2 EXPERIMENTAL

2.1 General Method

Melting points (uncorrected) were determined on a digital Electrothermal Melting Point Apparatus (Electrothermal 9100) and were recorded in °C. Ultraviolet spectra (UV) were measured with UV-160A spectrophotometer (SHIMADZU). Principle bands (λ_{max}) were recorded as wavelenghts (nm) and log ε in methanol solution. Infrared spectra (IR) were obtained on a FTS165 FT-IR spectrometer and were recorded in wave number (cm^{-1}). ^{1}H and ^{13}C-Nuclear magnetic resonance spectra (^{1}H and ^{13}C NMR) were recorded on a FTNMR Bruker Ultra Shield^{TM} 300 MHz 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. Spectra were recorded in deuterochloroform, tetradeutero-methanol or hexadeutero-dimethylsulphoxide solution and were recorded as δ value in ppm downfield from TMS (internal standard δ 0.00). Optical rotation was measured in methanol and chloroform solution with sodium D line (590 nm) on an AUTOPOL® II automatic polarimeter. Thin layer chromatography (TLC) aluminium sheets of silica gel 60 F_{254} (20×20 cm, layer thickness 0.2 mm, Merck) were used for analytical purposes and the compounds were visualized by UV light and/or anisaldehyde-sulfuric acid or $CeSO_4$-H_2SO_4 reagent. Column chromatography was performed on silica gel 100 (70-230 Mesh ASTM, Merck) or silica gel 60 (230-400 Mesh ASTM, Merck). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical (Merck) was used to determine the antioxidation activity and the absorptions were measured with a spectronic 21 (MILTON ROY).

2.2 Plant Material

The twigs of *Mangifera odorata* (Anacardiaceac) was collected from Phang-nga Province in the Southern part of Thailand. The plant was identified by Professor

Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University, Thailand.

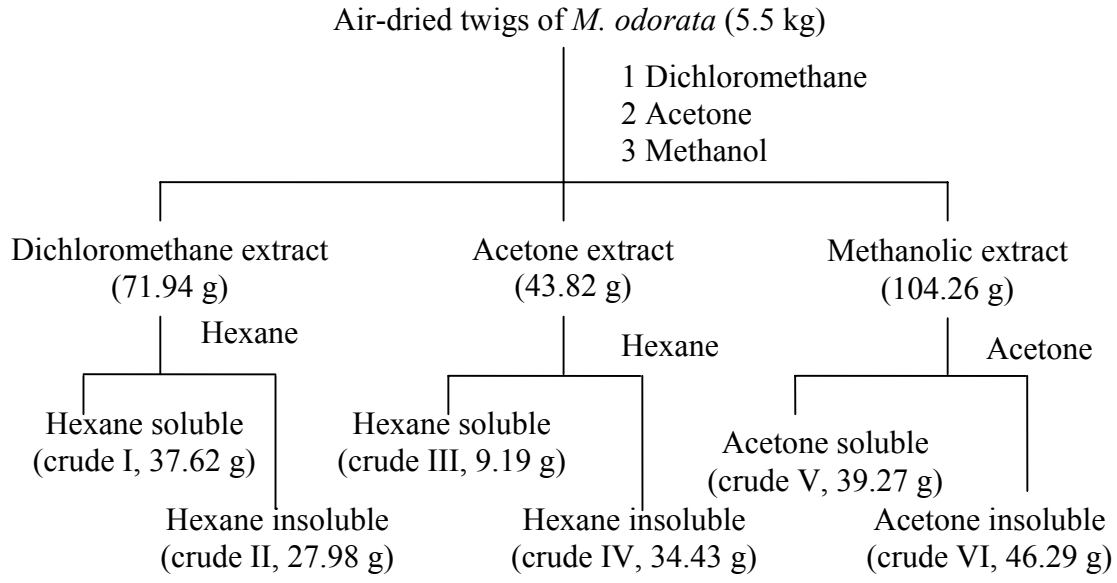
2.3 Extraction and Isolation

Air-dried twigs of *Mangifera odorata* (5.5 kg) were immersed in dichloromethane (7 days), acetone (5 days) and methanol (5 days), successively. After evaporation under vacuum, the viscous dichloromethane extract (71.94 g), acetone extract (43.82 g) and methanolic extract (104.26 g) were obtained.

Dichloromethane extract (71.94 g) was dissolved in hexane to give hexane-soluble (crude I, 37.62 g) and hexane-insoluble fractions (crude II, 27.98 g).

Acetone extract (43.82 g) was separated into two fractions by dissolving in hexane, the hexane-soluble (crude III, 9.19 g) and hexane-insoluble portions (crude IV, 34.43 g) were obtained.

Methanolic extract (104.26 g) was dissolved in acetone to give acetone-soluble (crude V, 39.27 g) and insoluble fractions (crude VI, 46.29 g). The process of extraction was shown in **Scheme 1**.



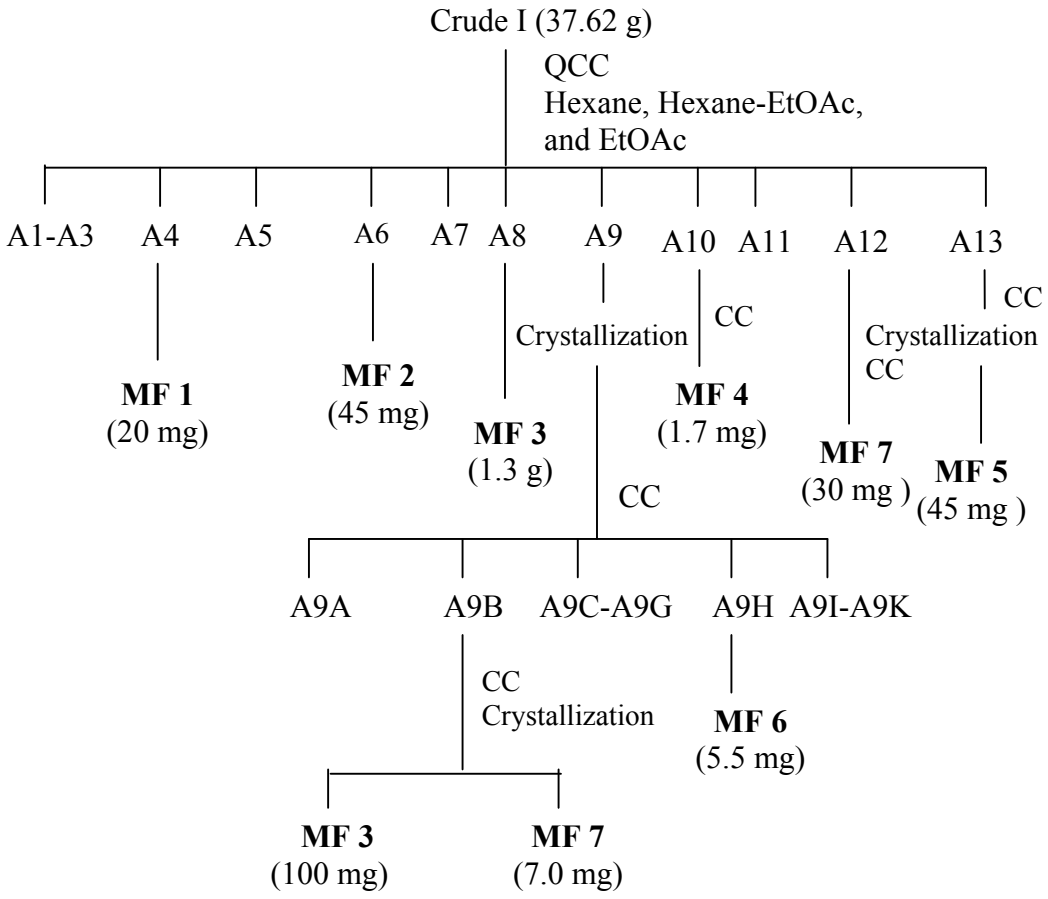
Scheme 1 Extraction of the twigs of *M. odorata*

2.3.1 Purification of crude I

Crude I (37.62 g) was subjected to quick column chromatography using silica gel as the stationary phase and eluted with hexane, hexane-EtOAc and finally with EtOAc. On the basis of their TLC characteristic, the collected fractions which contained the same major components were combined to afford 13 fractions (A1-A13) (**Table 2**) (**Scheme 2**). The selected fractions were further purified to give seven pure compounds (**MF 1-MF 7**).

Table 2 Fractions obtained from chromatographic separation of crude I

Fraction	Weight (g)	Physical characteristic
A1	1.351	white solid mixed with yellow viscous liquid
A2	1.443	white solid mixed with yellow viscous liquid
A3	1.055	white solid mixed with yellow viscous liquid
A4	2.065	white solid mixed with yellow viscous liquid
A5	1.423	white solid mixed with orange viscous liquid
A6	2.359	white solid mixed with orange viscous liquid
A7	2.262	white solid mixed with yellow viscous liquid
A8	2.345	white solid mixed with brown viscous liquid
A9	1.384	brown viscous liquid
A10	1.439	brown viscous liquid
A11	4.362	white solid mixed with brown viscous liquid
A12	2.511	white solid mixed with brown viscous liquid
A13	3.092	brown viscous liquid



Scheme 2 Purification of crude I

Isolation of MF 1

Fraction A4 was crystallized in the mixed solvent of haxane-EtOAc (97:3). A white solid of **MF 1** (20 mg) was obtained.

Optical rotation: $[\alpha]_D^{29}$ +0.72° (c 1.5×10^{-4} g/cm^3 in MeOH)

Melting point: 276-277 °C

FT-IR (neat) ν (cm^{-1}): 3479, 2934 and 2869

^{1}H NMR 500 MHz ($CDCl_3$) (δ ppm): 5.53 (1H, *dd*, *J* = 8.5, 3.5 Hz), 3.20 (1H, *dd*, *J* = 11.5, 5.0 Hz), 1.09 (3H, *s*), 0.97 (3H, *s*), 0.94 (3H, *s*), 0.92 (3H, *s*), 0.90 (2x3H each *s*), 0.82 (3H, *s*) and 0.80 (3H, *s*)

^{13}C NMR 125 MHz ($CDCl_3$) (δ ppm): 158.05 (C), 116.86 (CH), 79.05 (CH), 55.45

(CH), 49.24 (CH), 48.70 (CH), 41.28 (CH_2), 38.95 (C), 38.74 (C), 37.96 (C), 37.70 (CH_2), 37.69 (C), 37.68 (CH_2) 37.53 (C), 35.77 (C), 35.08 (CH_2), 33.66 (CH_2), 33.64 (CH_2), 33.33 (CH_3), 33.06 (CH_2), 29.90 (CH_3), 29.80 (CH_3), 28.78 (C), 27.97 (CH_3), 27.11 (CH_2), 25.89 (CH_3), 21.29 (CH_3), 18.77 (CH_2), 17.48 (CH_2), 15.44 (CH_3), and 15.41 (CH_3)

Isolation of MF 2

Fraction A6 was further chromatographed on column chromatography and eluted with hexane-EtOAc (98:2). The major component was obtained and crystallized in the mixture of EtOAc and hexane. The white solid of **MF 2** was collected (45 mg).

Optical rotation: $[\alpha]_D^{29}$ +17.0° (c 9.5×10^{-4} g/cm^3 in MeOH)

Melting point: 251.2-252.4 °C

FT-IR (KBr) ν (cm^{-1}): 2924 and 1702

^{1}H NMR 500 MHz ($CDCl_3$) (δ ppm): 2.39 (1H, *dddd*, *J* = 14.0, 7.5, 5.0, 2.5, Hz), 2.25 (1H, *q J* = 7.0 Hz), 1.85 (1H, *dt J* = 2.5, 13.0 Hz), 1.18 (3H, *s*), 1.05 (3H, *s*), 1.01 (3H, *s*), 1.00 (3H, *s*), 0.95 (3H, *s*), 0.87 (3H, *d J* = 6.0 Hz) and 0.72 (3H, *s*)

^{13}C NMR 125 MHz ($CDCl_3$) (δ ppm): 213.32 (C=O), 59.44 (CH), 58.20 (CH), 53.08 (CH), 42.75 (CH), 42.14 (C), 41.52 (CH_2), 41.26 (CH_2), 39.67 (C), 39.23 (CH_2), 38.27 (C), 37.41 (C), 35.98 (CH_2), 35.59 (CH_2), 35.32 (CH_2), 35.01 (CH_3), 32.73 (CH_2), 32.39 (CH_2), 32.07 (CH_3), 31.80 (CH_3), 30.49 (CH_2), 29.97 (C), 28.16 (C), 22.27 (CH_2), 20.25 (CH_3), 18.66 (CH_3), 18.21 (CH_2), 17.93 (CH_3), 14.64 (CH_3) and 6.82 (CH_3)

Isolation of MF 3

Fraction A8 was crystallized in the mixed solvent of hexane-EtOAc (92:8) to give a white solid of **MF 3** (1.3 g).

Optical rotation: $[\alpha]_D^{28}$ -55.48° (c 1.5×10^{-4} g/cm^3 in MeOH)

Melting point: 128-130 °C

FT-IR (neat) ν (cm^{-1}): 3418, 2936 and 2851

^{1}H NMR 500 MHz ($CDCl_3$) (δ ppm): 5.36 (1H, *dd J* = 3.0, 2.0 Hz), 3.53 (1H, *m*), 1.25 (3H, *s*), 1.00 (3H, *s*), 0.92 (3H, *s*), 0.85 (3H, *dd J* = 7.0, 4.0 Hz), 0.82 (3H, *d J* = 4.5 Hz), 0.81 (3H, *s*) and 0.69 (3H, *d J* = 9.5 Hz)

Isolation of MF 3 and MF 7

Fraction A9 was further separated by column chromatography on silica gel 100. Elution was conducted initially with hexane and gradually enriched with EtOAc. All fractions were collected and combined on the basis of TLC characteristic to give eleven subfractions A9A-A9K.

Fraction A9B was rechromatographed on column chromatography and eluted with EtOAc-hexane (2:98). **MF 3** and **MF 7** were obtained and crystallized in EtOAc-hexane mixture. The white solid of **MF 3** (100.0 mg) and **MF 7** (7.0 mg) were collected.

MF 7

Optical rotation: $[\alpha]_D^{29}$ +67° (c 1.4×10^{-4} g/cm^3 in MeOH)

Melting point: 201-203 °C

UV (CH_3OH) λ_{max} nm (log ε): 228 (4.09)

FT-IR (neat) ν (cm^{-1}): 3430, 2937, 2869 and 1686

^{1}H NMR 300 MHz ($CDCl_3$) (δ ppm): 9.40 (1H, *s*), 6.50 (1H, *t J* = 7.2 Hz), 3.30 (1H, *dd J* = 10.8, 4.5 Hz), 1.76 (3H, *s*), 0.98 (2×3H, each *s*), 0.97 (2×3H, each *s*), 0.93 (3H, *s*), 0.92 (3H, *d J* = 6.0 Hz), 0.83 (3H, *s*) 0.56 (1H, *d J* = 4.2 Hz), and 0.35 (1H, *d J* = 4.2 Hz)

^{13}C NMR 75 MHz ($CDCl_3$) (δ ppm): 195.45 (CHO), 155.64 (CH), 139.08 (C), 78.84 (CH), 52.19 (CH), 48.83 (C), 47.97 (CH), 47.09 (CH), 45.37 (C), 40.48 (C), 35.99 (CH), 35.52 (CH_2), 34.78 (CH_2), 32.90 (CH_2), 31.97 (CH_2), 30.35 (CH_2) 29.89 (CH_2), 28.20 (CH_2), 26.43 (C), 26.06 (CH_2), 26.00 (CH_2), 25.92 (CH_2), 25.42 (CH_3), 21.10 (CH_2), 19.94 (C), 19.32 (CH_3), 18.10 (CH_3), 18.06 (CH_3), 14.00 (CH_3) and 9.16 (CH_3)

Isolation of MF 4

Fraction A10 was crystallized in MeOH upon standing at room temperature to give a white solid. The white solid was further purified by column chromatography using hexane as an eluent to afford pure **MF 4** (1.7 mg).

Optical rotation: $[\alpha]_D^{29}$ +29° (c 1.7×10^{-4} g/cm^3 in MeOH)

Melting point: 168-170 °C

UV (CH_3OH) λ_{max} nm (log ε): 215 (3.17)

FT-IR (neat) ν (cm^{-1}): 3421, 2938, 2869, 2663 and 1686

^{1}H NMR 500 MHz ($CDCl_3$) (δ ppm): 6.90 (1H, *qt J* = 11.5, 8.0 Hz), 3.48 (1H, *t J* = 3.0 Hz) 1.85 (3H, *s*), 0.97 (3H, *s*), 0.95 (3H, *s*), 0.91 (2×3H, *d J* = 7.0 Hz), 0.52 (1H, *d J* = 4.5 Hz), 0.35 (1H, *d J* = 4.5 Hz)

^{13}C NMR 125 MHz ($CDCl_3$) (δ ppm): 172.49 (C=O), 145.81 (CH), 126.45 (C), 77.04 (CH), 52.14 (CH), 48.89 (C), 48.03 (CH), 45.27 (C), 41.07 (CH), 39.53 (C), 35.96 (CH), 35.47 (CH_2), 34.77 (CH_2), 32.86 (CH_2), 29.80 (CH_2), 28.55 (CH_2) 28.12 (CH_2), 27.46 (CH_2), 26.43 (C), 26.24 (CH_2), 25.90 (CH_2), 25.84 (CH_3), 25.64 (CH_2), 21.21 (CH_3), 21.07 (CH_2), 19.78 (C), 19.27 (CH_3), 18.08 (CH_3), 18.03 (CH_3) and 11.99 (CH_3)

Isolation of MF 5

Fraction A13 was chromatographed on column chromatography and eluted with hexane-EtOAc (95:5). The major component of this fraction was obtained and recrystallized in the mixed solvent of EtOAc and hexane. The white solid of **MF 5** was collected (45 mg).

Optical rotation: $[\alpha]_D^{29}$ +49° (c 1.6×10^{-4} g/cm^3 in MeOH)

Melting point: 181-183 °C

UV (CH_3OH) λ_{max} nm (log ε): 214 (4.08)

FT-IR (KBr) ν (cm^{-1}): 3389, 2944, 2866, 2633 and 1687

^{1}H NMR 300 MHz ($CDCl_3$) (δ ppm): 6.92 (1H, *qt J* = 6.6, 1.2 Hz), 3.31 (1H, *dd*

J = 11.5, 4.0 Hz), 1.86 (3H, *s*), 0.98 (2×3H, each *s*), 0.92 (3H, *d J* = 7.2 Hz), 0.83 (2×3H, each *s*), 0.57 (1H, *d J* = 4.2 Hz), and 0.35 (1H, *d J* = 4.2 Hz)

^{13}C NMR 75 MHz ($CDCl_3$) (δ ppm): 172.94 (C=O), 145.75 (CH), 126.60 (C), 78.88 (CH), 52.20 (CH), 48.81 (C), 47.96 (CH), 47.10 (CH), 45.34 (C), 40.47 (C), 35.97 (CH), 35.54 (CH_2), 34.80 (CH_2), 32.89 (CH_2), 31.96 (CH_2), 30.34 (CH_2), 29.89 (CH_2), 28.15 (CH_2), 26.44 (CH_2), 26.06 (CH_2), 26.01 (C), 25.92 (CH_2), 25.43 (CH_3), 21.11 (CH_2), 19.96 (C), 19.31 (CH_2), 18.11 (CH_3), 18.06 (CH_3), 14.00 (CH_3) and 11.97 (CH_3)

Isolation of MF 6

Fraction A9H was further chromatographed on column chromatography and eluted with hexane-EtOAc (95:5). The major component was obtained and crystallized in the mixture of EtOAc and hexane. The white solid of **MF 6** (5.5 mg) was collected.

Optical rotation: $[\alpha]_D^{29}$ +24° (c 1.6×10^{-4} g/cm^3 in MeOH)

Melting point: 186-188 °C

UV (CH_3OH) λ_{max} nm (log $\mathcal{E}$): 216 (3.92)

FT-IR (neat) ν (cm^{-1}): 3380, 2944, 2871, 2663, 2554, 1705 and 1686

^{1}H NMR 300 MHz ($CDCl_3$) (δ ppm): 6.91 (1H, *t J* = 7.2 Hz), 1.85 (3H, *s*), 1.10 (3H, *s*), 1.05 (2×3H, each *s*), 1.00 (3H, *s*), 0.92 (3H, *d J* = 6.3 Hz), 0.78 (1H, *d J* = 4.2 Hz), and 0.58 (1H, *d J* = 4.2 Hz)

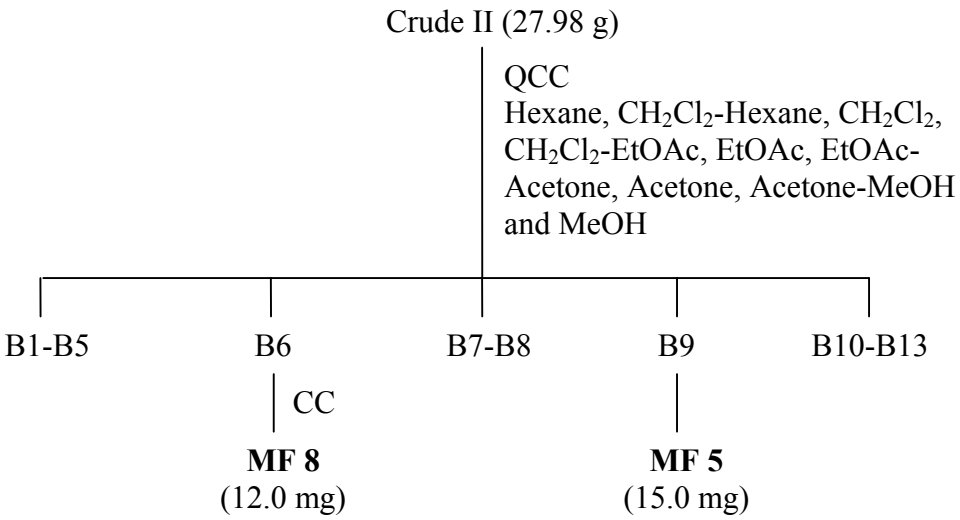
^{13}C NMR 75 MHz ($CDCl_3$) (δ ppm): 217.50 (C=O), 172.96 (C=O), 145.76 (CH), 126.13 (C), 52.22 (CH), 50.25 (C), 48.76 (C), 48.44 (CH), 47.88 (CH), 45.40 (C), 37.48 (CH_2), 35.96 (CH), 35.55 (CH_2), 34.78 (CH_2), 33.43 (CH_2), 32.81 (CH_2), 29.56 (CH_2), 28.16 (CH_2), 26.71 (CH_2), 26.01 (C), 25.88 (CH_2), 25.87 (CH_2), 22.18 (CH_3), 21.51 (C), 21.11 (CH_2), 21.07 (CH_3), 20.78 (CH_3), 19.30 (CH_3), 18.11 (CH_3) and 11.99 (CH_3)

2.3.2 Purification of crude II

Crude II (27.98 g) was subjected to quick column chromatography and eluted with hexane-methanol (98:2) to give 13 fractions (B1-B13) (**Table 3**) (**Scheme 3**).

Table 3 Fractions obtained from chromatographic separation of crude II

Fraction	Weight (g)	Physical characteristic
B1	1.583	brown viscous liquid
B2	1.463	brown viscous liquid
B3	1.203	brown viscous liquid
B4	2.074	brown viscous liquid
B5	2.020	brown viscous liquid
B6	2.230	white solid mixed with brown viscous liquid
B7	2.227	brown viscous liquid
B8	1.075	brown viscous liquid
B9	2.954	white solid mixed with brown viscous liquid
B10	1.777	brown viscous liquid
B11	3.010	brown viscous liquid
B12	1.431	brown viscous liquid
B13	2.885	brown viscous liquid



Scheme 3 Purification of crude II

Isolation of MF 5

Fraction B9 was chromatographed on column chromatography and eluted with hexane-EtOAc (9:1). The major component was obtained and recrystallized in the mixed solvent of EtOAc and hexane. A white solid of **MF 5** (15.0 mg) was collected.

Isolation of MF 8

Fraction B6 was chromatographed on column chromatography and eluted with hexane-EtOAc solvent system to afford pure **MF 8** (12.0 mg).

Optical rotation: $[\alpha]_D^{29}$ +57° (c 1.8×10^{-4} g/cm^3 in MeOH)

Melting point: 185-187 °C

FT-IR (neat) ν (cm^{-1}): 3418, 2937 and 2870

^{1}H NMR 300 MHz ($CDCl_3$) (δ ppm): 5.69 (1H, *ddd, J* =15.6, 9.9, 2.4 Hz), 5.54 (1H, *d, J* = 15.6 Hz), 3.30 (1H, *dd, J* = 10.8, 4.5 Hz), 1.35 (2×3H, each *s*), 0.97 (2×3H, each *s*), 0.87 (3H, *d J* = 8.1 Hz), 0.85 (3H, *s*), 0.81 (3H, *s*), 0.57 (1H, *d J* = 4.2 Hz) and 0.34 (1H, *d J* = 4.2 Hz)

^{13}C NMR 75 MHz ($CDCl_3$) (δ ppm): 134.54 (CH), 130.61 (CH), 78.87 (CH), 70.78 (C) 52.06 (CH), 47.97 (CH), 47.09 (CH), 45.31 (C), 40.47 (C), 39.37 (CH_2),

36.29 (CH), 35.56 (CH_2), 32.79 (C), 31.95 (CH_2), 30.92 (CH_2), 30.33 (CH_2), 29.87 (CH_2), 29.70 (CH_2), 28.08 (CH_2), 26.42 (CH_2), 26.10 (C), 25.99 (CH_2), 25.43 (CH_3), 24.42 (CH_3), 24.40 (CH_3), 21.10 (CH_2), 19.96 (C), 19.29 (CH_3), 18.34 (CH_3), 18.08 (CH_3) and 14.00 (CH_3)

2.3.3 Purification of crude Ⅲ

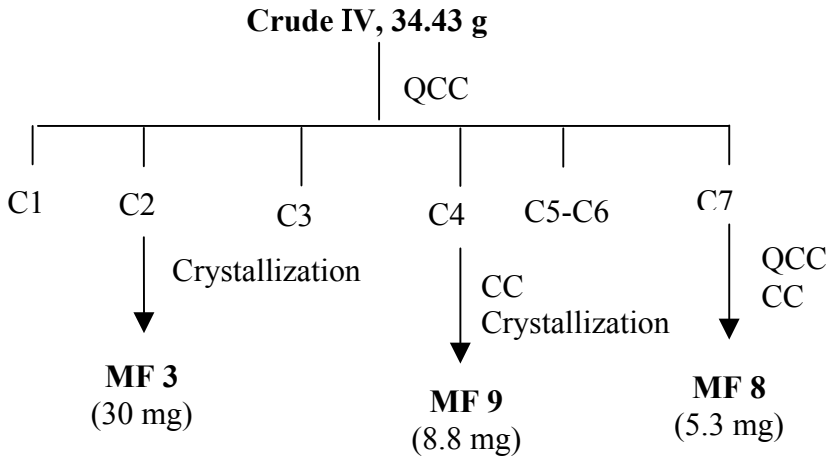
Brown viscous liquid of crude III (9.19 g) was purified by column chromatography using ethyl acetate in hexane (1:9) as an eluent to afford pure **MF 4** (1.7 mg) as a white solid.

2.3.4 Purification of crude Ⅳ

Crude IV (34.43 g) was further subjected to quick column chromatography using silica gel as the stationary phase and eluted with dicholoromethane, dicholoromethane -acetone, acetone, acetone-methanol and methanol. On the basis of their TLC characteristic, the collected fractions which contained the same major components were combined and fractions C1-C7 were obtained (**Table 4**). The selected fractions were further purified to afford three pure compounds as shown in **Scheme 4**.

Table 4 Fractions obtained from chromatographic separation of crude IV

Fraction	Weight (g)	Physical characteristic
C1	1.210	brown viscous liquid
C2	1.870	brown viscous liquid
C3	2.299	brown viscous liquid
C4	0.910	brown viscous liquid
C5	9.075	brown viscous liquid
C6	3.501	brown viscous liquid
C7	10.680	brown viscous liquid



Scheme 4 Purification of crude IV

Isolation of MF 3

Fraction C2 which contained one major component was further purified by crystallization in EtOAc-dichloromethane (1:9) to afford **MF 3** (30.0 mg) as a white solid upon standing at room temperature.

Isolation of MF 8

Fraction C7 (10.68 g) was subjected to a quick column chromatography and eluted with dichloromethane-methanol (9:1) to give six subfractions (C7A-C7G).

Fraction C7C (50.0 mg) was rechromatographed on column chromatography and further crystallized in the mixture of EtOAc and dichloromethane to afford a white solid of **MF 8** (5.3 mg).

Isolation of MF 9

Fraction C4 was a yellow solid. The solid was chromatographed on column chromatography over silica gel. Elution was conducted with acetone-dichloromethane solvent system. The fractions containing similar components were combined into five fractions. Crystallization of the fourth fraction gave pure yellow solid of **MF 9** (8.8 mg).

Optical rotation: $[\alpha]_D^{29}$ +105.2° (c 1.6×10^{-4} g/cm^3 in MeOH)

Melting point: 206-208 °C

UV (CH_3OH) λ_{max} nm (log ε): 289 (3.97) and 331 (2.85)

FT-IR (neat) ν (cm^{-1}): 3374 and 1638

^{1}H NMR 500 MHz ($CDCl_3$+DMSO-d_6) (δ ppm): 11.70 (1H, *s*), 10.54 (1H, *br s*), 8.59 (1H, *br s*), 8.49 (1H, *br s*), 7.01 (1H, s), 6.86 (1H, *d J* = 8 Hz), 6.84 (1H, *d J* = *8* Hz), 5.99 (1H, *d J* = 2.0 Hz), 5.94 (1H, *d J* = 2.0 Hz), 5.24 (1H, *d J* = 4.5 Hz), 4.94 (1H, *d J* = 12.0 Hz), and 4.49 (1H, *dd J* = 11.5, 4.0 Hz)

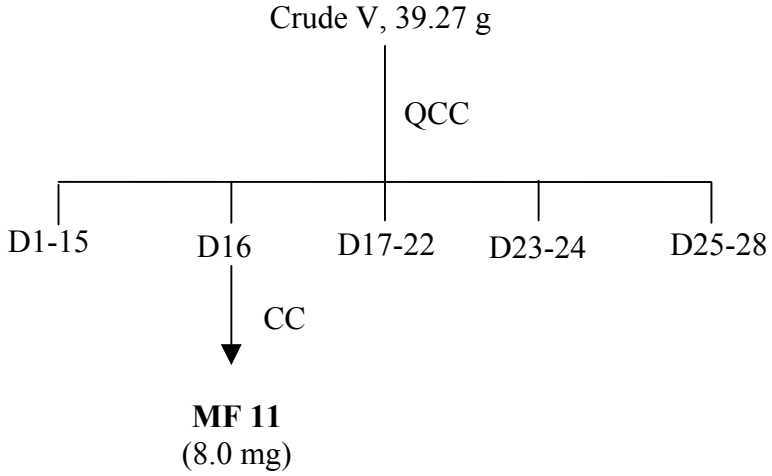
^{13}C NMR 125 MHz ($CDCl_3$+DMSO-d_6) (δ ppm): 197.58 (C=O), 167.14 (C), 162.73 (C), 145.98 (C), 145.15 (C), 136.66 (C), 128.19 (C), 119.51 (CH), 115.34 (CH), 100.63 (C), 96.34 (CH), 95.27 (CH), 83.38 (CH) and 71.96 (CH)

2.3.5 Purification of crude V

The acetone soluble was concentrated under reduced pressure to give a brown viscous-liquid (39.27 g). The solid was further separated by quick column chromatography on silica gel and eluted with a gradient of hexane/ethyl acetate (from 5 % to 100% ethyl acetate) followed by ethyl acetate/methanol (from 2 % to 100 % methanol). Fractions which showed similar TLC chromatograms were combined to afford 28 fractions (**Table 5)**. The selected fractions were further purified to give **MF 11** (**Scheme 5**).

Table 5 Fractions obtained from chromatographic separation of crude V

Fraction	Weight (g)	Physical characteristic
D1	0.010	white solid mixed with brown viscous liquid
D2	0.025	white solid mixed with brown viscous liquid
D3	0.017	brown viscous liquid
D4	0.139	brown viscous liquid
D5	0.095	brown viscous liquid
D6	0.012	brown viscous liquid
D7	0.015	brown viscous liquid
D8	0.030	brown viscous liquid
D9	0.050	brown viscous liquid
D10	2.001	brown viscous liquid
D11	4.003	brown viscous liquid
D12	0.023	brown viscous liquid
D13	0.023	brown viscous liquid
D14	0.197	brown viscous liquid
D15	3.100	brown viscous liquid
D16	2.500	yellow solid mixed with brown viscous liquid
D17	2.100	yellow solid mixed with brown viscous liquid
D18	1.292	brown viscous liquid
D19	0.102	brown viscous liquid
D20	0.123	brown viscous liquid
D21	0.077	brown viscous liquid
D22	4.000	brown viscous liquid
D23	0.423	brown viscous liquid
D24	3.353	brown viscous liquid
D25	3.800	brown viscous liquid
D26	5.526	brown viscous liquid
D27	2.352	brown viscous liquid
D28	0.222	brown viscous liquid



Scheme 5 Purification of crude V

Isolation of MF 11

Fraction D16 was further subjected to column chromatography on silica gel using a gradient system (CH_2Cl_2-EtOAc and EtOAc-MeOH) to yield **MF 11** (8.0 mg).

Optical rotation: $[\alpha]_D^{29}$ -18.5° (c 1.2×10^{-4} g/cm^3 in MeOH)

Melting point: 240-242 °C

UV (CH_3OH) λ_{max} nm (log ε): 225 (3.39), 286 (3.21) and 333 (2.53)

FT-IR (neat) ν (cm^{-1}): 3378, 2924, 2854 and 1637

^{1}H NMR 300 MHz ($CDCl_3$+DMSO-d_6) (δ ppm): 12.80 (1H, *s*), 10.40 (1H, *br s*), 9.30 (1H, *br s*), 7.31 (2H, *d J* = 8.4 Hz), 6.90 (2H, *d J* = 8.4 Hz), 5.95 (1H, *br s*), 5.94 (1H, *br s*), 5.31 (1H, *dd J* = 13.2, 2.7 Hz), 3.08 (1H, *dd J* = 17.1, 13.2 Hz) and 2.71 (1H, *dd J* = 17.1, 2.7 Hz)

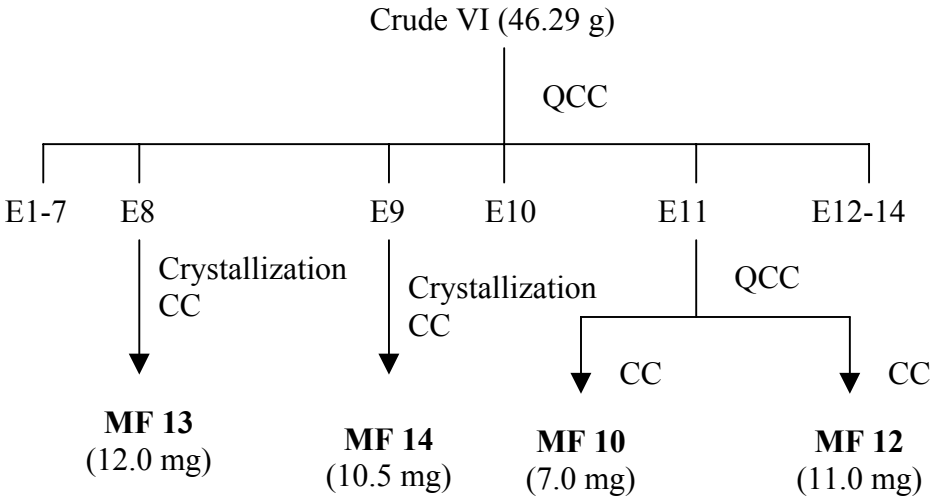
^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) (δ ppm): 200.54 (C=O), 171.79 (C), 168.83 (C), 167.90 (C), 162.73 (C), 133.76 (C), 132.64 (CH), 120.43 (CH), 107.01 (C), 101.26 (CH), 100.24 (CH), 83.66 (CH) and 47.70 (CH_2)

2.3.6 Purification of crude VI

Crude VI (46.29 g) was chromatographed on a quick column chromatography over silica gel. Elution was conducted with hexane-dichloromethane, dichloromethane, dichloromethane-ethyl acetate, ethyl acetate, ethyl acetate-acetone, acetone and finally with methanol. On the basis of their TLC characteristics, the collected fractions were combined based on the TLC characteristics to afford 14 fractions (E1-E14) (**Table 6**). The selected fractions were further purified to afford **MF 10**, **MF 12**, **MF 13**, and **MF 14** (**Scheme 6**).

Table 6 Fractions obtained from chromatographic separation of crude VI

Fraction	Weight (g)	Physical characteristic
E1	0.136	brown viscous liquid
E2	0.251	brown viscous liquid
E3	0.010	brown viscous liquid
E4	0.182	brown viscous liquid
E5	0.025	brown viscous liquid
E6	0.249	brown viscous liquid
E7	0.223	white solid mixed with brown viscous liquid
E8	1.050	yellow solid mixed with brown viscous liquid
E9	0.402	yellow solid mixed with brown viscous liquid
E10	0.108	brown viscous liquid
E11	19.202	brown viscous liquid
E12	9.978	brown viscous liquid
E13	2.764	white solid mixed with brown viscous liquid
E14	9.647	white solid mixed with brown viscous liquid



Scheme 6 Purification of crude VI

Isolation of MF 10 and MF 12

Fraction E11 was fractionated by quick column chromatography using dichloromethane, dichloromethane-ethyl acetate, ethyl acetate, ethyl acetate-acetone, acetone, acetone-methanol and methanol as the eluents. The fractions containing similar components were combined into seven fractions (E11A-E11G). The selected fractions were further purified to afford **MF 10** and **MF 12**.

Fraction E11C was further separated on column chromatography, eluted with dichloromethane, ethyl acetate, acetone, and then methanol to give ten fractions (E11C1-E11C10).

Fraction E11C6 was rechromatographed on column chromatography and eluted with dichloromethane-ethyl acetate, ethyl acetate, ethyl acetate-acetone, and acetone. The major component was obtained and recrystallized in dichloromethane-ethyl acetate mixture. A yellow solid of **MF 10** (7.0 mg) was collected.

Optical rotation: $[\alpha]_D^{29}$ -49° (c 1.7×10^{-4} g/cm^3 in MeOH)

Melting point: 250-252 °C

UV (CH_3OH) λ_{max} nm (log ε): 227 (4.08), 288 (4.04) and 330 (3.32)

FT-IR (neat) ν (cm^{-1}): 3255 and 1638

^{1}H NMR 300 MHz (DMSO-d_6) (δ ppm): 12.08 (1H, *s*), 6.94 (1H, *d, J* = 2.1 Hz), 6.83 (1H, *d J* = 8.0 Hz), 6.75 (1H, *dd J* = 8.0, 2.1 Hz), 5.94 (2H, *br s*), 5.28 (1H, *dd J* = 12.0, 3.0 Hz), 3.07 (1H, *dd J* = 12.0, 17.1 Hz) and 2.71 (1H, *dd J* = 17.1, 3.0 Hz),

^{13}C NMR 75 MHz (DMSO-d_6) (δ ppm): 200.76 (C=O), 171.82 (C), 168.75 (C), 167.89 (C), 150.50 (C), 150.22 (C), 134.50 (C), 122.85 (CH), 120.43 (CH), 119.01 (CH), 101.10 (CH), 100.18 (CH), 83.69 (CH) and 47.54 (CH_2)

Fraction E11F was dissolved in acetone and a few drops of ethyl acetate was added to give brown solid. The solid was filtered and further separated by column chromatography on silica gel. Elution was conducted initially with 30 % acetone in ethyl acetate, followed by increasing small amount of acetone in ethyl acetate. **MF 12** (11.0 mg) was obtained as a yellow solid.

Optical rotation: $[\alpha]_D^{29}$ +101° (c 1.3×10^{-4} g/cm^3 in MeOH)

Melting point: 210-211 °C

UV (CH_3OH) λ_{max} nm (log $\mathcal{E}$): 289 (3.53) and 331 (2.85)

FT-IR (neat) ν (cm^{-1}): 3353, 2937 and 1639

^{1}H NMR 300 MHz ($CDCl_3$+DMSO-d_6) (δ ppm): 11.70 (1H, *s*), 6.98 (1H, *br s*), 6.77 (2H, *br s*), 5.91 (1H, *d J* = 1.5 Hz), 5.87 (1H, *d J* = 1.5 Hz), 4.93 (1H, *d J* = 11.4 Hz) and 4.44 (2H, *d J* = 11.4 Hz)

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) (δ ppm): 201.44 (C=O), 172.21 (C), 167.50 (C), 150.53 (C), 149.61 (C), 133.01 (C), 124.51 (CH), 120.13 (CH), 119.76 (CH), 105.00 (C) 101.53 (CH), 100.49 (CH), 100.47 (C), 88.17 (CH) and 77.00 (CH)

Isolation of MF 13

Fraction E8 was dissolved in a small amount of acetone and filtered. The filtrate was concentrated and precipitated by gradually enriched with ethyl acetate. The solid was separated and was chromatographed on column chromatography. Elution was conducted initially with 10 % acetone in ethyl acetate, followed by increasing with

small amount of acetone in ethyl acetate. **MF 13** (12.0 mg) was obtained as a yellow solid from the fraction which was eluted with 70 % acetone in ethyl acetate.

Melting point: 106-107 °C

UV (CH_3OH) λ_{max} nm (log ε): 215 (3.62), 257 (3.33) and 291(3.02)

FT-IR (neat) ν (cm^{-1}): 3484, 2926 and 1681

^{1}H NMR 500 MHz ($CDCl_3$+DMSO-d_6) (δ ppm): 7.63 (1H, *dd J* = 8.0, 2.0 Hz), 7.57 (1H, *d J* = 2.0 Hz), 6.92 (1H, *d J* = 8.0 Hz) and 3.93 (3H, *s*)

^{13}C NMR 125 MHz ($CDCl_3$+DMSO-d_6) (δ ppm): 168.36 (C=O), 150.26 (C), 146.46 (C), 124.09 (CH), 122.31 (C), 114.25 (CH), 112.15 (CH) and 55.82 (CH_3)

Isolation of MF 14

Fraction E9 was dissolved in a small amount of methanol. A few drops of ethyl acetate was added to induce precipitation of a yellow solid. Further purification of a yellow solid by column chromatography gave a yellow solid of **MF 14** (10.5 mg).

Optical rotation: $[\alpha]_D^{29}$ -27° (c 1.5×10^{-4} g/cm^3 in MeOH)

Melting point: 276-278 °C

UV (CH_3OH) λ_{max} nm (log ε): 240 (2.94), 257 (3.00), 315 (2.68) and 366 (2.64)

FT-IR (neat) ν (cm^{-1}): 3375, 2920, 2851 and 1629

^{1}H NMR 500 MHz (DMSO-d_6) (δ ppm): 13.75 (1H, *s*), 7.37 (1H, *s*), 6.86 (1H, *s*), 6.36 (1H, *s*), 4.58 (1H, *d*, *J* = 10.0 Hz), 4.04 (1H, *t*, *J* = 9.0 Hz), 3.67 (1H, *d*, *J* = 10.5 Hz), 3.41 (2H, *m*), 3.19 (1H, *dd*, *J* = 17.0, 8.0 Hz) and 3.15 (1H, *m*)

^{13}C NMR 125 MHz (DMSO-d_6) (δ ppm): 179.43 (C=O), 164.16 (C), 162.09 (C), 156.58 (C), 154.40 (C), 151.14 (C), 144.07 (C), 112.02 (C), 108.38 (CH), 107.88 (C), 102.92 (CH), 101.62 (C), 93.64 (CH), 81.82 (CH), 79.28 (CH), 73.40 (CH), 70.93 (CH) and 61.80 (CH_2)

2.4 DPPH radical scavenging assay

The antioxidation activities of the crude material and pure compounds from the twigs of *Mangifera odorata* 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 solution with concentration of 10.0 mg/mL. The solution of each sample (30 μL) was mixed with 0.05 mM DPPH solution (3 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 7**.

Table 7 The scavenging activity of the crude extracts (30 μg/mL)

Sample	Average absorbances value (λ 517 nm)				
	0 min	15 min	30 min	45 min	60 min
DPPH	0.52	0.52	0.52	0.51	0.51
Dichloromethane extract	0.50	0.49	0.48	0.42	0.42
Acetone extract	0.48	0.32	0.20	0.15	0.06
Methanol extract	0.47	0.25	0.12	0.06	0.05

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

The DPPH solution (0.05 mM, 3mL) was mixed with the solution of sample to prepare the sample with the final concentrations of 60, 50, 40, 30, 20, 10, and 5 μg/mL. The mixed solution was allowed to stand at room temperature for 30 min

and the absorbance was measured at 517 nm. The results were shown in **Table 8**. The absorbances at each time point were plotted against the concentrations.

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).

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

Table 8 The average absorption and % inhibition of the crude extracts at various concentrations

Final concentrations (μg/mL)	Dichloromethane extract		Acetone extract		Methanol extract	
	A	% I	A	% I	A	% I
(DPPH)	0.52	0.00	0.52	0.00	0.52	0.00
5	0.51	1.92	0.44	15.38	0.35	32.69
10	0.50	3.85	0.35	32.69	0.30	42.31
20	0.49	5.77	0.31	40.38	0.22	57.69
30	0.48	7.69	0.20	62.50	0.12	76.92
40	0.47	9.62	0.12	76.92	0.09	82.69
50	0.46	11.54	0.08	84.62	0.07	86.54
60	0.44	15.38	0.07	86.54	0.04	92.31

A = Absorbances % I = % Inhibition

2.4.3 Screening on the free radical scavenging activity of pure compounds

The sample solution (3.535 mM in absolute ethanol 30 μL) and DPPH solution (0.05 mM, 3 mL) were mixed and allowed to stand at room temperature. The absorbances were measured every 15 minutes at 517 nm. The degree of loss of purple

color implied the activity. The residual absorbances at 517 nm were shown in **Table 9.**

Table 9 The absorbance value of the sample solution (35 μM)

Sample	Average absorbance value (λ 517 nm)				
	O min	15 min	30 min	45 min	60 min
DPPH	0.560	0.560	0.560	0.560	0.560
MF 9	0.550	0.420	0.310	0.290	0.280
MF 10	0.510	0.220	0.070	0.050	0.050
MF 11	0.520	0.300	0.170	0.140	0.140
MF 12	0.540	0.390	0.340	0.210	0.210
MF 13	0.550	0.550	0.540	0.540	0.540
MF 14	0.470	0.180	0.150	0.130	0.060

2.4.4 Evaluation of IC_{50} value of the pure compounds

MF 9 - MF 12 and **MF 14** 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 100, 70, 60, 50, 45, 40, 35, 25, 20, 15 and 10 μM. The absorbances at 517 nm were measured after incubation for at 30 minutes. The results were shown in **Table 10**.

The absorbances of the solution at each time period were plotted against the concentration. The concentrations needed to decrease the absorption of DPPH solution to 0.28 (the absorbance of 0.025 mM DPPH) were the IC_{50}.

Table 10 The average absorption and % inhibition of pure compounds at various concentrations

Final concentrations (μM)	**MF 9**		**MF 10**		**MF 11**		**MF 13**		**MF 14**	
	A	% I	A	% I	A	% I	A	% I	A	% I
DPPH	0.560	0.00	0.560	0.00	0.560	0.00	0.560	0.00	0.560	0.00
10.0	0.480	14.28	0.470	16.07	0.460	07.85	0.480	14.28	0.350	37.50
15.0	0.450	19.64	0.360	35.71	0.30	32.14	0.430	23.21	0.270	51.79
20.0	0.420	25.00	0.250	55.36	0.340	39.29	0.410	26.79	0.235	58.03
25.0	0.360	35.71	0.135	75.89	0.280	50.00	0.390	30.36	0.180	67.86
30.0	0.350	37.50	0.090	83.92	0.220	60.71	0.365	34.82	0.170	69.64
35.0	0.310	44.64	0.070	87.50	0.170	69.64	0.340	39.28	0.150	73.21
40.0	0.275	50.89	0.050	91.07	0.130	76.79	0.310	44.64	0.145	74.11
45.0	0.250	55.35	0.045	91.96	0.090	83.93	0.300	46.42	0.140	75.00
50.0	0.220	60.71	0.045	91.96	0.060	89.29	0.290	48.21	0.125	77.67
60.0	0.170	69.64	0.045	91.96	0.050	91.07	0.270	51.79	0.110	80.36
70.0	0.130	76.78	0.035	93.75	0.050	91.07	0.260	53.57	0.090	83.93
100.0	0.040	92.86	0.035	93.75	0.050	91.07	0.240	57.14	0.080	85.71

A = Absorbances % I = % Inhibition