

CHAPTER 2

EXPERIMENTAL

2.1 General Method

Column chromatography was performed by using silica gel 100 (70-230 Mesh ASTM, Merck) or silica gel 60 (230-400 Mesh ASTM, Merck) or SephadexTM LH-20 (Amersham Biosciences, Sweden). For thin-layer chromatography (TLC), aluminum sheets of silica gel 60 GF254 (20×20 cm, layer thickness 0.2 mm, Merck) were used for analytical purposes and the compounds were visualized under ultraviolet light. Solvents for extraction and chromatography were distilled at their boiling ranges prior to use. Melting points were recorded in °C on a digital Electrothermal Melting Point Apparatus (Electrothermal 9100). Ultraviolet spectra were measured with UV-160A spectrophotometer (SHIMADZU). Principle bands (λ_{\max}) were recorded as wavelengths (nm) and $\log \varepsilon$ in ethanol solution. Infrared spectra (IR) were obtained on a FTS165 FT-IR spectrophotometer and were recorded in wave number (cm^{-1}). ¹H and ¹³C-Nuclear magnetic resonance spectra were recorded on a FT-NMR Bruker Ultra ShieldTM 300 MHz or 500 MHz spectrometer at Department of Chemistry, Faculty of Science, Prince of Songkla University. Spectra were recorded in CDCl₃ and DMSO-*d*₆ and were recorded as δ value in ppm downfield from TMS (internal standard δ 0.00). Low and high resolution mass spectra were recorded on a MAT 95 XL at Scientific Equipment Center, Prince of Songkla University.

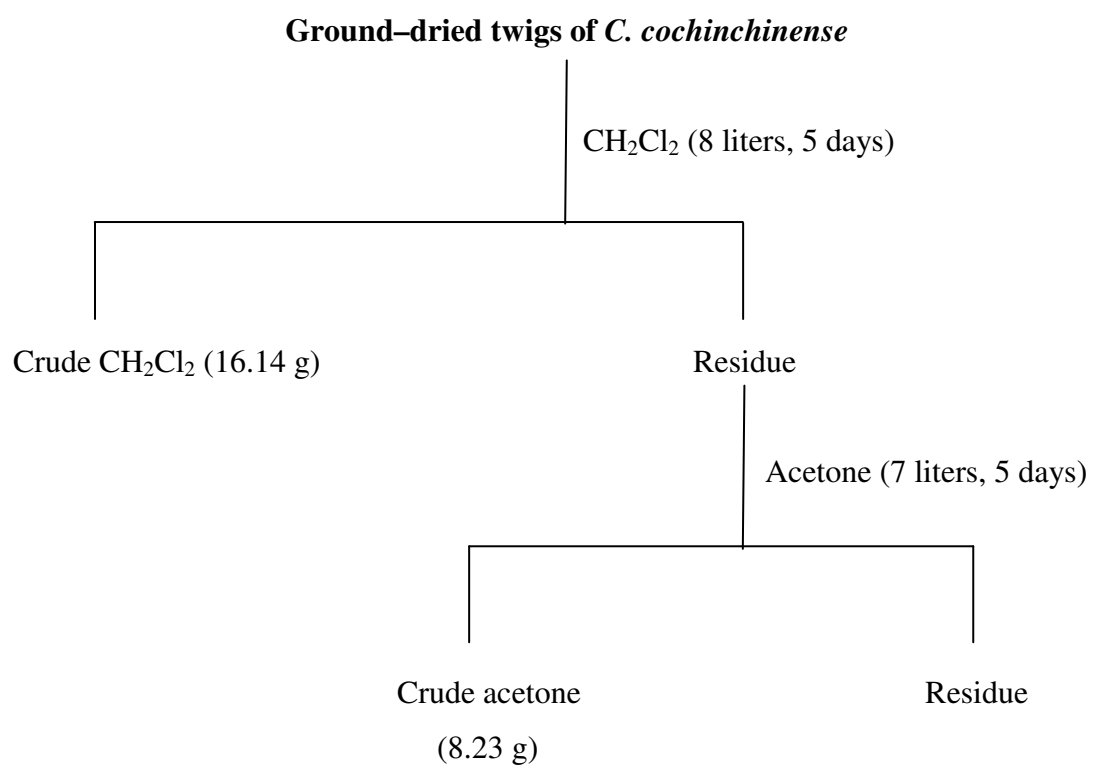
2.2 Plant Material

The twigs and fruits of *C. cochinchinense* (Guttiferae) were collected from Amphur Thungsong, Nakorn sri thammarat Province on October 2005, the roots was collected from Amphur Bannasan, Suratthani Province in the southern part of Thailand on February 2003. The herbarium specimen (W. Nuangnaowarat 1 Suratthani: Bannasan 31/3/04, respectively) have been deposited in the Herbarium of Department of Biology, Faculty of Science, Prince of Songkla University, Thailand.

2.3 Extraction and isolation

A. Twigs

Ground-dried twigs (4.6 kg) of *C. cochinchinense* were immersed in dichloromethane and acetone at room temperature (each extract time of 5 days). After evaporation, the yellow-brown viscous dichloromethane extract (16.14 g) and acetone extract (8.23 g) were obtained. The process of extraction was shown in **Scheme 1**.



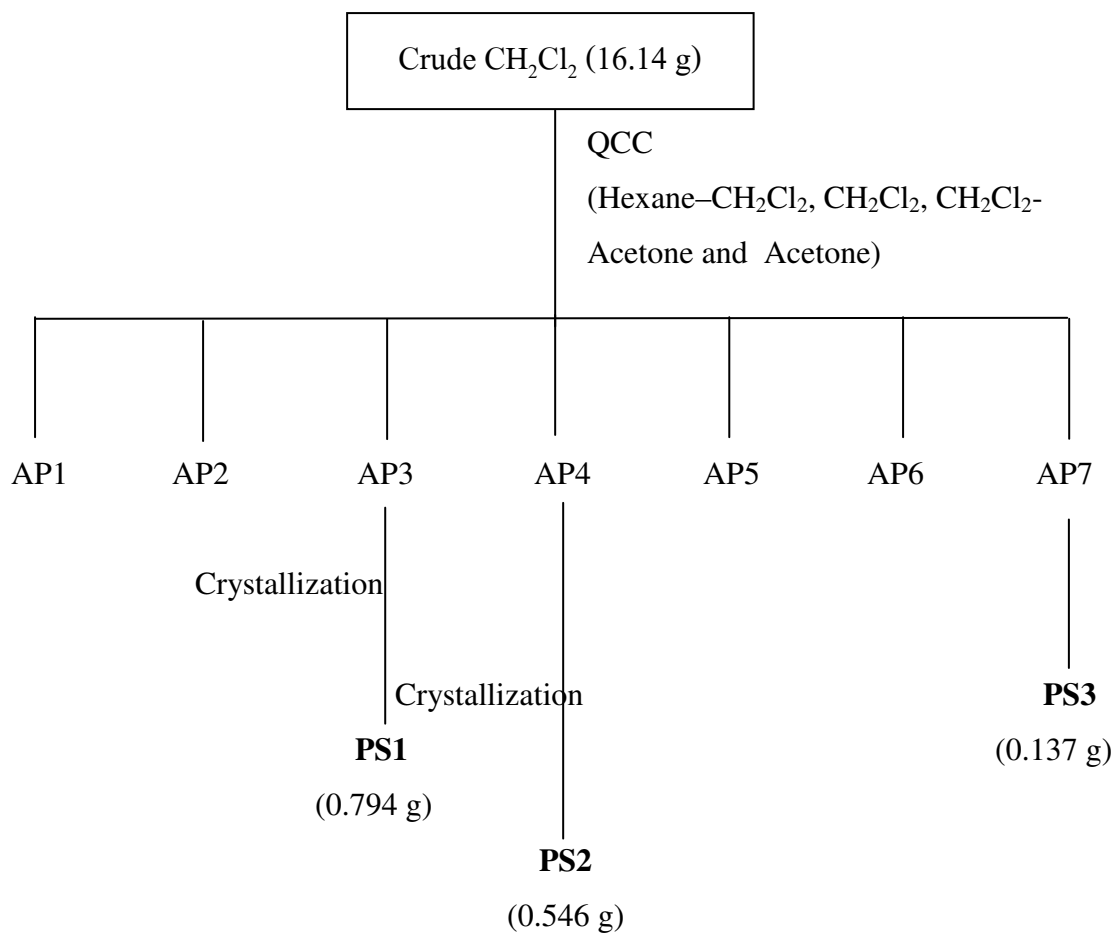
Scheme 1 Extraction of crude extracts from the twigs of *C. cochinchinense*

2.3.1 Purification of dichloromethane extract of twigs of *C. cochinchinense*

Dichloromethane extract (16.14 g) was separated by quick column chromatography over silica gel 60H using hexane–dichloromethane, dichloromethane, dichloromethane–acetone and acetone as eluents. On the basis of their TLC characteristic, the fractions which contained the same major components were combined to give fractions AP1– AP7 (**Table 2**).

Table 2 Physical characteristic and weight of fractions from CH₂Cl₂ extract

Fraction	Weight (g)	Appearance
AP1	2.014	yellow gel
AP2	2.101	yellow gel
AP3	0.939	yellow solid mixed with yellow liquid
AP4	0.949	yellow solid mixed with yellow liquid
AP5	0.730	yellow–brown viscous liquid
AP6	4.530	brown viscous liquid
AP7	2.424	yellow solid mixed with brown liquid



Scheme 2 Isolation of compounds **PS1-3** from dichloromethane extract of twigs of *C. cochinchinense*

Isolation of PS1

Fraction AP3 (0.929 g) which contained one major component was further purified by crystallization from hexane–dichloromethane (9:1). A yellow solid of **PS1** (0.794 g) which formed was filtered.

PS1

Melting point : 176-180 °C

¹H-NMR 300 MHz (CDCl₃) δ(ppm) : 13.53 (1H, *s*, 1-OH), 6.77 (1H, *s*, H-5), 6.44 (1H, *br s*, 6-OH), 6.28 (1H, *s*, H-4), 5.25 (1H, *br t*, *J* = 6.0 Hz, H-2''), 5.23 (1H, *br t*, *J* = 7.2 Hz, H-2'), 4.07 (2H, *d*, *J* = 6.0 Hz, H-1''), 3.87 (3H, *s*, 3-OCH₃), 3.77 (3H, *s*, 7-OCH₃), 3.32 (2H, *d*, *J* = 7.2 Hz, H-1'), 1.82 (3H, *s*, H-4''), 1.79 (3H, *s*, H-4'), 1.68 (3Hx2, *s*, H-5', H-5'')

Isolation of PS2

Fraction AP4 (0.949 g) was further purified by crystallization from hexane–dichloromethane to give a yellow solid (0.572 g) upon standing at room temperature and the filtrate (0.211 g). The yellow solid was further purified by column chromatography using hexane–dichloromethane (1:5) as an eluent to afford pure **PS2** as a yellow solid (0.546 g).

PS2

Melting point : 246-247 °C

¹H-NMR 300 MHz (CDCl₃) δ(ppm) : 13.48 (1H, *s*, 6-OH), 9.18 (1H, *br s*, 12-OH), 8.00 (1H, *d*, *J* = 10.2 Hz, H-4), 6.81 (1H, *s*, H-11), 6.35 (1H, *s*, H-9), 5.82 (1H, *d*, *J* = 10.2 Hz, H-3), 5.21 (1H, *br t*, *J* = 7.2 Hz, H-2'), 3.91 (3H, *s*, 8-OCH₃), 3.32 (2H, *d*, *J* = 7.2 Hz, H-1'), 1.78 (3H, *s*, H-4'), 1.67 (3H, *s*, H-5'), 1.50 (3Hx2, *s*, H-13, H-14)

Isolation of PS3

Fraction AP7 (2.424 g) was further purified by column chromatography over silica gel and eluted with hexane–acetone (9:1) solvent system. The fractions containing similar components were combined into five fractions. Crystallization of the fourth fraction from hexane–acetone gave a yellow solid of **PS3** (0.137 g).

PS3

Melting point : 119-120 °C

UV (EtOH) λ_{\max} nm (log ϵ) : 232 (4.44), 268 (4.42), 316 (4.04), 384 (3.70)

IR (KBr) ν_{\max} (cm⁻¹) : 3413 (O-H stretching), 1641 (C=O stretching)

¹H-NMR 300 MHz (CDCl₃) δ (ppm) : 12.96 (1H, *s*, 1-OH), 7.54 (1H, *d*, *J* = 3.0 Hz, H-8), 7.36 (1H, *d*, *J* = 9.0 Hz, H-5), 7.24 (1H, *dd*, *J* = 9.0, 3.0 Hz, H-6), 5.20 (2Hx2, *br t*, *J* = 7.0 Hz, H-2', H-2''), 5.00 (1H, *br t*, *J* = 7.0 Hz, H-6''), 3.48 (2H, *d*, *J* = 7.0 Hz, H-1'), 3.37 (2H, *d*, *J* = 7.0 Hz, H-1'), 1.93-2.10 (2Hx2, *m*), 1.83 (3H, *s*, H-9''), 1.79 (3H, *s*, H-4'), 1.69 (3H, *s*, H-5'), 1.60 (3H, *s*, H-10''), 1.50 (3H, *s*, H-8'')

¹³C NMR 75 MHz (CDCl₃) δ (ppm) : 180.9 (C=O), 161.1, 158.2, 152.9, 152.4, 150.3, 137.9, 134.9, 132.0, 124.1, 123.8, 121.5, 120.4, 118.8, 108.9, 108.8, 105.0, 102.9, 39.7, 26.4, 25.8, 25.6, 21.5, 21.5, 17.9, 17.6, 16.2

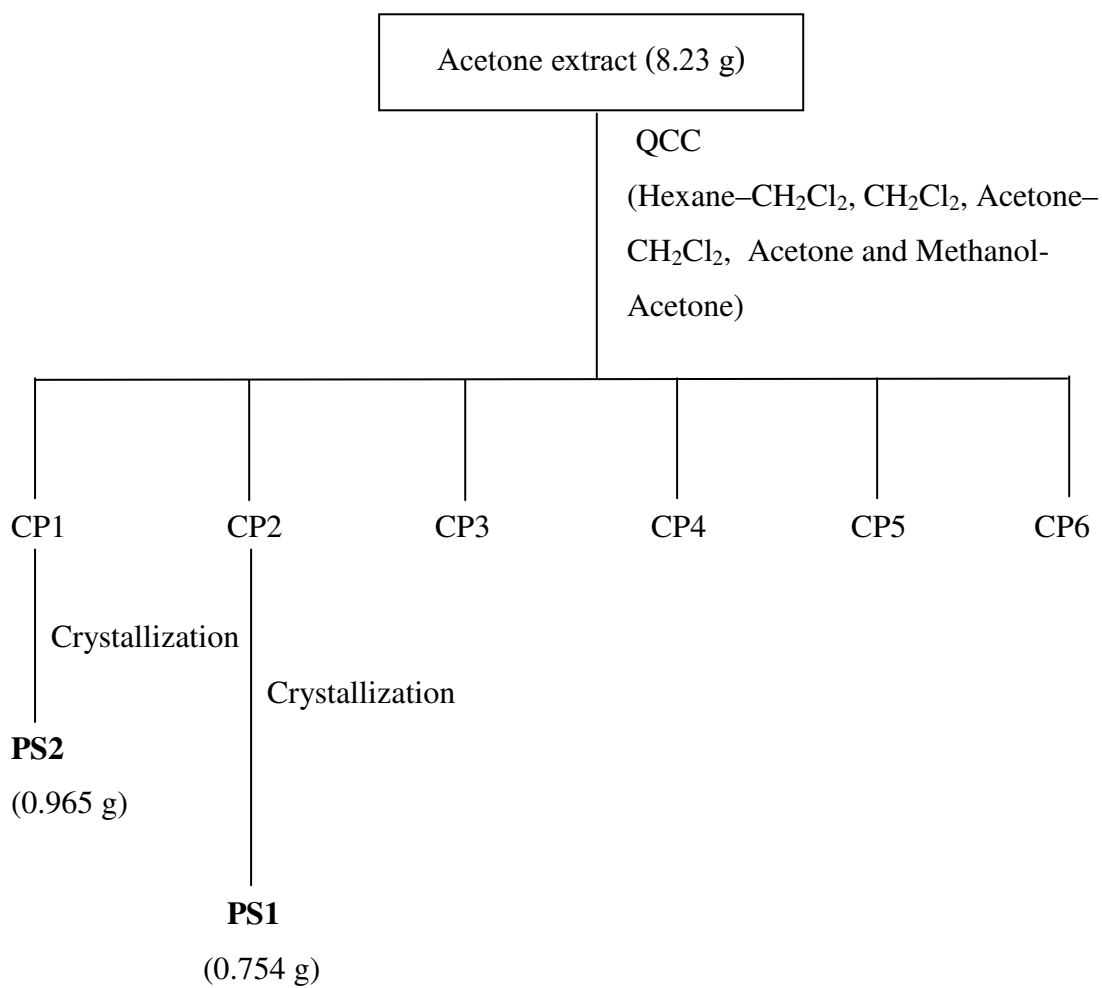
DEPT 135° (CDCl₃) δ (ppm) CH₃ : 25.8, 25.6, 17.9, 17.6, 16.2; CH₂: 39.7, 26.4, 21.5, 21.5; CH: 124.1, 123.8, 121.5, 121.5, 118.8, 108.8

2.3.2 Purification of acetone extract from twigs of *C. cochinchinense*

Acetone extract (8.23 g) was separated by quick column chromatography over silica gel 60H using hexane–dichloromethane, dichloromethane, dichloromethane–acetone, acetone and acetone-methanol as eluents. On the basis of their TLC characteristic, the fractions which contained the same major components were combined to give fractions CP1– CP6 (**Table 3**).

Table 3 Physical characteristic and weight of fractions from acetone extract

Fraction	Weight (g)	Appearance
CP1	2.050	yellow solid mixed with yellow gel
CP2	4.251	yellow solid mixed with yellow liquid
CP3	0.765	brown viscous liquid
CP4	0.034	brown viscous liquid
CP5	0.009	brown viscous liquid
CP6	0.031	dark–brown viscous liquid



Scheme 3 Isolation of compounds **PS1-2** from acetone extract of twigs of *C. cochinchinense*

Isolation of PS2

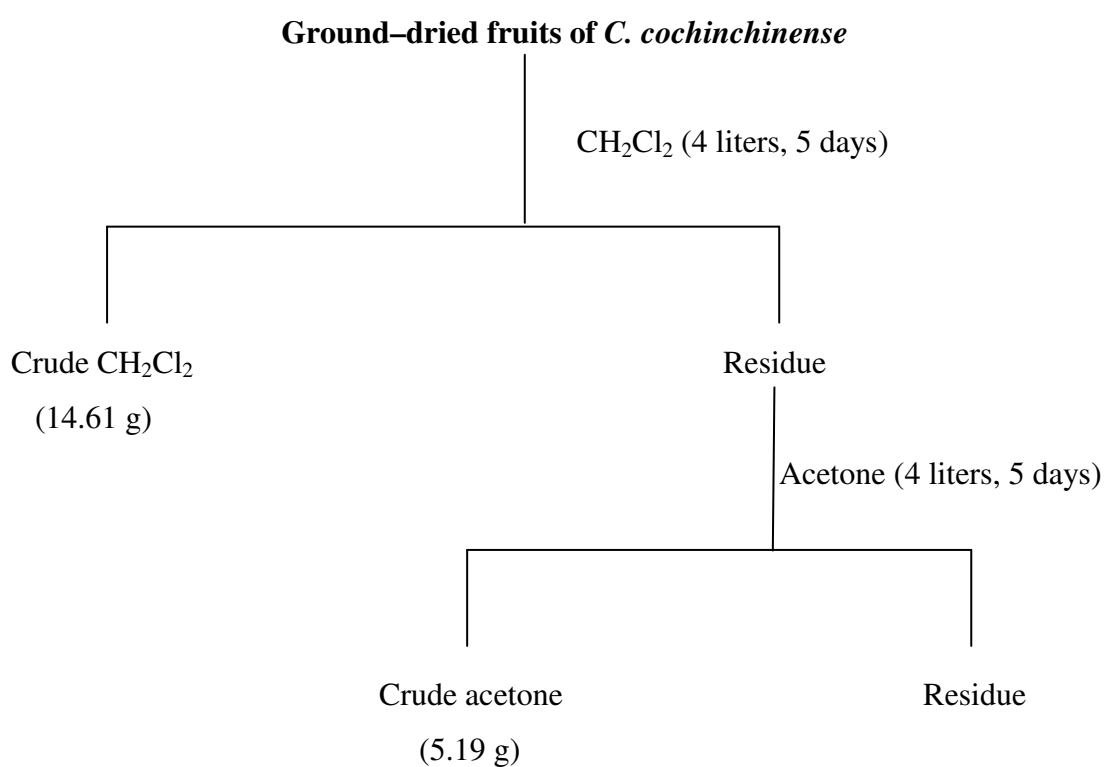
Fraction CP1 (2.050 g) was purified by crystallization from hexane–dichloromethane (8:2). The yellow solid of **PS2** (0.965 g) which formed was filtered.

Isolation of PS1

Fraction CP2 (4.251 g) was further purified by crystallization from hexane–dichloromethane upon standing at room temperature to give a yellow solid of **PS1** (0.754 g). The filtrate of CP2 (3.470 g) was further recrystallized from methanol. The white solid (2.143 g) which formed was filtered.

B. Fruits

Ground-dried fruits (0.84 kg) of *C. cochinchinense* were immersed in dichloromethane and acetone at room temperature (each extract time of 5 days). After evaporation, the yellow-brown viscous dichloromethane extract (14.61 g) and acetone extract (5.19 g) were obtained. The process of extraction was shown in **Scheme 4**.



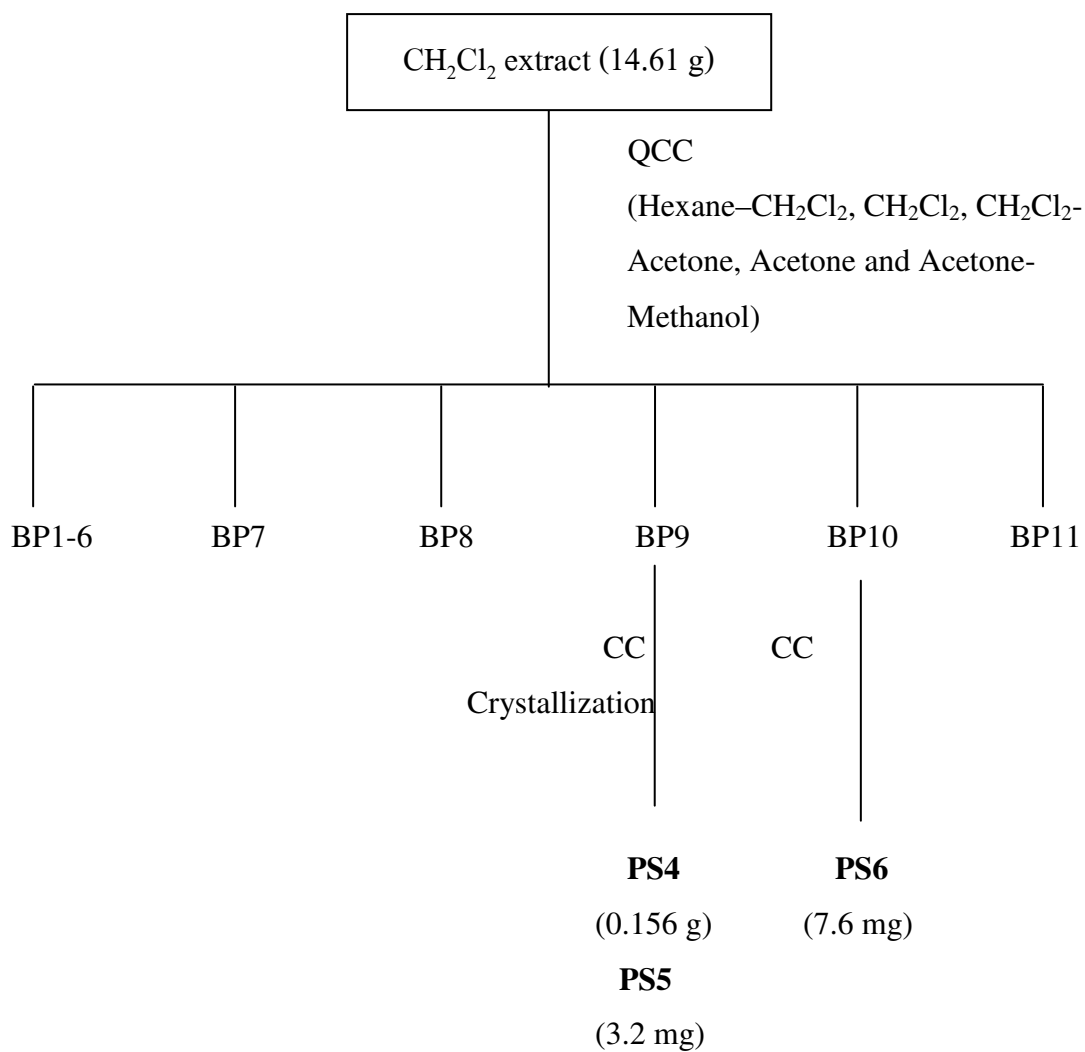
Scheme 4 Extraction of crude extracts from the fruits of *C. cochinchinense*

3.2.3 Purification of dichloromethane extract from the fruits of *C. cochinchinense*

Dichloromethane extract (14.61 g) was separated by quick column chromatography over silica gel 60H using hexane–dichloromethane, dichloromethane, dichloromethane–acetone, acetone and methanol-acetone as eluents. On the basis of their TLC characteristic, the fractions which contained the same major components were combined to give fractions BP1–BP11 (**Table 4**).

Table 4 Physical characteristic and weight of fractions from dichloromethane extract

Fraction	Weight (g)	Appearance
BP1	2.104	yellow gel
BP2	1.548	orange gel
BP3	0.419	yellow–brown viscous liquid
BP4	1.271	yellow–brown viscous liquid
BP5	1.045	yellow–brown viscous liquid
BP6	0.390	yellow–brown viscous liquid
BP7	1.410	yellow–brown viscous liquid
BP8	0.389	yellow–brown viscous liquid
BP9	0.497	yellow–brown viscous liquid
BP10	1.926	brown viscous liquid
BP11	3.111	dark-brown viscous liquid



Scheme 5 Isolation of compounds **PS4-6** from dichloromethane extract of the fruits of *C. cochinchinense*

Isolation of PS4 and PS5

Fraction BP9 (0.497 g) was further purified by crystallization from hexane–dichloromethane (1:1) upon standing at room temperature to give a yellow–brown solid (0.194 g) and the filtrate (0.321 g). The yellow–brown solid was further purified by column chromatography using hexane–dichloromethane (4:6) as an eluent to afford a yellow solid of **PS4** (0.156 g). The filtrate (0.321 g) was purified by crystallization from hexane–dichloromethane (4:6) to give a yellow solid (5.80 mg) upon standing at room temperature. The yellow solid was rechromatographed using hexane–dichloromethane (6:4) as an eluent to give a yellow solid of **PS5** (3.20 mg).

PS4

Melting point : 137-138 °C

UV (EtOH) λ_{\max} nm (log ϵ): 204 (4.74), 232 (4.66), 259 (4.68), 310 (4.32), 368 (4.01)

IR (KBr) ν_{\max} (cm⁻¹): 3137 (O-H stretching), 1650 (C=O stretching)

¹H-NMR 300 MHz (CDCl₃) δ (ppm) : 12.96 (1H, *s*, 1-OH), 7.61 (1H, *d*, *J* = 3.0 Hz, H-8), 7.35 (1H, *d*, *J* = 9.0 Hz, H-5), 7.32 (1H, *dd*, *J* = 9.0 Hz, 3.0 Hz H-6), 6.38 (1H, *d*, *J* = 3.0 Hz, H-4), 6.29 (1H, *d*, *J* = 3.0 Hz, H-2), 5.50 (1H, *t*, *J* = 6.6 Hz, H-2'), 5.09 (1H, *t*, *J* = 6.6 Hz, H-6'), 4.61 (2H, *d*, *J* = 6.6 Hz, H-1'), 2.12 (2x2H, *m*, H-3', H-4'), 1.76 (3H, *s*, H-9'), 1.67 (3H, *s*, H-10'), 1.60 (3H, *s*, H-8')

¹³C-NMR 75 MHz (CDCl₃) δ (ppm) : 180.67 (C=O), 163.5, 157.9, 155.3, 150.7, 142.0, 131.8, 125.5, 123.7, 120.6, 118.8, 118.7, 106.2, 103.6, 98.3, 94.1, 65.6, 39.5, 26.3, 25.6, 17.6, 16.7

DEPT 135° (CDCl₃) δ (ppm) : CH₃ : 16.7, 17.6, 25.6; CH₂ : 26.3, 39.5, 65.6; CH : 94.1, 98.3, 106.2, 118.7, 118.8, 123.7, 125.5

PS5

Melting point : 147-148 °C

UV (EtOH) λ_{\max} nm (log ϵ): 203 (4.49), 229 (4.30), 259 (4.31), 307 (3.99), 374 (3.63)

IR (KBr) ν_{\max} (cm⁻¹) : 3288 (O-H stretching), 1647 (C=O stretching)

¹H-NMR 300 MHz (CDCl₃) δ (ppm) : 12.70 (1H, *s*, 1-OH), 7.58 (1H, *d*, *J* = 3.0 Hz, H-8), 7.29 (1H, *d*, *J* = 9.0 Hz, H-5), 7.24 (1H, *dd*, *J* = 9.0, 3.0 Hz, H-6), 6.39 (1H, *d*, *J* = 3.0 Hz, H-4), 6.33 (1H, *d*, *J* = 3.0 Hz, H-2), 5.48 (1H, *br t*, *J* = 6.6 Hz, H-2'), 5.09 (1H, *t*, *J* = 6.6 Hz, H-6'), 4.62 (2H, *d*, *J* = 6.6 Hz, H-1'), 2.11 (2x2H, *m*, H-4', H-5'), 1.76 (3H, *s*, H-9'), 1.67 (3H, *s*, H-10'), 1.55 (3H, *s*, H-8')

¹³C-NMR 75 MHz (CDCl₃) δ (ppm) : 180.5 (C=O), 166.1, 163.2, 157.5, 152.3, 150.5, 142.3, 131.9, 124.1, 120.9, 119.0, 118.3, 123.6, 109.0, 103.5, 97.6, 93.2, 65.5, 39.5, 26.2, 25.6, 17.7, 16.7

DEPT 135° (CDCl₃) δ (ppm) : CH₃ : 16.7, 17.7, 25.6; CH₂ : 26.2, 39.5, 65.5; CH : 93.2, 97.6, 109.0, 118.3, 119.0, 123.6, 124.1

EI-MS *m/z* (% relative intensity) : 380 [M⁺] (2), 379 (6), 364 (1), 310 (3), 244 (29), 243 (100), 242 (2), 215 (4), 214 (8), 186 (2), 136 (5), 69 (17)

HR-MS *m/z* : 380.1634 for C₂₃H₂₄O₅ (calcd. 380.1624)

Isolation of PS6

Fraction BP10 (1.926 g) was separated by column chromatography over silica gel and eluted with hexane–dichloromethane (3:7) solvent system. The fractions containing similar components were combined into four fractions. Crystallization of the third fraction from acetone gave an orange solid of **PS6** (7.6 mg).

PS6

Melting point : 116-118 °C

UV (EtOH) λ_{\max} nm (log ϵ): 221 (3.83), 273 (3.88), 304 (3.43), 437 (3.56)

IR (Neat) ν_{\max} (cm⁻¹): 3406 (O-H stretching), 1668 (non-chelated C=O stretching),
1623 (chelated C=O stretching)

¹H-NMR 300 MHz (Acetone-*d*₆) δ (ppm) : 12.44 (1H, *s*, 8-OH), 12.05 (1H, *s*, 1-OH),
7.60 (1H, *d*, *J* = 0.5 Hz, H-5), 7.45 (1H, *s*, H-4), 7.16 (1H, *d*, *J* = 0.5 Hz,
H-7), 5.20 (1H, *t*, *J* = 8.0 Hz, H-2'), 4.09 (3H, *s*, 3-OCH₃), 3.45 (2H, *d*,
J = 8.0 Hz, H-1'), 2.48 (3H, *s*, 6-CH₃), 1.80 (3H, *s*, H-4'), 1.66 (3H, *s*, H-5')

¹³C-NMR 75 MHz (Acetone-*d*₆) δ (ppm) : 124.0, 120.8, 56.3, 24.7, 21.8, 21.0, 17.0

3.2.4 Purification of acetone extract from fruits of *C. cochinchinense*

Acetone extract (5.197 g) was obtained in a small amount and a proper solvent system to give separable spots could not be determined. Thus, this extract was not further purified.

C. Roots

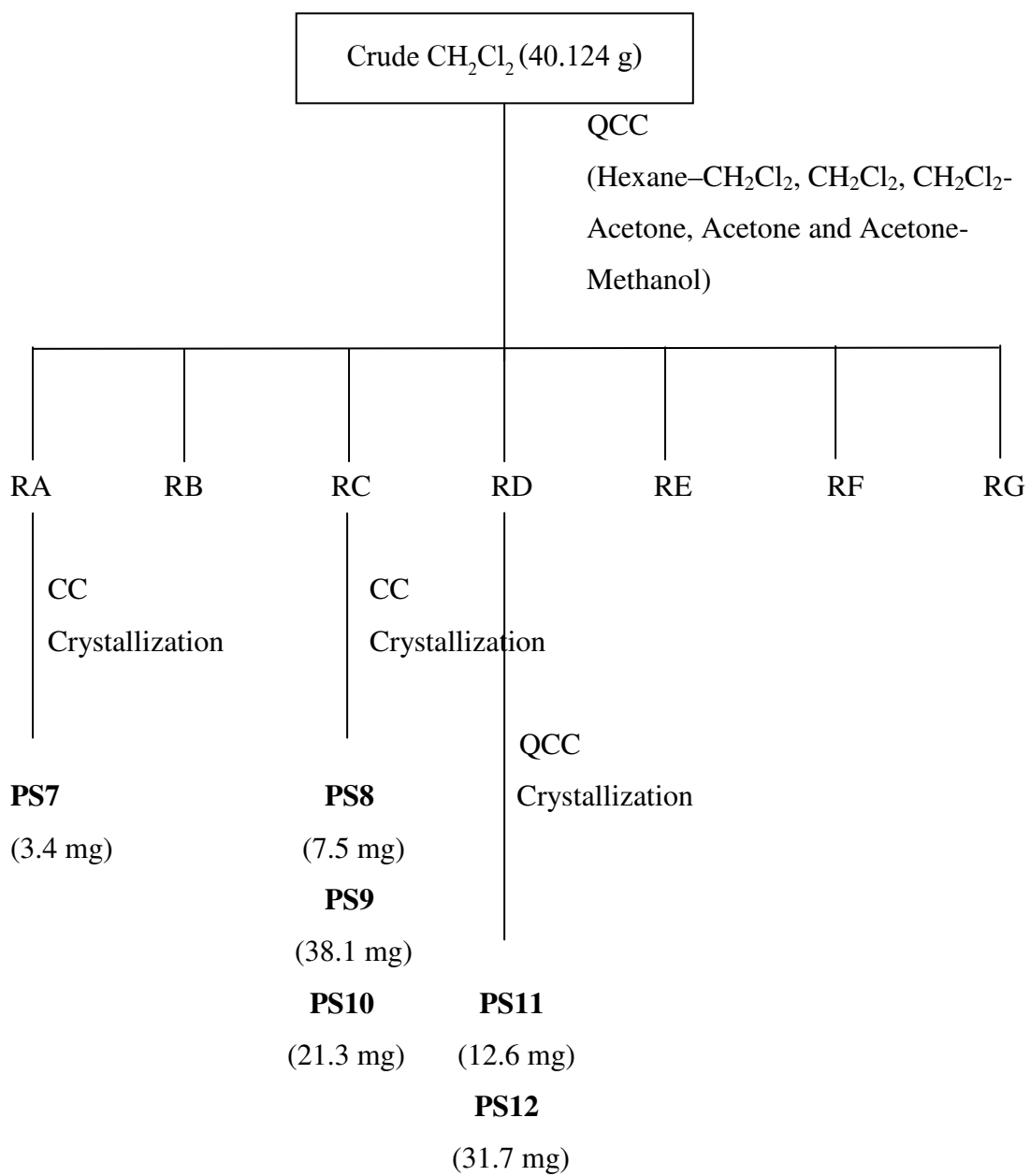
The minor low polarity fractions of the crude dichloromethane extract (40.124 g) and the methanol extract (22.17 g) of the roots were obtained from the previous work.

3.2.5 Purification of dichloromethane extract of the roots of *C. cochinchinense*

Dichloromethane extract (40.124 g) was separated by quick column chromatography over silica gel 60H using hexane–dichloromethane, dichloromethane, dichloromethane–acetone, acetone and methanol–acetone as eluents. On the basis of their TLC characteristic, the fractions which contained the same major components were combined to give fractions RA–RG (**Table 5**).

Table 5 Physical characteristic and weight of fractions from CH₂Cl₂ extract

Fraction	Weight (g)	Appearance
RA	0.906	yellow viscous liquid
RB	0.941	yellow–brown viscous liquid
RC	3.831	yellow–brown viscous liquid
RD	20.747	yellow–brown gel
RE	2.724	yellow–brown viscous liquid
RF	3.048	brown viscous liquid
RG	2.375	brown viscous liquid



Scheme 6 Isolation of compounds **PS7-12** from dichloromethane extract of the roots of *C. cochinchinense*

Isolation of PS7

Fraction RA (0.906 g) was further purified by crystallization from hexane–dichloromethane to give a yellow solid of **PS7** (3.40 mg) upon standing at room temperature.

PS7

$^1\text{H-NMR}$ 300 MHz (CDCl_3) δ (ppm) : 13.57 (1H, *s*, 1-OH), 7.78 (1H, *d*, $J = 9.0$ Hz, H-8), 6.97 (1H, *d*, $J = 9.0$ Hz, H-7), 6.76 (1H, *d*, $J = 10.2$ Hz, H-4), 6.67 (1H, *dd*, $J = 17.7, 10.8$ Hz, H-4'), 6.23 (1H, *s*, 10-OH), 5.59 (1H, *d*, $J = 10.2$ Hz, H-3), 5.19 (1H, *d*, $J = 17.7$ Hz, H-5'*E*), 5.03 (1H, *d*, $J = 10.8$ Hz, H-5'*Z*), 4.09 (3H, *s*, 9-OCH₃), 1.67 (3Hx2, *s*, H-2', H-3'), 1.53 (3Hx2, *s*, H-13, H-14)

$^{13}\text{C NMR}$ 125 MHz (CDCl_3) δ (ppm) : 180.9 (C=O), 159.1, 156.7, 154.1, 154.7, 151.5, 144.4, 133.6, 127.1, 116.8, 116.6, 114.4, 114.3, 108.4, 105.4, 104.6, 103.1, 79.2, 56.6, 41.8, 28.5, 28.5, 27.9, 27.2

DEPT 135° (CDCl_3) δ (ppm) CH₃: 27.2, 27.9, 28.5, 28.5; CH₂: 104.6; CH: 154.9, 127.1, 116.8, 116.6, 108.4

Isolation of PS8, PS9, PS10

Fraction RC (3.831 g) was chromatographed on column chromatography and elution was conducted with hexane, hexane–dichloromethane and dichloromethane to afford 3 portions RC1 (1.159g), RC2 (1.372 g) and RC3 (0.165 g), respectively. The yellow solid **PS8** (7.50 mg) and **PS9** (38.10 mg) were isolated from RC1 by column chromatography and eluted with hexane–dichloromethane (3:2). Fraction RC2 was crystallized in methanol to give a white solid (1.015 g). Fraction RC 3 was crystallized from hexane–dichloromethane (7:3) to give a yellow solid of **PS10** (21.30 mg).

PS8

Melting point : 208-209 °C

UV (EtOH) λ_{\max} nm (log ϵ): 205 (3.79), 225 (3.55), 281 (3.05), 350 (3.22)

IR (Neat) ν_{\max} (cm⁻¹): 3411 (O-H stretching), 1734 (C=O stretching), 1633 (chelated C=O stretching)

¹H-NMR 300 MHz (CDCl₃+DMSO-*d*₆) δ (ppm) : 12.48 (1H, *s*, 1-OH), 7.44 (1H, *d*, $J = 7.0$ Hz, H-8), 6.08 (1H, *d*, $J = 2.0$ Hz, H-4), 6.08 (1H, *d*, $J = 2.0$ Hz, H-2), 4.43 (1H, *br t*, $J = 7.5$ Hz, H-16), 3.53 (1H, *dd*, $J = 7.0, 4.0$ Hz, H-7), 2.64 (2H, *d*, $J = 7.5$ Hz, H-15), 2.49 (1H, *d*, $J = 9.6$ Hz, H-11), 2.37 (1H, *dd*, $J = 13.5, 4.0$ Hz, H_a-10), 1.71 (3H, *s*, H-13), 1.41 (3H, *s*, H-18), 1.33 (1H, *m*, H_b-10), 1.32 (3H, *s*, H-14), 1.12 (3H, *s*, H-19)

¹³C NMR 75 MHz (CDCl₃+DMSO-*d*₆) δ (ppm) : 203.1 (C=O), 178.7 (C=O), 167.5, 165.0, 161.0, 135.0, 134.0, 133.0, 118.0, 100.4, 96.8, 95.3, 89.8, 84.4, 83.3, 48.7, 46.7, 30.2, 28.9, 25.4, 25.1, 16.9

PS9

Melting point : 147- 148 °C

Optical rotation : $[\alpha]_D^{29} + 98^\circ$ (c 1.0 x 10⁻² g/cm⁻³ in CHCl₃)

¹H-NMR 300 MHz (CDCl₃) δ (ppm) : 12.00 (1H, *s*, 1-OH), 7.51 (1H, *s*, H-8), 7.41 (1H, *t*, $J = 8.4$ Hz, H-3), 6.54 (1H, *dd*, $J = 8.4, 1.0$ Hz, H-2), 6.51 (1H, *dd*, $J = 8.4$ Hz, 1.0 Hz, H-4), 4.40 (1H, *br t*, $J = 8.1$ Hz, H-16), 3.64 (3H, *s*, 7- OCH₃), 2.64 (2H, *d*, $J = 8.1$ Hz, H-15), 2.38 (1H, *d*, $J = 12.9$ Hz, H_a-10), 1.68 (3H, *s*, H-13), 1.59 (1H, *dd*, $J = 12.9$ Hz, 9.9 Hz, H_b-10), 1.37 (3H, *s*, H-18), 1.32 (3H, *s*, H-14), 1.01 (3H, *s*, H-19)

PS10

Melting point : 180-182 °C

¹H-NMR 300 MHz (CDCl₃) δ (ppm) : 13.80 (1H, *s*, 1-OH), 6.82 (1H, *s*, H-5), 6.30 (1H, *s*, 6-OH), 6.27 (1H, *s*, H-4), 6.15 (1H, *s*, 3-OH), 5.28 (1H, *t*, *J* = 7.0 Hz, H-2'), 5.27 (1H, *br t*, *J* = 7.0 Hz, H-2''), 4.10 (2H, *d*, *J* = 7.0 Hz, H-1''), 3.81 (3H, *s*, 7-OCH₃), 3.44 (2H, *d*, *J* = 7.0 Hz, H-1'), 1.85 (3H, *s*, H-4'), 1.82 (3H, *s*, H-4''), 1.78 (3H, *s*, H-5'), 1.70 (3H, *s*, H-5'')

Isolation of PS11 and PS12

Fraction RD (20.747 g) was chromatographed on quick column chromatography over silica gel 60H and eluted with hexane–dichloromethane (7:3) to obtain five fractions; RD1 (1.987 g), RD2 (1.786 g), RD3 (0.665 g), RD4 (1.571 g) and RD5 (10.356 g). Fraction RD5 was crystallized from hexane-dichloromethane (3:7) to afford a yellow solid **PS11** (12.6 mg). The filtrate of RD5 (10.329 g) was recrystallized from acetone-dichloromethane to give a yellow solid (74.10 mg) which was further rechromatographed on column chromatography using acetone-dichloromethane (1:19) as eluent to give a yellow solid of **PS12** (31.70 mg).

PS11

Melting point : 226-227 °C

UV (EtOH) λ_{\max} nm (log ϵ) : 204 (4.40), 237 (4.32), 253 (4.46), 287 (3.95), 328 (4.09)

IR (KBr) ν_{\max} (cm⁻¹) : 3417 (O-H stretching), 1647 (C=O stretching)

¹H-NMR 300 MHz (CDCl₃) δ (ppm) : 13.35 (1H, *s*, 1-OH), 7.69 (1H, *d*, *J* = 8.7 Hz, H-8), 6.95 (1H, *d*, *J* = 8.7 Hz, H-7), 6.70 (1H, *dd*, *J* = 17.7 Hz, 10.5 Hz, H-4'), 6.40 (1H, *s*, H-2), 5.21 (1H, *dd*, *J* = 17.7 Hz, 1.5 Hz, H-5'E), 5.03 (1H, *dd*, *J* = 10.5 Hz, 1.5 Hz, H-5'Z), 1.61 (2x3H, *s*, H-2', H-3')

¹³C-NMR 75 MHz (CDCl₃) δ (ppm) : 180.8 (C=O), 165.2, 162.4, 156.8, 154.0, 149.1, 131.0, 117.5, 113.6, 113.2, 112.6, 103.2, 103.0, 95.6, 55.7, 41.5, 29.6, 27.8

DEPT 135° (CDCl₃) δ (ppm) : CH₃ : 27.8, 27.8, 55.7; CH₂ : 103.2; CH: 95.6, 112.6, 117.5, 156.8

PS12

Melting point : 219-220 °C

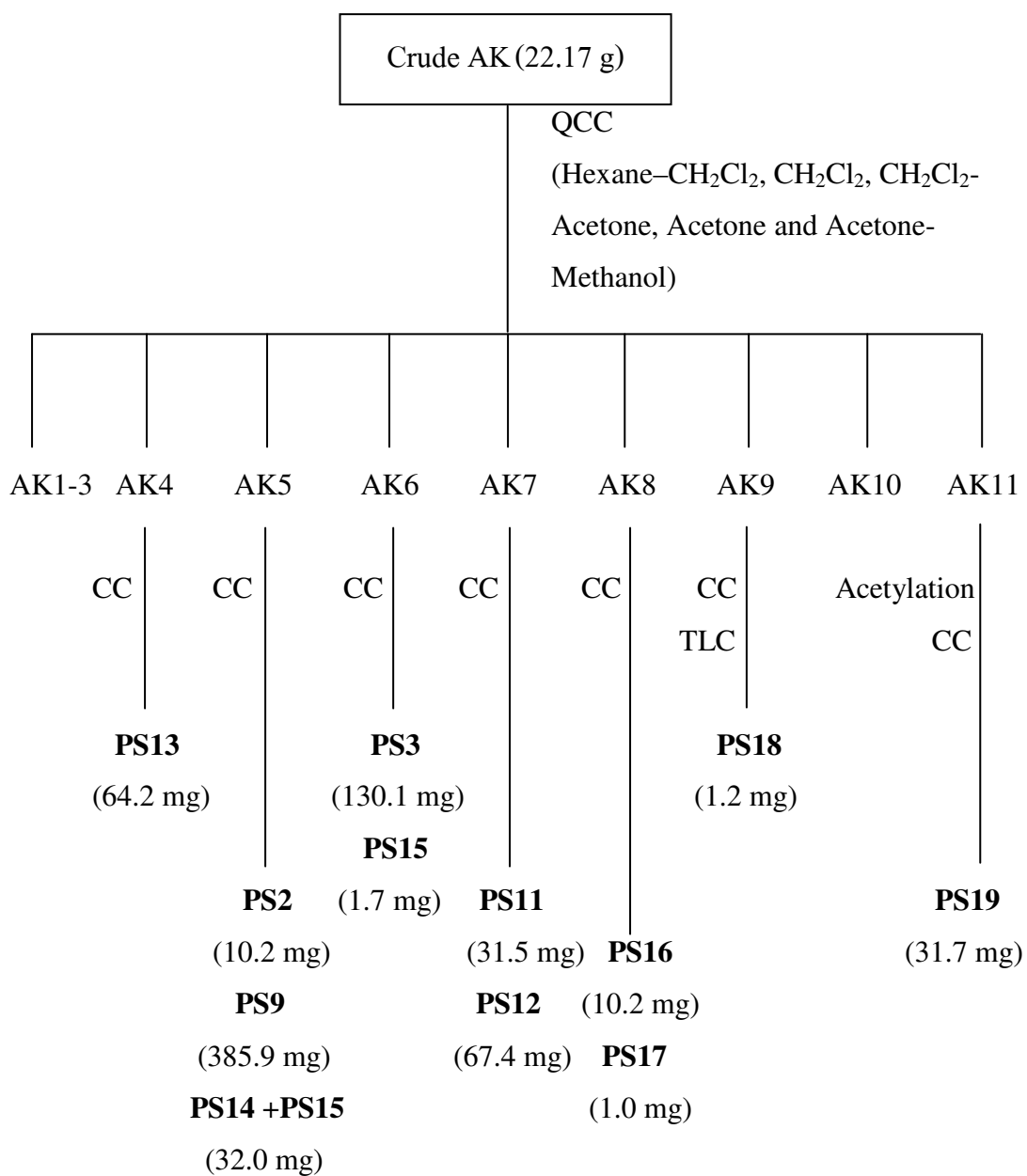
¹H-NMR 300 MHz (CDCl₃+ DMSO-*d*₆) δ (ppm) : 13.25 (1H, *s*, 1-OH), 7.49 (1H, *t*, *J* = 8.4 Hz, H-3), 6.99 (1H, *dd*, *J* = 8.4 Hz, 1.5 Hz, H-4), 6.72 (1H, *dd*, *J* = 8.4 Hz, 1.5 Hz, H-2), 5.25 (1H, *br t*, *J* = 6.3 Hz, H-2'), 4.05 (2H, *d*, *J* = 6.3 Hz, H1'), 3.83 (3H, *s*, 7-OCH₃), 1.84 (3H, *s*, H-4'), 1.68 (3H, *s*, H-5')

3.2.6 Purification of methanol extract from the roots of *C. cochinchinense*

The methanol extract (22.17 g) was subjected to quick column chromatography using silica gel 60H as the stationary phase and eluted with hexane, hexane-dichloromethane, dichloromethane, acetone-dichloromethane, acetone, acetone-methanol and methanol. On the basis of their TLC characteristic, the fractions which contained the same major components were combined to give fractions AK1-AK11 (**Table 6**). The selected fractions were further purified to give 11 pure compounds and a mixture as shown in **Scheme 7**.

Table 6 Physical characteristic and weight of fractions from methanol extract

Fraction	Weight (g)	Appearance
AK1	2.345	yellow gel
AK2	0.876	yellow viscous liquid
AK3	0.023	yellow–brown viscous liquid
AK4	0.508	yellow–brown viscous liquid
AK5	1.939	yellow–brown viscous liquid
AK6	1.149	yellow–brown viscous liquid
AK7	5.724	yellow–brown viscous liquid
AK8	5.760	yellow–brown viscous liquid
AK9	2.554	brown viscous liquid
AK10	0.043	brown viscous liquid
AK11	0.178	dark brown viscous liquid



Scheme 7 Isolation of compounds **PS2**, **PS3**, **PS9**, **PS11-PS19** from the crude methanol extract of the roots of *C. cochinchinense*.

Isolation of PS13

Fraction AK4 (0.508 g) was purified by column chromatography over silica gel and eluted with hexane-dichloromethane (4:6) solvent system. The fractions containing similar components were combined into four fractions. The fourth fraction (101.34 mg) was acetylated with acetic anhydride (2 ml) in pyridine (2 ml) at room temperature. The reaction mixture was purified by column chromatography using hexane-dichloromethane (6:4) as an eluent to afford pure **PS13Ac** as a yellow solid (64.20 mg).

PS13Ac

Melting point : 128-129 °C

UV (EtOH) λ_{\max} nm (log ϵ) : 204 (4.80), 233 (4.59), 257 (4.54), 288 (4.20), 309 (4.14), 369 (3.85)

IR (KBr) ν_{\max} (cm⁻¹) : 3411 (O-H stretching), 1779 and 1636 (C=O stretching)

¹H-NMR 300 MHz (CDCl₃) δ (ppm) : 13.39 (1H, *s*, 1-OH), 7.38 (1H, *d*, *J* = 9.3 Hz, H-6), 7.30 (1H, *d*, *J* = 9.3 Hz, H-5), 6.67 (1H, *s*, H-4), 5.15 (2x1H, *br t*, H-2', H-2''), 5.05 (1H, *br t*, *J* = 7.0 Hz, H-6''), 4.05 (2H, *d*, *J* = 7.0 Hz, H-1''), 3.32 (2H, *d*, *J* = 7.0 Hz, H-1'), 2.39 (3Hx2, *s*, 3-C(=O)CH₃, 7-C(=O)CH₃), 2.01 (2Hx2, *m*, H-4', H-5'), 1.81(3H, *s*, H-9''), 1.78 (3H, *s*, H-5'), 1.69 (3H, *s*, H-4'), 1.61 (3H, *s*, H-10''), 1.55 (3H, *s*, H-8'')

¹³C-NMR 75 MHz (CDCl₃) δ (ppm) : 183.4 (C=O), 169.4 (C=O), 168.4 (C=O), 161.0, 155.3, 155.0, 153.7, 144.9, 135.9, 132.2, 131.3, 129.8, 124.1, 121.5, 121.2, 118.8, 116.6, 116.2, 107.2, 100.4, 39.7, 39.7, 26.6, 26.3, 25.7, 25.6, 22.3, 20.9, 20.8, 17.8, 17.6, 16.5

DEPT 135° (CDCl₃) δ (ppm) CH₃ : 16.5, 17.6, 17.8, 20.8, 20.9, 25.6, 25.7;

CH₂ : 22.3, 26.3, 26.6, 39.7; CH : 100.4, 116.6, 121.2, 121.5, 124.1, 129.8

EI-MS *m/z* (% relative intensity) : 533 ([M+H]⁺, 4), 532 ([M]⁺, 14), 531 (36), 489 (2), 463 (18), 462 (62), 421 (9), 420 (31), 378 (9), 364 (100), 322 (31), 304 (29), 280 (14)

HR-MS *m/z* : 532.2469 for C₃₂H₃₆O₇ (calcd. 532.2461)

Isolation of PS2, PS9 and PS14 + PS15

Fraction AK5 (1.939 g) was isolated by column chromatography over silica gel and eluted with hexane-dichloromethane (6:4) solvent system. The fractions containing similar components were combined into ten fractions. The fifth, sixth and ninth fractions gave yellow solids of **PS2** (10.20 mg), **PS14+PS15** (32.00 mg) and **PS9** (385.90 mg), respectively.

PS14+PS15

UV (EtOH) λ_{\max} nm : 235 (4.03), 255 (4.30), 265 (4.21), 314 (3.36), 344 (3.84), 413 (3.54)

IR (Neat) ν_{\max} (cm⁻¹) : 3422 (O-H stretching), 1625 (C=O stretching)

¹H-NMR 300 MHz (CDCl₃) δ (ppm) : 13.27 (1H, *s*, 1-OH), 7.59 (1H, *s*, H-8), 6.96 (1H, *s*, H-5), 5.28 (2x1H, *m*, H-2', H-2''), 4.01 (3H, *s*, 7-OCH₃), 3.53 (2H, *d*, *J* = 7.2 Hz, H-1''), 3.47 (2H, *d*, *J* = 7.2 Hz, H-1'), 1.88 (3H, *s*, H-5''), 1.85 (3H, *s*, H-5'), 1.77 (3H, *s*, H-4''), 1.73 (3H, *s*, H-4')

¹³C-NMR 75 MHz (CDCl₃) δ (ppm) : 180.3 (C=O), 160.2, 158.1, 155.7, 152.7, 152.6, 144.2, 135.2, 133.6, 121.8, 121.6, 113.2, 110.2, 108.8, 104.6, 103.0, 102.5, 56.5, 25.8, 25.8, 21.8, 21.6, 17.9, 17.9

DEPT 135° (CDCl₃) δ (ppm) CH₃ : 25.8, 25.8, 17.9, 17.9; CH₂ : 21.8, 21.6; CH : 121.8, 121.6, 104.6, 102.5

EI-MS *m/z* (% relative intensity) : 367 (5), 366 (1), 243 (100), 215 (7), 186 (7), 69 (15)

Isolation of PS3 and PS15

The yellow solid of **PS3** (130.1 mg) and brown-yellow solid (3.57 mg) were isolated from fraction AK6 (1.149 g) by column chromatography over silica gel and eluted with dichloromethane. The brown-yellow solid (3.57 mg) which was rechromatographed on column chromatography using methanol-dichloromethane (3:97) as eluent to give a yellow solid of **PS15** (1.70 mg).

PS15

Melting point : 248-250 °C

UV (EtOH) λ_{\max} nm (log ϵ) : 203 (4.97), 230 (4.82), 255 (4.83), 265 (4.76), 312 (4.23), 343 (4.36), 411 (3.97)

IR (Neat) ν_{\max} (cm⁻¹) : 3411 (O-H stretching), 1627 (C=O stretching)

¹H-NMR 500 MHz (CDCl₃+DMSO-*d*₆) δ (ppm) : 11.19 (1H, *s*, 1-OH), 11.96 (1H, *s*, 8-OH), 7.62 (1H, *t*, *J* = 8.0 Hz, H-6), 7.32 (1H, *d*, *J* = 9.5 Hz, H-3), 7.04 (1H, *d*, *J* = 8.0 Hz, H-5), 6.79 (1H, *d*, *J* = 8.0 Hz, H-7), 6.66 (1H, *d*, *J* = 9.5 Hz, H-4)

¹³C-NMR 125 MHz (CDCl₃+DMSO-*d*₆) δ (ppm) : 186.3 (C=O), 161.3, 156.2, 153.2, 143.8, 137.3, 137.1, 124.4, 110.6, 109.5, 108.0, 107.8, 107.3

DEPT 135° and DEPT 90° (CDCl₃+DMSO-*d*₆) δ (ppm) CH : 137.3, 124.4, 110.6, 109.5, 107.3

Isolation of PS12 and PS13

Fraction AK7 (5.724 g) was further purified by column chromatography over silica gel and eluted with dichloromethane to give yellow solids of **PS12** (31.50 mg) and **PS13** (67.40 mg).

Isolation of PS 16 and PS17

Fraction AK8 (5.760 g) was chromatographed on column chromatography over silica gel using dichloromethane-methanol (49:1) as an eluent. The fractions containing similar components were combined into eleven fractions. The fourth and sixth fractions gave yellow solids of **PS16** (10.20 mg) and **PS17** (1.0 mg), respectively.

PS16

Melting point : 228-229 °C

UV (EtOH) λ_{\max} nm : 204 (4.68), 230 (4.49), 261 (4.40), 320 (4.12), 377 (4.01)

IR (KBr) ν_{\max} (cm⁻¹) : 3142 (O-H stretching), 1636 (C=O stretching)

¹H-NMR 300 MHz (CDCl₃+DMSO-*d*₆) δ (ppm) : 13.34 (1H, *s*, 1-OH), 7.52 (1H, *s*, H- 8), 6.83 (1H, *s*, H-5), 5.21 (1Hx2, *t*, H-2', H-2''), 3.45 (2H, *d*, *J* = 9.9 Hz, H-1'), 3.35 (2H, *d*, *J* = 9.9 Hz, H-1''), 1.84 (3H, *s*, H-5''), 1.78 (3H, *s*, H-5'), 1.66 (3H, *s*, H-4''), 1.62 (3H, *s*, H-4')

¹³C-NMR 75 MHz (CDCl₃+DMSO-*d*₆) δ (ppm) : 180.3 (C=O), 159.7, 158.0, 152.9, 152.7, 151.7, 142.5, 133.8, 132.7, 122.1, 121.9, 112.9, 108.9, 108.6, 105.2, 102.8, 102.6, 25.7, 25.7, 21.8, 21.5, 17.9, 17.8

DEPT 135° (CDCl₃+DMSO-*d*₆) δ (ppm) CH₃ : 17.8, 17.9, 25.7, 25.7; CH₂: 21.5, 21.8; CH: 102.6, 108.6, 121.9, 122.1

PS17

¹H-NMR 300 MHz (CDCl₃+DMSO-*d*₆) δ (ppm) : 13.89 (1H, *s*, 1-OH), 6.78 (1H, *s*, H- 4), 6.30 (1H, *s*, H-5), 5.28 (1Hx2, *br t*, H-2', H-2''), 4.18 (2H, *d*, *J* = 7.8 Hz, H-1'') 3.40 (2H, *d*, *J* = 7.8 Hz, H-1'), 1.87 (3H, *s*, H-4'), 1.82 (3H, *s*, H-4''), 1.72 (3Hx2, *s*, H-4')

Isolation of PS18

Fraction AK9 (2.554 g) was chromatographed on column chromatography over Sephadex LH-20 and eluted with methanol : water (9:1) to obtain five fractions. Fraction AK9.3 was further purified by column chromatography over silica gel using dichloromethane : methanol (98 : 2) as eluent to give three fractions. Fraction AK9.3.1 (2.0 mg) was further isolated by TLC to give **PS18** (1.2 mg).

PS18

Melting point : 219–220 °C

UV (EtOH) λ_{\max} nm : 212 (3.32), 237 (3.68), 251 (3.82), 284 (3.24), 329 (3.52)

IR (KBr) ν_{\max} (cm⁻¹) : 3400 (O-H stretching), 1650 (C=O stretching)

¹H-NMR 500 MHz (CDCl₃+DMSO-*d*₆) δ (ppm) : 7.56 (1H, *s*, H-8), 6.47 (1H, *d*,
 $J = 2.0$ Hz, H-2), 6.29 (1H, *d*, $J = 2.0$ Hz, H-4), 3.40 (2H, *t*, $J = 7.0$ Hz,
 H-1'), 5.37 (1H, *br t*, $J = 7.0$ Hz, H-2'), 1.76 (3H, *s*, H-4'), 1.73 (3H, *s*, H-5')

DEPTQGPSP 125 MHz (CD₃OD) δ (ppm) : 180.2, 163.5, 157.8, 150.1, 149.2, 145.0,
 133.1, 121.5, 115.2, 112.5, 102.3, 97.5, 93.6, 27.6, 24.6, 16.4

EI-MS m/z (% relative intensity) : 328 ([M]⁺, 15), 327 (75), 312 (19), 310 (16), 273
 (14), 272 (100), 271 (57), 259 (3), 242 (6), 214 (5)

HR-MS m/z : 328.0949 for C₃₂H₃₆O₇ (calcd. 328.0947)

Isolation of PS19

Compound **PS19** was isolated from fraction AK11 by column chromatography over Sephadex LH-20 and eluted with water-methanol (1:9).

PS19

Melting point : 330-331 °C

UV (EtOH) λ_{\max} nm : 204 (4.67), 237 (4.51), 254 (4.53), 269 (4.27), 313 (4.23), 365
 (4.11)

IR (KBr) ν_{\max} (cm⁻¹) : 3417 (O-H stretching), 1653 (C=O stretching)

¹H-NMR 300 MHz (CDCl₃+DMSO-*d*₆) δ (ppm) : 13.18 (1H, *s*, 1-OH), 7.55 (1H, *s*,
 H-8), 6.88 (1H, *s*, H-5), 6.37 (1H, *d*, $J = 2.7$ Hz, H-4), 6.23 (1H, *d*, $J = 2.7$
 Hz, H-2)

¹³C-NMR 75 MHz (CDCl₃+DMSO-*d*₆) δ (ppm) : 179.6 (C=O), 164.5, 163.0, 157.7,
 153.1, 151.5, 142.9, 112.8, 108.6, 102.6, 102.3, 97.9, 93.5

DEPT 135° and DEPT 90° (CDCl₃+DMSO-*d*₆) δ (ppm) CH : 108.6, 102.6, 97.9,
 93.5