CHAPTER III

EXPERIMENTAL

1. Source of Plant Material

The roots of *Prismatomeris sessiliflora* Pierre ex Pitard were collected from the Phuwua Wild Life Sanctuary, Nongkhai province, Thailand in March 1996. The plant was identified by comparison with herbarium specimens in the Royal Forest Department, Ministry of Agriculture and Co-operatives, Bangkok, Thailand.

The roots of *Diospyros montana* Roxb. were collected from Medicinal Plant Garden of Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand in September 1997.

2. General Techniques

2.1 Analytical Thin-layer Chromatography (TLC)

Technique	:	One dimension, ascending
Adsorbent	:	Silica gel 60 F_{254} (E. Merck) precoated plate
Layer thickness	:	0.2 mm
Distance	:	6 cm
Temperature	:	Laboratory temperature (30-35° C)
Detection	:	1. Ultraviolet light at wavelengths of 254 and 365 nm
	:	2. 10% Sulfuric acid in ethanol and heated at 105° C for 10 min.

2.2 Preparative Thin- Layer Chromatography (PLC)

Technique	:	One dimension, ascending
Adsorbent	:	Silica gel 60 F_{254} (E. Merck) precoated plate
Layer thickness	:	0.2 mm

Distance	15 cm
Temperature	Laboratory temperature (30-35°C)
Detection	Ultraviolet light at the wavelengths of 254 and 365 nm

2.3 Column Chromatography

2.3.1 Quick Column Chromatography

Adsorbent	Silica gel 60 (No.9385) particle size 0.040-0.063 nm (230-400 mesh ASTM) (E. Merck)
Packing Method	Dry packing
Sampling Loading	The sample was dissolved in a small amount of organic solvent, mixed with a small quantity of adsorbent, triturated, dried and then placed gently on the top of the column.
Detection	Fractions were examined by TLC observing under UV light at the wavelengths of 254 and 365 nm. The TLC plate was then sprayed with 10% sulfuric acid in ethanol and heated at 105°C for 10 min. Fractions of similar chromatographic pattern were combined.

2.3.2 Flash Column Chromatography

Adsorbent	Silica gel 60 (No.9385) particle size 0.040-0.063 nm (230-400 mesh ASTM) (E. Merck)
Packing method	Wet packing
Sampling loading	The sample was dissolved in a small amount of eluent, then apply gently on the top of the column.
Detection	Fractions was examined in the same manner as described in section 2.3.1

2.3.3 Gel Filtration Chromatography

Gel filter	:	Sephadex LH-20 (Pharmacia)
Packing method	:	Gel filter was suspended in the eluent and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tightly.
Sample loading	:	The sample was dissolved in a small volume of eluent and applied on top of the column.

2.4 Spectroscopyzz

2.4.1 Ultraviolet (UV) Absorption Spectra

UV (in methanol) spectra were obtained on a Milton Roy Spectronic 3000 Array Spectrometer (Pharmaceutical Research Equipment Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.4.2 Infrared (IR) Absorption Spectra

IR (KBr disc and film) spectra were obtained from a Perkin Elmer FT-IR 1760X spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University).

2.4.3 Mass Spectra (MS)

Electron Impact Mass Spectra (EIMS) of PS-A and PS-B were performed on a Finnigan MAT Incos 50 mass spectrometer (Department of Chemistry, Faculty of Science, Mahidol University). EIMS of DM-A, DM-B, DM-C, and DM-D were performed with Micromass (VG Platform II, Fisons Instrument) Spectrometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.4.4 Proton and Carbon-13 Nuclear Magnetic Resonance (^{1}H and ^{13}C-NMR) Spectra

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained with Avance DPX-300 FT-NMR Spectrometer, Bruker Spectro-Spin, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

NMR solvents used in this study were deuterated dimethylsulfoxide (DMSO-d_6) and deuterated chloroform (chloroform-d). Chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal.

2.5 Physical Properties

2.5.1 Melting Points

Melting points were obtained on a Fisher/Johns Melting Point Apparatus (Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn Univesity).

2.5.2 Optical Rotations

Optical rotations were measured on a Perkin Elmer Polarimeter 341 (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.6 Solvents

Throughout this work, all organic solvents were of commercial grade and were redistilled prior to use.

3. Extraction

3.1 Extraction of *Prismatomeris sessiliflora* (Scheme 1)

The dried roots of *Prismatomeris sessiliflora* (1.25 kg) were chopped and blended into small pieces. They were extracted repeatedly with methanol five times (4 L, 3 days, each). The filtrates were pooled and evaporated under reduced pressure at temperature not exceeding 40°C to yield a methanol extract (syrupy mass 53.46 g, 4.28% based on dried weight of root).

The methanol extract was partitioned between chloroform and water. The chloroform fraction (1 L) was evaporated under reduced pressure to give a chloroform extract (7.50 g, 0.60 % based on dried weight of root).

The water extract (11.30 g, 0.90 % based on dried weight of root) was obtained from the water fraction (300 ml) after removal of water by lyophilization.

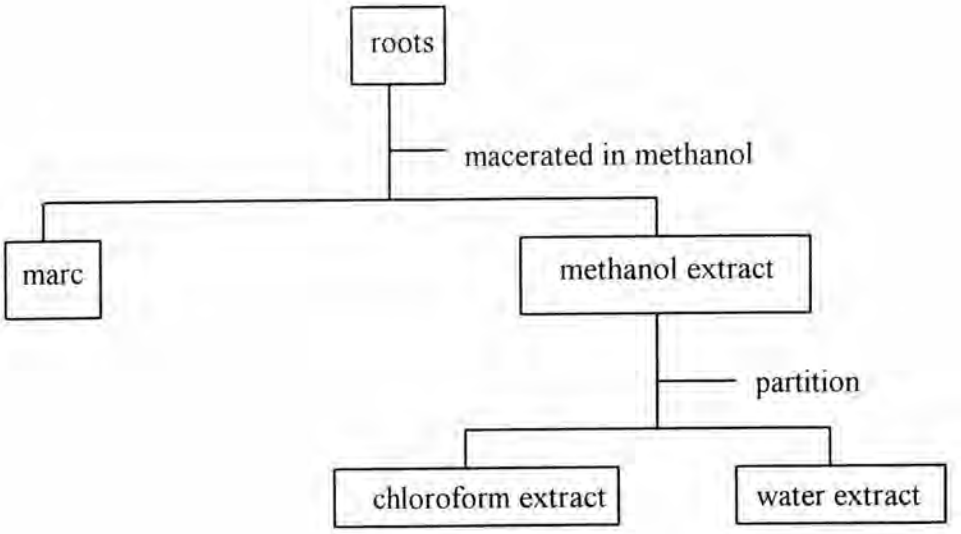
3.2 Extraction of *Diospyros montana* (Scheme 2)

The dried roots of *Diospyros montana* (2.6 kg) were chopped and blended into small pieces. They were extracted with petroleum ether three times (10 L, 3 days, each). The filtrates were pooled and evaporated under reduced pressure at temperature not exceeding 40°C to yield a petroleum extract (5g, 0.19 % based on dried weight of root).

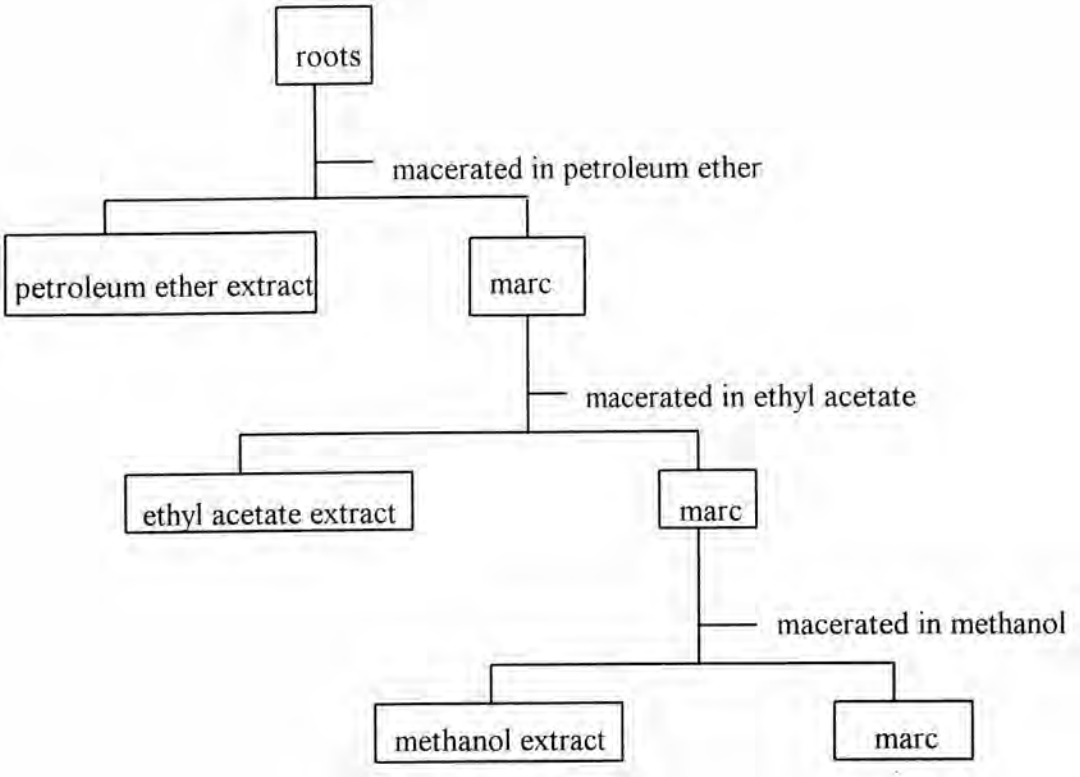
The marc (after extracted with petroleum ether) was extracted three times with ethyl acetate (10 L, 3 days, each). Removal of the organic solvent gave an ethyl acetate extract (12.34g, 0.47% based on dried weight of root).

The marc (after extracted with petroleum ether and ethyl acetate, respectively) was extracted three times with methanol (10 L, 3 days, each). The

obtained extract was evaporated under reduced pressure to yield a methanol extract (90g, 3.46% based on dried weight of root).



Scheme 1 Separation of *Prismatomeris sessiliflora* Pierre ex Pitard



Scheme 2 Separation of *Diospyros montana* Roxb.

4. Isolation

4.1 Isolation of Chemical Compounds from *Prismatomeris sessiliflora*

The chloroform extract (7 g) was dissolved in a small amount of chloroform, triturated with silica gel 60 (No. 9385) and dried under the vacuum. It was then fractionated by the quick column chromatographic technique using a sintered glass filter column of silica gel 60 (No. 9385) (400g). Elution was performed in a polarity gradient manner with chloroform (9.76 L) and methanol (0.64 L) as the solvents. The ratio and volume of solvents used in this column chromatography are summarized in Table 3.

Table 3 The ratio and volume of solvents for quick column chromatography of chloroform extract from *P. sessiliflora*

Fractions	Ratio (%) of $CHCl_3$: MeOH	Volume of solvents (ml)	
		$CHCl_3$	MeOH
1-19	100 : 0	7600	0
20	99 : 1	396	4
21	98 : 2	392	8
22	96 : 4	384	16
23	92 : 8	368	32
24	84 : 16	336	64
25	70 : 30	280	120
26	0 : 100	0	400

The eluents were examined by TLC using 10% petroleum ether in chloroform and 10% ethyl acetate in toluene as developing solvents. Fractions (26 fractions, 400 ml, each) with similar chromatographic pattern were combined to yield eight fractions, as shown in Table 4.

Table 4 Combination of fractions from quick column chromatography of chloroform extract from *P. sessiliflora*

Fractions	Combined fractions	Weight (g)	Volume of solvents (ml)	
			$CHCl_3$	MeOH
1-2	P-1	0.61	800	0
3-5	P-2	0.11	1200	0
6-9	P-3	0.45	1600	0
10-11	P-4	0.48	800	0
12-18	P-5	0.74	2800	0
19-21	P-6	0.10	1188	12
22-25	P-7	0.27	1368	232
26	P-8	0.90	0	400

4.1.1 Isolation of PS-A

Fraction P-5 (0.74 g) was equally divided into six portions. Each was fractionated by quick column chromatographic technique using a sintered glass filter column of silica gel 60 (No. 9385) (20 g, 3×3 cm). First, fraction P-5 (120 mg, each) was dissolved in a small amount of chloroform, triturated with silica gel 60 H (No. 9385) and dried under vacuum. The mixture was then applied on the top of the column. Separation was carried out with gradient elution, using mixtures of hexane and ethyl acetate (10:0 to 0:10). Fractions, approximately 50 ml each, were collected based on the color bands. Fractions showing similar TLC pattern were combined. The ratio and volume of solvents used in six portions of quick column chromatography are summarized in Table 5.

Table 5 The ratio and volume of solvents in six portions of quick column chromatography

Portions	Fractions	Ratio (%) of Hexane : EtOAc	Volume of solvents (ml)	
			Hexane	EtOAc
First	1	100 : 0	50	0
	2-39	90 : 10	1710	190
	40-41	80 : 20	80	20
	42-43	70 : 30	70	30
	44	60 : 40	30	20
	45	50 : 50	25	25
	46	0 : 100	0	50
Second	1	100 : 0	50	0
	2-39	90 : 10	1710	190
	40-41	80 : 20	80	20
	42-43	70 : 30	70	30
	44	60 : 40	30	20
	45	50 : 50	25	25
	46	0 : 100	0	50
Third	1	100 : 0	50	0
	2-47	90 : 10	2070	230
	48-49	80 : 20	80	20
	50	70 : 30	35	15
	51	60 : 40	30	20
	52	0 : 100	0	50
Fourth	1	100 : 0	50	0
	2-37	90 : 10	1620	180
	38-40	80 : 20	120	30
	41-42	70 : 30	70	30
	43	60 : 40	30	20
	44	0 : 100	0	50

Table 5 (continued)

Portions	Fractions	Ratio (%) of Hexane : EtOAc	Volume of solvents (ml)	
			Hexane	EtOAc
Fifth	1	100 : 0	50	0
	2-38	90 : 10	1665	185
	39-41	80 : 20	120	30
	42	70 : 30	35	15
	43	60 : 40	30	20
	44	0 : 100	0	50
Sixth	1	100 : 0	50	0
	2-39	90 : 10	1710	190
	40-41	80 : 20	80	20
	42	70 : 30	35	15
	43	60 : 40	30	20
	44	0 : 100	0	50

Fractions 6-11 (11.8 mg) of the first portion (46 fractions), fractions 13-16 (9.60 mg) of the second portion (46 fractions), fractions 15-23 (15.0 mg) of the third portion (52 fractions), fractions 12-17 (12.0 mg) of the fourth portion (44 fractions), fraction 9-14 (11.8 mg) of the fifth portion (44 fractions), and fractions 8-14 (26.3 mg) of the sixth portion (44 fractions) were combined. The TLC chromatogram of these fractions using various developing solvent systems showed only one spot. Evaporation of the combined fractions under reduced pressure gave 86.5 mg of compound PS-A as orange-yellow plates [R_f 0.16 (Toluene : EtOAc = 95 : 5), 6.92×10^{-3}% based on dried weight of root]. It was subsequently identified as rubiadin [**1**].

4.1.2 Isolation of PS-B

After the separation of PS-A from fraction P-5, the remaining fractions of P-5 : fractions 12-23 (22.6 mg) of the first portion (46 fractions), fractions

17-35 (42.0 mg) of the second portion (46 fractions), fractions 24-29 (11.7 mg) of the third portion (52 fractions), fractions 18-35 (37.4 mg) of the fourth portion (44 fractions), fractions 15-28 (38.9 mg) of the fifth portion (44 fractions) and fractions 15-31 (54.6 mg) of the sixth portion (44 fractions) were combined and further separated by preparative TLC (59 plates) on precoated silica gel 60 F_{254} (0.2 mm, 15×20 cm) plate with triple development in toluene : ethyl acetate (90 : 10) and then recrystallized from acetone to give compound PS-B as yellow plates (dark spot under UV light at 254 nm, R_f 0.22 (Toluene : EtOAc = 90 : 10), 53.1 mg, 4.25×10^{-3}% based on the dried weight of root). It was subsequently identified as rubiadin-1-methyl ether [**2**].

4.2 Isolation of Chemical Compounds from *Diospyros montana*

4.2.1 Isolation of DM-A

The ethyl acetate extract (12 g) was dissolved in a small amount of chloroform, triturated with silica gel 60 H (No. 9385) and dried under the vacuum. It was then fractionated by the quick column chromatographic technique using a sintered glass filter column of silica gel 60 H (No. 9385) (400 g). Elution was performed in a polarity gradient manner with petroleum ether (8.2 L), chloroform (14.7 L) and methanol (1.1 L) as the solvents. The ratio and volume of solvents that used in quick column chromatography are summarized in Table 6.

Table 6 The ratio and volume of solvents for quick column chromatography of ethyl acetate extract from *D. montana*

Fractions	Ratio (%) of Pet. ether : $CHCl_3$: MeOH	Volume of solvents (ml)		
		Pet. ether	$CHCl_3$	MeOH
1	95 : 5 : 0	475	25	0
2-3	90 : 10 : 0	900	100	0
4	85 : 15 : 0	425	75	0
5	80 : 20 : 0	400	100	0
6	75 : 25 : 0	375	125	0

Table 6 (continued)

Fractions	Ratio (%) of Pet. ether : $CHCl_3$: MeOH	Volume of solvents (ml)		
		Pet. ether	$CHCl_3$	MeOH
7	70 : 30 : 0	350	150	0
8	65 : 35 : 0	325	175	0
9	60 : 40 : 0	300	200	0
10-12	50 : 50 : 0	750	750	0
13-16	40 : 60 : 0	800	1200	0
17-34	30 : 70 : 0	2700	6300	0
35-38	20 : 80 : 0	400	1600	0
39	0 : 100 : 0	0	500	0
40-43	0 : 90 : 10	0	1800	200
44-47	0 : 80 : 20	0	1600	400
48	0 : 0 : 100	0	0	500

The eluates was collected approximately 500 ml per fraction and were examined by TLC using 100% chloroform as the developing system. Fractions (48 fractions) with similar chromatographic pattern were combined to yield six fractions, as shown in Table 7.

Table 7 Combination of fractions from quick column chromatography of ethyl acetate extract from *D. montana*

Fractions	Combined fractions	Weight (g)	Volume of solvents (ml)		
			Pet. ether	$CHCl_3$	MeOH
1-15	D-1	0.0572	4300	1700	0
16-19	D-2	0.0059	650	1350	0
20	D-3	0.0342	150	350	0
21-30	D-4	3.4800	1500	3500	0
31-40	D-5	0.7400	1000	3950	50
41-48	D-6	6.6400	0	2950	1050

Fraction D-4 (3.48 g) was equally divided into ten portions. Each was fractionated on a column using silica gel 60 (No. 9385) (4.5x20 cm) as the adsorbent. Gradient elution was performed using mixtures of petroleum ether and chloroform (100:0 to 0:100). Fractions of 50 ml were collected. The ratio and volume of solvents used in this column are summarized in Table 8. Similar fractions were combined after examining with TLC, using 100% chloroform and 30% petroleum ether in chloroform as the developing systems.

Table 8 The ratio and volume of solvents in ten portions of column chromatography

Portions	Fractions	Ratio (%) of Pet. ether : $CHCl_3$	Volume of solvents (ml)	
			Pet. ether	$CHCl_3$
First	1	100 : 0	50	0
	2	90 : 10	45	5
	3	80 : 20	40	10
	4	70 : 30	35	15
	5	60 : 40	30	20
	6	50 : 50	25	25
	7-25	40 : 60	380	570
	26-27	0 : 100	0	100
Second	1	100 : 0	50	0
	2	90 : 10	45	5
	3	80 : 20	40	10
	4-7	70 : 30	140	60
	8-10	60 : 40	90	60
	11-25	40 : 60	300	450
	26-28	0 : 100	0	150
Third	1	100 : 0	50	0
	2	90 : 10	45	5
	3	80 : 20	40	10

Table 8 (continued)

Portions	Fractions	Ratio (%) of Pet. ether : $CHCl_3$	Volume of solvents (ml)	
			Pet. ether	$CHCl_3$
	4	70 : 30	35	15
	5	60 : 40	30	20
	6-15	40 : 60	200	300
	16-18	0 : 100	0	150
Fourth	1	100 : 0	50	0
	2-3	90 : 10	90	10
	4-5	80 : 20	80	20
	6-7	70 : 30	70	30
	8-9	60 : 40	60	40
	10-13	50 : 50	100	100
	14-28	40 : 60	300	450
	29-33	0 : 100	0	250
Fifth	1	100 : 0	50	0
	2-3	90 : 10	90	10
	4-6	80 : 20	120	30
	7-9	70 : 30	105	45
	10-11	60 : 40	60	40
	12-13	50 : 50	50	50
	14-27	40 : 60	280	420
	28-31	0 : 100	0	200
Sixth	1	100 : 0	50	0
	2	80 : 20	40	10
	3	60 : 40	30	20
	4-8	40 : 60	100	150
	9-11	0 : 100	0	150
Seventh	1	100 : 0	50	0
	2	80 : 20	40	10

Table 8 (continued)

Portions	Fractions	Ratio (%) of Pet. ether : $CHCl_3$	Volume of solvents (ml)	
			Pet. ether	$CHCl_3$
	3-4	60 : 40	60	40
	5-13	40 : 60	180	270
	14-17	0 : 100	0	200
Eighth	1	100 : 0	50	0
	2	80 : 20	40	10
	3	60 : 40	30	20
	4-13	40 : 60	200	300
	14-16	0 : 100	0	150
Ninth	1	100 : 0	50	0
	2	80 : 20	40	10
	3-4	60 : 40	60	40
	5-10	40 : 60	120	180
	11-13	0 : 100	0	150
Tenth	1	100 : 0	50	0
	2-3	80 : 20	80	20
	4-5	60 : 40	60	40
	6-19	40 : 60	280	420
	20-23	0 : 100	0	200

Fractions 10-11 of the first portion (27 fractions), fractions 16-18 of the second portion (28 fractions), fractions 18 of the fourth portion (33 fractions) were combined and further purified by recrystallizing from methanol to give DM-A as orange plates (R_f 0.25 ($CHCl_3$ 100%), 0.13 g, 5 x 10^{-3}% based on dried weight of root). It was subsequently identified as diospyrin [**6**].

4.2.2 Isolation of DM-B

After the separation of DM-A from fraction D-4, the remaining fractions of D-4 were combined. The combined fractions (70 mg) from fractions 5-8 (21 mg) of the first portion (27 fractions), fractions 8-10 (18 mg) of the second portion (28 fractions), fractions 10-14 (15 mg) of the fourth portion (33 fractions), fractions 7-10 (4 mg) of the fifth portion (31 fractions), fractions 2-3 (7 mg) of the seventh portion (17 fractions), fraction 3 (2 mg) of the ninth portion (13 fractions), and fraction 4 (3 mg) of the tenth portion (23 fractions), showing fluorescent green-yellow color under UV light at 365 nm, R_f 0.62 ($CHCl_3$ 100%) were further examined. They were combined and further purified by gel filtration chlomatography using a column of Sephadex LH-20 (100 g, 2.5x80 cm) with methanol (500 ml) as the eluent. The purification of DM-B by gel filtration chromatography was monitored by UV light at 365 nm. Nine fractions, approximately 50 ml each, were collected. The TLC chromatogram of fraction 5 using 100% chloroform as the developing system showed only one green-yellow fluorescent spot under UV light at 365 nm, Rf 0.62 ($CHCl_3$ 100%). Evaporation of this fraction under reduced pressure gave 39 mg of compound DM-B as colorless needles (1.5x10-3% based on dried weight of root). It was subsequently identified as 5-hydroxy-4-methoxy-2-naphthaldehyde.

4.2.3 Isolation of DM-C

The petroleum ether extract (5 g) was dissolved in a small amount of chloroform, triturated with silica gel 60 (No. 9385) and dried under the vacuum. It was then fractionated by quick column chromatography using a sintered glass filter column of silica gel (No. 9385, 200 g, 13 x 5 cm). Elution was performed in a polarity gradient manner with petroleum ether (3 L), chloroform (4.7 L) and methanol (0.39 L) as the solvents. The ratio and volume of solvents used in the quick column are summarized in Table 9.

Table 9 The ratio and volume of solvents for quick column chromatography of petroleum ether extract from *D. montana*

Fractions	Ratio (%) of Pet. ether : $CHCl_3$: MeOH	Volume of solvents (ml)		
		Pet. ether	$CHCl_3$	MeOH
1-3	70 : 30 : 0	630	270	0
4	60 : 40 : 0	180	120	0
5	50 : 50 : 0	150	630	0
6-22	40 : 60 : 0	2040	180	0
23-24	0 : 100 : 0	0	150	0
25	0 : 90 : 10	0	2040	30
26	0 : 80 : 20	0	0	60
27	0 : 0 : 100	0	0	300

The eluates were examined by TLC using 40% petroleum ether in chloroform, observing under ultraviolet light at the wavelengths of 254 and 365 nm and followed by spraying with 10% sulphuric acid in ethanol before heating at 105°C for 10 min. Fractions of similar chromatographic pattern were combined to give seven fractions, as shown in Table 10.

Table 10 Combination of fractions from quick column chromatography of petroleum ether extract from *D.montana*

Fractions	Combined fractions	Weight (g)	Volume of solvents (ml)		
			Pet. ether	$CHCl_3$	MeOH
1-4	T-1	0.0081	810	390	0
5-7	T-2	0.5794	390	510	0
8-10	T-3	0.0150	360	540	0
11-13	T-4	0.9090	360	540	0
14-16	T-5	1.0952	360	540	0

Table 10 (continued)

Fractions	Combined fractions	Weight (g)	Volume of solvents (ml)		
			Pet. ether	$CHCl_3$	MeOH
17-22	T-6	1.0322	720	1080	0
23-27	T-7	1.3393	0	1110	390

Isolate DM-C (0.1108 g) was obtained as colorless needles from fraction T-5 through recrystallization from methanol. The yield was $4.3x10^{-3}$% based on dried weight of root. It was identified as lupeol [**16**].

4.2. 4 Isolation of DM-D

Isolate DM-D (0.048 g) was obtained as colorless needles from fraction T-6 through recrystallization from methanol. The yield was $1.8x10^{-3}$% based on dried weight of the root. It was identified as betulinic acid [**17**].

5. Physical and Spectral data of Isolated Compounds

5.1 Compound PS-A [1]

Compound PS-A was obtained as orange-yellow plates (86.5 mg). It was soluble in chloroform.

Melting Point : >300°C

EIMS : *m/z* (% relative intensity); Figure 3
254 (M^+, 100), 236 (10), 226 (25), 197 (29), 180 (17), 169 (13), 152 (42), 141 (29), 115 (50), 105 (30), 76 (62), 65 (41), 55 (55).

UV : λ_{max} nm (log ε), in methanol; Figure 4
244 (4.34), 278 (4.41), 412 (3.75).

IR : ν_{max} cm^{-1}, KBr disc; Figure 5
3398, 2923, 1664, 1624, 1589, 1433, 1339, 1311, 1123, 713.

^{1}H NMR : δ ppm, 500 MHz, in DMSO-d_6; Figures 6a-6b
2.04 (3H, s, 2-CH_3), 7.21 (1H, s, H-4), 7.85 (1H, ddd, *J*=1.5, 7.5, 7.5, H-6), 7.88 (1H, ddd, *J*=1.5, 7.5, 7.5, H-7), 8.09 (1H, dd, *J*=1.5, 7.5, H-5), 8.16 (1H, dd, *J*=1.5, 7.5, H-8), 13.08 (1H, s,1-OH).

^{13}C NMR : δ ppm, 75 MHz, in DMSO-d_6; Figures 7a-7b
9.06 (q, 2-CH_3), 108.32 (d, C-4), 109.53 (s, C-9a), 118.03 (s, C-2), 127.01 (d, C-8), 127.35 (d, C-5), 132.36 (s, C-4a), 133.56 (s, C-10a), 133.71 (s, C-8a), 135.00 (d, C-6), 135.14 (d, C-7), 163.10 (s, C-1), 163.82 (s, C-3), 182.35 (s, C-10), 186.57 (s,C-9).

5.1 Compound PS-B [2]

Compound PS-B was obtained as yellow plates (53.1 mg). It was soluble in chloroform.

Melting Point : 290°C

EIMS : *m/z* (% relative intensity); Figure 12
268 (M^+, 100), 253 (42), 239 (27), 221 (34), 194 (13), 165 (20), 147 (35), 111 (17), 97 (30), 83 (41), 73 (68), 55 (69).

UV : λ_{max} nm (log ε), in methanol; Figure 13
238 (4.39), 279 (4.68), 361 (3.72).

IR : ν_{max} cm^{-1}, KBr disc; Figure 14
3315, 2925, 1673, 1566, 1336, 1121, 713

^{1}H NMR : δ ppm, 500 MHz, in DMSO-d_6; Figures 15a-15b

2.13 (3H, s, 2-CH_3), 3.77 (3H, s, 1-OCH_3), 7.45 (1H, s, H-4), 7.80 (1H, ddd, *J*=1.5, 7.5, 7.5, H-6), 7.86 (1H, ddd, *J*=1.5, 7.5, 7.5, H-7), 8.08 (1H, dd, *J*=1.5, 7.5, H-5), 8.13 (1H, dd, *J*=1.5, 7.5, H-8).

^{13}C NMR : δ ppm, 75 MHz, in DMSO-d_6; Figures 16a-16b

9.91 (q, 2-CH_3), 61.37 (q, 1-OCH_3), 110.20 (d, C-4), 117.81 (s, C-9a), 126.84 (d, C-5), 127.06 (s, C-2), 127.45 (d, C-8), 132.93 (s, C-8a), 134.09 (s, C-6), 134.58 (s, C-4a), 135.33 (d, C-7), 135.45 (s, C-10a), 161.50 (s, C-1), 163.54 (s, C-3), 180.83 (s, C-9), 183.64 (s, C-10).

5.3 Compound DM-A [6]

Compound DM-A was obtained as orange plates (0.13 g). It was soluble in chloroform.

Melting Point : 253-255°C

EIMS : *m/z* (% relative intensity); Figure 21

374 (M^+, 100), 359 (22), 357 (13), 328 (10), 189 (12), 163 (18), 135 (31), 134 (30), 128 (11), 107 (16), 106 (64), 105 (12), 83 (15), 78 (17), 77 (48), 76 (16), 75 12), 63 (21), 51 (27).

UV : λ_{max} nm (log ε), in methanol; Figure 22

216 (4.48), 253 (4.08), 432 (3.65).

IR : ν_{max} cm^{-1}, KBr disc; Figure 23

3438, 3058, 1670, 1664, 1611, 1382, 1333, 1259

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Figure 24

2.29 (3H, s, 7′-CH_3), 2.44 (3H, s, 7-CH_3), 6.88 (1H, s, H-3), 6.94 (2H, s, H-2′, H-3′), 7.11 (1H, d, *J*=1 Hz, H-6), 7.49 (1H, d, *J*=1 Hz, H-8), 7.54 (1H, s, H-8′), 11.86 (1H, s, 5-OH), 12.12 (1H, s, 5′-OH).

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Figure 25

21.21 (q, 7′-CH3), 22.40 (q, 7-CH3), 112.91 (s, C-9′), 113.10 (s, C-9), 120.66 (d, C-8′), 121.17 (d, C-8), 124.12 (d, C-6), 128.73(s,C-6′), 131.22 (s, C-10′), 131.49 (s, C-10), 138.62 (d, C-3), 138.68 (d, C-3′), 139.28 (d, C-2′), 145.57 (s, C-2), 146.30 (s, C-7′), 148.47 (s, C-7), 158.96 (s, C-5′), 161.52 (s, C-5), 182.27 (s, C-1), 183.85 (s, C-1′), 188.64 (s, C-4), 189.48 (s, C-4′).

5.4 Compound DM-B

Compound DM-B was obtained as colorless needles (39 mg). It was soluble in chloroform.

Melting Point : 91°C

EIMS : *m/z* (% relative intensity); Figure 30

202 (M^+, 100), 187 (97), 159 (94), 131 (97), 115 (65), 103 (71), 102 (80), 77 (81), 63 (66).

UV : λ_{max} nm (log ε), in methanol; Figure 31

217 (4.26), 257 (4.27), 299 (3.42), 377 (3.57).

IR : ν_{max} cm^{-1}, film; Figure 32

3390, 1691, 1613, 1587, 1514, 1398, 1363, 1248, 1124, 1080.

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Figure 33
4.06 (3H, s, 4-OCH_3), 7.02 (1H, dd, *J*=2.57, 7.71 Hz, H-6), 7.13 (1H, d, *J*=1.03 Hz, H-3), 7.42 (2H, m, H-7, H-8), 7.82 (1H, d, *J*=1.03 Hz, H-1), 9.24 (1H, s, 5-OH), 9.97 (1H, s, 2-CHO).

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Figure 37
56.38 (q, 4-OCH_3), 98.50 (d, C-3), 114.26 (d, C-6), 117.32 (s, C-4a) 120.56 (d, C-8), 128.77 (d, C-7), 130.16 (d, C-1), 134.09 (s, C-2), 135.48 (s, C-8a), 154.48 (s, C-5), 156.87 (s, C-4), 191.35 (s, 2-C=0).

5.5 Compound DM-C [16]

Compound DM-C was obtained as colorless needles (0.1108 g). It was soluble in chloroform.

Melting Point: 215°C

$[\alpha]^{20}_D$: +24.3° (*c* 0.23, $CHCl_3$)

EIMS : *m/z* (% relative intensity); Figure 42
426 (M^+,11), 411 (5), 315 (6), 218 (23), 207 (37), 203 (22), 198 (50), 175 (21), 149 (25), 147 (30), 135 (65), 121 (61), 109 (70), 95 (97), 93 (78), 91 (42), 81 (86), 79 (51), 69 (89), 67 (79), 57 (34).

IR : ν_{max} cm^{-1}, KBr disc; Figure 43
3350 (br), 2944, 2872, 1641, 1455, 1382, 1042, 822.

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Figures 44a-44b
0.73 (3H, s, H-24), 0.76 (3H, s, H-28), 0.80 (3H, s, H-25), 0.92 (3H, s,H-27), 0.94 (3H, s, H-23), 1.00 (3H, s, H-26), 1.65 (3H, s, H-30),

2.35 (1H, m, H-19), 3.16 (1H, dd, *J*=5.10, 10.50 Hz, H-3), 4.54 (1H, br s, H-29), 4.66 (1H, br s, H-29).

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Figures 45a-45b

14.51 (q, C-27), 15.36 (q, C-24), 15.941 (q, C-26), 16.10 (q, C-25), 17.97 (q, C-28), 18.28 (t, C-6), 19.28 (q, C-30), 20.89 (t, C-11), 25.08 (t, C-12), 27.36 (t, C-2), 27.40 (t, C-15), 27.96 (q, C-23), 29.80 (t,C-21), 34.23 (t, C-7), 35.54 (t, C-16), 37.12 (s, C-10), 58.00 (d, C-13), 38.66 (t, C-1), 38.82 (s, C-4), 39.96 (t, C-22), 40.78 (s, C-8), 42.78 (s,C-14), 42.96 (s, C-17), 47.94 (d, C-19), 48.25 (d, C-18), 50.37 (d, C-9), 55.25 (d, C-5), 78.95 (d, C-3), 109.31 (t, C-29), 150.92 (s, C-20).

5.6 Compound DM-D [17]

Compound DM-D was obtained as colorless needles (48 mg). It was soluble in chloroform.

Melting Point: 290°C

$[\alpha]^{20}_D$: +7.7° (*c* 0.26, pyridine).

EIMS : *m/z* (% relative intensity); Figure 48

456 (M^+,9), 438 (6), 411 (6), 248 (22), 207 (34), 190 (27), 189 (80), 175 (32), 161 (21), 136 (29), 135 (47), 119 (53), 107 (54), 95 (67), 91 (49), 81 (81), 67 (94), 57 (43).

IR : ν_{max} cm^{-1}, KBr disc; Figure 49

3446 (br), 2944, 2871, 1712, 1642, 1453, 1375, 1033, 884.

^{1}H NMR : δ ppm, 300 MHz, in DMSO-d_6; Figures 50a-50b

0.64 (3H, s, H-24), 0.76 (3H, s, H-25), 0.86 (3H, s, H-23), 0.92 (3H, s, H-26), 0.97 (3H, s, H-27), 1.64 (3H, s, H-30), 2.97 (1H, m, H-19), 3.17 (1H, m, H-3), 4.55-4.68 (2H, br s, H-29).

^{13}C NMR : δ ppm, 75 MHz, in DMSO-d_6; Figures 51a-51b

14.51 (q, C-27), 15.86 (q, C-24), 15.92 (q, C-25), 16.07 (q, C-26), 18.09 (t, C-6), 19.05 (q, C-30), 20.58 (t, C-11), 25.19 (t, C-12), 27.24 (t, C-2), 28.19 (q, C-23), 29.30 (t, C-21), 30.20 (t, C-15), 31.82 (t, C-16), 34.00 (t, C-7), 36.44 (t, C-22), 36.79 (s, C-10), 37.66 (d, C-13), 38.34 (t, C-1), 38.57 (s, C-4), 40.32 (s, C-8), 42.06 (s, C-14), 46.67 (d, C-18), 48.58 (d, C-19), 49.98 (d, C-9), 54.93 (d, C-5), 55.45 (s, C-17), 76.78 (d, C-3), 109.52 (t, C-29), 150.12 (s, C-20), 176.99 (s, C-28).