2 EXPERIMENTAL

2.1 General Method

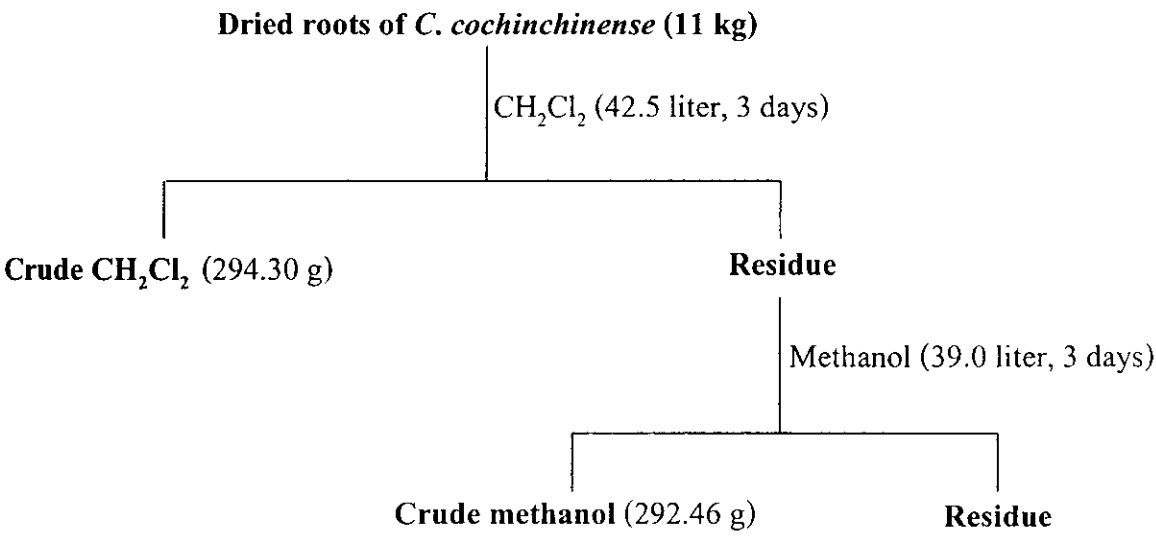
Column chromatography was performed by using silica gel 100 (70-230 Mesh ASTM, Merck) or silica gel 60 (230-400 Mesh ASTM, Merck). Thin layer chromatography (TLC) aluminium sheets of silica gel 60 F_{254} (20x20 cm, layer thickness 0.2 mm, Merck) were used for analytical purposes and the spots were visualized under ultraviolet light. Melting points (uncorrected) were determined on a digital Electrothermal Melting Point Apparatus (Electrothermal 9100) and were recorded in ^{o}C. Ultraviolet spectra were measured with UV-160A spectrophotometer (SHIMADZU). Principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in ethanol solution. Infrared spectra (IR) were obtained on a FTS165 FT-IR spectrophotometer and were recorded in wave number (cm^{-1}). ^{1}H and ^{13}C-Nuclear magnetic resonance spectra were recorded on a FT-NMR Bruker Ultra Shield™ 300 MHz spectrometor at Department of Chemistry, Faculty of Science, Prince of Songkla University or Varian UNITY INOVA spectrometer 500 MHz at Central Instrument Facilities, Prince of Songkla University. Spectrum were recorded in $CDCl_3$, C_6D_6 and DMSO-d_6 and were recorded as δ value in ppm down field from TMS (internal standard δ 0.00). High resolution mass spectra were recorded on a MAT 95 XL at Central Instrument Facilities, Prince of Songkla University. Solvent for extraction and chromatography were distilled at their boiling ranges prior to use. Optical rotation was measured in $CHCl_3$ solution with sodium D line (590 nm) on an AUTOPOL® II automatic polarimeter. DPPH radical (Merck) was used to determine the antioxidative activity and the absorptions were measured with a spectronic 21 (MILTON ROY).

2.2 Plant Material

The roots of *C. cochinchinense* (Guttiferae) was collected from Amphur Bannasan, Suratthani Province in the southern part of Thailand in February 2003. A herbarium specimen (W. Nuangnaowarat 1 Suratthani: Bannasan 31/3/04) has been deposited in the herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University, Thailand.

2.3 Extraction and Isolation

Choped, dried roots of *C. cochinchinense* (11 kg) were sequently extracted with dichloromethane and methanol (each extract time of 3 days) at room temperature. After removal of solvents, a yellow-brown viscous dichloromethane extract (294.30 g) and methanolic extract (292.46 g) were obtained, respectively. The process of extraction was shown in scheme 1.



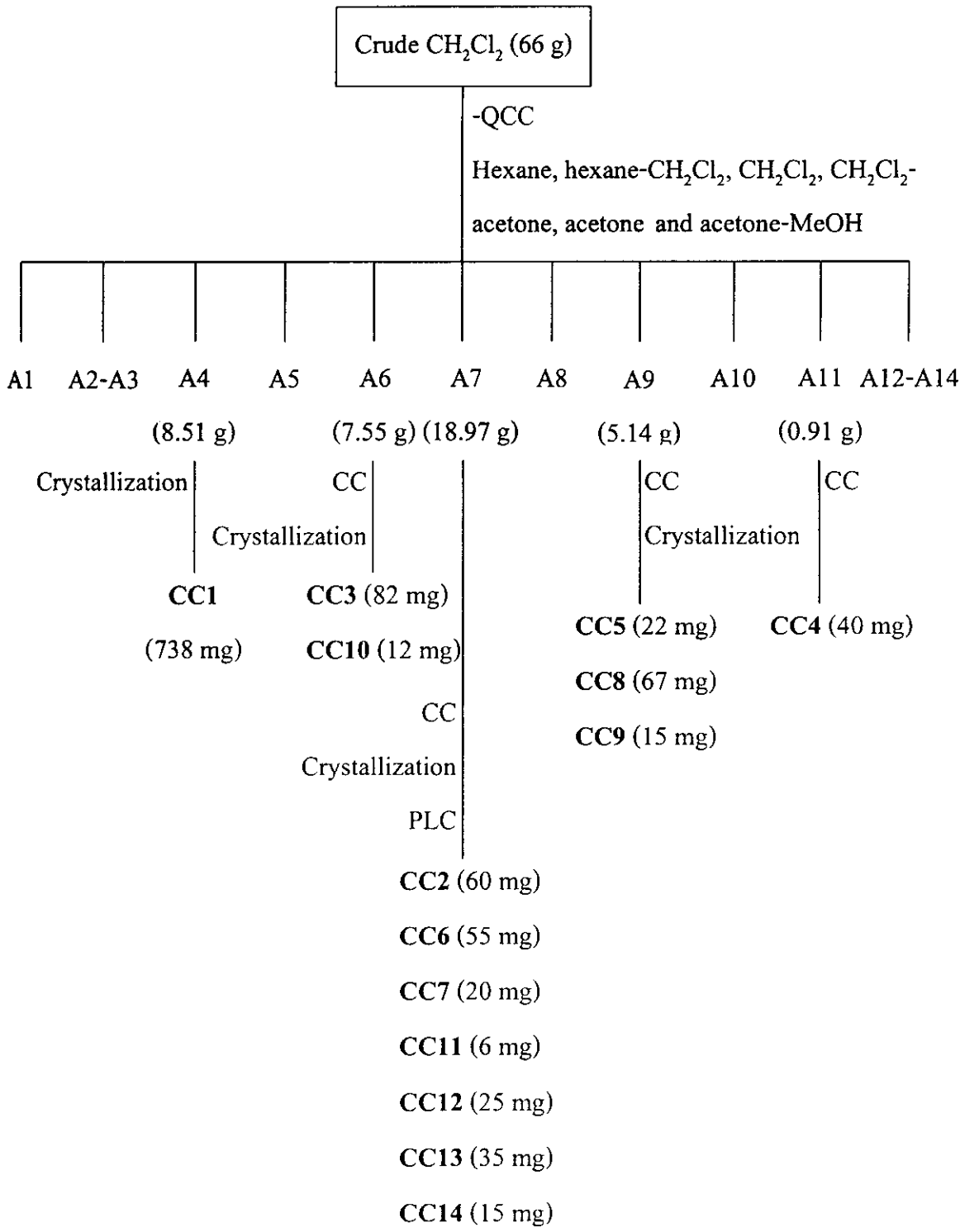
Scheme 1 Extraction of the crude extracts from the roots of *C. cochinchinense*

2.3.1 Isolation of chemical constituents from the dichloromethane extract

The dichloromethane extract (66 g) was chromatographed on quick column chromatography over silica gel 60H using hexane, hexane-dichloromethane, dichloromethane, dichloromethane-acetone, acetone and acetone-methanol as eluents. Fractions with the similar characteristic on TLC were combined to afford fourteen fractions (A1-A14) (Table 2).

Table 2 Fractions from dichloromethane extract

Fraction	Weight (g)	Appearance
A1	0.201	turbid white viscous liquid
A2	3.964	orange viscous liquid
A3	4.225	orange viscous liquid
A4	8.515	orange-yellow solid
A5	0.162	orange-yellow solid
A6	7.548	orange-yellow solid
A7	18.967	yellow solid
A8	7.641	yellow solid
A9	5.136	brown viscous liquid
A10	1.020	brown viscous liquid
A11	0.913	brown viscous liquid
A12	0.996	brown viscous liquid
A13	1.124	dark brown solid
A14	0.272	dark brown solid



Scheme 2 Isolation of compounds **CC1-14** from the roots of *C. cochinchinense*

Isolation of CC1

Fraction A4 (8.515 g) which contained one major component was further purified by crystallization in hexane-dichloromethane (9:1). The yellow solid of **CC1** (0.738 g) which formed was filtered.

CC1

Melting point : 176-180 °C

UV (EtOH) λ_{max} nm : 244 (4.43), 258 (4.36), 316 (4.22), 354 (3.90)

IR (KBr) ν (cm^{-1}) : 3380 (O-H stretching), 1642 (C=O stretching)

^{1}H NMR 300 MHz ($CDCl_3$) (δ ppm) : 13.54 (1H, *s*, 1-OH), 6.77 (1H, *s*, H-5), 6.45 (1H, *brs*, 6-OH), 6.28 (1H, *s*, H-4), 5.26 (1H, *brt*, J = 6.0 Hz, H-2″), 5.23 (1H, *brt*, J = 7.2 Hz, H-2′), 4.07 (2H, *d*, J = 6.0 Hz, H-1″), 3.88 (3H, *s*, 3-OCH_3), 3.79 (3H, *s*, 7-OCH_3), 3.33 (2H, *d*, J = 7.2 Hz, H-1′), 1.83 (3H, *s*, H-4″), 1.79 (3H, *s*, H-4′) 1.68 (2x3H, *s*, H-5′, H-5″)

^{13}C NMR 75 MHz ($CDCl_3$) (δ ppm) : 181.86 (C=O), 163.47, 159.67, 155.62, 155.16, 154.42, 142.59, 136.99, 131.98, 131.62, 123.26, 122.37, 112.31, 111.45, 103.74, 101.50, 88.79, 61.98, 55.77, 26.52, 25.80, 21.34, 18.20, 17.76

DEPT 135° ($CDCl_3$) (δ ppm) CH_3: 61.98, 55.77, 25.80, 25.80, 18.20, 17.76; CH_2: 26.52, 21.34; CH : 123.26, 122.37, 101.50, 88.79

Isolation of CC3, CC10

Fraction A6 (7.548 g) was further purified by crystallization in hexane-dichloromethane (1:4), after filtration, to give a yellow solid of **CC3** (82 mg) and filtrate (7.440 g). The filtrate (0.150 g) was chromatographed on column chromatography and the elution was conducted with hexane, hexane-dichloromethane (19:1) and hexane-dichloromethane (9:1) to afford four portions (A6.1 20 mg, A6.2 39 mg, A6.3 43 mg, A6.4 24 mg and A6.5 12 mg). Fraction A6.3 (43 mg) was rechromatographed by using hexane as eluent to give a yellow solid **CC10** (12 mg).

CC3

Melting point : 180-181 °C

UV EtOH λ_{max} nm (log ε) : 245 (4.38), 261 (4.44), 318 (4.04), 370 (4.01)

IR (KBr) ν (cm^{-1}) : 3360 (O-H stretching), 1648 (C=O stretching)

^{1}H NMR 300 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 13.65 (1H, *s*, 1-OH), 6.77 (1H, *s*, H-5), 6.25 (1H, *s*, H-4), 5.30 (1H, *brt*, J = 6.3 Hz, H-2″), 5.21 (1H, *brt*, J = 7.2 Hz, H-2′), 4.13 (2H, *d*, J = 6.3 Hz, H-1″), 3.85 (3H, *s*, 3-OCH_3), 3.31 (2H, *d*, J = 7.2 Hz, H-1′), 1.85 (3H, *s*, H-4″), 1.78 (3H, *s*, H-4′), 1.69 (3H, *s*, H-5″), 1.65 (3H, *s*, H-5′)

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 182.24 (C=O), 162.99, 159.48, 155.10, 152.74, 151.08, 140.33, 131.50, 131.21, 127.72, 123.22, 122.52, 111.28, 110.66, 103.64, 100.54, 88.50, 55.64, 25.91, 25.74, 21.22, 18.11, 17.70

DEPT 135°($CDCl_3$) (δ ppm) CH_3: 55.64, 25.74, 25.74, 18.11, 17.70; CH_2: 25.91, 21.22; CH : 123.22, 122.52, 100.54, 88.50

EIMS *m/z* (% relative intensity) : 411 ($[M+H]^+$, 19), 410 ($[M]^+$, 76), 395 (15), 367 (55), 355 (91), 339 (61), 337 (18), 311 (80), 299 (33), 281 (10), 178 (20), 167 (14), 149 (47), 129 (15), 111 (19), 105 (24), 97 (32), 84 (91), 69 (74), 66 (100)

HR-MS *m/z* : 410.1711 for $C_{24}H_{26}O_6$ (calcd. 410.1729)

CC10

Melting point : 246-247 °C

UV EtOH λ_{max} nm (log ε) : 245 (4.39), 267 (4.41), 321 (4.28), 332 (4.30), 382 (3.77)

IR (KBr) ν (cm^{-1}) : 3483 (O-H stretching), 1631 (C=O stretching)

^{1}H NMR 300 MHz ($CDCl_3$) (δ ppm) : 13.48 (1H, *s*, 6-OH), 9.19 (1H, *brs*, 12-OH), 8.00 (1H, *d*, *J* = 10.2 Hz, H-4), 6.81 (1H, *s*, H-11), 6.35 (1H, *s*, H-9), 5.81 (1H, *d*, *J* = 10.2 Hz, H-3), 5.20 (1H, *brt*, *J* = 7.2 Hz, H-2′), 3.90 (3H, *s*, 8-OCH_3), 3.32 (2H, *d*, *J* = 7.2 Hz, H-1′), 1.78 (3H, *s*, H-4′), 1.67 (3H, *s*, H-5′), 1.50 (2x3H, s, H-13, H-14)

^{13}C NMR 75 MHz ($CDCl_3$) (δ ppm) : 182.20 (C=O), 163.29, 159.35, 155.30, 152.87, 152.45, 138.01, 132.08, 131.31, 122.27, 120.94, 119.94, 115.45, 110.98, 107.89, 103.71, 102.63, 88.80, 75.70, 55.73, 26.98, 25.68, 21.16, 17.66

DEPT 135° ($CDCl_3$) (δppm) CH_3: 55.73, 26.98, 26.98, 25.68, 17.66; CH_2: 21.16; CH: 132.08, 122.27, 120.94, 102.63, 88.80

EIMS *m/z* (% relative intensity) : 409 ($[M+H]^+$, 16), 408 ($[M]^+$, 55), 393 (51), 366 (16), 365 (65), 353 (100), 351 (13), 335 (12), 323 (18), 189 (10), 169 (15), 154 (10), 57 (11)

HR-MS *m/z* : 408.1555 for $C_{24}H_{24}O_6$ (calcd. 408.1573)

Isolation of CC2, CC6, CC7, CC11, CC12, CC13, CC14

Fraction A7 (18.967 g) was absorbed on silica gel 100 (242.57 g). The absorbed silica gel was sequentially eluted with hexane, dichloromethane and acetone. The eluents were evaporated to afford fractions A7A (5.337 g), A7B (10.778 g) and A7C (1.949 g), respectively. Fraction A7A (2.006 g) was chromatographed on column chromatography and eluted with hexane-acetone (17:3) to obtain eight portions (A7.1 30 mg, A7.2 675 mg, A7.3 575 mg, A7.4 53 mg, A7.5 152 mg, A7.6 23 mg, A7.7 233 mg and A7.8 24 mg). The yellow solids **CC6** (55 mg), **CC7** (20 mg) and **CC14** (15 mg) were isolated from A7.3 (306 mg) by column chromatography and eluted with hexane-acetone (9:1). Fraction A7.7 (233 mg) was crystallized in hexane-dichloromethane (4:1) to give a yellow solid of **CC2** (60 mg). Fraction A7B (1.203 g) was chromatographed on column chromatography and eluted with hexane-acetone (99:1) to hexane-acetone (17:3) to afford eleven fractions; B7.1 (19 mg), B7.2 (22 mg), B7.3 (48 mg), B7.4 (67 mg), B7.5 (321 mg), B7.6 (212 mg), B7.7 (69 mg), B7.8 (296 mg), B7.9 (53 mg), B7.10 (142 mg) and B7.11 (8.3 mg). Fraction B7.1 (19 mg) was further purified by preparative chromatography. Elution was conducted with hexane-dichloromethane (2:3) to afford **CC11** (6 mg). Fraction B7.7 (69.3 mg) which was the mixture of two major fluorescent spots was chromatographed on column chromatography and using hexane-dichloromethane (3:2) as an eluent to give the yellow solids **CC12** (25 mg) and **CC13** (35 mg).

CC2

Melting point : 180-182 $^{\circ}C$

UV EtOH λ_{max} nm : 243 (4.30), 258 (4.19), 318 (4.10), 352 (3.77)

IR (Neat) ν (cm^{-1}) : 3419 (O-H stretching), 1644 (C=O stretching)

^{1}H NMR 300 MHz ($CDCl_3$) (δ ppm) : 13.80 (1H, *s*, 1-OH), 6.83 (1H, *s*, H-5), 6.41 (1H, *brs*, 6-OH), 6.29 (1H, *s*, H-4), 6.28 (1H, *brs*, 3-OH), 5.29 (1H, *brt*, J = 7.2 Hz, H-2′), 5.26 (1H, *brt*, J = 6.3 Hz, H-2″), 4.09 (2H, *d*, J = 6.3 Hz, H-1″), 3.81 (3H, *s*, 7-OCH_3), 3.45 (2H, *d*, J = 7.2 Hz, H-1′), 1.84 (2x3H, *s*, H-4′, H-4″), 1.77 (3H, *s*, H-5′), 1.69 (3H, *s*, H-5″)

CC6

Melting point : 172-173 °C

^{1}H NMR 300 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 13.26 (1H, *s*, 1-OH), 9.08 (1H, *s*, 7-OH), 7.63 (1H, *d*, J = 3.0 Hz, H-8), 7.33 (1H, *d*, J = 9.0 Hz, H-5), 7.26 (1H, *dd*, J = 9.0, 3.0 Hz, H-6), 7.03 (1H, *s*, 3-OH), 5.28 (1H, *m*, H-2′), 5.27 (1H, *m*, H-2″), 3.55 (2H, *d*, J = 6.9 Hz, H-1″), 3.47 (2H, *d*, J = 6.9 Hz, H-1′), 1.91 (3H, *s*, H-4″), 1.85 (3H, *s*, H-4′), 1.77 (3H, *s*, H-5′), 1.73 (3H, *s*, H-5″)

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 181.06 (C=O), 160.55, 158.24, 53.52, 153.00, 149.90, 134.34, 133.12, 124.16, 121.97, 121.80, 120.74, 118.50, 108.97, 105.27, 103.16, 25.81, 21.84, 21.57, 17.92

DEPT 135° ($CDCl_3$+DMSO-d_6) (δ ppm) CH_3: 25.81, 25.81, 17.92, 17.92; CH_2: 21.84, 21.57; CH : 124.16, 121.97, 121.80, 118.50, 108.97

CC7

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 (cm^{-1}) : 3413 (O-H stretching), 1641 (C=O stretching)

^{1}H NMR 500 MHz ($CDCl_3$+C_6D_6+DMSO-d_6) (δ ppm) : 12.95 (1H, *s*, 1-OH), 7.59 (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), 5.29 (1H, *brt*, J = 7.0 Hz, H-2′), 5.27 (1H, *brt*, J = 7.0 Hz, H-2″), 5.05 (1H,

brt, J = 7.0 Hz, H-6″), 3.57 (2H, *d*, J = 7.0 Hz, H-1″), 3.47 (2H, *d*, J = 7.0 Hz, H-1′), 2.11-2.08 (2H, *m*, H-5″), 2.06- 2.03 (2H, *m*, H-4″), 1.88 (3H, *s*, H-9″), 1.84 (3H, *s*, H-4′), 1.76 (3H, *s*, H-5′), 1.64 (3H, *s*, H-10″), 1.57 (3H, *s*, H-8″)

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 180.90 (C=O), 161.13, 158.27, 152.97, 152.43, 150.34, 137.94, 134.92, 132.00, 124.12, 123.85, 121.58, 120.45, 118.87, 108.90, 108.89, 105.00, 102.96, 39.72, 26.43, 25.86, 25.66, 21.59, 21.58, 17.94, 17.69, 16.27

DEPT 135° ($CDCl_3$+DMSO-d_6) (δ ppm) CH_3: 25.86, 25.66, 17.94, 17.69, 16.27; CH_2: 39.72, 26.43, 21.59, 21.58; CH : 124.12, 123.85, 121.58, 121.58, 118.87, 108.89

FABMS *m/z* (% relative intensity) : 449 ($[M+H]^+$, 53), 448 ($[M]^+$, 15), 447 (10), 393 (18), 325 (12), 323 (30), 309 (13), 281 (10), 277 (18), 270 (23), 269 (100), 257 (13), 253 (10), 185 (75), 93 (60)

HR-MS *m/z* : 448.2299 for $C_{28}H_{32}O_5$ (calcd. 448.2250)

CC11

Melting point : 170-172 °C

^{1}H NMR 300 MHz ($CDCl_3$) (δ ppm) : 13.56 (1H, *s*, 5-OH), 7.71 (1H, *d*, J = 9.0 Hz, H-7), 6.97 (1H, *d*, J = 9.0 Hz, H-8), 6.80 (1H, *d*, J = 10.2 Hz, H-4), 6.76 (1H, *dd*, J = 17.7, 10.8 Hz, H-4′), 5.64 (1H, *d*, J = 10.2 Hz, H-3), 5.24 (1H, *dd*, J = 17.7, 1.2 Hz, H-5′*Z*), 5.07 (1H, *dd*, J = 10.8, 1.2 Hz, H-5′*E*), 1.67 (2x3H, *s*, H-2′, H-3′), 1.53 (2x3H, *s*, H-13, H-14)

^{13}C NMR 125 MHz ($CDCl_3$) (δ ppm) : 180.79 (C=O), 158.94, 156.90, 156.79, 154.11, 146.01, 144.55, 131.05, 127.16, 117.53, 116.14, 113.74, 113.07, 112.76, 105.59, 103.29, 103.08, 78.26, 41.46, 28.20, 27.94

DEPT 135° ($CDCl_3$) (δ ppm) CH_3: 28.20, 28.20, 27.94, 27.94; CH_2: 103.29; CH : 156.90, 127.16, 117.53, 116.14, 112.76

CC12

Optical rotation : $[\alpha]_D^{29}$ -63° (c 1.19x10^{-3} g/cm^3 in $CHCl_3$)

Melting point : 208-209 °C

UV EtOH λ_{max} nm (log ε) : 213 (4.63), 326 (4.37), 350 (4.42)

IR (KBr) ν (cm^{-1}) : 3558 (O-H stretching), 1738 (C=O stretching), 1635 (C=O stretching)

^{1}H NMR 300 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 12.48 (1H, *s*, 1-OH), 7.44 (1H, *d*, *J* = 7.0 Hz, H-8), 7.07 (1H, *brs*, 3-OH), 6.12 (1H, *d*, *J* = 2.0 Hz, H-4), 6.06 (1H, *d*, *J* = 2.0 Hz, H-2), 4.44 (1H, *brt*, *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), 2.48 (1H, *d*, *J* = 9.6 Hz, H-11), 2.36 (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)

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 202.96 (C=O), 179.22 (C=O), 165.15, 161.15, 135.31, 133.83, 133.70, 118.11, 101.08, 96.87, 95.23, 90.14, 84.49, 83.71, 48.77, 46.80, 30.29, 28.98, 28.97, 25.52, 25.16, 16.90

DEPT 135° ($CDCl_3$+DMSO-d_6) (δ ppm) CH_3: 30.29, 28.98, 25.52, 16.90; CH_2: 28.97, 25.16; CH : 133.83, 118.11, 96.87, 95.23, 48.77, 46.80

EIMS *m/z* (% relative intensity) : 369 (23), 368 (93), 353 (20), 300 (12), 299 (48), 272 (32), 271 (74), 257 (27), 255 (12), 230 (19), 229 (100), 215 (19), 173 (13), 153 (20), 91 (11), 69 (34)

HR-MS *m/z* : 396.1563 for $C_{23}H_{24}O_6$ (calcd. 396.1573)

CC13

Optical rotation : $[\alpha]_D^{29}$ -58° (c 6.90x10^{-4} g/cm^3 in $CHCl_3$)

Melting point : 218-219 °C

UV EtOH λ_{max} nm : 212 (4.47), 275 (4.09), 332 (4.06), 357 (4.10)

IR (KBr) ν (cm^{-1}) : 3392 (O-H stretching), 1738 (C=O stretching), 1646 (C=O stretching)

^{1}H NMR 300 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 12.39 (1H, *s*, 1-OH), 8.05 (1H, *brs*, 3-OH), 7.44 (1H, *s*, H-8), 6.05 (1H, *d*, *J* = 2.1 Hz, H-4), 6.03 (1H, *d*, *J* = 2.1 Hz, H- 2), 4.43 (1H, *brt*, *J* = 7.5 Hz, H-16), 3.62 (3H, *s*, 7-OCH_3), 2.63 (2H, *d*, *J* = 7.5 Hz, H-15), 2.50 (1H, *d*, *J* = 9.6 Hz, H-11), 2.35 (1H, *d*, *J* = 13.2 Hz, H_a-10), 1.66 (3H, *s*, H-13), 1.59 (1H, *dd*, *J* = 13.2, 9.6 Hz, H_b-10), 1.40 (3H, *s*, H-18), 1.31 (3H, *s*, H-14), 1.13 (3H, *s*, H-19)

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 201.00 (C=O),178.00 (C=O), 167.88, 164.50, 160.50, 135.50, 133.25, 132.25, 117.86, 100.50, 96.95, 95.49, 88.50, 84.50, 3.78, 83.76, 53.84, 49.27, 30.26, 29.87, 28.98, 28.94, 25.46, 16.88

DEPT 135° ($CDCl_3$+DMSO-d_6) (δ ppm) CH_3: 53.84, 30.26, 28.94, 25.46, 16.88; CH_2: 28.98, 25.46; CH : 133.25, 117.86, 96.95, 95.49, 49.27

CC14

Optical rotation : $[\alpha]_D^{29}$ +50° (c 8.90x10^{-4} g/cm^3 in $CHCl_3$)

Melting point : 147-148 °C

UV EtOH λ_{max} nm (log ε) : 206 (4.46), 222 (4.30), 307 (3.98), 346 (3.70)

IR (KBr) ν (cm^{-1}) : 3467 (O-H stretching), 1749 (C=O stretching), 1642 (C=O stretching)

^{1}H NMR 300 MHz ($CDCl_3$) (δ ppm) : 12.00 (1H, *s*, 1-OH), 7.51 (1H, *s*, H-8), 7.41 (1H, *t*, *J* = 8.4 Hz, H-3), 6.55 (1H, *dd*, *J* = 8.4, 0.9 Hz, H-2), 6.52 (1H, *dd*, *J* = 8.4,

0.9 Hz, H-4), 4.39 (1H, *brt*, J = 8.1 Hz, H-16), 3.65 (3H, *s*, 7-OCH_3), 2.64 (2H, *d*, J = 8.1 Hz, H-15), 2.54 (1H, *d*, J = 9.9 Hz, H-11), 2.39 (1H, *d*, J = 12.9 Hz, H_a-10), 1.69 (3H, *s*, H-13), 1.59 (1H, *dd*, J = 12.9, 9.9 Hz, H_b-10), 1.37 (3H, *s*, H-18), 1.33 (3H, *s*, H-14), 1.01 (3H, *s*, H-19)

^{13}C NMR 125 MHz ($CDCl_3$) (δ ppm) : 201.16 (C=O), 180.73 (C=O), 162.90, 159.44, 138.97, 135.73, 135.25, 132.14, 118.48, 109.57, 107.41, 106.15, 88.75, 84.86, 84.18, 83.96, 54.09, 49.43, 30.37, 29.73, 29.21, 29.04, 25.51, 16.69

DEPT 135° ($CDCl_3$) (δ ppm) CH_3: 54.09, 30.37, 29.04, 25.51, 16.69; CH_2: 29.73, 29.21; CH : 138.97, 135.25, 118.48, 109.57, 107.41

Isolation of CC5, CC8, CC9

Fraction A9 (4.067 g) was further separated by column chromatography and eluted with hexane-acetone (4:1) to obtain fourteen fractions; A9A (0.181 g), A9B (0.199 g), A9C (0.244 g), A9D (0.246 g), A9E (0.281 g), A9F (0.411 g), A9G (0.390 g), A9H (0.422 g), A9I (0.152 g), A9J (0.202 g), A9K (0.246 g), A9L (0.288 g), A9M (0.526 g), A9N (0.091 g). Fraction A9B (0.199 g) was crystallized by using hexane-dichloromethane (1:1) as solvent to give a yellow solid **CC9** (15 mg). Fraction A9C (0.244 g) which contained a major component was induced precipitation by addition of dichloromethane. The yellow solid **CC8** (67 mg) which formed was filtered. Fraction A9I (0.152 g) was chromatographed on column chromatography and eluted with dichloromethane and dichloromethane-MeOH (99:1) to afford a yellow solid **CC5** (22 mg).

CC5

Melting point : 219-220 °C

UV EtOH λ_{max} nm (log ε) : 241 (4.40), 249 (4.40), 333 (4.08), 362 (3.90)

IR (KBr) ν (cm^{-1}) : 3521 (O-H stretching), 1639 (C=O stretching)

^{1}H NMR 300 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 13.19 (1H, *s*, 1-OH), 7.51 (1H, *t*, *J* = 8.4 Hz, H-3), 6.91 (1H, *dd*, *J* = 8.4, 1.5 Hz, H-2), 6.75 (1H, *dd* , *J* = 8.4, 1.5 Hz, H-4), 5.24 (1H, *brt*, *J* = 6.3 Hz, H-2′), 4.04 (2H, *d*, *J* = 6.3 Hz, H-1′), 3.84 (3H, *s*, 7-OCH_3), 1.84 (3H, *s*, H-4′), 1.69 (3H, *s*, H-5′)

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 183.40 (C=O), 161.84, 155.16, 144.89, 144.60, 143.39, 135.46, 131.36, 131.04, 127.58, 123.89, 110.92, 109.92, 108.82, 106.20, 60.97, 25.80, 25.53, 18.09

DEPT 135° ($CDCl_3$+DMSO-d_6) (δppm) CH_3: 25.80, 18.09; CH_2: 25.53; CH : 135.46, 123.89, 109.92, 106.20

CC8

Melting point : 221-222 °C

UV EtOH λ_{max} nm (log ε) : 243 (4.35), 259 (4.32), 323 (4.10), 372 (3.98)

IR (KBr) ν (cm^{-1}) : 3350 (O-H stretching), 1640 (C=O stretching)

^{1}H NMR 500 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 13.33 (1H, *s*, 1-OH), 7.48 (1H, *s*, H-8), 6.40 (1H, *s*, H-4), 5.26 (2x1H, *brt*, *J* = 7.0 Hz, H-2′, H-2″), 5.00 (1H, *brt*, *J* = 7.0 Hz, H-6″), 3.56 (2H, *d*, *J* = 7.0 Hz, H-1″), 3.35 (2H, *d*, *J* = 7.0 Hz, H-1′), 2.03-1.99 (2H, *m*, H-5″), 1.94- 1.91 (2H, *m*, H-4″), 1.84 (3H, *s*, H-9″), 1.78 (3H, *s*, H-4′), 1.66 (3H, *s*, H-5′), 1.56 (3H, *s*, H-10″), 1.50 (3H, *s*, H-8″)

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 180.11 (C=O), 162.28, 160.00, 155.73, 150.23, 149.76, 141.60, 135.41, 131.40, 131.19, 124.12, 122.63, 121.43,

115.32, 112.85, 110.02, 105.95, 102.33, 93.34, 39.66, 26.56, 25.72, 25.52, 22.32, 21.28, 17.79, 17.57, 16.23

DEPT 135° ($CDCl_3$+DMSO-d_6) (δ ppm) CH_3: 25.72, 25.52, 17.79, 17.57, 16.23; CH_2: 39.66, 26.56, 22.32, 21.28; CH : 124.12, 122.63, 121.43, 105.95, 93.34

EIMS *m/z* (% relative intensity) : 465 ($[M+H]^+$, 18), 464 ($[M]^+$, 63), 422 (10), 421 (39), 409 (76), 379 (17), 353 (11), 342 (24), 339 (31), 325 (24), 297 (46), 285 (45), 257 (25), 207 (13), 178 (58), 161 (32), 121 (37), 108 (50), 91 (76), 79 (77), 69 (96), 57 (100)

HR-MS *m/z* : 464.2189 for $C_{28}H_{32}O_6$ (calcd. 464.2199)

CC9

Melting point : 190-192 °C

^{1}H NMR 300 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 13.69 (1H, *s*, 6-OH), 8.03 (1H, *d*, *J* = 10.5 Hz, H-4), 6.82 (1H, *s*, H-11), 6.31 (1H, *s*, H-9), 5.83 (1H, *d*, *J* = 10.5 Hz, H-3), 5.30 (1H, *brt*, *J* = 7.2 Hz, H-2′), 3.46 (2H, *d*, *J* = 7.2 Hz, H-1′), 1.84 (3H, *s*, H-4′), 1.78 (3H, *s*, H-5′), 1.50 (2x3H, *s*, H-13, H-14)

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 182.28 (C=O), 162.27, 160.45, 155.04, 152.92, 151.42, 138.00, 132.02, 131.31, 122.54, 121.14, 119.92, 110.11, 106.40, 102.68, 102.48, 92.86, 76.27, 27.16 (2-CH_3), 25.77, 21.37, 17.83

DEPT 135° ($CDCl_3$+DMSO-d_6) (δ ppm) CH_3: 27.16, 27.16, 25.77, 17.83; CH_2: 21.37; CH : 132.02, 122.54, 121.14, 102.48, 92.86

Isolation of CC4

Fraction A11 (300 mg) was chromatographed on column chromatography and eluted with dichloromethane and dichloromethane-MeOH (49:1) to afford **CC4** (40 mg).

CC4

Melting point : 205-206 ^{o}C

UV EtOH λ_{max} nm : 244 (4.42), 258 (4.31), 318 (4.24), 354 (3.90)

IR (Neat) ν (cm^{-1}) : 3400 (O-H stretching), 1646 (C=O stretching)

^{1}H NMR 300 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 13.60 (1H, *s*, 1-OH), 9.52 (1H, *s*, 6-OH), 9.49 (1H, *s*, 3-OH), 6.67 (1H, *s*, H-5), 6.26 (1H, *s*, H-4), 5.19 (1H, *brt*, *J* = 6.0 Hz, H-2′), 3.76 (3H, *s*, 7-OCH_3), 3.42 (2H, *m*, H-1″), 3.35 (2H, *d*, *J* = 6.0 Hz, H-1′), 1.79 (3H, *s*, H-4′), 1.71 (2H, *m*, H-2″), 1.66 (3H, *s*, H-5′), 1.29 (2x3H, *s*, H-4″, H-5″)

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) (δ ppm) : 181.87 (C=O), 162.22, 160.52, 156.18, 155.54, 154.80, 143.16, 138.51, 131.53, 122.57, 111.13, 110.17, 102.98, 101.96, 92.67, 70.50, 61.29, 44.49, 29.19, 25.77, 21.95, 21.36, 17.81

DEPT 135^{o} ($CDCl_3$+DMSO-d_6) (δ ppm) CH_3: 61.29, 29.19, 29.19, 25.77, 17.81; CH_2: 44.49, 21.95, 21.36; CH : 122.57, 101.96, 92.67

2.4 Evaluation of Antioxidative Activity

The potential antioxidation activities of the crude material and pure compounds isolated from the roots of *C. cochinchinense* were assessed on the basis of the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical.

2.4.1 Screening on the free radical scavenging activity of the crude extracts

The crude materials were dissolved in absolute ethanol to prepare the solutions with concentration of 10.0 mg/mL. The solution of each sample (50 μL) was mixed with 0.05 mM DPPH solution (3 mL) to give the sample solution with the final concentration of 40 μg/mL. The trapping effect was determined by measuring the absorbance change of the solution at 517 nm against 0.05 mM DPPH ethanolic solution every 15 min. The measurements were performed at least in triplicate. The degree of loss of purple color implied the activity. The results were shown in Table 3.

Table 3 DPPH radical scavenging activity of the crude extracts (40 μg/mL)

Sample	Average absorbances (517 nm)				
	0 min	15 min	30 min	45 min	60 min
DPPH	0.58	0.58	0.58	0.57	0.57
Dichloromethane extract	0.40	0.22	0.16	0.15	0.14
Methanolic extract	0.39	0.13	0.11	0.10	0.10

2.4.2 Evaluation of IC_{50} value of the crude extracts

The DPPH solution (0.05 mM, 3 mL) was mixed with the solution of sample to prepare the sample with the final concentrations of 40, 30, 20, 10 and 5 μg/mL. The mixed solutions were allowed to stand at room temperature for 30 min and the absorbance were measured at 517 nm. The results were shown in Table 4.

IC_{50} (the concentration of the sample at 50% inhibition) was obtained by linear regression analysis of dose response curve, which was plotted between % inhibition and concentration (μg/mL).

$$\% \text{ inhibition} = \frac{\mathbf{A}\text{control} - \mathbf{A}\text{sample}}{\mathbf{A}\text{control}} \times 100$$

Table 4 Average absorption and % inhibition of the crude extracts at various concentrations

Final concentrations (μg/mL)	Dichloromethane extract		Methanolic extract		BHT	
	A	% I	A	% I	A	% I
DPPH	0.58	-	0.58	-	0.58	-
5	0.51	12.07	0.50	13.79	0.30	48.28
10	0.47	18.96	0.44	24.14	0.13	77.59
20	0.37	36.21	0.30	48.28	0.09	84.48
30	0.25	56.90	0.17	70.69	0.08	86.21
40	0.16	72.41	0.11	81.03	0.07	87.93

A = Absorbances % I = % Inhibition

2.4.3 Screening on the free radical scavenging activity of pure compounds

The testing was performed as in 2.4.2 except the final concentrations of the solution were made at 50 and 100 μM. The results were shown in Table 5.

Table 5 The average absorption and % inhibition of the solutions

Sample	50 μM		100 μM	
	absorbances	% inhibition	absorbances	% inhibition
DPPH	0.58	-	0.58	-
CC1	0.57	1.7	0.57	1.7
CC2	0.55	5.2	0.55	5.2
CC3	0.11	81.0	0.11	81.0
CC4	0.54	6.9	0.52	10.3
CC5	0.12	79.3	0.10	82.8
CC6	0.46	20.7	0.41	29.3
CC7	0.46	20.7	0.40	31.0
CC8	0.12	79.3	0.11	81.0
CC9	0.54	6.9	0.53	8.6
CC10	0.56	3.4	0.56	3.4
CC11	0.14	75.9	0.12	79.3
CC12	0.55	5.2	0.54	6.9
CC13	0.55	5.2	0.55	5.2
CC14	0.57	1.7	0.56	3.4
BHT	0.28	51.7	0.20	65.5

2.4.4 Evaluation of IC_{50} value of pure compounds

CC3, CC5, CC8 and **CC11** were selected for further study. The DPPH solution (0.05 mM, 3 ml) was mixed with the sample to give the solution with final concentrations of 40, 30, 25, 20, 15, 10 and 5 μM. The absorbances at 517 nm were measured after incubation for 30 minutes. The results were shown in Table 6. Percentage inhibition of the solution were plotted against the concentration to obtain IC_{50}.

Table 6 The average absorption and % inhibition of pure compounds at various concentrations

Final concentrations (μM)	CC3		CC5		CC8		CC11		BHT	
	A	% I	A	% I	A	% I	A	% I	A	% I
DPPH	0.55	-	0.55	-	0.55	-	0.55	-	0.55	-
5.0	0.48	12.73	0.45	18.18	0.41	25.45	0.48	12.73	0.52	5.45
10.0	0.40	27.27	0.35	36.36	0.24	56.36	0.41	25.45	0.48	12.73
15.0	0.32	41.82	0.21	61.82	0.15	72.73	0.33	40.00	0.42	23.64
20.0	0.24	56.36	0.15	72.73	0.13	76.36	0.27	50.91	0.38	30.91
25.0	0.17	69.09	0.13	76.36	0.12	78.18	0.21	61.82	0.32	41.82
30.0	0.12	78.18	0.13	76.36	0.12	78.18	0.16	70.91	0.26	52.73
40.0	0.10	81.82	0.12	78.18	0.11	80.00	0.14	74.54	0.17	69.09

A = Absorbances % I = % Inhibition