 cm) eluted with hexane-acetone (5:1) to give thirty-seven fractions (30 ml each). After being examined by TLC (solvent system: hexane-acetone = 4:1), they were combined into five fractions (J-51 – J-55). Fraction J-54 (735.6 mg) recrystallized in hexane-acetone (4:1) yielded an additional amount of compound AE-1 (248.7 mg, 0.025% yield).

Fraction J-52 (674.7 mg) was chromatographed on a silica gel column (30 g, 2.3 × 18 cm) eluting with hexane-acetone (6:1) to give thirty-seven fractions (20 ml each), which were combined, after TLC (solvent system: hexane-acetone = 4:1), into five fractions (J-521 – J-525). Compound AE-2 (23 mg, 0.0023% yield) was obtained from fraction J-523.

3.5.3.3 Isolation of compound AE-8 (scopoletin)

Fraction J-6 (4.97 g) was further separated on a silica gel column (75 g, 2.9 × 24 cm) eluted with hexane - acetone (2:1). The eluates (20 ml each) were examined by TLC (solvent system: hexane-acetone = 3:1) and then combined to yield five fractions: (J-61 – J-65). Fraction J-65 (450.8 mg) was separated on a Sephadex

LH-20 column, using $CHCl_3$-MeOH (1:1) as the eluent. Five combined fractions (J-651 – J-655) were collected. Compound AE-8 was obtained as yellow needle crystals(35.7 mg, 0.0036% yield) from fraction J-654. Finally, both compounds AE-8 and CP-10 were interpreted as the same compound.

3.6 Extraction and Isolation of compounds from the seeds of cf. *Aglaia erythrosperma*

3.6.1 Extraction

The dried, powdered seeds (200 g) were macerated with 95% ethanol. After evaporation under reduced pressure, the residue was pre-adsorbed on kieselguhr and washed down with hexane (5 × 3 L), $CHCl_3$ (5 × 3 L) and MeOH (5 × 3 L), respectively. Evaporation under reduced pressure afforded the hexane extract (6.13 g, 3.065% based on dried weight of fruits), $CHCl_3$ extract (2.0 g, 1.0% of dried weight), and MeOH extract (22.2 g, 11.1% of dried weight).

3.6.2 Isolation of Compounds from the Hexane Extract of cf. *Aglaia erythrosperma* Seeds

The hexane extract (6.13 g) was dissolved in a small amount of $CHCl_3$, triturated with kieselguhr, dried then applied on top of a silica gel column (180 g, 5 × 22 cm), eluted with hexane-acetone gradient (10:1 to 0:1). Sixty fractions (50 ml each) were collected and fractions with similar TLC pattern (solvent system: hexane-acetone = 5:1) were combined to yield nine fractions (K-1-K-9, Table 16).

Table 15. Combined fractions from hexane extract of *Aglaia erythrosperma*) seeds

Fraction Code	Weight (mg)	Fraction Code	Weight (mg)
K-1	450.3	K-6	564.7
K-2	654.1	K-7	654.7
K-3	507.4	K-8	543.2
K-4	675.3	K-9	475.9
K-5	437.1		

3.6.2.1 Isolation of Compound AE-9 (Cabralealactone)

Fraction K-1 (450.3 mg) was subjected to silica gel column chromatography (75 g, 3.2 × 20 cm). Hexane-acetone (5:1) was used as the mobile phase. Thirty-two collected fractions (30 ml each) were combined (TLC solvent system: hexane-acetone = 3:1) into eight fractions (K-11 - K-18). Fraction K-16 (179.6 mg) was recrystallized in hexane-acetone (3:1) to yield compound AE-9 as white amorphous powder (76.8 mg, 1.25% yield). TLC comparison and NMR analysis revealed that compounds AE-9 and CP-7 were the same compound.

3.6.2.2 Isolation of Compound AE-1 (Cabraleadiol)

Fraction K-2 (654.1 mg) was fractionated on a silica gel column (40 g, 2.5 × 20 cm) eluted with hexane-acetone (5:1) to give thirty-five fractions (20 ml each), later combined into six fractions (K-21 - K-26). Fraction K-24 (375.5 mg) was futher purified on a silica gel column (75 g, 3.2 × 20 cm) eluting and monitoring with hexane-acetone (4:1) to give thirty-seven fractions (10 ml each), which were then combined into six fractions (K-241 - K-246). Compound AE-1 (97.8 mg, 1.59% yield) was obtained from fraction K-244.

3.6.2.3 Isolation of Compound AE-2 (Cabraleahydroxylactone)

Further separation of fraction K-4 (675.3 mg) on a silica gel column (20 g, 2.1 × 15 cm) using hexane-acetone (5:1) gave eight combined fractions (K-41 - K-48). Compound AE-2 (58.6 mg, 0.956% yield) precipitated from fraction K-46.

3.6.2.4 Isolation of Compound AE-6 (Eichlerialactone)

Fraction K-6 (564.7 mg) was fractionated by silica gel column chromatography (50 g, 2.9 × 15 cm) using hexane-acetone (3:1) to afford fifty-six fractions (20 ml each). After each fraction was examined by TLC, using the same solvent system, they were combined into eight fractions: (K-61 - K-68). Compound AE-6 (58.7 mg, 0.957% yield) was obtained from fraction K-63.

3.6.3 Isolation of Compound AE-10 from the $CHCl_3$ and MeOH Extracts of cf. *Aglaia erythrosperma*

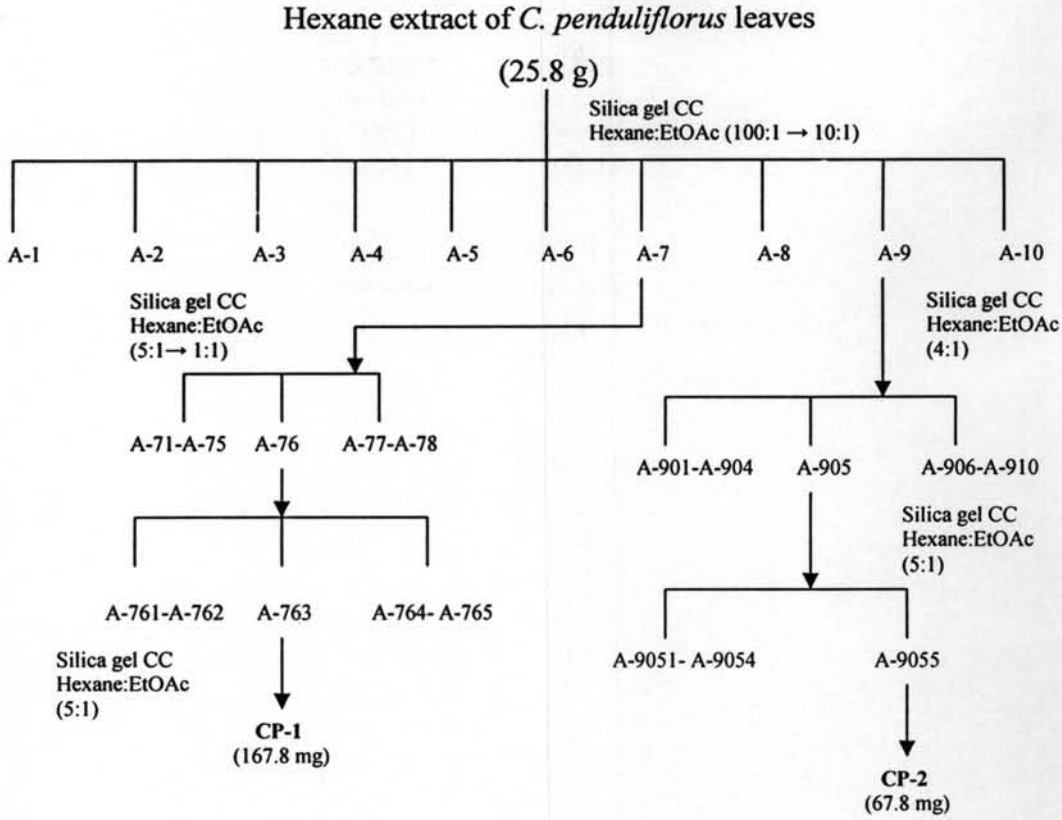
Seeds

The $CHCl_3$ extract (2.0 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 (75 g, 2.5 × 21 cm), eluted stepwise with a gradient of hexane-acetone (2:1 to 0:1). The eluates were examined by TLC (solvent system: hexane-acetone = 3:1). Fifty-seven fractions (50 ml each) were collected and fractions with similar chromatographic pattern were combined to yield eight fractions (L-1-L-6).

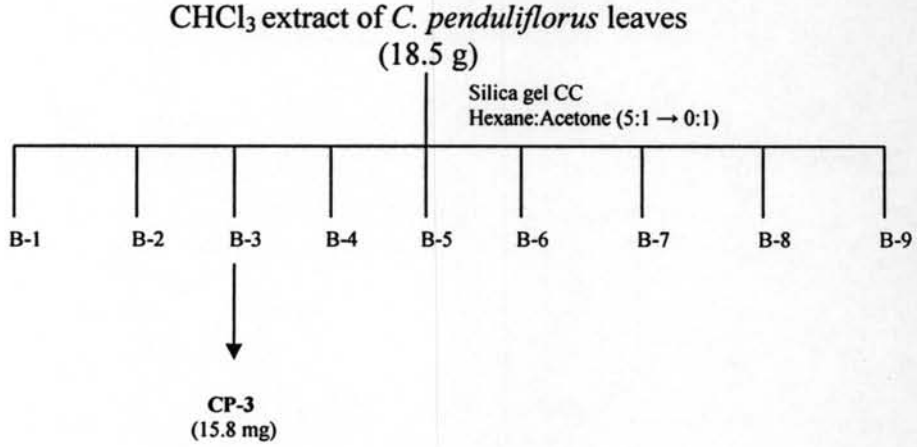
Fraction L-5 (324 mg) was separated on a silica gel column chromatography (20 g, 2.1 × 15 cm) using $CHCl_3$-MeOH (30:1) to give five combined fractions (L-51 – L-55). Fraction L-54 (167.9 mg) was further separated on another silica gel column (20 g, 2.1 × 15 cm), using hexane-acetone (4:1) as the mobile phase. Ten fractions (10 ml each) were collected and combined according to their behaviour on TLC (solvent system: hexane-acetone = 3:1) into 4 fractions (L-541 – L-544). After removal of the solvent, fraction L-543 yield compound AE-10 as white amorphous powder (37.5 mg, 0.019% yield).

The MeOH extract (22.2 g) was dry – packed on top of a silica gel column (400 g, 13 × 6 cm), then eluted stepwise with $CHCl_3$-MeOH gradient (4:1 to 0:1). Fifty-seven fractions (200 ml each) were collected and fractions with similar chromatographic pattern (solvent system: $CHCl_3$-MeOH = 30:1) were combined to yield six fractions (M-1-M-6).

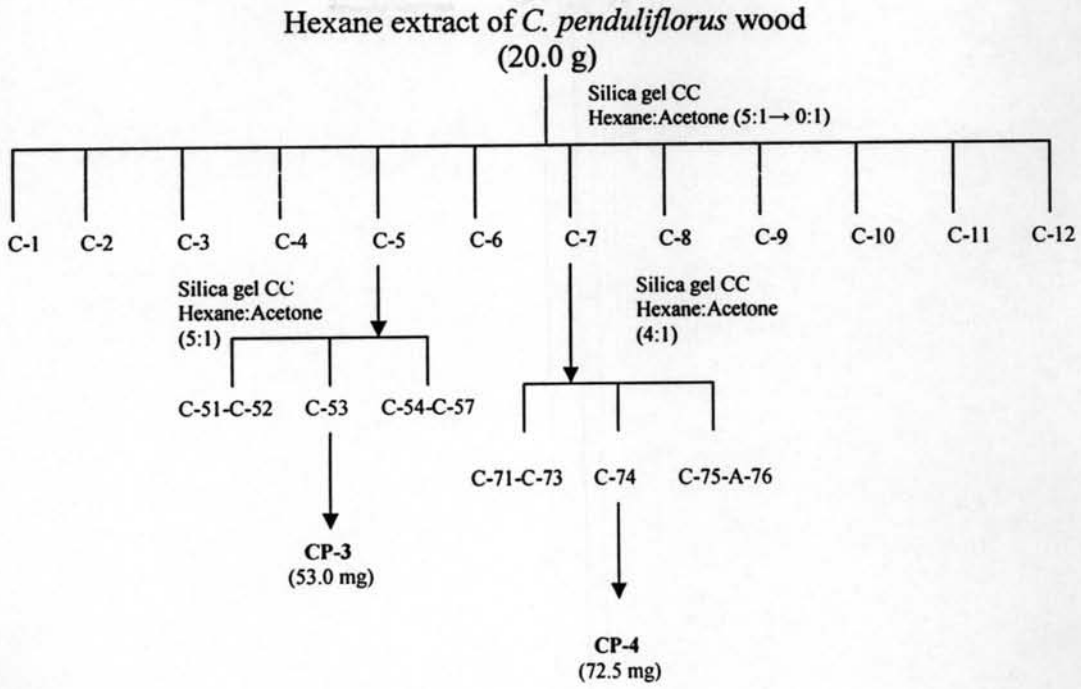
Fraction M-2 (3.28 mg) was separated on a silica gel column (75 g, 2.9 × 24 cm) using $CHCl_3$-MeOH (30:1) to give five combined fractions (M-21 – M-25). Fraction M-24 (867.9 mg) was further separated on another silica gel column (20 g, 2.1 × 15 cm), using hexane-acetone (4:1) as the mobile phase. Twenty fractions (20 ml each) were collected and combined according to their behaviour on TLC (solvent system: hexane-acetone = 3:1) into 5 fractions (M-241 –M-245). After removal of the solvent, fraction M-243 yielded an additional quantity of compound AE-10 (56.0 mg, 0.028% yield).



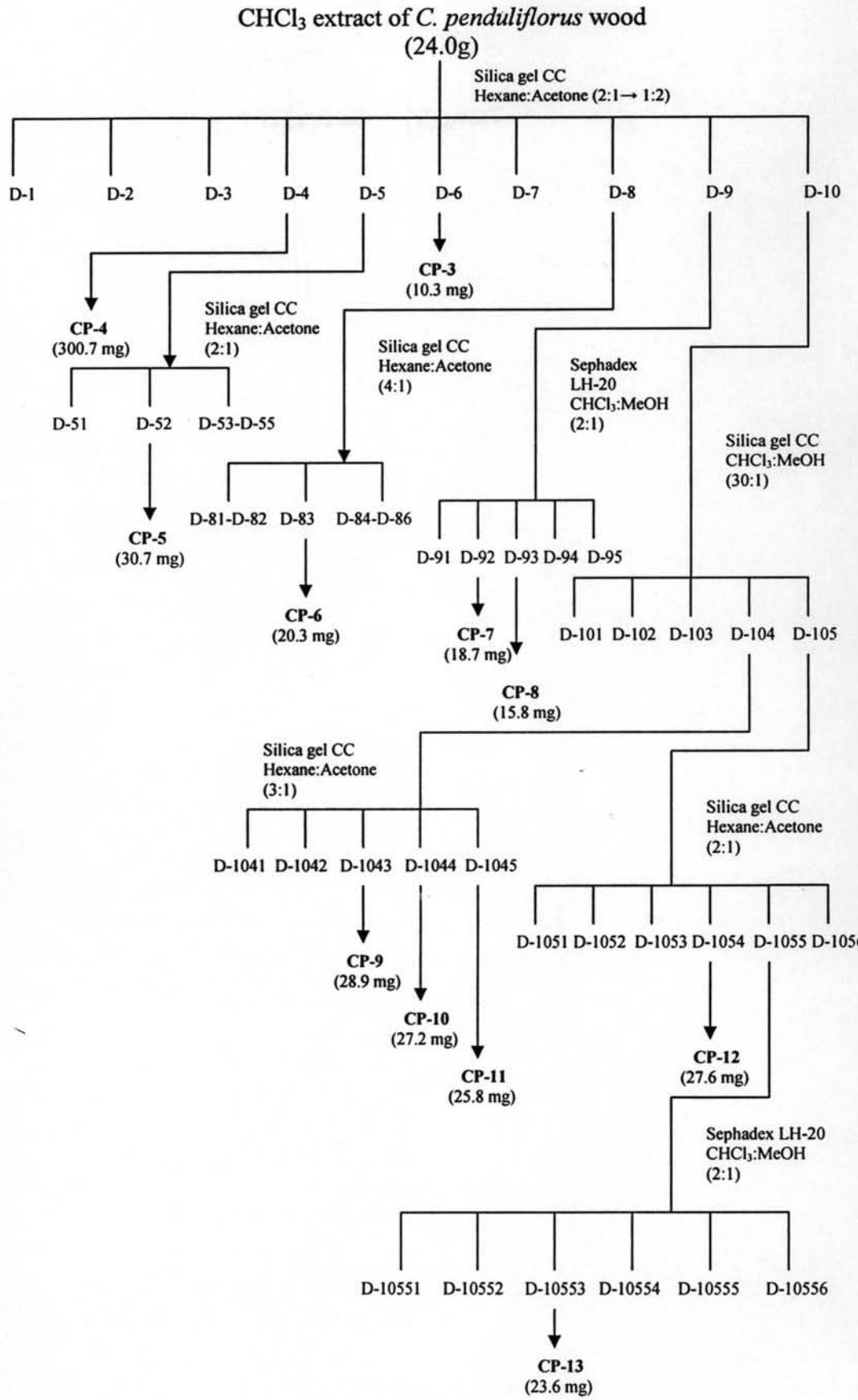
Scheme 1. Isolation of compounds from the hexane extract of *Chisocheton penduliflorus* leaves



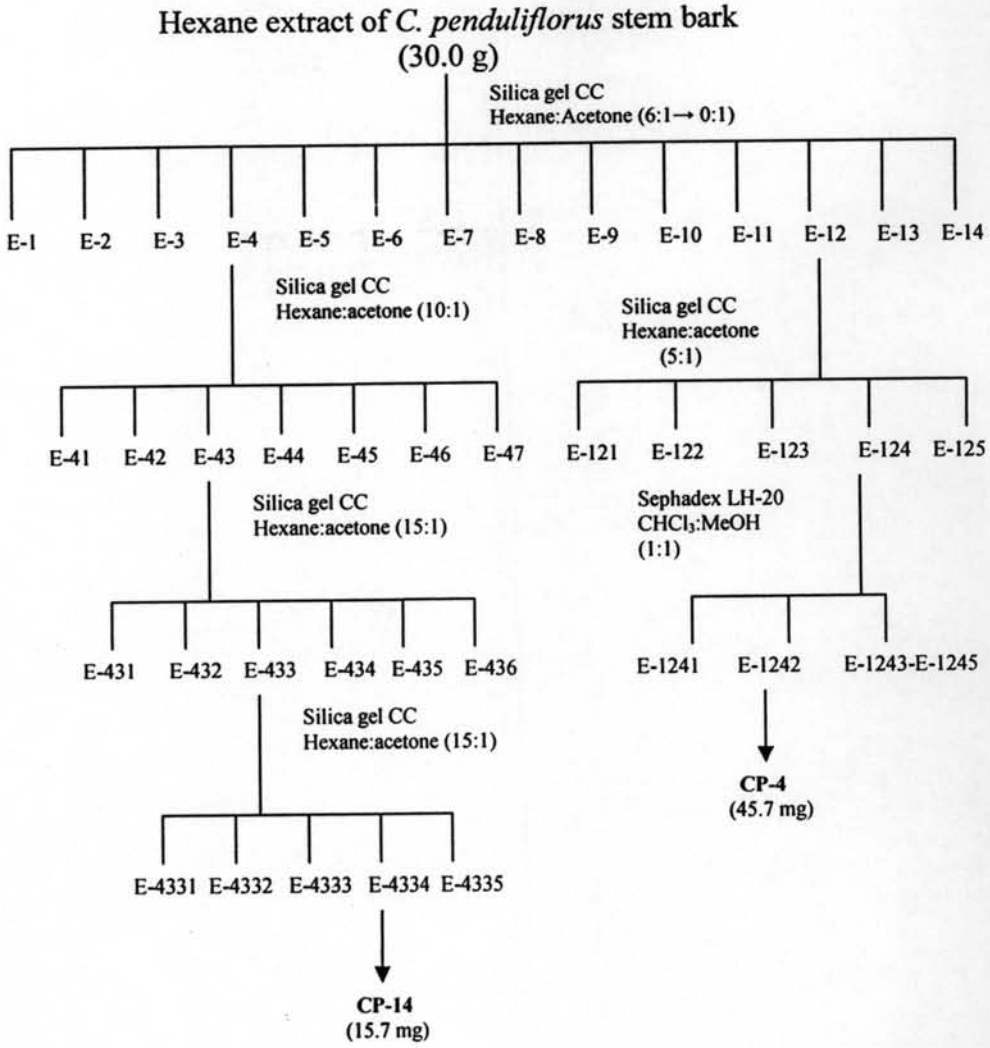
Scheme 2. Isolation of compound CP-3 from the $CHCl_3$ extract of *C. penduliflorus* leaves



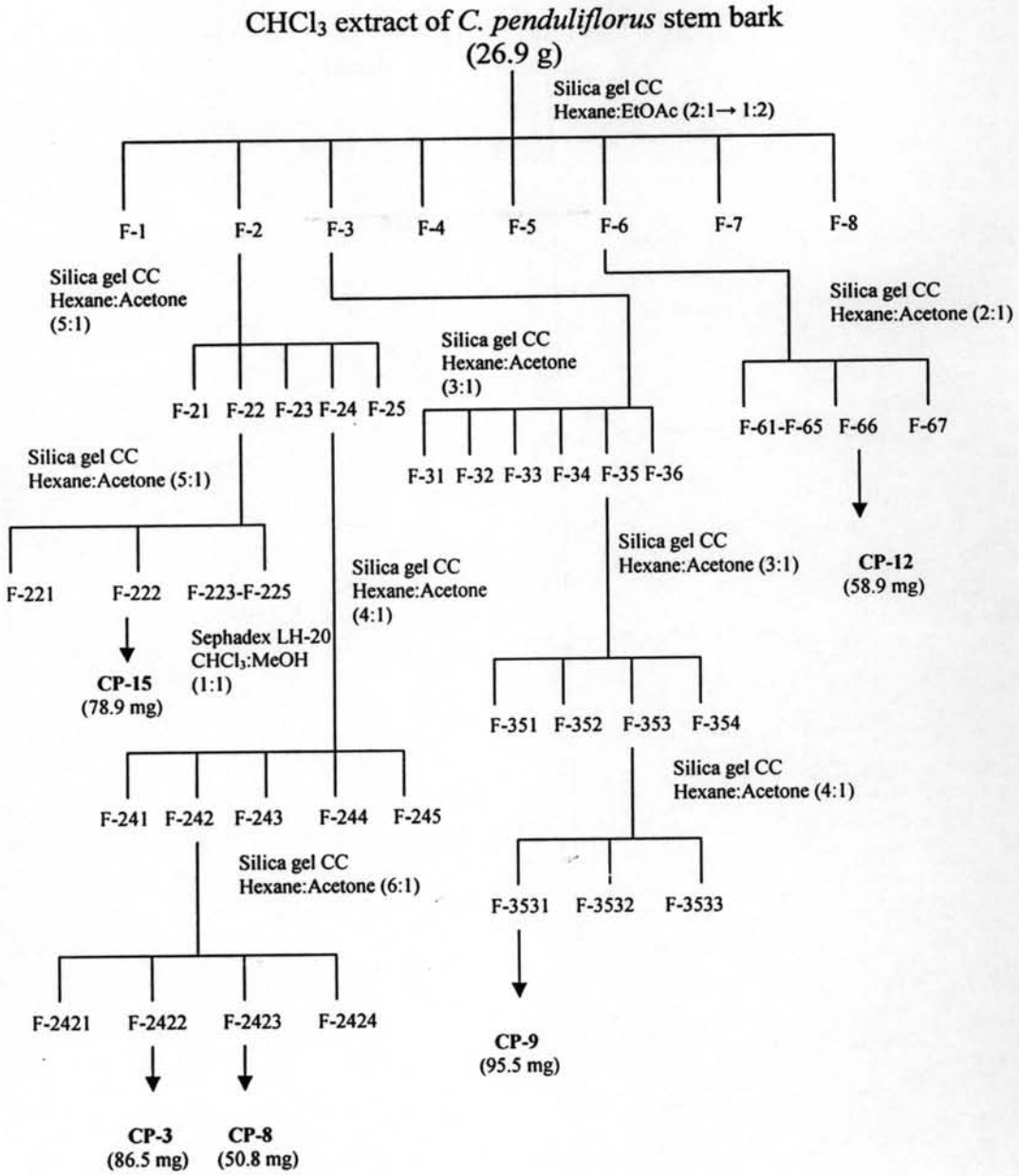
Scheme 3. Isolation of compounds from the hexane extract of *C. penduliflorus* wood



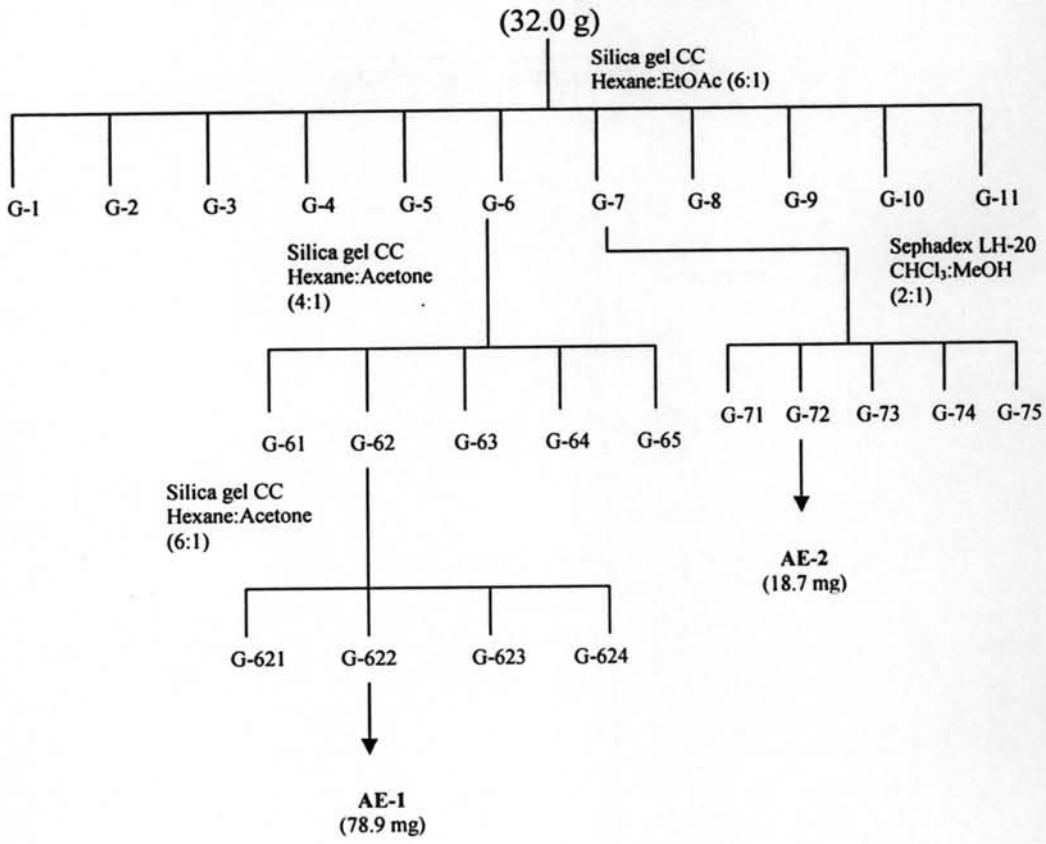
Scheme 4. Isolation of compounds from the $CHCl_3$ extract of *C. penduliflorus* wood



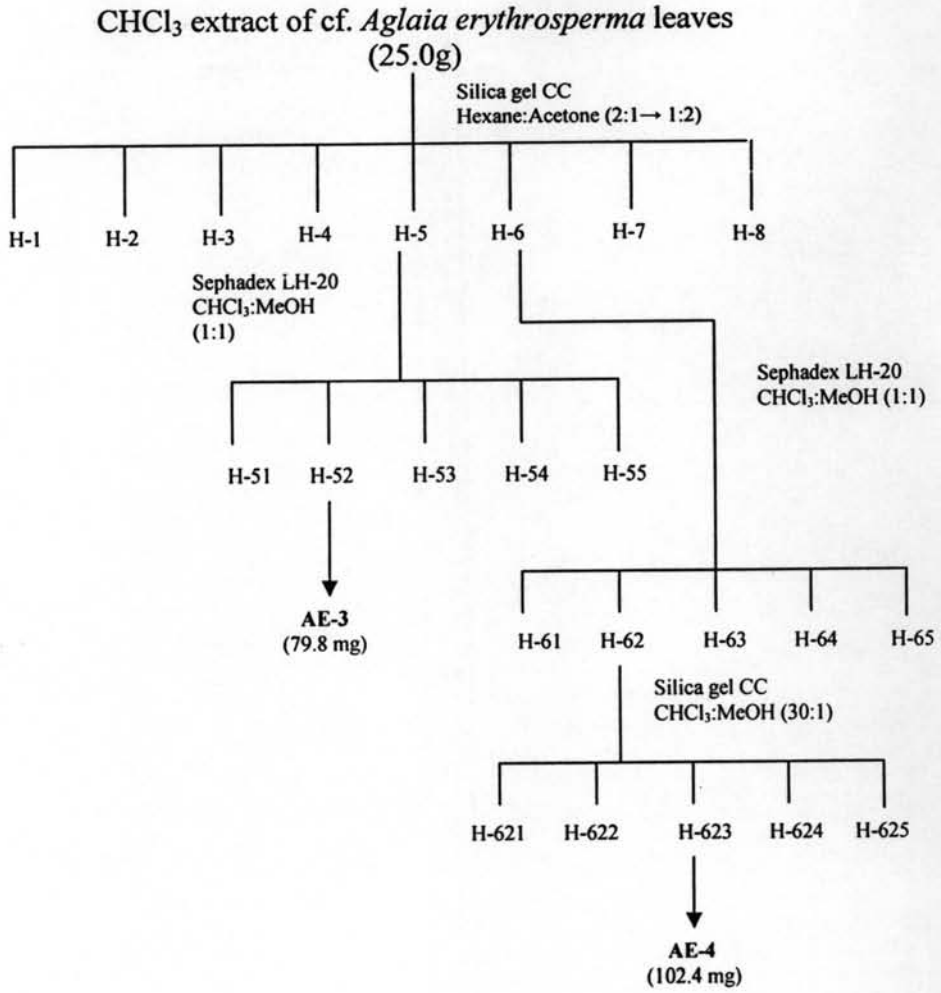
Scheme 5. Isolation of compounds from the Hexane extract of *C. penduliflorus* stem bark



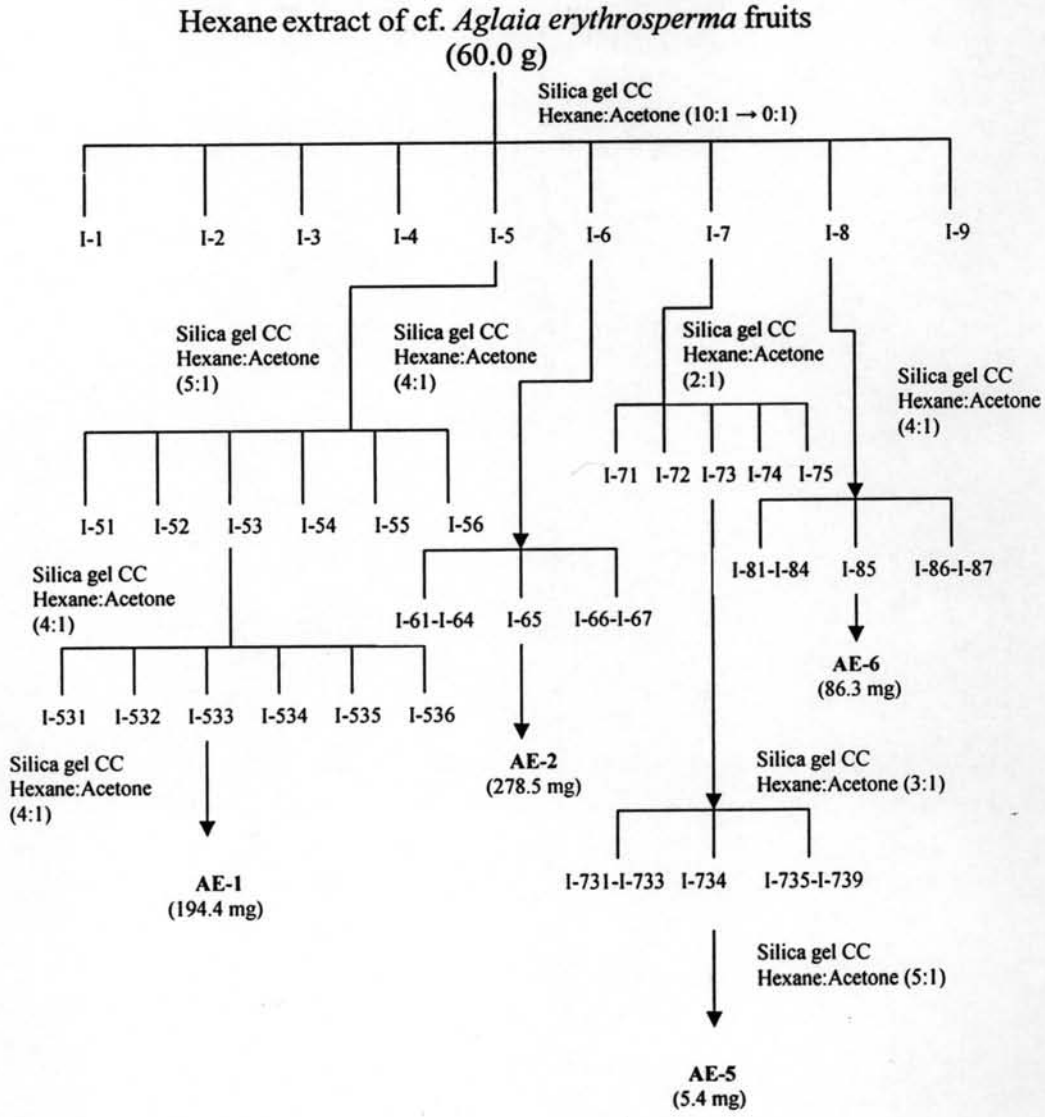
Scheme 6. Isolation of compounds from the $CHCl_3$ extract of *C. penduliflorus* from stem bark



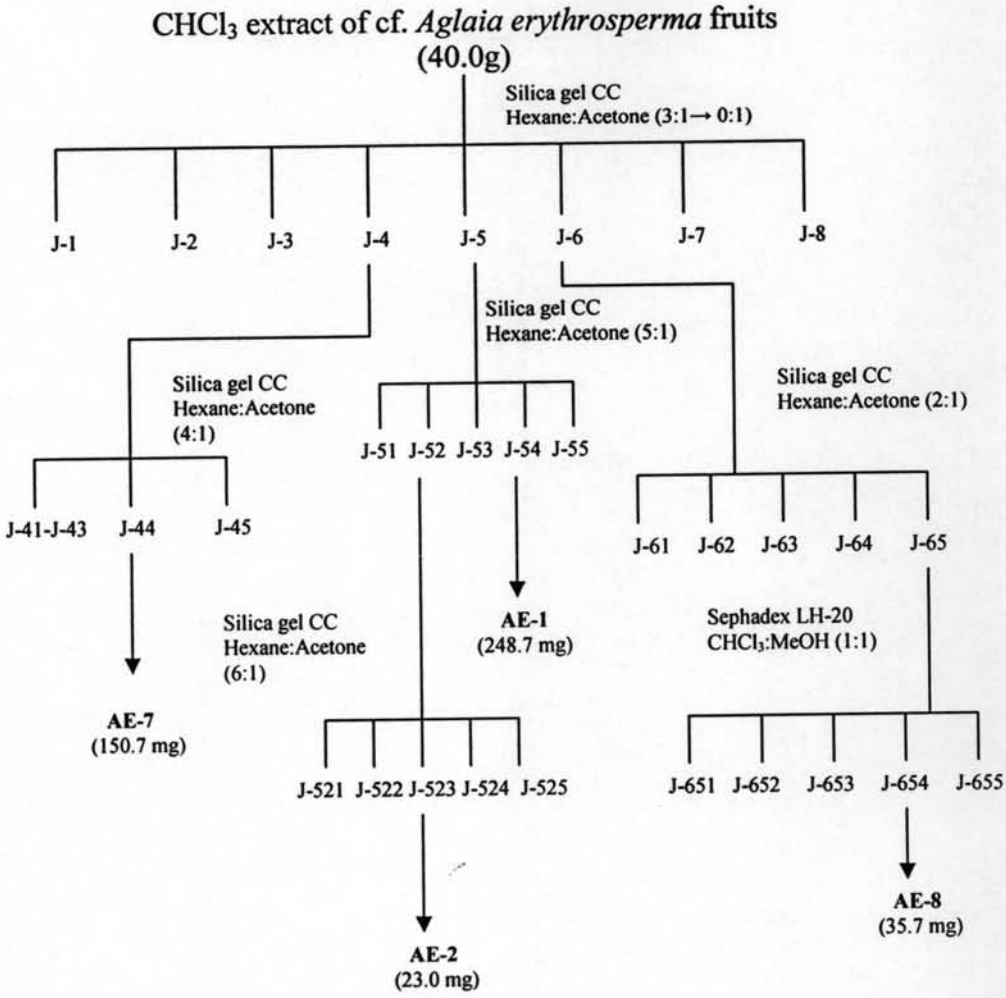
Scheme 7. Isolation of compounds from the hexane extract of cf. *Aglaia erythrosperma* leaves



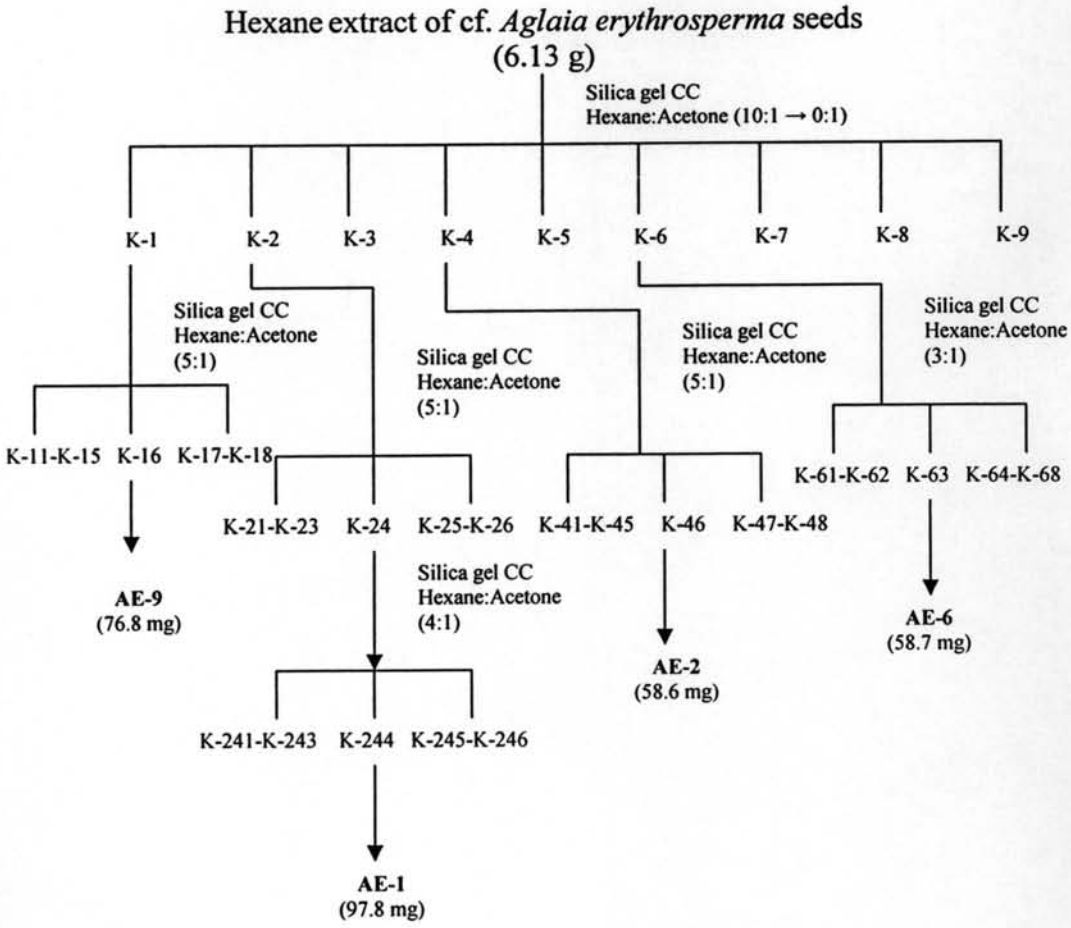
Scheme 8. Isolation of compounds from the $CHCl_3$ extract of cf. *Aglaia erythrosperma* leaves



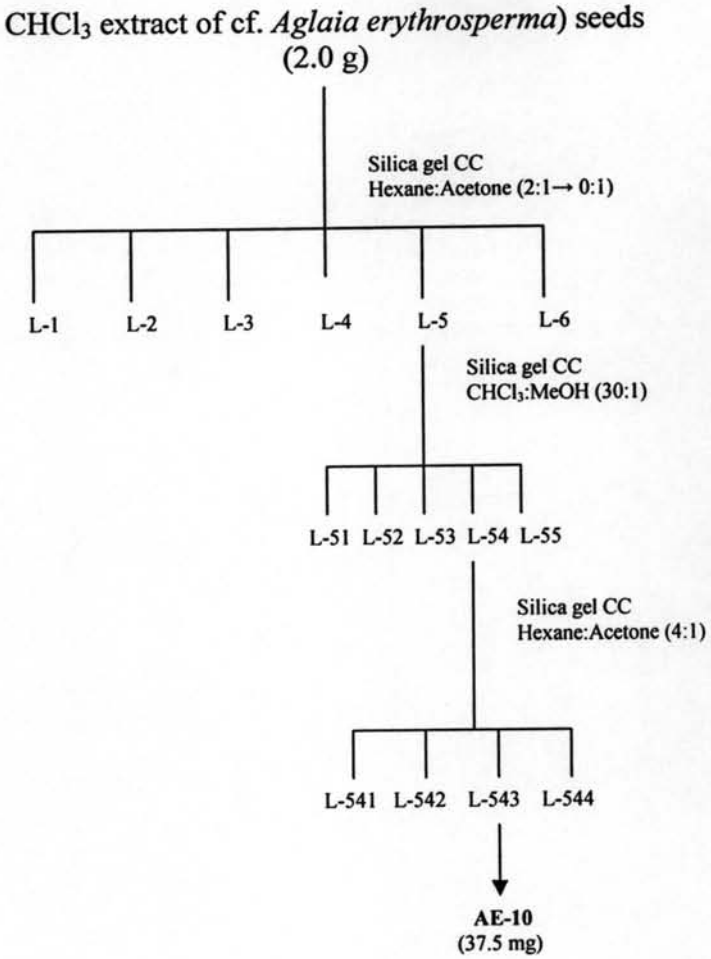
Scheme 9. Isolation of compounds from the hexane extract of cf. *Aglaia erythrosperma* pericarp



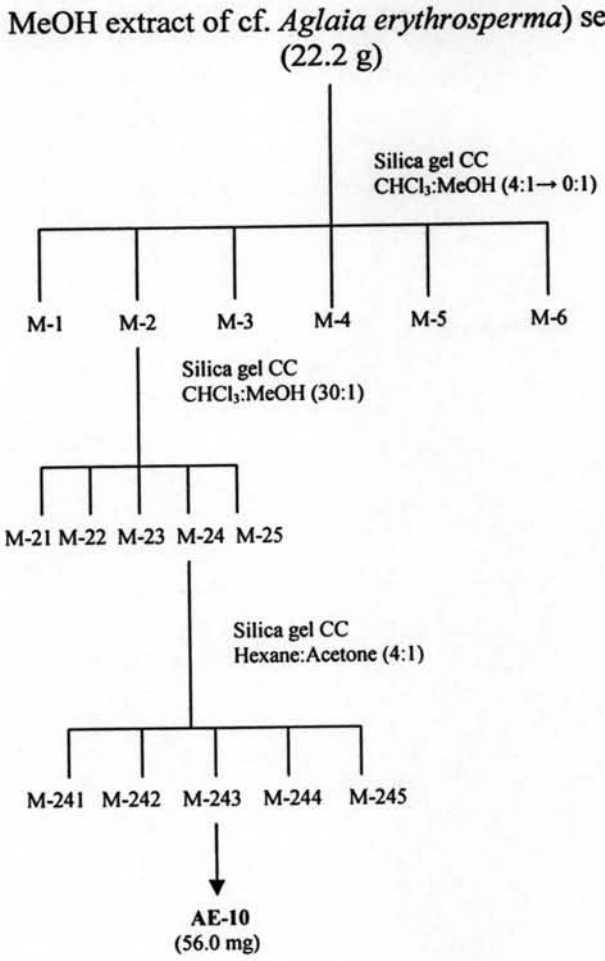
Scheme 10. Isolation of compounds from the $CHCl_3$ extract of cf. *Aglaia erythrosperma* pericarp



Scheme 11. Isolation of compounds from the hexane extract of cf. *Aglaia erythrosperma* seeds



Scheme 12. Isolation of compounds from the $CHCl_3$ extract of cf. *Aglaia erythrosperma* seeds



Scheme 13. Isolation of compounds from the MeOH extract of cf. *Aglaia erythrosperma* seeds

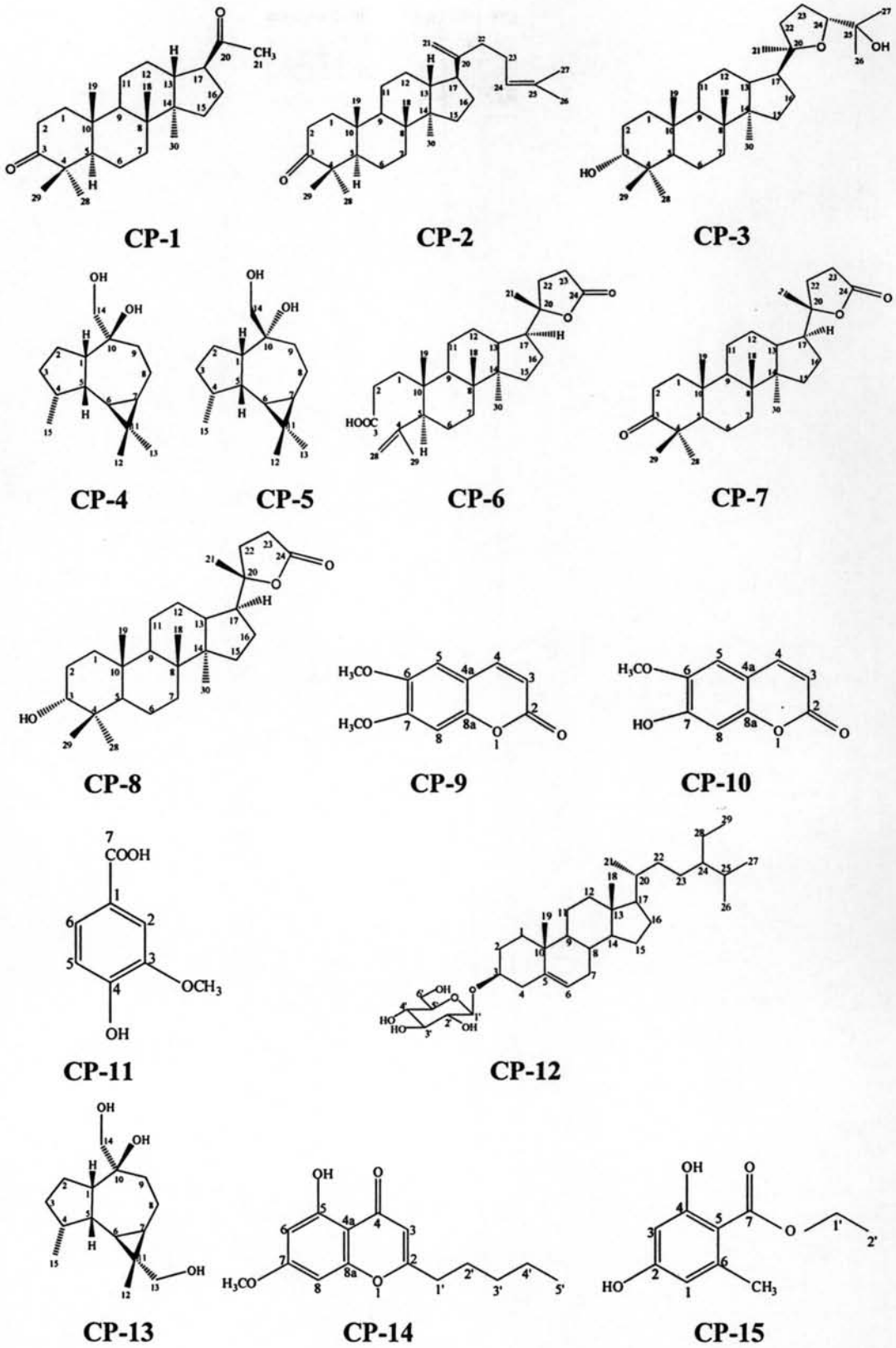
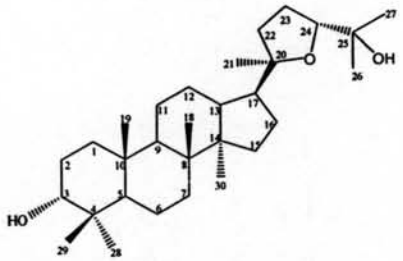
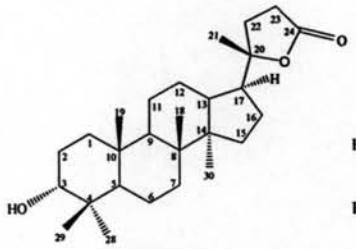


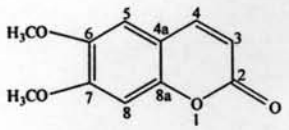
Figure 7. Structure of compounds isolated from leaves, wood and stem bark of *C. penduliflorus*



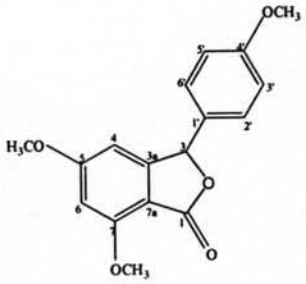
AE-1 (CP-3)



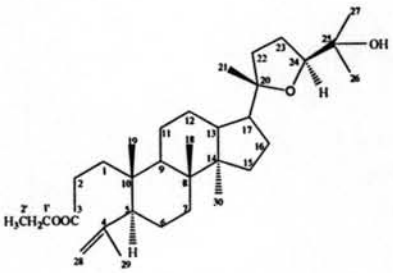
AE-2 (CP-8)



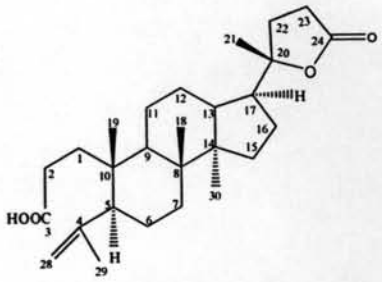
AE-3 (CP-9)



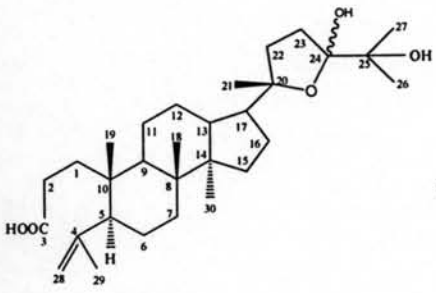
AE-4



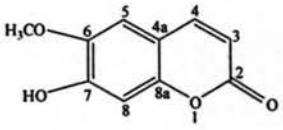
AE-5



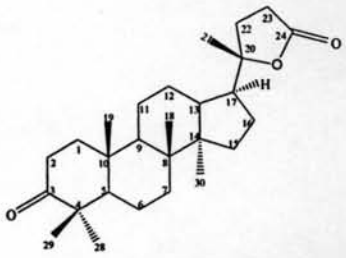
AE-6 (CP-6)



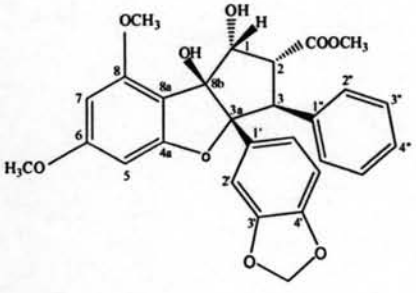
AE-7



AE-8 (CP-10)



AE-9 (CP-7)



AE-10

Figure 8. Structure of compounds isolated from leaves, pericarp and seeds of cf. *Aglaia erythrosperma*

4. Physical and Spectral Data of Isolated Compounds

4.1 Compound CP-1 (Hollongdione)

White amorphous powder

ESIMSMS : *m/z*; 381 $[M+Na]^+$; see **Figure 10**

Mp : 183-185 °C

$[\alpha]^{25}_D$: +87.6° (*c* 0.36,$CHCl_3$)

IR : ν_{max}^{cm-1}, KBr; 2931, 1700, 1455, 1365, 1176; see **Figure 9**

^1H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Table 16** and **Figure 11a-11c**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Table 16** and **Figure 12**

4.2 Compound CP-2 (Dammaradienone)

White amorphous powder

EIMS : *m/z*; 424 $[M]^+$; see **Figure 19**

Mp : 75-76 °C

$[\alpha]^{25}_D$: +90° (*c* 0.6,$CHCl_3$)

IR : ν_{max}^{cm-1}, KBr; 2944, 1706, 1455, 1375, 1218, 671; see **Figure 18**

^1H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Table 17** and **Figure 20a-20c**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Table 17** and **Figure 21**

4.3 Compound CP-3 (AE-1) (Cabraleadiol)

White amorphous powder

EIMS : *m/z*; 442 $[M-H_2O]^+$; see **Figure 28**

Mp : 175°C

$[\alpha]^{25}_D$: +19° (*c* 0.1,$CHCl_3$)

IR : ν_{max}^{cm-1}, KBr; 3457, 3183, 2948, 2861, 1710, 1461, 1380, 1305, 1139, 1047, 993; see **Figure 27**

^1H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Table 18** and **Figure 29a-29c**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Table 18** and **Figure 30**

4.4 Compound CP-4 (14-Hydroxyviridiflorol)

Coloeless needle crystals

ESI-TOFMS : *m/z*; 261.37 $[M+Na]^+$; see **Figure 37**

Mp : 75-76°C

$[\alpha]^{25}_D$: +2.6° ($CHCl_3$)

IR : ν_{max} $^{cm-1}$, KBr; 3517, 3295, 2925, 2867, 1737, 1455, 1375, 1238, 1064, 1029, 883, 676; see **Figure 36**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Table 19** and **Figure 38a-38d**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Table 19** and **Figure 39**

4.5 Compound CP-5 (14-Hydroxyepiviridiflorol)

Colorless needle crystals

HRESITOFMS: *m/z*; 261.1830 $[M+Na]^+$; see **Figure 46**

Mp : 100 - 105°C

IR : ν_{max} $^{cm-1}$, KBr; 3544, 3372, 2923, 2867, 1704, 1457, 1375, 1251, 1160, 1066, 877, 665; see **Figure 45**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Table 20** and **Figure 47a** and **47b**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Table 20** and **Figure 48**

4.6 Compound CP-6 (AE-6) (Eichlerialactone)

White amorphous powder

EIMS : *m/z*; 430 $[M]^+$; see **Figure 55**

IR : ν_{max} $^{cm-1}$, KBr; 2944, 1743, 1710, 1455, 1375, 1218, 937, 755, 655; see **Figure 54**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Table 21** and **Figure 56a** and **56b**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Table 21** and **Figure 57a and 57b**

4.7 Compound CP-7 (AE-9) (Cabralealactone)

White amorphous powder

EIMS : *m/z*; 414 $[M]^+$; see **Figure 64**

Mp : 181-183°C

$[\alpha]^{25}_{D}$: +70° (*c* 0.6,$CHCl_3$)

IR : ν_{max} $^{cm-1}$, KBr; 2944, 2854, 1739, 1704, 1454, 1375, 1216, 1139, 1076, 946, 667; see **Figure 63**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Table 22** and **Figure 65**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Table 22** and **Figure 66**

4.8 Compound CP-8 (AE-2) (Cabraleahydroxylactone)

White amorphous powder

EIMS : *m/z*; 416 $[M]^+$; see **Figure 69**

Mp : 240-242°C

$[\alpha]^{25}_{D}$: +24° (*c* 0.6,$CHCl_3$)

IR : ν_{max}^{cm-1}, KBr; 3463, 2944, 1747, 1704, 1454, 1376, 1216, 1072, 933; see **Figure 68**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Table 23** and **Figure 70a-70c**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Table 23** and **Figure 71**

4.9 Compound CP-9 (AE-3) (Scoparone)

Yellow needle crystals

EIMS : *m/z*; 207 $[M+H]^+$; see **Figure 79**

Mp : 147°C

UV : λ_{max}nm (log ε), in MeOH; 228 (4.32), 296 (4.06), 343 (3.90), **Figure 77**

IR : ν_{max}^{cm-1}, KBr; 1712, 1609, 1567, 1463, 1414, 1275, 821; see **Figure 78**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Table 24** and **Figure 80**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Table 24** and **Figure 81**

4.10 Compound CP-10 (AE-8) (Scopoletin)

Yellow needle crystals

EIMS : *m/z*; 192 $[M]^+$; see **Figure 88**

Mp : 205°C

UV : λ_{max}nm (log ε), in MeOH; 222 (4.13), 266 (3.06), 328 (4.13), **Figure 86**

IR : ν_{max}^{cm-1}, KBr; 3297, 1707, 1607, 1453, 1292, 1141, 1012, 853; see **Figure 87**

^{1}H NMR : δ ppm, 500 MHz, in DMSO-d_6; **Table 25** and **Figure 89**

^{13}C NMR : δ ppm, 125 MHz, in DMSO-d_6; **Table 25** and **Figure 90**

4.11 Compound CP-11 (Vanillic acid)

Colorless needle crystals (25.8 mg, 0.005 % based on dried weight of woods).

EIMS : *m/z*; 168 $[M]^+$; see **Figure 97**

Mp : 209-212°C

UV : λ_{max}nm (log ε), in MeOH; 222 (3.24), 260 (4.12), 290 (3.42),

Figure 95

IR : ν_{max} $^{cm-1}$, KBr; 3484, 3098, 2857, 1681, 1282, 1206; see **Figure 96**

^{1}H NMR : δ ppm, 500 MHz, in DMSO-d_6; **Table 26** and **Figure 98a-98b**

^{13}C NMR : δ ppm, 125 MHz, in DMSO-d_6; **Table 26** and **Figure 99**

4.12 Compound CP-12 (*β*-sitosterol glucoside)

Colorless needle crystals

Mp : 304-306°C

^{1}H NMR : δ ppm, 500 MHz, in DMSO-d_6; **Figure 104**

^{13}C NMR : δ ppm, 125 MHz, in DMSO-d_6; **Table 27** and **Figure 105a-105c**

4.13 Compound CP-13 [(-)-10*β*,13,14-trihydroxy-*allo*-aromadendrane]

Yellow needle crystals

HRESIMS : *m/z*; 277.4772 $[M+Na]^+$; see **Figure 108**

Mp : 135°C

$[\alpha]^{25}_{D}$: -31.3° (*c* 1,$CHCl_3$)

IR : ν_{max} $^{cm-1}$, KBr; 3391, 2945, 2926, 2871, 1789, 1456, 1378, 1060, 756; see **Figure 107**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Table 28** and **Figure 109**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Table 28** and **Figure 110**

4.14 Compound CP-14 (5-Hydroxy-7-methoxy-2-pentylchromone)

Colorless oil

ESI-TOFMS : *m/z*; 263.21 $[M+H]^+$; see **Figure 118**

Mp : 55-57°C

UV : λ_{max}nm (log ε), in MeOH; 246 (3.34), 272 (4,05), 328 (3.48), **Figure 116**

IR : ν_{max} $^{cm-1}$, KBr; 2927, 2855, 1687, 1650, 1622, 1573, 1238, 1164; see **Figure 117**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Table 29** and **Figure 119**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Table 29** and **Figure 120**

4.15 Compound CP-15 (Ethyl orsellinate)

Colorless needle crystals

ESIMS : *m/z*; 195 $[M-H]^+$ (Negative mode) ; see **Figure 127**

Mp : 130°C

UV : λ_{max}nm (log ε), in MeOH; 209 (4.11), 220 (3.58), 238 (3.44), 262 (3.44), 283 (4.27), 300 (3.12), **Figure 125**

IR : ν_{max}^{cm-1}, KBr; 3367, 1643, 1317, 1271, 1177, 838; see **Figure 126**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Table 30** and **Figure 128a-128c**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Table 30** and **Figure 129**

4.16 Compound AE-4 (Aglaialactone or 5,6-desmethylenedioxy-5-methoxy-aglalactone)

White amorphous powder

EIMS : *m/z*; 300 $[M]^+$; see **Figure 136**

Mp : 145°C

$[\alpha]^{25}_D$: -13° (*c* 1.1,$CHCl_3$)

UV : λ_{max}nm (log ε), in MeOH; 211 (4.26), 222 (4.37), 257 (3.99), **Figure 134**

IR : ν_{max}^{cm-1}, KBr; 1751, 1608, 1513, 1459, 1330, 1218, 1157, 1058; see **Figure 135**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Table 31** and **Figure 137a-137b**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Table 31** and **Figure 138**

4.17 Compound AE-5 (20*S*,24*R*-Epoxy-25-hydroxy-3,4-seco-5*α*-dammar-4(28)-en-3-ethylester) or (Ethyl eichlerianoate)

White amorphous powder

ESI-TOFMS : *m/z*; 525.4 $[M+Na]^+$; see **Figure 144**

Mp : 179°C

IR : ν_{max}^{cm-1}, KBr; 3489, 2964, 1735, 1456, 1384, 1178, 756; see **Figure 143**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Table 32** and **Figure 145a-145c**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Table 32** and **Figure 146**

4.18 Compound AE-7 (Aglinin A)

White amorphous powder

ESIMS : *m/z*; 490.6 $[M]^+$; see **Figure 153**

Mp : 182°C

IR : ν_{max} $^{cm-1}$, KBr; 3412, 2970, 1710, 1636, 1385, 1098, 796; see **Figure 152**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Table 33** and **Figure 154a-154b**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Table 33** and **Figure 155**

4.19 Compound AE-10 (4′-Demethoxy-3′,4′-methylenedioxy-methyl rocaglate)

White amorphous powder

ESI-TOFMS : *m/z*; 488.93 $[M-H_2O]^+$; see **Figure 162**

Mp : 267°C

$[\alpha]^{20}_D$: -59.8° (*c* 0.12,$CHCl_3$)

UV : λ_{max}nm (log ε), in MeOH; 208 (4.58), 221 (4.43), 257 (3.45), 280 (2.47), **Figure 160**

IR : ν_{max} $^{cm-1}$, KBr; 3501, 2924, 1744, 1624, 1599, 1503, 1455, 1437, 1148; see **Figure 161**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Table 34** and **Figure 163a-163c**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Table 34** and **Figure 164a-164c**

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 were 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 0.1ml 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 falciparum (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, Milroy and Kaye, 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 25µl of Sorensen's 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 the previous section (5.3.2.). If % cell viability $\geq$ 50%, IC_{50} was reported as > 50 µg/ml, and if % cell viability < 50%, IC_{50} would be reported 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 previously mentioned. 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}).