CHAPTER 2.2

EXPERIMENTAL

2.2.1 Chemicals and Instruments

Melting points were recorded in °C and were measured on a digital Electrothermal 9100 Melting Point Apparatus. Infrared spectra were recorded using FTS165 FT-IR spectrometer. Major bands (ν) were recorded in wave number (cm^{-1}). Ultraviolet (UV) absorption spectra were recorded using UV-160A spectrophotometer (SHIMADZU). Principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in chloroform solution. Nuclear magnetic resonance spectra were recorded on either 300 MHz Brucker FTNMR Ultra Shield™ spectrometer or 500 MHz Varian UNITY INOVA spectrometer. Spectra were recorded in deuterochloroform, tetradeuteromethanol, hexadeuteroacetone or pentadeuteropyridine solution and were recorded as δ value in ppm downfield from TMS (internal standard δ 0.00). Optical rotation was measured in methanol, chloroform, ethanol or pyridine solution with sodium D line (590 nm) on either an AUTOPOL^{R} II automatic polarimeter or a JASCO DIP-370 digital polarimeter by using a 50 mm microcell (1 mL). Solvent for extraction and chromatography were distilled at their boiling point ranges prior to use except for petroleum ether and diethyl ether which were analytical grade reagent. Quick column chromatography, thin-layer chromatography (TLC) and precoated thin-layer chromatography were performed on silica gel 60 GF_{254} (Merck) or reverse-phase C-18. Column chromatography was performed on silica gel (Merck) type 100 (70–223 mesh ASTM) or reverse-phase C-18.

2.2.2 Plant Material

The twigs of *M. kunstleri* were collected from Songkhla Province in Thailand. The plant was identified by Dr. Kitichate Sridith, Department of Biology, Faculty of Science, Prince of Songkla University. A voucher specimen has been deposited at the Prince of Songkla University Herbarium.

2.2.3 Extraction

The twigs (6,200 g) of *M. kunstleri* were extracted with methanol (16 L) over the period of 7 days at room temperature for 3 times. The filtered solution was then evaporated to dryness under reduced pressure to afford crude methanol extract as a brown viscous liquid in 354.49 g.

2.2.4 Isolation and Chemical Investigation of the Crude Methanol Extract from the Twigs of M. kunstleri

The crude methanol extract of *M. kunstleri* (96.80 g) was dissolved in chloroform (8.20 L). The chloroform soluble portion was evaporated to dryness to give a brown viscous liquid (17.29 g). The chloroform extract was further purification by quick column chromatography over silica gel eluted with hexane and increasing the polarity with acetone to afford thirteen fractions, as shown in **Table 23**.

Table 23 Fractions obtained from the chloroform extract by quick column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
A	0.141	yellow viscous liquid
B	0.017	yellow viscous liquid
C	1.600	yellow liquid
D	2.840	yellow liquid
E	0.727	yellow viscous liquid
F	0.285	yellow viscous liquid
G	0.284	green viscous liquid
H	0.446	green viscous liquid
I	1.201	green solid
J	0.560	green solid
K	1.498	green solid
L	1.027	brown solid
M	1.392	brown solid

Fractions A-B contained many spots, none of which were major components. Therefore, they were not investigated further.

Fraction C contained approximately eight spots on normal phase TLC with 5% ethyl acetate in hexane which were visualized as purple spots in vanilin sulfuric acid reagent and subsequently heating. Further separation by column chromatography over silica gel. Elution was conducted initially with hexane, followed by increasing amount of ethyl acetate in hexane and finally with 10% ethyl acetate in hexane. Fractions with the

similar TLC chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions, as shown in **Table 24**.

Table 24 Subfractions obtained from **fraction C** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C1	0.865	yellow solid
C2	0.188	yellow viscous liquid
C3	0.065	yellow viscous liquid
C4	1.600	yellow semi-liquid

Subfraction C1 was separated by column chromatography over silica gel. Elution was conducted initially with petroleum ether followed by increasing amount of ethyl acetate in petroleum ether and finally with 30% ethyl acetate in petroleum ether. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness under reduced pressure to afford five subfractions, as shown in **Table 25**.

Table 25 Subfractions from **subfraction C1** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C1.1	0.653	yellow liquid
C1.2	0.004	yellow viscous liquid
C1.3	0.005	Yellow viscous liquid

Table 25 (continued)

Subfraction	Weight (g)	Physical appearance
C1.4	0.035	Yellow viscous liquid
C1.5	0.024	yellow viscous liquid

Subfraction C1.1 was separated by column chromatography over silica gel, eluting with 2% diethyl ether in hexane. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness *in vacuo* to afford five subfractions, as shown in **Table 26**.

Table 26 Subfractions obtained from **subfraction C1.1** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C1.1A	0.517	Yellow liquid
C1.1B	0.024	yellow liquid
C1.1C	0.073	yellow viscous liquid
C1.1D	0.007	yellow viscous liquid
C1.1E	0.003	yellow viscous liquid

Subfraction C1.1A contained many spots on normal phase TLC. These spots were not well-separated. Further purification was not attempted.

Subfraction C1.1B showed no definite spot on normal phase TLC using 1% ethyl acetate in hexane as a mobile phase and spray the TLC plate with

vanilin in sulfuric reagent and subsequently heating. Therefore, it was not further investigated .

Subfraction C1.1C showed no definite spot on normal phase TLC using 1% diethyl ether in hexane as a mobile phase and spray the TLC plate with vanilin in sulfuric reagent and subsequently heating. Therefore, it was not further investigated.

Subfraction C1.1D was a yellow viscous liquid which contained one major purple spot on normal phase TLC using 1% diethyl ether in hexane as a mobile phase (4 runs) with the R_f value of 0.32 after spray the TLC plate with vanilin in sulfuric reagent and subsequently heating. The ^{1}H NMR spectra data indicated that it was a long chain compound.

Subfraction C1.1E showed no definite spot on normal phase TLC using 1% diethyl ether in hexane as a mobile phase and spray the TLC plate with vanilin in sulfuric reagent and subsequently heating. No further investigation was pursued.

Subfraction C1.2 contained approximately four compounds on normal phase TLC using 1% diethyl ether in hexane as a mobile phase. Because of a low quantity, no further purification was performed.

Subfraction C1.3 showed no definite spot on normal phase TLC. Thus, it was further investigated.

Subfraction C1.4 showed a single purple spot with the R_f value of 0.49 on normal phase TLC with 15% ethyl acetate in hexane as a mobile phase in vanillin sulfuric acid reagent and subsequently heating. It was named as **MKT6**.

Compound MKT6 (Yellow viscous liquid)

$[\alpha]_D^{30}$	**:**	+44.22° (c = 0.19, $CHCl_3$)
FT-IR (neat) ν (cm^{-1})	**:**	3386 (O-H stretching), 2949, 2867 (C-H stretching), 1704 (C=O stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	**:**	4.70 (*brd*, *J* = 2.1 Hz, 1H), 4.58 (*brqd*, *J* = 2.1 and 1.2 Hz,1H), 2.45 (*m*, 1H), 2.39 (*m*, 1H), 1.93 (*m*, 1H), 1.89 (*m*, 1H), 1.71 (*m*, 2H), 1.69 (*brs*, 3H), 1.68 (*m*, 1H), 1.50 (*m*, 1H), 1.47 (*m*, 2H), 1.46 (*m*, 4H), 1.42 (*m*, 1H), 1.38 (*m*, 1H), 1.37 (*m*, 3H), 1.32 (*m*, 1H), 1.27 (*m*, 1H), 1.19 (*m*, 2H), 1.08 (*s*, 6H), 1.04 (*s*, 3H), 0.97 (*s*, 3H), 0.94 (*s*, 3H), 0.81 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)	**:**	218.15, 150.84, 109.41, 54.93, 49.79, 48.25, 47.96, 47.33, 42.99, 42.90, 40.78, 39.98, 39.62, 38.17, 36.88, 35.53, 34.15, 33.57, 29.84, 27.44, 26.66, 25.16, 21.48, 21.04, 19.69, 19.32, 18.02, 15.97, 15.79, 14.49
DEPT 135°($CDCl_3$) **CH_3**	**:**	26.66, 21.04, 19.32, 18.02, 15.97, 15.79, 14.49
CH_2	**:**	109.41, 39.98, 39.62, 35.53, 34.15, 33.57, 29.64, 27.44, 25.16, 21.48, 19.69
CH	**:**	54.93, 49.79, 48.25, 47.96, 38.17

Subfraction C1.5 showed no definite spot on normal phase TLC. Thus, it was not investigated further.

Subfraction C2 showed no definite spot on normal phase TLC. Therefore, it was not investigated further.

Subfraction C3 contained many spots on normal phase TLC. No further separation was carried out.

Subfraction C4 was recrystallized from the chloroform to give **MKT1** as a white solid (1.371 g). It showed a single spot on normal phase TLC with 15% ethyl acetate in hexane with the R_f value of 0.27 which was visualized as a purple spot in vanillin sulfuric acid reagent and subsequently heating.

Compound MKT1 (White solid)

$[\alpha]_D^{30}$	**:**	+19.36° (c = 0.16, EtOH)
mp	**:**	204.5 - 205.7 °C
FT-IR (KBr) ν (cm^{-1})	**:**	3417 (O-H stretching), 2937, 2863 (C-H stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	**:**	4.69 (*d*, *J* = 2.1 Hz, 1H), 4.57 (*brqd*, *J* = 2.1 and 1.2 Hz, 1H), 3.19 (*dd*, *J* = 10.8 and 5.4 Hz, 1H), 2.38 (*dt*, *J* = 11.1 and 5.7 Hz, 1H), 1.94 (*m*, 1H), 1.69 (*m*, 2H), 1.68 (*s*, 3H), 1.68 (*m*, 1H), 1.67 (*m*, 2H), 1.55 (*m*, 2H), 1.53 (*m*, 1H), 1.50 (*m*, 1H), 1.45 (*m*, 1H), 1.41 (*m*, 1H), 1.40 (*m*, 2H), 1.37 (*m*, 1H), 1.35 (*m*, 1H), 1.27 (*m*, 1H), 1.26 (*m*, 1H), 1.19 (*m*, 1H), 1.03 (*s*, 3H), 0.97 (*s*, 3H), 0.95 (*s*, 3H), 0.91 (*m*, 2H), 0.83 (*s*, 3H), 0.79 (*s*, 3H), 0.76 (*s*, 3H), 0.69 (*brd*, *J* = 9.0 Hz, 1H)
^{13}C NMR ($CDCl_3$) (δ ppm)	**:**	150.95, 109.33, 78.99, 55.32, 50.45, 48.32,

(75 MHz)			47.99, 43.01, 42.84, 40.84, 40.01, 38.86, 38.73, 38.07, 37.18, 35.60, 34.30, 29.86, 28.00, 27.46, 27.42, 25.16, 20.94, 19.32, 18.33, 18.01, 16.12, 15.99, 15.38, 14.56
DEPT 135°	**CH_3**	**:**	28.00, 19.32, 18.01, 16.12, 15.99, 15.38, 14.56
	CH_2	**:**	109.33, 40.01, 38.73, 35.56, 34.30, 29.86, 27.46, 27.42, 25.16, 20.94, 18.33
	CH	**:**	78.99, 55.32, 50.45, 48.32, 47.99, 38.07

The mother liquor of **subfraction C4** was fractionated by column chromatography over silica gel with 50% dichloromethane in hexane. Fractions with the similar TLC chromatogram were combined and evaporated to dryness under reduced pressure to afford seven subfractions, as shown in **Table 27**.

Table 27 Subfractions obtained from the mother liquor of **subfraction C4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
MC4.1	0.041	yellow viscous liquid
MC4.2	0.010	yellow viscous liquid
MC4.3	0.010	yellow viscous liquid
MC4.4	0.088	white-yellow liquid
MC4.5	0.021	yellow viscous liquid
MC4.6	0.015	yellow viscous liquid
MC4.7	0.284	yellow viscous liquid

Subfraction MC4.1 was therefore rechromatography by preparative TLC on silica gel plates, with (0.2:2:8) ethyl acetate, chloroform and hexane as a mobile phase to give two isolated bands. The first band was a yellow viscous liquid (10.0 mg). The ^{1}H NMR spectrum data indicated that it was a mixture. Therefore, it was not further purified. The second band was a yellow viscous liquid (7.0 mg). Its ^{1}H NMR spectrum showed two signals of olefinic proton in a ratio 1:0.5, indicating that it was a mixture of at least two compounds.

Subfractions MC4.2-MC4.7 showed no definite spot on normal phase TLC and they were not investigated further.

Fraction D was recrystallized from the dichloromethane in methanol (8:2) to give **MKT1** as a white solid (1.970 g) and the mother liquor (0.577 g) which was contained **MKT1** as a major component.

Fraction E was recrystallized from the dichloromethane in methanol (8:2) to give **MKT2** as a white solid (28.0 mg). It showed a single spot on normal phaseTLC with 15% ethyl acetate in hexane with R_f value of 0.14 which was visualized as a purple spot in vanillin sulfuric acid reagent and subsequently heating.

Compound MKT2 (White solid)

$[\alpha]_D^{28}$	**:**	-28.75° (c = 0.16, $CHCl_3$)
mp	**:**	133.0 - 135.5 °C
FT-IR (KBr) ν (cm^{-1})	**:**	3439 (O-H stretching), 2952, 2929, 2863 (C-H stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	**:**	5.28 (*d*, *J* = 5.1 Hz), 5.08 (*dd*, *J* = 15.0 and 8.4 Hz), 4.94 (*dd*, *J* = 15.0 and 8.4 Hz), 3.45 (*m*, 2H), 2.29-2.10 (*m*), 2.03-1.88 (*m*), 1.84-1.73 (*m*), 1.67-

1.56 (*m*), 1.50-1.36 (*m*), 1.26-0.98 (*m*), 0.94 (*s*), 0.85 (*d*, *J* = 6.6 Hz), 0.80-0.69 (*m*), 0.63 (*s*), 0.61 (*s*)

The mother liquor of fraction E was separated by column chromatography over silica gel. Elution was conducted initially with hexane, followed by increasing amount of diethyl ether and finally with 50% diethyl ether in hexane. Fractions with the similar TLC chromatogram were combined and evaporated to dryness under reduced pressure to afford eight subfractions, as shown in **Table 28**.

Table 28 Subfractions obtained from the mother liquor of **fraction E** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
ME1	0.002	yellow viscous liquid
ME2	0.001	white solid
ME3	0.016	white solid
ME4	0.030	white solid
ME5	0.028	yellow solid
ME6	0.154	yellow viscous liquid
ME7	0.242	yellow semi-solid
ME8	0.005	yellow semi-solid

Subfraction ME1 showed no definite spot on normal phase TLC. It was not investigated further.

Subfraction ME2 consisted of **MKT1** as a major component separated from **fraction D** and **subfraction ME3**. Therefore, further purification was not carried out.

Subfraction ME3 was **MKT1**.

Subfraction ME4 showed two major compounds on normal phase TLC with 10% ethyl acetate in hexane (7 runs). The first compound was **MKT1** and the second compound was a major component separated from **subfraction ME5**. Therefore, further purification was not carried out.

Subfraction ME5 was recrystallized from the hexane to give **MKT8** as a white needle (7.0 mg). It showed a single spot on normal phase TLC with 30% ethyl acetate in hexane with the R_f value of 0.46 which was visualized as a purple spot in vanillin sulfuric acid reagent and subsequently heating.

Compound MKT8 (White needles)

$[\alpha]_D^{30}$	**:**	+21.17° (c = 0.34, $CHCl_3$)
mp	**:**	141.9 - 143.7 °C
FT-IR (KBr) ν (cm^{-1})		3402 (O-H stretching), 2944, 2 870 (C-H stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	**:**	9.68 (*d*, *J* = 1.5 Hz, 1H), 4.76 (*brd*, *J* = 2.1 Hz, 1H), 4.62 (*brqd*, *J* = 2.1 and 1.5 Hz, 1H), 3.18 (*dd*, *J* = 11.0 and 5.3 Hz, 1H), 2.86 (*dt*, *J* = 11.1 and 7.5 Hz, 1H), 2.12 (*m*, 2H), 2.04 (*m*, 1H), 1.80 (*m*, 2H), 1.78 (*m*, 1H), 1.73 (*m*, 1H), 1.70 (*s*, 3H), 1.68 (*m*, 3H), 1.63 (*m*, 2H), 1.51 (*m*, 1H), 1.49 (*m*, 2H), 1.45 (*m*, 1H), 1.38 (*m*, 4H), 1.36

			(*m*, 1H), 1.26 (*m*, 1H), 1.20 (*m*, 1H), 0.97 (*s*, 3H), 0.96 (*s*, 3H), 0.91 (*s*, 3H), 0.82 (*s*, 3H), 0.75 (*s*, 3H), 0.67 (*m*, 1H)
^{13}C NMR ($CDCl_3$) (δppm) (75 MHz)		**:**	206.73, 149.73, 110.16, 78.99, 59.33, 55.32, 50.47, 48.07, 47.54, 42.56, 40.83, 38.71, 38.71, 38.71, 37.16, 34.33, 33.23, 29.87, 29.26, 28.81, 27.98, 27.39, 25.54, 19.00, 18.27, 16.13, 15.90, 15.35, 14.27
DEPT 135°	**CH_3**	**:**	27.98, 19.00, 16.13, 15.90, 15.35, 14.27
	CH_2	**:**	110.71, 38.71, 34.33, 33.23, 29.87, 29.26, 28.81, 27.39, 25.54, 20.75, 18.27
	CH	**:**	206.73, 78.99, 55.32, 50.47, 48.07, 47.54, 38.71

Subfraction ME6 showed two major compounds on normal phase TLC with 10% ethyl acetate in hexane (7 runs). The first compound was **MKT8** and the second compound was a major component separated from subfraction **ME7**. Therefore, further purification was not carried out.

Subfraction ME7 was recrystallized from the dichloromethane in methanol (8:2) to give **MKT2** (142.0 mg). The mother liquor contained many spots on normal phase TLC. These spots were not well-separated. Further purification was not attempted.

Subfraction ME8 showed no definite spot on normal phase TLC and it was not investigated further.

Fraction F contained many spots without major component. No further separation was conducted.

Fraction G contained many spots on normal phase TLC. These spots were not well-separated. Further purification was not attempted.

Fraction H was recrystallized from the dichloromethane in methanol (8:2) to give a white solid (19.0 mg) which contained two major spots on normal phase TLC which was major component in **fraction I**. Therefore, purification was not carried out.

Fraction I was recrystallized from the dichloromethane in methanol (8:2) to give white-yellow solid (0.741 g) which contained two major purple spots on normal phase TLC with 15% ethyl acetate in petroleum ether as a mobile phase in vanilin sulfuric acid reagent and subsequently heating. Further it was then separated by column chromatography over silica gel. Elution was conducted initially with petroleum ether, followed by increasing amount of ethyl acetate in petroleum ether and finally with 30% ethyl acetate in petroleum ether. Fractions with the similar TLC chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions, as shown in **Table 29**.

Table 29 Subfractions obtained from **fraction I** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
SI1	0.001	yellow needles
SI2	0.001	yellow solid
SI3	0.064	white solid
SI4	0.159	white solid
SI5	0.435	yellow solid
SI6	0.008	white-yellow solid

Subfractions SI1-SI2 showed no definited spot on normal phase TLC and they were not carried out.

Subfraction SI3 was recrystallized from the dichloromethane, methanol and hexane (6:1:3) to give **MKT5** as a white solid (33.0 mg). It showed a single spot on normal phase TLC with 50% ethyl acetate in hexane with the R_f value of 0.53 which was visualized as a purple spot in vanillin sulfuric acid reagent and subsequently heating.

Compound MKT5 (White solid)

$[\alpha]_D^{28}$	**:**	+22.67° (c = 0.16, Pyridine)
mp	**:**	248.4 - 248.8 °C
FT-IR (KBr) ν (cm^{-1})	**:**	3424 (O-H stretching), 2937, 2863 (C-H stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	**:**	4.68 (*d*, *J* = 1.8 Hz, 1H), 4.58 (*brs*, 1H), 3.80 (*dd*, *J* = 10.8 and 1.5 Hz, 1H), 3.33 (*d*, *J* = 10.8 Hz, 1H), 3.19 (*dd, J* = 10.9 and 5.2 Hz, 1H), 2.39 (*dt*, *J* = 10.5 and 5.8 Hz, 1H), 2.00, (*m*, 2H) 1.92 (*m*, 1H), 1.90 (*m*, 1H), 1.68 (*s*, 3H), 1.67 (*m*, 1H), 1.66 (*m*, 3H), 1.62 (*m*, 1H), 1.58 (*m*, 1H), 1.55 (*m*, 2H), 1.52 (*m*, 1H), 1.43 (*m*, 1H), 1.39 (*m*, 1H), 1.38 (*m*, 2H), 1.24 (*t*, *J* = 12.4 Hz, 1H), 1.22 (*m*, 1H), 1.20 (*m*, 1H), 1.07 (*m*, 1H), 1.02 (*s*, 3H), 1.02 (*m*, 1H), 0.98 (*s*, 3H), 0.96 (*s*, 3H), 0.96 (*m*, 2H), 0.89 (*m*, 1H), 0.82 (*s*, 3H), 0.76 (*s*, 3H), 0.68 (*brd*, *J* = 9.3 Hz, 1H)

^{13}C NMR ($CDCl_3$) (δppm) (75 MHz)		**:**	150.48, 109.68, 78.98, 60.56, 55.29, 50.40, 48.77 47.79, 47.79, 42.72, 40.92, 38.84, 38.70, 37.31, 37.16, 34.24, 33.97, 29.76, 29.18, 27.98, 27.39, 27.05, 25.22, 20.83, 19.09, 18.30, 16.10, 15.98, 15.35, 14.76
DEPT 135°	**CH_3**	**:**	27.98, 19.09, 16.10, 15.98, 15.35, 14.76
	CH_2	**:**	109.68, 60.56, 38.70, 34.24, 33.97, 29.76, 29.18, 27.39, 27.05, 25.22, 20.83, 18.30
	CH	**:**	78.98, 55.29, 50.04, 48.77, 47.39, 37.31

Subfraction SI4 consisted of **MKT5** and **MKT7** as a major component separated from **subfractions SI3** and **SI5**, respectively. Therefore, further purification was not carried out.

Subfraction SI5 was recrystallized from the dichloromethane, methanol and hexane (6:1:3) to give white solid (0.308 g) which was identical to **subfraction SI4** by its TLC. The mother liquor showed the single spot on normal phase TLC with 50% ethyl acetate in hexane with the R_f value of 0.45 which was visualized as a purple spot in vanillin sulfuric acid reagent and subsequently heating. It was named as **MKT7**.

Compound MKT7 (White solid)

$[\alpha]_D^{28}$	**:**	15.61° (c = 0.21, $CHCl_3$)
mp	**:**	195.5 - 197.5 °C
FT-IR (KBr) ν(cm^{-1})	**:**	3386 (O-H stretching), 2942, 2867 (C-H stretching)

^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)		**:**	4.71 (*brd*, *J* = 2.1 Hz, 1H), 4.60 (*brqd*, *J* = 2.1 and 1.2 Hz, 1H), 3.61 (*dd*, *J* = 11.1 and 4.8 Hz, 1H), 3.19 (*dd*, *J* = 10.8 and 5.7 Hz, 1H), 2.50 (*dt*, *J* = 10.8 and 5.7 Hz, 1H), 1.97 (*m*, 2H), 1.78 (*m*, 1H), 1.68 (s, 3H), 1.64 (*m*, 1H), 1.60 (*m*, 2H), 1.57 (*m*, 1H), 1.53 (*m*, 3H), 1.51 (*m*, 1H), 1.45 (*m*, 1H), 1.39 (*m*, 3H), 1.37 (*m*, 1H), 1.26 (*m*, 1H), 1.23 (*m*, 1H), 1.04 (*s*, 3H), 1.01 (*m*, 1H), 0.99 (*s*, 3H), 0.97 (*s*, 3H), 0.88 (*m*, 1H), 0.83 (*s*, 3H), 0.80 (*s*, 3H), 0.76 (*s*, 3H), 0.68 (*brd*, *J* = 9.0 Hz, 1H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		**:**	149.98, 109.80, 78.95, 77.09, 55.33, 50.02, 48.60, 47.72, 47.60, 44.08, 40.94, 38.87, 38.75, 37.72, 37.26, 37.13, 36.90, 34.25, 29.92, 27.99, 27.38, 24.79, 20.88, 19.34, 18.30, 16.18, 16.12, 15.99, 15.36, 11.68
DEPT 135°	**CH_3**	**:**	27.99, 19.34, 16.18, 16.12, 15.99, 15.36, 11.68
	CH_2	**:**	109.80, 38.75, 37.72, 36.90, 34.25, 29.92, 27.38, 24.79, 20.88, 18.30
	CH	**:**	78.95, 77.09, 55.33, 50.02, 47.71, 47.60, 37.26

The mother liquors of **fractions H** and **I** were combined and further separated by column chromatography over silica gel. Elution was conducted initially with chloroform followed by increasing amount of ethyl acetate in chloroform and finally with 30% ethyl acetate in chloroform. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to

dryness under reduced pressure to afford **subfractions MHI1- MHI5**, as shown in **Table 30**.

Table 30 Subfractions obtained from the mother liquor of **fractions H** and **I** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
MHI1	0.016	colourless viscous liquid
MHI2	0.003	yellow viscous liquid
MHI3	0.007	yellow solid
MHI4	0.491	green solid
MHI5	0.172	green viscous liquid

Subfraction MHI1 showed no definite spot on normal phase TLC. No further investigation was conducted.

Subfraction MHI2 showed many spots, none of which were major components. Therefore, it was not investigated further.

Subfraction MHI3 contained approximately three spots on normal phase TLC with 20% diethyl ether in hexane (8 runs) which were visualized as a purple spot in vanilin sulfuric acid reagent and subsequently heating. The major compound of this subfraction was **MKT5**. Therefore, further purification was not carried out.

Subfraction MHI4 consisted of **MKT5** and **MKT8** as a major component separated from **subfractions SI3** and **SI5**, respectively. Therefore, it was not investigated further.

Subfraction MHI5 was recrystallized from the chloroform to give **MKT9** as a yellow needle (0.003 g). It showed a single UV-active spot on normal phase TLC with 30% ethyl acetate in hexane with the R_f value of 0.31.

Compound MKT9 (Yellow needles)

Mp	**:**	239.8 - 240.8 °C
UV (MeOH) λ_{max} **(nm) (log** ε**)**	**:**	235 (3.72), 259 (3.81), 287 (3.09), 389 (3.15)
FT-IR (KBr) ν**(cm^{-1})**	**:**	1639 (C=O stretching), 1606, 1580 (C=C) stretching)
^{1}H NMR (Acetone-d_6+$CDCl_3$) (δ**ppm) (300 MHz)**	**:**	12.71 (*s*, 1H), 9.01 (*s*, 1H)7.64 (*t*, *J* = 8.4 Hz,1H), 7.61(*dd*, *J* = 3.0 and 0.4 Hz,1H), 7.46 (*dd*, *J* = 9.0 and 0.4 Hz, 1H), 7.39 (*dd*, *J* = 9.0 and 3.0 Hz, 1H), 6.74 (*dd*, *J* = 8.4 and 0.9 Hz, 1H)
^{13}C NMR (Acetone-d_6+$CDCl_3$) (δ**ppm) : (75 MHz)**	**:**	182.06, 161.86, 156.40, 153.98, 150.13, 136.61, 25.17, 120.97, 119.09, 109.61, 108.50 (2xC), 106.81
DEPT 90 CH	**:**	136.61, 125.17, 119.09, 109.61, 108.50, 106.81

The mother liquor of **subfraction MHI5** was fractionated by column chromatography over silica gel. Elution was conducted initially with hexane followed by increasing amount of diethyl ether in hexane. All fractions were examined by TLC and combined on the basis of their chromatograms. The solvents were evaporated to dryness *in vacuo* to afford seven subfractions, as shown in **Table 31**.

Table 31 Subfractions obtained from the mother liquor of **subfraction MHI5** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
MHI5.1	0.005	yellow needles
MHI5.2	0.001	yellow needles
MHI5.3	0.001	white solid
MHI5.4	0.003	white-yellow solid
MHI5.5	0.031	yellow semi-solid
MHI5.6	0.028	white-yellow semi-solid
MHI5.7	0.021	green solid

Subfraction MHI5.1 was recrystallized from chloroform to give **MKT9** as yellow needles (4.0 mg).

Subfractions MHI5.2-MHI5.4 were a mixture of compounds which found in **subfractions MHI5.1** and **MHI5.5**. No further purifications were then carried out.

Subfraction MHI5.5 was crystallized from 30% diethyl ether in hexane to give **MKT10** as a white solid (4.0 mg). It showed a single spot on normal phase TLC with 30% ethyl acetate in hexane with the R_f value of 0.34 which was visualized as a purple spot in vanilin sufuric acid reagent and subsequently heating.

Compound MKT10 (White solid)

$[\alpha]_D^{27}$: +9.23° (c = 0.07, Acetone)

mp		**:**	284.0 - 286.0°C
IR (KBr) ν (cm^{-1})		**:**	3417 (O-H stretching), 2945, 2863 (C-H stretching)
^{1}H NMR (Acetone-d_6 + $CDCl_3$) (δ ppm) (300 MHz)		**:**	4.73 (*brd*, *J* = 2.1 Hz, 1H), 4.59 (*brqd*, *J* = 2.1 and 1.2 Hz, 1H), 3.18 (*dd*, *J* = 10.2 and 6.0 Hz, 1H), 3.04 (*dt*, *J* = 11.1 and 4.5 Hz, 1H), 2.28 (*m*, 1H), 2.21 (*m*, 2H), 2.20 (*m*, 2H), 2.08 (*m*, 2H), 1.99 (*m*, 1H), 1.98 (*m*, 2H), 1.72 (*m*, 2H), 1.69 (*s*, 3H), 1.68 (*m*, 1H), 1.60 (*m*, 3H), 1.55 (*m*, 1H), 1.54 (*m*, 2H), 1.47 (*m*, 1H), 1.43 (*m*, 1H), 1.40 (*m*, 3H), 1.28 (*m*, 1H), 1.24 (*m*, 1H), 1.19 (*m*, 1H), 0.99 (*s*, 3H), 0.97 (*s*, 3H), 0.94 (*s*, 3H), 0.83 (*s*, 3H), 0.77 (*s*, 3H), 0.70 (*brd*, *J* = 10.5 Hz, 1H)
^{13}C NMR (Acetone-d_6 + $CDCl_3$) (δ ppm) (75 MHz)		**:**	177.67, 150.58, 109.36, 78.41, 55.98, 55.34, 50.50, 49.17, 46.83, 42.36, 40.65, 38.76, 38.71, 38.18, 37.11, 36.94, 34.30, 32.12, 30.51, 29.63,27.89, 27.23, 25.48, 20.81, 19.20, 18.23, 16.01, 15.90, 15.33, 14.56
DEPT 135°	**CH_3**	**:**	27.89, 19.20, 16.01, 15.90, 15.33, 14.56
	CH_2	**:**	109.36, 38.71, 36.94, 34.30, 32.12, 30.51, 29.63, 27.23, 25.48, 20.81, 18.23
	CH	**:**	78.41, 55.34, 50.50, 49.17, 46.83, 38.18

Subfractions MHI5.6-MHI5.7 showed no definite spot on normal phase TLC. No further investigations were conducted.

Fraction J was recrystallized from the dichloromethane in hexane (8:2) to give **MKT3** as a colourless needle (19.0 mg). It showed a single spot on normal phase TLC with 60% ethyl acetate in hexane with the R_f value of 0.33 which was visualized as a purple spot in vanillin sulfuric acid reagent and subsequently heating.

Compound MKT3 (Colourless needles)

$[\alpha]_D^{27}$	**:**	+15.00° (c = 0.12, Acetone)
mp	**:**	277.3 - 279.5 °C
FT-IR (KBr) ν (cm^{-1})	**:**	3446 (O-H stretching), 2937, 2863 (C-H stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	**:**	4.71 (*brd*, *J* = 1.5 Hz, 1H), 4.62 (*brs*, 1H), 4.19 (*brd*, *J* = 11.6 Hz, 1H), 3.84 (*dd*, *J* = 11.2 and 5.0 Hz, 1H), 3.41 (*brd*, *J* = 11.6 Hz, 1H), 3.20 (*dd*, *J* = 10.9 and 5.3 Hz, 1H), 2.43 (*m*, 1H), 2.36 (*m*, 1H), 2.05 (*m*, 2H), 1.84 (*m*, 1H), 1.70 (*s*, 3H), 1.64 (*m*, 1H), 1.62 (*m*, 2H), 1.60 (*m*, 2H), 1.55 (*m*, 2H), 1.54 (*m*, 1H), 1.53 (*m*, 1H), 1.45 (*m*, 1H), 1.44 (*m*, 1H), 1.43 (*m*, 1H), 1.42 (*m*, 2H), 1.24 (*m*, 1H), 1.21 (*m*, 1H), 1.18 (*m*, 1H), 1.07 (*s*, 3H), 1.01 (*s*, 3H), 0.99 (*s*, 3H), 0.89 (*m*, 1H), 0.85 (*s*, 3H), 0.78 (*s*, 3H), 0.68 (*brd*, *J* = 10.2 Hz, 1H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)	**:**	149.59, 109.91, 78.74, 78.55, 61.00, 55.29, 51.02, 49.86, 47.88, 47.60, 44.47, 40.91, 38.77, 38.70, 37.22, 37.03, 36.61, 34.15, 32.08, 29.74,

			27.83, 26.99, 24.84, 20.68, 19.11, 18.21, 16.00, 15.88 (2×C), 15.27
DEPT 135°	**CH_3**	**:**	27.83, 19.11, 16.00, 15.88 (2×C), 15.27
	CH_2	**:**	109.91, 61.00, 38.70, 37.22, 34.15, 32.08, 29.74, 26.99, 24.84, 20.68, 18.21
	CH	**:**	78.74, 78.58, 55.29, 49.86, 47.88, 47.60, 36.61

Fraction K was separated by column chromatography over silica gel, eluting with proportion of ethyl acetate-hexane and finally with 70% ethyl acetate in hexane. Fractions with the similar TLC chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions, as shown in **Table 32**.

Table 32 Subfractions obtained **fraction K** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
K1	0.015	green viscous- liquid
K2	0.027	green viscous- liquid
K3	0.216	green viscous- liquid
K4	0.414	green viscous- liquid
K5	0.131	green viscous- liquid
K6	0.119	green viscous- liquid

Subfractions K1-K2 were contained many spots without major component. Further separations were not carried out.

Subfraction K3 was recrystallized from 40% ethyl acetate in hexane to afford **MKT13** as a yellow solid (16.0 mg). It showed only one UV-active spot on normal phase TLC with 50% ethyl acetate in hexane with the R_f value of 0.27.

Compound MKT13 (Yellow solid)

$[\alpha]_D^{27}$		**:**	+50.00° (c = 0.01, Acetone)
mp		**:**	175.6 - 177.0 °C
UV (MeOH) λ_{max} **(nm) (log** ε**)**		**:**	220 (2.67), 244 (2.67), 312 (2.51), 358 (2.04)
FT-IR (KBr) ν**(cm^{-1})**		**:**	3469 (O-H stretching), 1653 (C=O stretching), 1218 (C-O stretching)
^{1}H NMR (Acetone-d_6) (δ**ppm) (300 MHz)**		**:**	13.13 (*s*, 1H), 9.35 (*brs*, 2H), 7.67 (*dd*, *J* = 7.8 and 1.8 Hz, 1H), 7.35 (*dd*, *J* = 7.8 and 1.8 Hz, 1H), 7.28 (*t*, *J* = 7.8 Hz, 1H), 6.54 (*s*, 1H), 3.89 (*s*, 3H)
^{13}C NMR (Acetone-d_6) (δ**ppm) (75 MHz)**		**:**	182.14, 159.29, 155.34, 153.85, 146.94, 146.11, 131.57, 124.81, 121.75, 121.31, 116.14, 103.96, 94.78, 60.76
DEPT 135°	**CH_3**	**:**	60.76
	CH	**:**	124.81, 121.31, 116.14, 94.78

The mother liquor of **subfraction K3** was separated by column chromatography over reversed-phase silica gel. Elution was conducted initially with 40% methanol in water, followed by increasing amount of methanol in water and finally with methanol. Fractions with the similar TLC chromatogram were combined

and evaporated to dryness *in vacuo* to afford thirteen subfractions, as shown in **Table 33**.

Table 33 Subfractions obtained from the mother liquor of **subfraction K3** by column chromatography over reversed-phase silica gel

Subfraction	Weight (g)	Physical appearance
MK3.1	0.011	yellow needles
MK3.2	0.011	brown viscous-liquid
MK3.3	0.004	brown viscous-liquid
MK3.4	0.006	brown viscous-liquid
MK3.5	0.015	yellow viscous-liquid
MK3.6	0.018	yellow viscous-liquid
MK3.7	0.008	yellow semi-solid
MK3.8	0.019	yellow viscous-liquid
MK3.9	0.033	yellow solid
MK3.10	0.019	white-yellow solid
MK3.11	0.012	green solid
MK3.12	0.016	green solid
MK3.13	0.040	green solid

Subfraction MK3.1 was **MKT11** (11.0 mg) as a yellow needle. It showed a single UV-active spot on normal phase TLC with 50% acetone in hexane with the R_f value of 0.46.

Compound MKT11 (Yellow needles)

Mp	**:**	+125.5 – 127.6 °C
UV (MeOH)	**:**	219 (4.03), 261 (3.81), 295 (3.58)
λ_{max} (nm) (log ε)		
FT-IR (KBr) ν(cm^{-1})	**:**	3454, 3255 (O-H stretching), 1690 (C=O stretching)
^{1}H NMR (Acetone-d_6)	**:**	7.49 (*d*, *J* = 1.8 Hz, 1H), 7.44 (*dd*, *J* = 8.1 and
(δppm) (300 MHz)		1.8 Hz, 1H), 6.90 (*d*, *J* = 8.1 Hz, 1H), 3.81 (*s*, 3H)
^{13}C NMR (Acetone-d_6)	**:**	166.21, 149.95, 144.79, 122.36, 121.91,
(δppm) (75 MHz)		116.26, 114.86, 50.96
DEPT 90° **CH**	**:**	122.36, 116.26, 114.86

Subfraction MK3.2 consisted of **MKT11** as a major component separated from **subfracton MK3.1** together with a compound with the R_f value of 0.22. No further purification was performed.

Subfractions 3.3-3.4 showed no definite spot on normal phase TLC and they were not investigated further.

Subfraction MK3.5 was separated by preparative TLC on silica gel plates, with 1% methanol in dichloromethane as a mobile phase to give two isolated bands. The first band was a yellow solid (5.0 mg). The ^{1}H NMR spectral data indicated that it was **MKT13** separated from **subfraction K3**. The second band was a yellow solid (5.0 mg). It' s ^{1}H NMR spectrum showed two signals of chelated hydroxy proton in a ratio 1:0.7, indicated that it was a mixture of at least two compounds.

Subfraction MK3.6 showed no definite spot on normal phase TLC and it was not investigated further.

Subfractions 3.7-3.8 showed no definite spot on TLC. They were then not investigated further.

Subfraction 3.9 was fractionated by column chromatography over silica gel. Elution was conducted initially with chloroform, followed by increasing amount of acetone in chloroform and finally with 15% acetone in chloroform. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness under reduced pressure to afford **subfractions MK3.9A- MK3.9D**.

Subfraction MK3.9A showed a single UV-active spot on normal phase TLC with 40% ethyl acetate in hexane with the R_f value of 0.31. The ^1H NMR spectrum data indicated that it contained some impurities.

Subfraction MK3.9B contained two purple spots on normal phase TLC with 40% ethyl acetate in hexane as a mobile phase with the R_f value of 0.45 and 0.42, respectively. Because of a low quantity, purification was then not performed further.

Subfraction MK3.9C showed no definite spot on normal phase TLC. It was then not investigated further.

Subfraction MK3.9D was **MKT12** (12.0 mg) as a white solid. It showed a purple spot on normal phase TLC with 40% ethyl acetate in hexane with the R_f value of 0.33.

Compound MKT12 (White solid)

$[\alpha]_D^{27}$: -32.00° (c = 0.03, $CHCl_3$)

mp		**:**	278.1 - 279.0 °C
FT-IR (KBr) ν (cm^{-1})		**:**	3395 (O-H stretching), 2945, 2871 (C-H stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)		**:**	4.74 (*brd*, *J* = 2.1 Hz, 1H), 4.61 (*m*, 1H), 3.68 (*ddd*, *J* = 11.1, 9.6 and 4.5 Hz, 1H), 3.01 (*m*, 1H), 2.98 (*d*, *J* = 9.6 Hz, 1H), 2.30 (*m*, 1H), 2.21 (*m*, 1H), 2.05 (*m*, 1H), 2.00 (*m*, 2H), 1.97 (*m*, 1H), 1.72 (*m*, 2H), 1.69 (*s*, 3H), 1.60 (*t*, *J* = 10.4 Hz, 1H), 1.55 (*m*, 2H), 1.44 (*m*, 1H), 1.43 (*m*, 1H), 1.42 (*m*, 1H), 1.40 (*m*, 2H), 1.36 (*m*, 1H), 1.35 (*m*, 2H), 1.19 (*m*, 1H), 1.01 (*s*, 3H), 0.98 (*s*, 3H), 0.93 (*s*, 3H), 0.90 (*s*, 3H), 0.85 (*m*, 1H), 0.83 (*m*, 1H), 0.80 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		**:**	180.22, 150.25, 109.79, 83.91, 69.25, 56.28, 55.45, 50.47, 49.24, 46.88, 46.73, 42.50, 40.77, 39.18, 38.58, 38.31, 37.00, 34.22, 32.13, 30.54, 29.64, 28.46, 25.37, 20.97, 19.37, 18.27, 17.37, 16.50, 16.06, 14.66
DEPT 135°	**CH_3**	**:**	28.46, 19.37, 17.37, 16.50, 16.06, 14.66
	CH_2	**:**	109.79, 46.73, 37.00, 34.22, 32.13, 30.54, 29.64, 25.37, 20.97, 18.27
	CH	**:**	83.91, 69.25, 55.45, 50.47, 49.24, 46.88, 38.31

Subfractions MK3.10-MK3.13 showed many spots, overlapping with other. Therefore, they were not further carried out.

Subfractions K4-K6 contained many spots, none of which were major compounds. Therefore, they were not investigated further.

Fractiom L upon standing at room temperature, afforded **MKT4** as a white solid (27.0 mg). It showed a purple spot on normal phase TLC with 10% methanol in dichloromethane with the R_f value of 0.38.

Compound MKT4 (White solid)

$[\alpha]_D^{29}$	**:**	-51.43° (c = 0.10, Pyridine)
mp	**:**	277.0 - 280.0 °C
FT-IR (KBr) ν (cm^{-1})	**:**	3410 (O-H stretching), 2959, 2930 (C-H stretching)
^{1}H NMR (Pyridine-d_5+$CDCl_3$) (δ ppm) (300 MHz)	**:**	5.33-5.31 (*m*), 5.21 (*dd*, *J* = 15.3 and 8.4 Hz), 5.06 (*dd*, *J* = 15.3 and 8.4 Hz), 4.92 (*d*, *J* = 7.5 Hz), 4.45 (*dd*, *J* = 11.7 and 2.4 Hz), 4.29 (*dd*, *J* = 11.7 and 5.1 Hz), 4.19-4.09 (*m*), 3.95-3.87 (*m*), 2.66-2.62 (*m*), 2.43-2.35 (*m*), 2.10-1.85 (*m*), 1.75-1.66 (*m*), 1.57-1.54 (*m*), 1.50-1.21 (*m*), 1.53-1.06 (*m*), 0.98 (*d*, *J* = 6.3 Hz), 0.93(*s*), 0.89 (*d*, *J* = 6.9 Hz), 0.86 (*d*, *J* =6.6 Hz), 0.67 (*s*)

Fraction M showed no definite spot on normal phase TLC. No further investigation was conducted.