2 EXPERIMENTAL

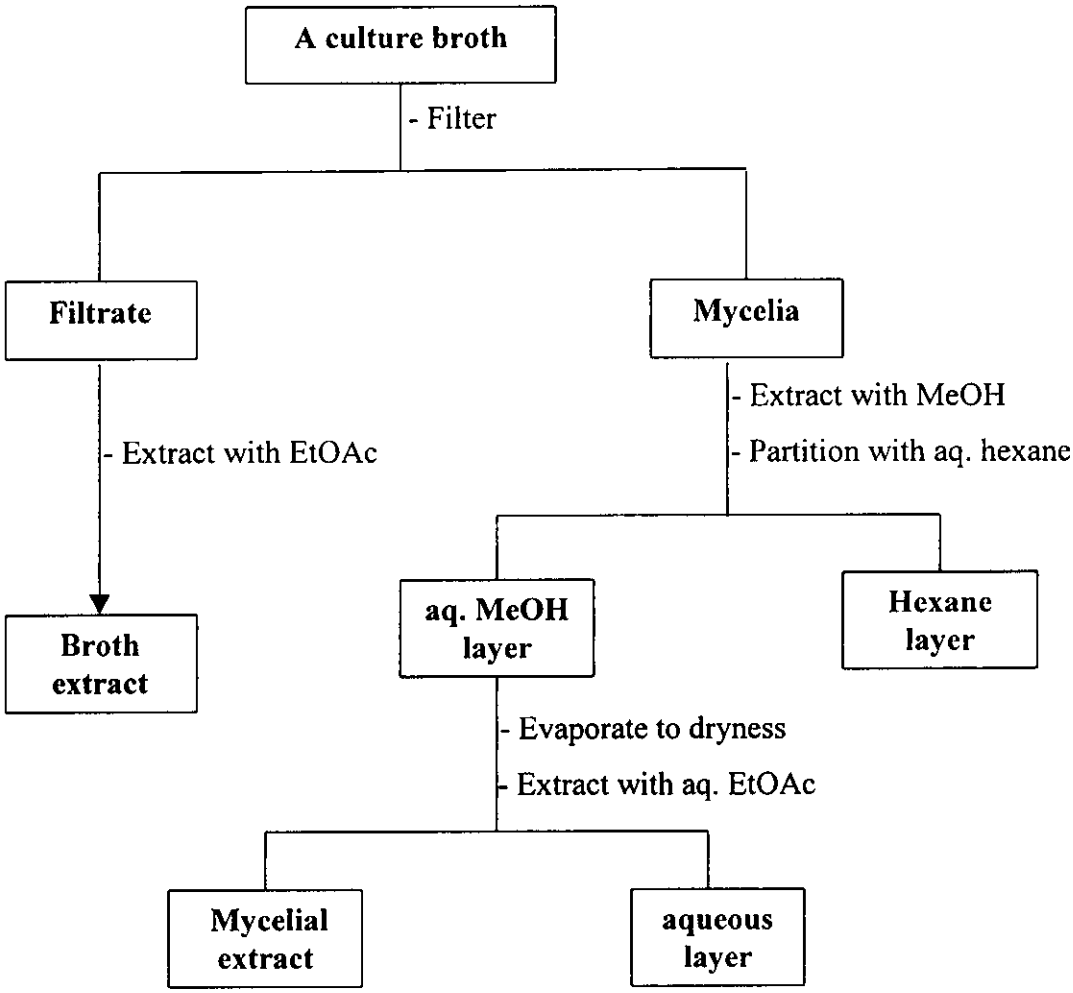
2.1 Chemical and instruments

Melting points were determined on an electrothermal melting point apparatus (Electrothermal 9100) and reported without correction. Infrared spectra (IR) were obtained on a FTS165 FT-IR spectrometer and Perkin Elmer Spectrum GX FT-IR system and recorded on wavenumber (cm^{-1}). ^{1}H and ^{13}C-Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on a FTNMR, Varian UNITY INOVA 500 MHz or Bruker ADVANCE 300MHz using a solution in either deuterochloroform or deuteromethanol with tetramethylsilane (TMS) as an internal standard. Spectra were recorded as chemical shift parameter (δ) value in ppm down field from TMS (δ 0.00). Ultraviolet spectra (UV) were measured with UV-160A spectrophotometer (SHIMUDZU). Principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in methanol solution. Optical rotation was measured in methanol solution with sodium D line (590 nm) on an AUTOPOL®II automatic polarimeter. 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 (70-230 Mesh ASTM) or reverse-phase C-18. The solvents for extraction and chromatography were distilled at their boiling point range prior to use while petroleum ether (bp. 40-60), diethyl ether and ethyl acetate were analytical grade reagent.

2.2 Extraction

The cultures of *Penicillium* sp. BCC 7540, *Cordyceps militaris* BCC 2816 and *C. militaris* BCC 2819 were chemically investigated as follows (Scheme 1). Each culture was filtered to give two parts; filtrate and mycelia. The filtrate was extracted with EtOAc to give an EtOAc extract which was evaporated under reduced pressure to dryness to afford a broth extract. The mycelia were extracted with MeOH followed by partition with aqueous hexane to obtain aqueous MeOH extract. The extract was

evaporated under reduced pressure and then extracted with aqueous EtOAc to give a crude mycelial extract after evaporation under reduced pressure to dryness.



Scheme 1 Extraction procedure of a culture broth

2.3 Chemical investigation of *Penicillium* sp. BCC 7540

2.3.1 Purification of the broth extract

The crude material (1.886 g) was separated by column chromatography over Sephadex LH20. Elution was conducted with MeOH. Fractions with similar were combined and evaporated under reduced pressure to dryness to afford five fractions, as shown in **Table 3**.

Table 3 Fractions obtained form the broth extract by column chromatography over Sephadex LH20

Fraction	Weight (g)	Physical appearance
A1	0.0546	Black solid with black gum
A2	1.5096	Black solid with black gum
A3	0.1768	Brown gum
A4	0.1310	Brown gum
A5	0.0672	Brown gum

Fraction A1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 3 times) showed no definite spots. Thus, it was not further investigated.

Fraction A2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 3 times) showed three UV-active spots with the R_f values of 0.84, 0.50 and 0.43 and one yellow spot with the R_f value of 0.32. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 60%MeOH/$CHCl_3$ Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford nine subfractions, as shown in **Table 4**.

Table 4 Subfractions obtained from **A2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A2-1	0.0506	Yellow gum
A2-2	0.0288	Brown gum
A2-3	0.0225	Brown gum
A2-4	0.0043	Brown gum
A2-5	0.0145	Brown gum
A2-6	0.0661	Black gum
A2-7	0.0501	Black gum

Table 4 (Continued)

Subfraction	Weight (g)	Physical appearance
A2-8	0.3386	Yellow gum
A2-9	0.5495	Black solid with black gum

Subfraction A2-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 2 times) showed no definite spots. Thus, it was not further investigated.

Subfraction A2-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 2 times) showed many spots without any major spots. Because it was obtained in low quantity, it was not further investigated.

Subfraction A2-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 2 times) showed one major yellow spot with the R_f value of 0.63 and two yellow minor spots with the R_f values of 0.76 and 0.70. It was further separated by column chromatography over reverse-phase C-18 silica gel. Elution was conducted initially with 20% MeOH/H_2O and decreased the polarity until pure methanol. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford six subfractions, as shown in **Table 5**.

Table 5 Subfractions obtained from **A2-3** by column chromatography over reverse-phase C-18 silica gel

Subfraction	Weight (g)	Physical appearance
A2-3-1	0.0080	Brown gum
A2-3-2	0.0020	Brown gum
A2-3-3	0.0017	Yellow gum
A2-3-4	0.0011	Yellow gum
A2-3-5	0.0017	Yellow gum
A2-3-6	0.0006	Yellow gum

Subfraction A2-3-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 2 times) showed no definite spots. Thus, it was not further investigated.

Subfraction A2-3-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 2 times) showed one major UV-active spot with the R_f value of 0.75. The ^1H NMR spectrum indicated that the mixture contained no major component. Because it was obtained in low quantity. Thus, it was not further investigated.

Subfraction A2-3-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 2 times) showed no definite spots. Thus, it was not further investigated.

Subfraction A2-3-4 (VR-JOY1) The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 2 times) showed one yellow spot with the R_f value of 0.65.

^1H NMR ($CDCl_3$) (δ ppm) (300 MHz)	10.98 (*s*, 1H), 6.81 (*d*, J = 3.0 Hz, 1H), 6.70 (*brs*, 1H), 6.58 (*d*, J = 3.0 Hz, 1H), 3.80 (*s*, 3H), 3.76 (*s*, 3H), 3.58 (*s*, 3H), 2.35 (*s*, 3H)

Subfraction A2-3-5 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 2 times) showed one major yellow spot with the R_f value of 0.65 and one minor yellow spot with the R_f value of 0.45. Because it was obtained in low quantity, it was not further investigated.

Subfraction A2-3-6 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 2 times) showed many spots without any major spots. Because it was obtained in low quantity, it was not further investigated.

Subfraction A2-4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 2 times) showed no definite spots. Thus, it was not further investigated.

Subfraction A2-5 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 2 times) showed one major yellow spot with the R_f value of 0.58 and two minor yellow spots with the R_f values of 0.70 and 0.65. It was further separated by column chromatography on silica gel. Elution was conducted initially with 5%

EtOAc/Petrol and gradually increased the polarity until 70% EtOAc/Petrol. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford five subfractions, as shown in **Table 6**.

Table 6 Subfractions obtained from **A2-5** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A2-5-1	0.0027	Yellow gum
A2-5-2	0.0021	Yellow gum
A2-5-3	0.0051	Colorless gum
A2-5-4	0.0024	White solid
A2-5-5	0.0008	Brown gum

Subfraction A2-5-1 The chromatogram on normal phase TLC (40% EtOAc/Petrol, 3 times) showed no definite spots. Thus, it was not further investigated.

Subfraction A2-5-2 The chromatogram on normal phase TLC (40% EtOAc/Petrol, 3 times) showed two major UV-active spots with the R_f values of 0.85 and 0.82. Because it was obtained in low quantity, it was not further investigated.

Subfraction A2-5-3 (VR-JOY2) The chromatogram on normal phase TLC (40% EtOAc/Petrol, 3 times) showed one yellow spot with the R_f value of 0.58.

UV λ_{max}nm (MeOH) (logε)	281 (3.63), 355 (1.35)
IR (neat) ν cm^{-1}	3414 (O-H stretching), 1715 (C=O stretching), 2923, 2852 (C-H stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)	7.03 (*d*, *J* = 2.5 Hz, 1H), 6.64 (*d*, *J* = 2.5 Hz, 1H), 3.72 (*s*, 3H), 3.71 (*s*, 3H), 2.51 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)	166.75, 158.66, 157.37, 142.32, 125.52, 113.14, 108.37, 103.61, 56.17, 52.48, 19.10

Subfraction A2-5-4 (VR-JOY3) The chromatogram on normal phase TLC (40% EtOAc/Petrol, 3 times) showed one UV-active spot with the R_f value of 0.67.

$[\alpha]^{29}_D$	+ 182° (c = 2.2x10^{-2} g/100 cm^3, MeOH)
UV λ_{max}nm (MeOH) (logε)	219 (3.22), 272 (2.97), 308 (2.49)
IR (KBr) ν cm^{-1}	3496 (O-H stretching), 2922, 2852 (C-H stretching), 1660 (C=O stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)	11.13 (*s*, 1H), 6.56 (*s*, 1H), 4.65 (*dq*, *J* = 2.5 and 7.0 Hz, 1H), 4.51 (*brs*, 1H), 3.96 (*s*, 3H), 3.91 (*s*, 3H), 1.58 (*d*, *J* = 7.0 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)	168.97, 158.80, 156.21, 136.93, 102.67, 102.66, 101.54, 78.26, 67.57, 60.78, 56.29, 16.00

Subfraction A2-5-5 The chromatogram on normal phase TLC (40% EtOAc/Petrol, 3 times) showed no definite spots. Thus, it was not further investigated.

Subfraction A2-6 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 2 times) showed many yellow spots without any major spots. Because it was obtained in low quantity, it was not further investigated.

Subfraction A2-7 , The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 2 times) showed one major yellow spot with the R_f value of 0.40 and two minor yellow spots with the R_f values of 0.61 and 0.45. It was further separated by column chromatography on silica gel. Elution was conducted initially with 10% EtOAc/Petrol and gradually increased the polarity until 90% EtOAc/Petrol. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford five subfractions, as shown in **Table 7**.

Table 7 Subfractions obtained from **A2-7** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A2-7-1	0.0006	Brown solid
A2-7-2	0.0007	Brown gum
A2-7-3	0.0012	Brown gum
A2-7-4	0.0135	White solid
A2-7-5	0.0075	Brown gum

Subfraction A2-7-1 The chromatogram on normal phase TLC (30% EtOAc/Petrol, 3 times) showed no definite spots. Thus, it was not further investigated.

Subfraction A2-7-2 The chromatogram on normal phase TLC (30% EtOAc/Petrol, 3 times) showed one identical yellow spot to that of **VR-JOY3**.

Subfraction A2-7-3 The chromatogram on normal phase TLC (30% EtOAc/Petrol, 3 times) showed no definite spots. Thus, it was not further investigated.

Subfraction A2-7-4 (VR-JOY4) The chromatogram on normal phase TLC (30% EtOAc/Petrol, 3 times) showed one yellow spot with the R_f value of 0.45.

^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	6.29 (*s*, 1H), 5.22 (*s*, 1H), 4.76 (*brs*, 1H), 4.45 (*dq*, J = 4.5 and 6.3 Hz, 1H), 3.04 (*dq*, J = 4.5 and 6.9 Hz, 1H), 2.10 (*s*, 3H), 1.35 (*d*, J = 6.3 Hz, 3H), 1.26 (*d*, J = 6.9 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)	147.90, 139.11, 137.97, 131.80, 112.07, 102.63, 87.58, 44.45, 20.88, 19.27, 11.37

Subfraction A2-7-5 The chromatogram on normal phase TLC (30% EtOAc/Petrol, 3 times) showed many yellow spots without any major spots. Because it was obtained in low quantity, it was not further investigated.

Subfraction A2-8 (VR-JOY5) The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 2 times) showed one major active UV-spot with the R_f value of 0.27.

$[\alpha]^{29}_D$	+ 11° (c = $2.8x10^{-2}$ g/100 cm^3, MeOH)
UV λ_{max}nm (MeOH) (logε)	274 (3.00)
IR (neat) ν cm^{-1}	3370 (O-H stretching), 2910, (C-H stretching), 1692 (C=O stretching), 1637, 1575 (C=C stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	6.82 (*qd*, *J* = 6.9 and 15.6 Hz, 1H), 6.40 (*dd*, *J* = 1.8 and 15.6 Hz, 1H), 5.98 (*brs*, 1H), 4.80 (*d*, *J* = 2.7 Hz, 1H), 4.23 (*d*, *J* = 2.7 Hz, 1H), 1.95 (*dd*, *J* = 1.8 and 6.9 Hz, 1H)
^{13}C NMR (Acetone-d_6) (δ ppm) (75 MHz)	204.14, 169.00, 139.85, 125.98, 125.24, 81.25, 76.89, 19.58

Subfraction A2-9 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 2 times) showed many yellow spots without any major spots. Therefore, it was not further investigated.

Fraction A3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 3 times) showed one major brown spot with the R_f value of 0.80. It was further separated by column chromatography on silica gel. Elution was conducted initially with 5% MeOH/ $CHCl_3$ and gradually increased the polarity until 50%MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford four subfractions, as shown in **Table 8**.

Table 8 Subfractions obtained from **A3** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A3-1	0.0006	Brown gum
A3-2	0.0377	Brown gum

Table 8 (Continued)

Subfraction	Weight (g)	Physical appearance
A3-3	0.0741	Brown solid
A3-4	0.0301	Black solid

Subfraction A3-1 The chromatogram on normal phase TLC (5%MeOH/$CHCl_3$, 3 times) showed no definite spots. Thus, it was not further investigated.

Subfraction A3-2 The chromatogram on normal phase TLC (5%MeOH/$CHCl_3$, 3 times) showed one major brown spot with the R_f value of 0.72. It was further separated by column chromatography on silica gel. Elution was conducted initially with 100% Petrol and gradually increased the polarity until 50%EtOAc/Petrol. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford five subfractions, as shown in **Table 9**.

Table 9 Subfractions obtained from **A3-2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A3-2-1	0.0245	Brown solid
A3-2-2	0.0011	Orange solid
A3-2-3	0.0004	Black gum
A3-2-4	0.0059	Colorless gum
A3-2-5	0.0110	Black gum

Subfraction A3-2-1 The chromatogram on normal phase TLC (25% EtOAc/Petrol, 3 times) showed no definite spots. Thus, it was not further investigated.

Subfraction A3-2-2 (VR-JOY6) The chromatogram on normal phase TLC (25% EtOAc/Petrol, 3 times) showed one yellow spot with the R_f value of 0.48.

UV λ_{max}nm (MeOH) (logε) 216 (3.93), 223 (5.14), 283 (4.60), 355 (4.26), 424 (4.22)

IR (KBr) ν cm^{-1}	3456 (O-H stretching), 2924, (C-H stretching), 1719, 1626 (C=O stretching), 1589 (C=C stretching)
^1H NMR ($CDCl_3$+CD_3OD) (δ ppm) (500 MHz)	13.24 (*s*, 1H), 7.54 (*brs*, 1H), 7.30 (*d*, *J* = 2.5 Hz, 1H), 7.08 (*brs*, 1H), 6.77 (*d*, *J* = 2.5 Hz, 1H), 4.01 (*s*, 3H), 2.43 (*s*, 3H)
^{13}C NMR ($CDCl_3$+CD_3OD) (δ ppm) (125 MHz)	186.52, 184.51, 163.58, 156.23, 152.15, 146.65, 137.23, 132.29, 124.67, 119.86, 115.50, 113.00, 107.41, 104.81, 56.35, 21.77
EIMS (*m*/z) (% rel. int.)	284 ($M^{+\cdot}$) (100), 266 (42), 255 (31), 238 (51), 210 (12), 197 (14), 181 (12), 128 (20)

Subfraction A3-2-3 The chromatogram on normal phase TLC (25% EtOAc/Petrol, 3 times) showed no definite spots. Thus, it was not further investigated.

Subfraction A3-2-4 The chromatogram on normal phase TLC (25% EtOAc/Petrol, 3 times) showed one brown spot with the R_f value of 0.19. The ^1H NMR spectrum indicated that it was **VR-JOY2**.

Subfraction A3-2-5 The chromatogram on normal phase TLC (25% EtOAc/Petrol, 3 times) showed one major active-UV spot with the R_f value of 0.09 . The ^1H NMR spectrum indicated that it was long chain hydrocarbons. Therefore, it was not further studied.

^1H NMR ($CDCl_3$) (δ ppm) (300 MHz)	3.70-3.95 (*m*), 3.50 (*brs*), 1.20 (*brs*)

Fraction A4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 3 times) showed one major yellow spot with the R_f value of 0.89 and one minor brown spot with the R_f value of 0.78. It was further separated by column chromatography on silica gel. Elution was conducted initially with 3% MeOH/$CHCl_3$ and gradually

increased the polarity until 60% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford four subfractions, as shown in **Table 10**.

Table 10 Subfractions obtained from **A4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A4-1	0.0035	Yellow gum
A4-2	0.0036	Yellow gum
A4-3	0.0034	Brown gum
A4-4	0.0885	Black solid

Subfraction A4-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 3 times) showed no definite spots. Thus, it was not further investigated.

Subfraction A4-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 3 times) showed one yellow spot with the R_f value of 0.67. The ^1H NMR spectrum indicated that it was **VR-JOY6**

Subfraction A4-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 3 times) showed one yellow spot with the R_f value of 0.64. The ^1H NMR spectrum indicated that it was **VR-JOY2**

Subfraction A5 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 3 times) showed many UV- active spots without any major spots. Thus, it was not further investigated.

2.3.2 Purification of the mycelial extract

The crude material (0.6493 g) was separated by column chromatography over Sephadex LH20. Elution was conducted with pure MeOH. Fractions with similar chromatogram were obtained and evaporated under reduced pressure to dryness to afford five subfractions, as shown in **Table 11**.

Table 11 Fractions obtained from the mycelial extract by column chromatography over Sephadex LH20

Fraction	Weight (g)	Physical appearance
B1	0.1216	Black gum
B2	0.2521	Black gum
B3	0.1087	Black gum
B4	0.0371	Brown gum
B5	0.1023	Black solid with black gum

Fraction B1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed many UV-active spots without any major spots. Thus, it was not further investigated.

Fraction B2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one UV-active spot with the R_f value of 0.25. It was further separated by column chromatography over Sephadex LH20 silica gel. Elution was conducted pure MeOH. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford four subfractions, as shown in **Table 12**.

Table 12 Subfractions obtained from **B2** by column chromatography over Sephadex LH20

Subfraction	Weight (g)	Physical appearance
B2-1	0.0855	Brown gum
B2-2	0.0345	Yellow gum
B2-3	0.0152	Yellow gum
B2-4	0.0895	Brown gum

Subfraction B2-1 The chromatogram on normal phase TLC (2%MeOH/$CHCl_3$, 4 times) showed one major active-UV spot. The ^1H NMR spectrum indicated that it might be a mixture of hydrocarbons. Thus, it was not further investigated.

^{1}H NMR ($CDCl_3$ + CD_3OD) (δ ppm) (300 MHz)	5.30-5.45 (*m*), 3.65 (*brs*), 2.70-2.90 (*m*), 2.20-2.35 (*m*), 1.95-2.10 (*m*), 1.50-1.70 (*m*), 1.15-1.45 (*m*), 0.80-0.95 (*m*)

Subfraction B2-2 The chromatogram on normal phase TLC (2% MeOH/$CHCl_3$, 4 times) showed one active-UV spot with the R_f value of 0.35. It was further separated by column chromatography on silica gel. Elution was conducted initially with 100%$CHCl_3$ and gradually increased the polarity until 50% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford three subfractions, as shown in **Table 13**.

Table 13 Subfractions obtained from **B2-2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
B2-2-1	0.0159	Yellow gum
B2-2-2	0.0012	White solid
B2-2-3	0.0133	Yellow gum

Subfraction B2-2-1 The chromatogram on normal phase TLC (2%MeOH/$CHCl_3$, 3 times) showed no definite spots. Thus, it was not further investigated.

Subfraction B2-2-2 The chromatogram on normal phase TLC (2% MeOH/$CHCl_3$, 3 times) showed one UV-active spot with the R_f value of 0.26. The ^{1}H NMR spectrum indicated that it was **VR-JOY5**.

Subfraction B2-2-3 The chromatogram on normal phase TLC (2% MeOH/$CHCl_3$, 3 times) showed one major UV-active spot. The ^{1}H NMR spectrum indicated that the major components might be a mixture of hydrocarbons.

^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	7.10 (*brs*), 6.70 (*brs*), 3.70-3.85(*m*), 2.50-2.60 (*m*), 1.60 (*brs*), 1.25 (*brs*), 0.80-1.00 (*m*)

Subfraction B2-3 The chromatogram on normal phase TLC (2% MeOH/$CHCl_3$, 4 times) showed one major brown spot with the R_f value of 0.69 and two minor UV-active spots with the R_f values of 0.49 and 0.35. It was further separated by column chromatography on silica gel. Elution was conducted initially with 100%$CHCl_3$ and gradually increased the polarity until 60% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford three subfractions, as shown in **Table 14**.

Table 14 Subfractions obtained from **B2-3** by column chromatography on silica gel

Subfraction	Weight (g)	Physical appearance
B2-3-1	0.0017	Yellow gum
B2-3-2	0.0010	Yellow gum
B2-3-3	0.0052	Yellow gum

Subfraction B2-3-1 The chromatogram on normal phase TLC (30% EtOAc/Petrol, 3 times) showed many UV- active spots without any major spots. Thus, it was not further investigated.

Subfraction B2-3-2 The chromatogram on normal phase TLC (30% EtOAc/Petrol, 3 times) showed one brown spot with the R_f value of 0.40. The ^1H NMR spectrum indicated that it was **VR-JOY6**.

Subfraction B2-3-3 The chromatogram on normal phase TLC (30% EtOAc/Petrol, 3 times) showed one major UV- active spot with the R_f value of 0.15 which was identified by TLC comparision to be **VR-JOY5**.

Subfraction B2-4 The chromatogram on normal phase TLC (2% MeOH/$CHCl_3$, 4 times) showed one major yellow spot with the R_f value of 0.55. The ^1H NMR spectrum indicated that it was a mixture of **VR-JOY5** and **VR-JOY6**.

Fraction B3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major yellow spot with the R_f value of 0.59 and two minor UV-active spots with the R_f values of 0.85 and 0.34. It was further separated by column chromatography on silica gel. Elution was conducted initially with 100%$CHCl_3$ and

gradually increased the polarity until 50% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford four subfractions, as shown in **Table 15**.

Table 15 Subfractions obtained from **B3** by column chromatography on silica gel

Subfraction	Weight (g)	Physical appearance
B3-1	0.0102	Brown gum
B3-2	0.0039	Yellow gum
B3-3	0.0052	Yellow gum
B3-4	0.0789	Brown gum

Subfraction B3-1 The chromatogram on normal phase TLC (2% MeOH/$CHCl_3$, 3 times) showed many UV- active spots without any major spots. Thus, it was not further investigated.

Subfraction B3-2 The chromatogram on normal phase TLC (2% MeOH/$CHCl_3$, 3 times) showed one major yellow spot with the R_f value of 0.45 and one minor UV-active spot with the R_f value of 0.36. It was further separated by precoated TLC, using 30% EtOAc/Petrol as a mobile phase (24 times), to afford two bands.

Band B3-2-1 It was obtained as a yellow gum (0.0010 g). The ^{1}H NMR spectrum indicated that it was **VR-JOY2**.

Band B3-2-2 It was obtained as a yellow gum (0.0004 g). The ^{1}H NMR spectrum indicated that it was a mixture. Because it was obtained in low quantity, it was not further investigated.

Subfraction B3-3 The chromatogram on normal phase TLC (2%MeOH/$CHCl_3$, 3 times) showed no definite spots. Thus, it was not further investigated.

Subfraction B3-4 The chromatogram on normal phase TLC (2%MeOH/$CHCl_3$, 3 times) showed one major active UV-spot with the R_f value of 0.23. The ^{1}H NMR spectrum indicated that it was a mixture of **VR-JOY5** and **VR-JOY6**

Fraction B4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major yellow spot with the R_f value of 0.38 and two minor UV-active spots with the R_f values of 0.26 and 0.13. It was further separated by column chromatography on silica gel. Elution was conducted initially with 20% EtOAc/Petrol and gradually increased the polarity until 80% EtOAc/Petrol. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford four subfractions, as shown in **Table 16**.

Table 16 Subfractions obtained from **B4** by column chromatography on silica gel

Subfraction	Weight (g)	Physical appearance
B4-1	0.0037	Brown solid
B4-2	0.0026	Yellow solid
B4-3	0.0035	Yellow solid
B4-4	0.0125	Brown solid

Subfraction B4-1 The chromatogram on normal phase TLC (30% EtOAc/Petrol, 5 times) showed no definite spots. Thus, it was not further investigated.

Subfraction B4-2 The chromatogram on normal phase TLC (30% EtOAc/Petrol, 5 times) showed one major yellow spot with the R_f value of 0.45. The ^1H NMR spectrum indicated that it was a mixture of **VR-JOY2** and long chain hydrocarbons.

Subfraction B4-3 The chromatogram on normal phase TLC (30% EtOAc/Petrol, 5 times) showed two active-UV spots with the R_f values of 0.35 and 0.19. Because it was obtained in low quantity, it was not further investigated.

Subfraction B4-4 The chromatogram on normal phase TLC (30% EtOAc/Petrol, 5 times) showed one major active-UV spot with the R_f value of 0.26. The ^1H NMR spectrum indicated that it was a mixture. Because it was obtained in low quantity. Thus, it was not further investigated.

Fraction B5 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$,) showed many UV-active spots without any major spots. Thus, it was not further investigated.

2.4 Chemical investigation of *Cordyceps militaris* BCC 2816

2.4.1 Purification of the broth extract

The crude material (2.25 g) was separated by column chromatography over Sephadex LH20. Elution was conducted with pure MeOH. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford five fractions, as shown in **Table 17**.

Table 17 Fractions obtained form the broth extract by column chromatography over Sephadex LH20

Fraction	Weight (g)	Physical appearance
C1	0.1546	Brown gum
C2	0.4784	Brown gum
C3	1.2547	Black gum
C4	0.2366	Brown gum
C5	0.0792	Brown gum

Fraction C1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Fraction C2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed three yellow spots with the R_f values of 0.65, 0.62 and 0.46 after dipping the TLC plate in ASA reagent and subsequently heating. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 10% MeOH/$CHCl_3$. Fractions with

similar chromatogram were combined and evaporated under reduced pressure to dryness to afford five subfractions, as shown in **Table 18.**

Table 18 Subfractions obtained from **C2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C2-1	0.0383	Yellow gum
C2-2	0.0856	Yellow gum
C2-3	0.0263	Yellow gum
C2-4	0.0783	Yellow gum
C2-5	0.2135	Brown gum

Subfraction C2-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction C2-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed two yellow spots with the R_f values of 0.62 and 0.58 after dipping the TLC plate in ASA reagent and subsequently heating. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure Petroleum ether and gradually increased the polarity until 80% EtOAc/Petrol. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford three subfractions, as shown in **Table 19.**

Table 19 Subfractions obtained from **C2-2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C2-2-1	0.0383	Yellow gum
C2-2-2	0.0256	White solid
C2-2-3	0.0123	Colorless gum

Subfraction C2-2-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction C2-2-2 (VRJOY7) The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.60 after dipping the TLC plate in ASA reagent and subsequently heating.

$[\alpha]^{29}_D$		+ 70° (c = 1.4×10^{-2} g/100 cm^3, MeOH)
IR (KBr) ν cm^{-1}		2991, 2963, 2922 (C-H stretching), 1771 (C=O stretching)
^{1}H NMR (CD_3OD) (δ ppm) (300 MHz)		5.24 (*dd*, *J* = 6.0 and 6.0 Hz, 1H), 4.91 (*dd*, *J* = 6.0 and 7.8 Hz, 1H), 4.10-4.20 (*m*, 1H), 2.85 (*dd*, *J* = 7.8 and 18.6 Hz, 1H), 2.50 (*d*, *J* = 18.6 Hz, 1H), 2.33 (*d*, *J* = 14.1 Hz, 1H), 2.21 (*dd*, *J* = 6.0 and 14.1 Hz, 1H), 2.09-2.16 (*m*, 1H), 1.98-2.08 (*m*, 1H), 1.85-1.92 (*m*, 1H), 1.38-1.45 (*m*, 1H), 1.16 (*d*, *J* = 6.0 Hz, 3H)
^{13}C NMR (CD_3OD) (δ ppm) (75 MHz)		177.50, 115.25, 84.21, 77.71, 74.84, 41.07, 37.28, 33.80, 30.97, 19.90
DEPT (135°) (CD_3OD)	CH	84.21, 77.71, 74.84
	CH_2	41.07, 37.28, 33.80, 30.97
	CH_3	19.90
TOF MS (*m*/z) (% rel. int.)		221 $(M+Na)^+$ (60), 199 $(M+H)^+$ (100)

Subfraction C2-2-3 (VRJOY8) The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.58 after dipping the TLC plate in ASA reagent and subsequently heating.

$[\alpha]^{29}_D$		-56° (c = $1.8x10^{-2}$ g/100 cm^{-2}, MeOH)
IR (neat) ν cm^{-1}		2991, 2963, 2922 (C-H stretching), 1771 (C=O stretching)
^{1}H NMR ($CDCl_3$+CD_3OD) (δ ppm) (300 MHz)		5.03 (*ddd*, *J* = 2.1, 4.5 and 6.6 Hz, 1H), 4.72 (*dd*, *J* = 4.5 and 5.7 Hz, 1H), 4.07-4.18 (*m*, 1H), 2.70 (*dd*, *J* = 5.7 and 18.3 Hz, 1H), 2.65 (*d*, *J* = 18.3 Hz, 1H), 2.44 (*dd*, *J* = 6.6 and 15.0 Hz, 1H), 2.25 (*dd*, *J* = 2.1 and 15.0 Hz, 1H), 2.02-2.10 (*m*, 1H), 1.88-1.93 (*m*, 1H), 1.60-1.71 (*m*, 1H), 1.88-2.01 (*m*, 1H), 1.20 (*d*, *J* = 6.0 Hz, 3H)
^{13}C NMR ($CDCl_3$+CD_3OD) (δ ppm) (75 MHz)		175.75, 115.41, 83.79, 76.76, 76.52, 42.00, 36.86, 35.91, 32.31, 22.68
DEPT (135°) ($CDCl_3$+CD_3OD)	CH	83.79, 76.76, 76.52
	CH_2	42.00, 36.86, 35.91, 32.31
	CH_3	22.68
TOF MS (*m*/z) (% rel. int.)		221 $(M+Na)^+$ (25), 199 $(M+H)^+$ (100)

Subfraction C2-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction C2-4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major yellow spot with the R_f value of 0.42 and two minor brown spots with the R_f values of 0.39 and 0.35 after dipping the TLC plate in ASA reagent and subsequently heating. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure Petrol and gradually increased the polarity until 80% EtOAc/Petrol. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford three subfractions, as shown in **Table 20**.

Table 20 Subfractions obtained from **C2-4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C2-4-1	0.0285	Yellow gum
C2-4-2	0.0456	Colorless gum
C2-4-3	0.0123	Yellow gum

Subfraction C2-4-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction C2-4-2 (VRJOY9) The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.58 after dipping the TLC plate in ASA reagent and subsequently heating.

$[\alpha]^{29}_D$		+68° (c = 2.9×10^{-2} g/100 cm^3, MeOH)
UV λ_{max}nm (MeOH) (logε)		249 (3.52), 273 (2.83), 355 (2.16)
IR (neat) ν cm^{-1}		3466 (O-H stretching), 2942, 2930 (C-H stretching), 1735, 1728 (C=O stretching)
^{1}H NMR ($CDCl_3$+CD_3OD) (δ ppm) (300 MHz)		5.04-5.15 (*m*, 1H), 4.17 (*ddd*, *J* = 3.0 Hz, 10.0 and 12.0, 1H), 3.37-3.44 (*m*, 1H), 2.88 (*dd*, *J* = 3.0 and 9.0 Hz, 1H), 2.81 (*dd*, *J* = 6.0 and 18.0 Hz, 1H), 2.70 (*dd*, *J* = 3.0 and 18.0 Hz, 1H), 2.41 (*dd*, *J* = 12.0 and 18.0 Hz, 1H), 2.32-2.36 (*m*, 2H), 2.00-2.15 (*m*, 2H), 1.27 (*d*, *J* = 6.0 Hz, 3H)
^{13}C NMR ($CDCl_3$+CD_3OD) (δ ppm) (75 MHz)		213.94, 169.62, 74.34, 72.16, 68.48, 43.81, 40.42, 39.20, 33.45, 19.29
DEPT (135°) ($CDCl_3$+CD_3OD)	CH	74.34, 72.16, 68.48
	CH_2	43.81, 40.42, 39.20, 33.45
	CH_3	19.29
TOF MS (*m*/z) (% rel. int.)		239 $(M+Na)^+$ (100), 221 $(M+H)^+$ (30)

Subfraction C2-4-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed many brown spots without any major spots after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it might be a mixture of long chain hydrocarbons. Thus, it was not further investigated.

^1H NMR ($CDCl_3$) (δ ppm) (300 MHz)	5.30-5.45 (*m*), 3.65 (*brs*), 2.70-2.85 (*m*), 2.22-2.35 (*m*), 1.90-2.10 (*m*), 1.50-1.70 (*m*), 1.15-1.45 (*m*), 0.82-0.95 (*m*)

Subfraction C2-5 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Fraction C3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed three major yellow spots with the R_f values of 0.65, 0.62 and 0.46 and one minor yellow spot with the R_f value of 0.56 after dipping the TLC plate in ASA reagent and subsequently heating. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 10% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford five subfractions, as shown in **Table 21**.

Table 21 Subfractions obtained from **C3** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C3-1	0.2766	Yellow gum
C3-2	0.0463	Yellow gum
C3-3	0.0523	Yellow gum

Table 21 (Continued)

Subfraction	Weight (g)	Physical appearance
C3-4	0.2365	Brown gum
C3-5	0.5132	Brown gum

Subfraction C3-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction C3-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.68 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was **VRJOY7**

Subfraction C3-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.58 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was **VRJOY8**.

Subfraction C3-4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major yellow spot with the R_f value of 0.46 and one minor yellow spot with the R_f value of 0.54 after dipping the TLC plate in ASA reagent and subsequently heating. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 40%MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford four subfractions, as shown in **Table 22**.

Table 22 Subfractions obtained from **C3-4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C3-4-1	0.0563	Yellow gum
C3-4-2	0.0023	Yellow gum

Table 22 (Continued)

Subfraction	Weight (g)	Physical appearance
C3-4-3	0.0837	Yellow gum
C3-4-4	0.1986	Brown gum

Subfraction C3-4-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction C3-4-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major yellow spot with the R_f value of 0.59 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was a mixture of **VRJOY8** and an aromatic compound. Further purification was performed on precoated TLC, using 10% EtOAc/Petrol as mobile phase (29 times) to afford two bands.

Band C3-4-2-1 It was obtained as a pale yellow gum (0.0007 g). The ^1H NMR spectrum indicated that it was **VRJOY8**.

Band C3-4-2-2 It was obtained as a pale yellow gum (0.0014 g). The ^1H NMR spectrum indicated that it was a mixture of **VRJOY8** and an aromatic compound. Because it was obtained in low quantity, it was not further investigated.

Subfraction C3-4-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.42 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was **VRJOY9**.

Subfraction C3-4-4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction C3-5 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed many brown spots without any major spots after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it might be a mixture of long chain hydrocarbons. Thus, it was not further investigated.

^{1}H NMR ($CDCl_3$ + CD_3OD) (δ ppm) (300 MHz)	3.40-3.90 (*m*), 2.60 (*brs*), 1.58-2.40 (*m*), 1.10-1.45 (*m*), 0.80-1.00 (*m*)

Fraction C4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major UV-active spot with the R_f value of 0.14 and three minor yellow spots with the R_f values of 0.69, 0.67 and 0.51 after dipping the TLC plate in ASA reagent and subsequently heating. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 65% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford five subfractions, as shown in **Table 23**.

Table 23 Subfractions obtained from **C4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C4-1	0.0383	Yellow gum
C4-2	0.0753	Yellow gum
C4-3	0.0123	Yellow gum
C4-4	0.0963	Yellow gum
C4-5	0.135	Brown gum

Subfraction C4-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction C4-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed two yellow spots with the R_f values of 0.67 and 0.65 after dipping the TLC plate in ASA reagent and subsequently heating. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 25% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford three subfractions, as shown in **Table 24**.

Table 24 Subfractions obtained from **C4-2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C4-2-1	0.0297	Colorless gum
C4-2-2	0.0162	Colorless gum
C4-2-3	0.0463	Colorless gum

Subfraction C4-2-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.65 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was **VRJOY7**.

Subfraction C4-2-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.62 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was **VRJOY8**.

Subfraction C4-2-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction C4-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major yellow spot with the R_f value of 0.48 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was **VRJOY9**.

Subfraction C4-4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major UV-active spot with the R_f value of 0.15. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 55% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford three subfractions, as shown in **Table 25**.

Table 25 Subfractions obtained from **C4-4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C4-4-1	0.0136	Yellow gum
C4-4-2	0.0365	White solid
C4-4-3	0.0281	Yellow gum

Subfraction C4-4-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction C4-4-2 (VRJOY10) The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one UV-active spot with the R_f value of 0.12.

$[\alpha]^{29}_D$ λ_{max}		+ 38° (c = $5.3x10^{-2}$ g/100 cm^3, MeOH)
UV λ_{max}nm (MeOH) (logε)		260 (2.55)
IR (KBr) ν cm^{-1}		3225 (O-H stretching), 2945, 2923 (C-H stretching), 1623 (C=N stretching)
^{1}H NMR (CD_3OD) (δ ppm) (500 MHz)		8.42 (*s*, 1H), 8.20 (*s*, 1H), 5.95 (*d*, *J* = 3.0 Hz, 1H), 4.71 (*ddd*, *J* = 3.0, 3.0 and 6.0, 1H), 4.52 (*tdd*, *J* = 3.0, 6.0 and 9.0 Hz, 1H), 3.92 (*dd*, *J* = 3.0 and 12.0 Hz, 1H), 3.66 (*dd*, *J* = 3.0 and 12.0 Hz, 1H), 2.37 (*ddd*, *J* = 3.0, 6.0 and 12.0 Hz, 1H), 2.05 (*ddd*, *J* = 6.0, 9.0 and 12.0 Hz, 1H)
^{13}C NMR (CD_3OD) (δ ppm) (125 MHz)		157.39, 153.87, 149.87, 141.14, 120.65, 93.57, 82.56, 76.58, 64.17, 34.47
DEPT (135°) ($CDCl_3$+CD_3OD)	CH	153.87, 141.14, 93.57, 82.56, 76.58
	CH_2	64.17, 34.47
EIMS (*m*/z) (% rel. int.)		179 (M- CH_2O)$^+$ (13), 164 (55), 136 (100), 108 (19)

Subfraction C4-4-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed many spots without any major spots after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was a mixture of long chain hydrocarbons. Thus, it was not further investigated.

^1H NMR ($CDCl_3$) (δ ppm) (300 MHz)	5.30-5.45 (*m*), 2.70-2.85 (*m*), 2.70-2.85 (*m*), 2.30-2.40 (*m*), 1.95-2.10 (*m*), 1.60-1.75 (*m*), 1.15-1.45 (*m*), 0.82-0.95 (*m*)

Subfraction C4-5 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed many spots without any major spots after dipping the TLC plate in ASA reagent and subsequently heating. Because it was obtained in low quantity, it was not further studied.

Fraction C5 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

2.4.2 Purification of the mycelial extract

The crude material (0.579 g) was separated by column chromatography over Sephadex LH20. Elution was conducted with pure MeOH. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford seven fractions, as shown in **Table 26**.

Table 26 Fractions obtained form the mycelial extract by column chromatography over Sephadex LH20

Fraction	Weight (g)	Physical appearance
D1	0.0863	Yellow gum

Table 26 (Continued)

Fraction	Weight (g)	Physical appearance
D2	0.1261	Yellow gum
D3	0.2456	Brown gum
D4	0.0931	Brown gum
D5	0.0349	Yellow gum
D6	0.0358	Yellow gum with white solid
D7	0.8520	Brown gum

Fraction D1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Fraction D2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major brown spot with the R_f value of 0.07 and three minor yellow spots with the R_f values of 0.67, 0.65 and 0.50 after dipping the TLC plate in ASA reagent and subsequently heating. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 65% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford six subfractions, as shown in **Table 27**.

Table 27 Subfractions obtained from **D2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
D2-1	0.0186	Yellow gum
D2-2	0.0331	Colorless gum
D2-3	0.0179	Colorless gum
D2-4	0.0193	Colorless gum

Table 27 (Continued)

Subfraction	Weight (g)	Physical appearance
D2-5	0.0448	Yellow gum
D2-6	0.0163	Yellow gum

Subfraction D2-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction D2-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed two yellow spots with the R_f values of 0.68 and 0.66 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was a mixture of **VRJOY7** and **VRJOY8**. Thus, it was not further purified.

Subfraction D2-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major yellow spot with the R_f value of 0.49 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was **VRJOY9**.

Subfraction D2-4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed many spots without any major spots after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it might be a mixture of long chain hydrocarbons. Thus, it was not further investigated.

^1H NMR ($CDCl_3$) (δ ppm) (300 MHz)	5.30-5.45 (*m*), 4.10-4.20 (*m*), 3.30-3.95 (*m*), 3.60-3.70 (*m*), 3.40 (*s*), 2.75-2.80 (*m*), 2.30-2.45 (*m*), 1.95-2.10 (*m*), 1.40-1.75 (*m*), 1.15-1.45 (*m*), 0.82-0.95 (*m*)

Subfraction D2-5 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major brown spot with the R_f value of 0.09. The ^1H NMR

spectrum indicated that it was not pure. It was further separated by column chromatography over Sephadex LH20. Elution was conducted with pure MeOH. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford three fractions, as shown in **Table 28**.

Table 28 Subfractions obtained from **D2-5** by column chromatography over Sephadex LH20

Subfraction	Weight (g)	Physical appearance
D2-5-1	0.0136	Yellow gum
D2-5-2	0.0365	White solid with yellow gum
D2-5-3	0.0281	Yellow gum

Subfraction D2-5-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction D2-5-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one brown spot with the R_f value of 0.09 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was not pure. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 35% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford three subfractions, as shown in **Table 29**.

Table 29 Subfractions obtained from **D2-5-2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
D2-5-2-1	0.0065	Yellow gum
D2-5-2-2	0.0163	White solid

Table 29 (Continued)

Subfraction	Weight (g)	Physical appearance
D2-5-2-3	0.0132	Yellow gum

Subfraction D2-5-2-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction D2-5-2-2 (VR-JOY11) The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one brown spot with the R_f value of 0.09 after dipping the TLC plate in ASA reagent and subsequently heating.

$[\alpha]^{29}_D$		-55° (c = $3.6x10^{-2}$ g/100 cm^3, MeOH)
IR (KBr) ν cm^{-1}		3348 (O-H stretching), 2944, 2917 (C-H stretching), 1720 (C=O stretching)
^1H NMR (CD_3OD) (δ ppm) (300 MHz)		5.76 (*dd*, *J* = 3.0 and 16.0 Hz, 1H), 5.63 (*ddd*, *J* = 1.2, 8.1 and 16.0 Hz, 1H), 4.72-4.82 (*m*, 1H), 4.61-4.65 (*m*, 1H), 4.08-4.15 (*m*, 1H), 2.53 (*dd*, *J* = 3.9 and 12.0 Hz, 1H), 2.48 (*dd*, *J* = 3.6 and 12.0 Hz, 1H), 1.91-2.00 (*m*, 1H), 1.71-1.82 (*m*, 1H), 1.62-1.70 (*m*, 1H), 1.53-1.61 (*m*, 1H), 1.14 (*d*, *J* = 6.0 Hz, 3H)
^{13}C NMR (CD_3OD) (δ ppm) (75 MHz)		170.25, 132.97, 130.31, 74.25, 72.87 66.78, 43.98, 36.99, 31.34, 20.60
DEPT (135°) (CD_3OD)	CH	132.97, 130.31, 74.25, 72.87, 66.78
	CH_2	43.98, 36.99, 31.34
	CH_3	20.60
TOFMS (*m*/z) (% rel. int.)		201 $(M+H)^+$ (4), 183 (40), 182 (100) 167 (13), 165 (23), 164 (17), 158 (100)

Subfraction D2-5-2-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major brown spot with the R_f value of 0.09 and one minor brown spot with the R_f value of 0.08 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it contained **VR-JOY11**

Subfraction D2-5-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction D2-6 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed many spots without any major spots after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it might be a mixture of fatty acids. Thus, it was not further investigated.

^1H NMR ($CDCl_3$) (δ ppm) (300 MHz)	5.30-5.45 (*m*), 5.00-5.10 (*m*), 4.10-4.15 (*m*), 3.60-3.70 (*m*), 2.75-2.85 (*m*), 2.50-2.60 (*m*), 2.30-2.40 (*m*), 2.00-2.10 (m), 1.50-1.70 (*m*), 1.20-1.45 (*m*), 0.82-0.95 (*m*)

Fraction D3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed three major yellow spots with the R_f values of 0.67, 0.65 and 0.50 and one minor brown spot with the R_f value of 0.09 after dipping the TLC plate in ASA reagent and subsequently heating. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 55% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford four subfractions, as shown in **Table 30**.

Table 30 Subfractions obtained from **D3** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
D3-1	0.0163	Yellow gum

Table 30 (Continued)

Subfraction	Weight (g)	Physical appearance
D3-2	0.0651	Yellow gum
D3-3	0.0262	Yellow gum
D3-4	0.0753	Blown gum

Subfraction D3-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction D3-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed two yellow spots with the R_f values of 0.66 and 0.65 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was a mixture of **VRJOY7** and **VRJOY8**.

Subfraction D3-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.49. The ^1H NMR spectrum indicated that it contained **VRJOY9**. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 15% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford three subfractions, as shown in **Table 31**.

Table 31 Subfractions obtained from **D3-3** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
D3-3-1	0.0013	Yellow gum
D3-3-2	0.0159	Colorless gum
D3-3-3	0.0121	Yellow gum

Subfraction D3-3-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction D3-3-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.46. The ^1H NMR spectrum indicated that it contained **VRJOY9**. Further purification was performed on precoated TLC, using 30% EtOAc/Petroleum ether as mobile phase (36 times) to afford two bands.

Band D3-3-2-1 It was obtained as a colorless gum (0.0035 g). The ^1H NMR spectrum indicated that it was not pure. Further purification was performed on precoated TLC, using 20% EtOAc/Petroleum ether as mobile phase (63 times) to afford two bands.

Band D3-3-2-1-1 It was obtained as a colorless gum (0.0007 g). The ^1H NMR spectrum indicated that it was **VR-JOY12**.

$[\alpha]^{29}_D$		+59° (c = $1.7x10^{-2}$ g/100 cm^{-3}, MeOH)
IR (neat) ν cm^{-1}		3462 (O-H stretching), 2931 (C-H stretching), 1740, 1732 (C=O stretching)
^1H NMR ($CDCl_3$) (δ ppm) (500 MHz)		5.05-5.12 (*m*, 1H), 4.11 (*ddd*, *J* = 3.0, 9.0 and 12.0 Hz, 1H), 3.43 (*s*, 3H), 3.35 (*ddd*, *J* = 3.0, 7.5 and 9.0 Hz, 1H), 2.93 (*dd*, *J* = 7.5 and 18.0 Hz, 1H), 2.86 (*dd*, *J* = 3.0 and 18.0 Hz, 1H), 2.63 (*dd*, *J* = 3.0 and 18.0 Hz, 1H), 2.44 (*dd*, *J* = 12.0 and 18.0 Hz, 1H), 2.42 (*ddd*, *J* = 3.5, 6.5 and 13.5 Hz, 1H), 2.33 (*ddd*, *J* = 3.5, 11.0 and 13.5 Hz, 1H), 2.07-2.16 (*m*, 1H), 1.98-2.06 (*m*, 1H), 1.25 (*d*, *J* = 6.0 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		208.56, 169.19, 81.92, 71.64, 68.28, 57.38, 41.69, 40.35, 39.74, 33.15, 19.64
DEPT (135°) ($CDCl_3$+CD_3OD)	CH	81.92, 71.64, 68.28
	CH_2	41.69, 40.35, 39.74, 33.15

	CH_3	57.38, 19.64
EIMS (*m*/z) (% rel. int.)		198 (M-CH_3OH) (1), 170 (3), 142 (27), 127 (15), 111 (25), 101 (100), 83 (35), 71 (13), 55 (33)

Band D3-3-2-1-2 It was obtained as a colorless gum (0.0016 g). The ^1H NMR spectrum indicated that it was **VRJOY9**.

Band D3-3-2-2 It was obtained as a colorless gum (0.0063 g). The ^1H NMR spectrum indicated that it was **VRJOY9**.

Subfraction D3-4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major brown spot with the R_f value of 0.07 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it contained **VR-JOY11**. Thus, it was not further purified.

Fraction D4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed three major yellow spots with the R_f values of 0.66, 0.65 and 0.49 after dipping the TLC plate in ASA reagent and subsequently heating. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 50% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford five subfractions, as shown in **Table 32**.

Table 32 Subfractions obtained from **D4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
D4-1	0.0033	Yellow gum
D4-2	0.0093	Colorless gum
D4-3	0.0026	Colorless gum
D4-4	0.0359	Colorless gum
D4-5	0.0681	Brown gum

Subfraction D4-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction D4-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed two yellow spots with the R_f values of 0.67 and 0.65 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was a mixture of **VRJOY7** and **VRJOY8**. Thus, it was not further purified.

Subfraction D4-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.65 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was **VRJOY8**.

Subfraction D4-4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.47 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it contained **VRJOY9**. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 45% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford three subfractions, as shown in **Table 33**.

Table 33 Subfractions obtained from **D4-4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
D4-4-1	0.0029	Colorless gum
D4-4-2	0.0152	Colorless gum
D4-4-3	0.0096	Colorless gum

Subfraction D4-4-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction D4-4-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major yellow spot with the R_f value of 0.46. The ^1H NMR spectrum indicated that it was not pure. Further purification was performed on precoated TLC, using 20% EtOAc/Petrol as mobile phase (43 times) to afford two bands.

Band D4-4-2-1 It was obtained as a colorless gum (0.0059 g). The ^1H NMR spectrum indicated that it was not pure. Further purification was performed on precoated TLC, using 20% EtOAc/Petrol as mobile phase (58 times) to afford two bands.

Band D4-4-2-1-1 (VR-JOY13) It was obtained as a colorless gum (0.0043 g) with the R_f value of 0.53.

$[\alpha]^{29}_D$		-67° (c = 1.5×10^{-2} g/100 cm^{-3}, MeOH)
IR (neat) ν cm^{-1}		3464 (O-H stretching), 2931 (C-H stretching), 1735, 1729 (C=O stretching)
^1H NMR ($CDCl_3$) (δ ppm) (500 MHz)		5.00-5.07 (*m*, 1H), 4.28 (*ddd*, *J* = 3.0, 3.0 and 11.0 Hz, 1H), 3.98 (*ddd*, *J* = 3.0, 4.5 and 11.0 Hz, 1H), 3.41 (*s*, 3H), 2.81 (*dd*, *J* = 4.5 and 18.0 Hz, 1H), 2.70 (*dd*, *J* = 3.0 and 18.0 Hz, 1H), 2.57 (*dd*, *J* = 11.0 and 18.0 Hz, 1H), 2.51 (*dd*, *J* = 11.0 and 18.0 Hz, 1H), 2.41 (*ddd*, *J* = 3.0, 11.0 and 13.0 Hz, 1H), 2.30 (*ddd*, *J* = 3.0, 7.5 and 13.0 Hz, 1H), 1.96-2.02 (*m*, 1H), 2.05-2.13 (*m*, 1H), 1.23 (*d*, *J* = 6.0 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		209.72, 169.86, 78.84, 71.96, 66.68, 57.37, 42.89, 40.49, 37.43, 33.98, 19.51
DEPT (135°) ($CDCl_3$)	CH	78.84, 71.96, 66.68
	CH_2	42.89, 40.49, 37.43, 33.98
	CH_3	57.37, 19.51

EIMS (*m*/z) (% rel. int.)	198 (M-CH_3OH) (3), 180 (4), 170 (3), 154 (10), 142 (34), 127 (20), 111 (40), 101 (100)

Band D4-4-2-1-2 It was obtained as a colorless gum (0.0011 g). The ^{1}H NMR spectrum indicated that it was **VRJOY9**.

Band D4-4-2-2 It was obtained as a colorless gum (0.0072 g). The ^{1}H NMR spectrum indicated that it was **VRJOY9**.

Subfraction D4-4-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$, 1 times) showed many spots without any major spots after dipping the TLC plate in ASA reagent and subsequently heating. The ^{1}H NMR spectrum indicated that it was a mixture of long chain hydrocarbons. Thus, it was not further purified.

Subfraction D4-5 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed many spots without any major spots after dipping the TLC plate in ASA reagent and subsequently heating. The ^{1}H NMR spectrum indicated that it was a mixture of long chain hydrocarbons. Thus, it was not further purified.

^{1}H NMR ($CDCl_3$ + CD_3OD) (δ ppm) (300 MHz)	5.60-5.80 (*m*), 4.85-5.00 (*m*), 4.65-4.75 (*m*), 4.20-4.30 (*m*), 2.50-2.70 (*m*), 1.55-2.10 (*m*), 1.20-1.30 (*m*)

Fraction D5 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major UV-active spot with the R_f value of 0.14 and many minor pink spots. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 60% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford three subfractions, as shown in **Table 34**.

Table 34 Subfractions obtained from **D5** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
D5-1	0.0053	Colorless gum
D5-2	0.0186	White solid
D5-3	0.0072	Colorless gum

Subfraction D5-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction D5-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one UV-active spot with the R_f value of 0.13. The ^1H NMR spectrum indicated that it was **VRJOY10**.

Subfraction D5-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed many pink spots without any major spots after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it might be a mixture of long chain hydrocarbons. Thus, it was not further purified.

^1H NMR ($CDCl_3$) (δ ppm) (300 MHz)	4.90-5.30 (*m*), 4.15-4.30 (*m*), 2.85-2.95 (*m*), 2.70-2.80 (*m*), 2.30-2.50 (*m*), 1.90-2.20 (*m*), 1.40-1.80 (*m*), 1.20-1.40 (*m*)

Fraction D6 (VR-JOY14) The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major UV-active spot with the R_f value of 0.01 and many minor pink spots, chloroform was added to dissolve a yellow gum to afford a white solid (0.0086 g). The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major pink spot with the R_f value of 0.26 after dipping the TLC plate in ASA reagent and subsequently heating.

UV λ_{max}nm (MeOH) (logε)	205 (3.49), 263 (3.06), 269 (3.09)
IR (KBr) ν cm^{-1}	2800-3400 (O-H stretching),
	1706 (C=O stretching)
^{1}H NMR (CD_3OD) (δ ppm)	8.34 (*d*, *J* = 7.8 Hz, 2H),
(300 MHz)	8.19 (*t*, *J* = 7.8 Hz, 1H)
^{13}C NMR (CD_3OD) (δ ppm)	165.86, 147.47, 139.41,
(75 MHz)	127.64

The chloroform soluble part (0.0231 g) was further purified by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 70% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford three subfractions, as shown in **Table 35**.

Table 35 Subfractions obtained from chloroform soluble part of **D6** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
D6-1	0.0009	Yellow gum
D6-2	0.0132	Yellow gum
D6-3	0.0056	Yellow gum

Subfraction D6-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction D6-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed three pink spots with the R_f values of 0.45, 0.41 and 0.23 after dipping the TLC plate in ASA reagent and subsequently heating. The ^{1}H NMR spectrum indicated that it was a mixture of long chain hydrocarbons. Thus, it was not further purified.

^{1}H NMR ($CDCl_3$) (δ ppm)	4.90-5.40 (*m*), 3.10-4.50 (*m*),
(300 MHz)	2.70-2.80 (*m*), 2.30-2.40 (*m*),
	1.90-2.20 (*m*), 1.40-1.70 (*m*),
	0.70-1.00 (*m*)

Subfraction D6-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed many pink spots without any major spots after dipping the TLC plate in ASA reagent and subsequently heating. Because it was obtained in low quantity, it was not further investigated.

Fraction D7 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed many spots without any major spots after dipping the TLC plate in ASA reagent and subsequently heating. The ^{1}H NMR spectrum indicated that it was a mixture of long chain hydrocarbons. Thus, it was not further purified.

^{1}H NMR ($CDCl_3$) (δ ppm)	5.60 (*brs*), 4.40 (*brs*), 4.00 (*s*),
(300 MHz)	3.60-3.80 (*m*), 1.90-2.50 (*m*),
	0.75-1.60 (*m*)

2.5 Chemical investigation of *Cordyceps militaris* BCC 2819

2.5.1 Purification of the broth extract

The crude material (0.7380 g) was separated by column chromatography over Sephadex LH20. Elution was conducted with pure MeOH. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford five fractions, as shown in **Table 36**.

Table 36 Fractions obtained form the broth extract by column chromatography over Sephadex LH20

Fraction	Weight (g)	Physical appearance
E1	0.0759	Brown gum
E2	0.1532	Brown gum
E3	0.2851	Brown gum
E4	0.0936	Brown gum
E5	0.0831	Brown gum

Fraction E1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated

Fraction E2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed three yellow spots with the R_f values of 0.67, 0.65 and 0.48 and one brown spot with the R_f value of 0.07 after dipping the TLC plate in ASA reagent and subsequently heating. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 60% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford four subfractions, as shown in **Table 37**.

Table 37 Subfractions obtained from **E2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
E2-1	0.0237	Yellow gum
E2-2	0.0674	Colorless gum
E2-3	0.0452	Colorless gum
E2-4	0.0265	Yellow gum

Subfraction E2-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction E2-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed two yellow spots with the R_f values of 0.68 and 0.67 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was a mixture of **VRJOY7** and **VRJOY8**. Thus, it was not further purified.

Subfraction E2-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major yellow spot with the R_f value of 0.49 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was **VRJOY9**.

Subfraction E2-4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major brown spot with the R_f value of 0.08 and one minor brown spot with the R_f value of 0.12 after dipping the TLC plate in ASA reagent and subsequently heating. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 65% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford three subfractions, as shown in **Table 38**.

Table 38 Subfractions obtained from **E2-4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
E2-4-1	0.0032	Yellow gum
E2-4-2	0.0851	White solid
E2-4-3	0.0136	Yellow gum

Subfraction E2-4-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction E2-4-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one brown spot with the R_f value of 0.08 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was **VR-JOY11**.

Subfraction E2-4-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one brown spot with the R_f value of 0.13 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it might be a mixture of long chain hydrocarbons. Thus, it was not further investigated.

^1H NMR ($CDCl_3$) (δ ppm) (300 MHz)	5.30-5.45 (*m*), 2.70-2.90 (*m*), 2.30-2.40 (*m*), 1.95-2.15 (*m*), 1.50-1.70 (*m*), 1.20-1.45 (*m*), 0.80-0.95 (*m*)

Fraction E3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed four yellow spots with the R_f values of 0.67, 0.65, 0.48 and 0.08 after dipping the TLC plate in ASA reagent and subsequently heating. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 60% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford five subfractions, as shown in **Table 39**.

Table 39 Subfractions obtained from **E3** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
E3-1	0.0363	Yellow gum
E3-2	0.0856	Colorless gum
E3-3	0.0263	Colorless gum
E3-4	0.0792	Colorless gum
E3-5	0.0598	Brown gum

Subfraction E3-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction E3-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed two yellow spots with the R_f values of 0.67 and 0.65 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was a mixture of **VRJOY7** and **VRJOY8**. Thus, it was not further purified.

Subfraction E3-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction E3-4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.47 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was **VRJOY9**.

Subfraction E3-5 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.08 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was not pure. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 60% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford three subfractions, as shown in **Table 40**.

Table 40 Subfractions obtained from **E3-5** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
E3-5-1	0.0093	Yellow gum
E3-5-2	0.0293	Yellow gum
E3-5-3	0.0175	Yellow gum

Subfraction E3-5-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction E3-5-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.09 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was not pure. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 20% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford four subfractions, as shown in **Table 41**.

Table 41 Subfractions obtained from **E3-5-2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
E3-5-2-1	0.0061	Yellow gum
E3-5-2-2	0.0096	Yellow gum
E3-5-2-3	0.0118	Yellow gum
E3-5-2-4	0.0056	Yellow gum

Subfraction E3-5-2-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction E3-5-2-2 (VR-JOY15) The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.09 after dipping the TLC plate in ASA reagent and subsequently heating.

$[\alpha]^{29}_D$	-52° (c = 1.9×10^{-2} g/100 cm^3, MeOH)
UV λ_{max}nm (MeOH) (logε)	268 (4.96)
IR (neat) ν cm^{-1}	3400-2900 (O-H stretching),

	2971, 2930 (C-H stretching),
	1713 (C=O stretching)
^{1}H NMR ($CDCl_3$) (δ ppm)	6.05 (*d*, *J* = 3.0 Hz, 1H), 5.92 (*d*, *J* = 3.0
(300 MHz)	Hz, 1H), 3.83 (*sextet*, *J* = 6.0 Hz, 1H),
	3.53 (*s*, 2H), 2.65-2.70 (*m*, 2H),
	1.69-1.78, (*m*, 2H), 1.18 (*d*, *J* = 6.0 Hz,
	3H)
^{13}C NMR ($CDCl_3$) (δ ppm)	175.37, 154.81, 147.96, 107.40, 105.15,
(75 MHz)	66.37, 37.04, 35.00, 23.93, 22.02

Subfraction E3-5-2-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major yellow spot with the R_f value of 0.09 after dipping the TLC plate in ASA reagent and subsequently heating. The ^{1}H NMR spectrum indicated that it contained **VR-JOY15**. Thus, it was not further purified.

Subfraction E3-5-2-4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed many spots without any major spots after dipping the TLC plate in ASA reagent and subsequently heating. The ^{1}H NMR spectrum indicated that it was a mixture of long chain hydrocarbons. Thus, it was not further investigated.

^{1}H NMR ($CDCl_3$) (δ ppm)	4.70-4.90 (*brs*), 3.40-3.50 (*m*),
(300 MHz)	1.00-2.20 (*m*)

Subfraction E3-5-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequent heating. Thus, it was not further investigated.

Fraction E4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed four major yellow spots with the R_f values of 0.67, 0.65, 0.48 and 0.08 and one minor pink spot with the R_f value of 0.26 after dipping the TLC plate in ASA reagent and subsequently heating. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure $CHCl_3$ and gradually increased the polarity until 55% MeOH/$CHCl_3$. Fractions with similar chromatogram were

combined and evaporated under reduced pressure to dryness to afford four subfractions, as shown in **Table 42**.

Table 42 Subfractions obtained from **E4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
E4-1	0.0165	Yellow gum
E4-2	0.0235	Colorless gum
E4-3	0.0169	Colorless gum
E4-4	0.0395	Yellow gum

Subfraction E4-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction E4-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed two yellow spots with the R_f values of 0.66 and 0.65 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was a mixture of **VRJOY7** and **VRJOY8**. Thus, it was not further purified.

Subfraction E4-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major yellow spot with the R_f value of 0.47 and one minor pink spot with the R_f value of 0.26 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was a mixture of **VRJOY9** and long chain hydrocarbons. Thus, it was not further purified.

Subfraction E4-4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.07 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was a mixture of **VR-JOY15** and long chain hydrocarbons. Thus, it was not further purified.

Fraction E5 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed many pink spots without any major spots after dipping the TLC plate in ASA

reagent and subsequently heating. The ^{1}H NMR spectrum indicated that it might be a mixture of long chain hydrocarbons. Thus, it was not further purified.

^{1}H NMR ($CDCl_3$ + CD_3OD) (δ ppm) (300 MHz)	5.00-5.15 (*m*), 3.90-4.70(*m*), 3.60-3.85 (*m*), 2.20-2.80 (*m*), 1.90-2.20 (*m*), 0.80-1.45 (*m*)

2.5.2 Purification of the mycelial extract

The crude material (0.428 g) was separated by column chromatography over Sephadex LH20. Elution was conducted with pure MeOH. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford five fractions, as shown in **Table 43**.

Table 43 Fractions obtained form the mycelial extract by column chromatography over Sephadex LH20.

Fraction	Weight (g)	Physical appearance
F1	0.0756	Yellow gum
F2	0.1568	Yellow gum
F3	0.0813	Yellow gum
F4	0.0796	Yellow gum
F5	0.0124	Yellow gum

Fraction F1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Fraction F2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed four yellow spots with the R_f values of 0.65, 0.63, 0.45 and 0.07 after dipping the TLC plate in ASA reagent and subsequently heating. It was further separated by column chromatography on silica gel. Elution was conducted initially with pure

$CHCl_3$ and gradually increased the polarity until 65% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford five subfractions, as shown in **Table 44**.

Table 44 Subfractions obtained from **F2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
F2-1	0.0298	Yellow gum
F2-2	0.0651	Yellow gum
F2-3	0.0262	Yellow gum
F2-4	0.0254	Yellow gum
F2-5	0.0115	Yellow gum

Subfraction F2-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction F2-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed two yellow spots with the R_f values of 0.64 and 0.63 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was a mixture of **VRJOY7** and **VRJOY8**. Thus, it was not further purified.

Subfraction F2-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one yellow spot with the R_f value of 0.43 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was **VRJOY9**.

Subfraction F2-4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one major yellow spot with the R_f value of 0.06 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it was a mixture of **VR-JOY15** and long chain hydrocarbons. Thus, it was not further purified.

Subfraction F2-5 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed many brown spots without any major spots after dipping the

TLC plate in ASA reagent and subsequently heating. The ^{1}H NMR spectrum indicated that it might be a mixture of long chain hydrocarbons. Thus, it was not further investigated.

^{1}H NMR ($CDCl_3$ + CD_3OD) (δ ppm) (300 MHz)	5.80-6.00 (*m*), 5.50-5.55(*m*), 5.05-5.10 (*m*), 4.60-4.90 (*m*), 4.45-4.55 (*m*), 2.50-2.65 (*m*), 1.20-2.10 (*m*)

Fraction F3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed many brown spots without any major spots after dipping the TLC plate in ASA reagent and subsequently heating. The ^{1}H NMR spectrum indicated that the mixture contained no major component. Thus, it was not further investigated.

Fraction F4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed three green spots with the R_f values of 0.51, 0.47 and 0.32 after dipping the TLC plate in ASA reagent and subsequently heating. It was further separated by column chromatography on silica gel. Elution was conducted initially pure 100% $CHCl_3$ and gradually increased the polarity until 45% MeOH/$CHCl_3$. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford four subfractions, as shown in **Table 45**.

Table 45 Subfractions obtained from **F4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
F4-1	0.0102	Yellow gum
F4-2	0.0358	Yellow gum
F4-3	0.0235	Yellow gum
F4-4	0.0278	Yellow gum

Subfraction F4-1 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed no definite spots after dipping the TLC plate in ASA reagent and subsequently heating. Thus, it was not further investigated.

Subfraction F4-2 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one green spot with the R_f value of 0.49 after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it might be a mixture of long chain hydrocarbons.

^1H NMR ($CDCl_3$) (δ ppm)	4.70-4.90 (*brs*), 3.40-3.50 (*m*),
(300 MHz)	1.00-2.20 (*m*)

Subfraction F4-3 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one green spot with the R_f value of 0.46 after dipping the TLC plate in ASA reagent and subsequently heating. The same ^1H NMR spectrum as subfraction F4-2 indicated that it might be a mixture of long chain hydrocarbons.

^1H NMR ($CDCl_3 + CD_3OD$) (δ ppm)	5.80-6.00 (*m*), 5.50-5.55(*m*), 5.05-5.10
(300 MHz)	(*m*), 4.60-4.90 (*m*), 4.45-4.55 (*m*),
	2.50-2.65 (*m*), 1.20-2.10 (*m*)

Subfraction F4-4 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed one green spot with the R_f value of 0.30 after dipping the TLC plate in ASA reagent and subsequently heating. The same ^1H NMR spectrum as **subfraction F4-2** indicated that it might be a mixture of long chain hydrocarbons.

^1H NMR ($CDCl_3 + CD_3OD$) (δ ppm)	5.00-5.15 (*m*), 3.90-4.70(*m*), 3.60-3.85
(300 MHz)	(*m*), 2.20-2.80 (*m*), 1.90-2.20 (*m*),
	0.80-1.45 (*m*)

Fraction F5 The chromatogram on normal phase TLC (5% MeOH/$CHCl_3$) showed many pink spots without any major spots after dipping the TLC plate in ASA reagent and subsequently heating. The ^1H NMR spectrum indicated that it might be a mixture of long chain hydrocarbons. Thus, it was not further purified.