CHAPTER III

EXPERIMENTAL

As a part of our study on chemical constituents from Thai plants which had interesting structure and/or exhibited bioactivity, we have investigated the bioactivities of crude extracts of seven Thai plants.

The screening test of the activities of the seven species are shown in **Table 3.1**. Upon the screening of bioactivities, dichloromethane extract from root of *Caesalpinia pulcherrima* and hexane, ethyl acetate extracts from root of *Acronychia pedunculata* displayed both antituberculous activity against *Mycobacterium tuberculosis* and anti-tumor with KB and BC.

Therefore, in this work, we focused on isolation and purification of the dichloromethane extract from root of *Caesalpinia pulcherrima* and hexane and ethyl acetate extract from root of *Acronychia pedunculata* to search for some chemical constituents which exhibited bioactivities, especially for antituberculous and cytotoxic activities with KB and BC cell lines as well as possessing interesting molecular structure.

Table 3.1 Screening test of bioactivities of crude extracts from some Thai plants

Plants	Plant part	Crude extract	% yield of crude extract	Antituberculous Activity (MIC; µg/ml)	Cytotoxic activities ED_{50} (µg/ml)		Antimalarial activity ED_{50} (µg/ml)	Antifungal activity ED_{50} (µg/ml)
					KB	BC		
Hydrolea Zeylanica (L.) Vahl.	flower	CH_2Cl_2	6.32	inactive	inactive	inactive	inactive	inactive
		MeOH	6.79	inactive	inactive	inactive	inactive	inactive
Polygonum odoratum Lour.	leave	CH_2Cl_2	4.02	inactive	inactive	inactive	inactive	inactive
		MeOH	4.57	inactive	inactive	inactive	inactive	inactive
	stem	CH_2Cl_2	9.27	200	inactive	inactive	inactive	inactive
		MeOH	10.21	inactive	inactive	inactive	inactive	inactive
Coleus amboinicus Lour.	leave	CH_2Cl_2	5.77	200	inactive	inactive	inactive	inactive
		MeOH	2.84	inactive	inactive	inactive	inactive	inactive
	stem	CH_2Cl_2	2.92	200	inactive	inactive	inactive	inactive
		MeOH	5.74	inactive	inactive	inactive	inactive	inactive

Table 3.1 Screening test of bioactivities of crude extracts from some Thai plants (cont.)

Plants	Plant part	Crude extract	% yield of crude extract	Antituberculous Activity (MIC; µg/ml)	Cytotoxic activities ED_{50} (µg/ml)		Antimalarial activity ED_{50} (µg/ml)	Antifungal activity ED_{50} (µg/ml)
					KB	BC		
Millettia brandisiana Kurz.	stem	CH_2Cl_2	0.40	inactive	inactive	inactive	inactive	inactive
		MeOH	0.95	inactive	inactive	inactive	inactive	inactive
	stem bark	CH_2Cl_2	1.48	200	inactive	inactive	inactive	inactive
		MeOH	2.36	inactive	inactive	inactive	inactive	inactive
Caesalpinia mimosoides Lamk.	stem	CH_2Cl_2	1.16	inactive	inactive	inactive	inactive	inactive
		MeOH	3.59	inactive	inactive	inactive	inactive	inactive
Caesalpinia pulcherrima	root	CH_2Cl_2	3.10	25	18.57	14.62	inactive	inactive
		MeOH	3.33	inactive	inactive	inactive	inactive	inactive
Acronychia pedunculata	root	Hexane	0.15	200	2.69	2.08	1.60	inactive
		EtOAc	0.54	200	2.56	2.20	2.10	inactive
		MeOH	1.48	inactive	inactive	inactive	inactive	inactive

3.1 Plant materials

Root of *Acronychia pedunculata* were collected in May 2001 from Chiang Mai Province.

Root (without rootbark) of *Caesalpinia pulcherrima* were collected in June 2001 from Lumphun Province.

3.2 General method

3.2.1 Gel Filtration Column Chromatography (GFC)

For Gel Filtration Column Chromatography, porous particles was used as stationary phase. In this work, Sephadex LH-20 (Code Number 17-0090-01) was employed as packed material and methanol as eluent. ^{1}H-NMR spectroscopy was used for following almost eluate fractions. The Sephadex LH-20 column could be regenerated for further use by washing with enough volume of MeOH.

3.2.2 Classical Column Chromatography (CCC)

Classical column liquid chromatography was packed by the slurry method. Silica gel 60 for column chromatography, mesh size 0.0633-0.200 mm, E.Merck was used as absorbent. Distillated solvents were used as mobile phase. There were two types of sample loading in this experiment; a portion of sample was dissolved in a small amount of mobile phase, or else mixed with a small quantity of absorbent, dried under vacuum before adding to the top of the column. The fractions of eluate were examined by ^{1}H-NMR spectroscopy together with Thin Layer Chromatography that used UV light and developing agents for spots detector. Those fractions which showed similar chromatographic patterns were combined prior to further purification stage.

3.2.3 Flash Column Chromatography (FCC)

Flash column chromatography was carried out using the adaptation of classical column chromatography by applying the pressure from the air pump to the head of column. In a typical separation, the column was packed by silica gel 60 for thin layer chromatography, mesh size < 0.200 mm by slurry method. The suitable ratio of absorbent to sample loaded and size of column were important factors for the good separation.

3.2.4 Detection of Chromatographic plate

a) Ultraviolet light (UV) at 254 nm

b) Chromatogenic agents

p-Anisaldehyde reagent (12.5 ml conc. sulphuric acid was added to a solution of 9.23 ml of anisaldehyde in 3.8 ml acetic acid and 340 ml ethanol). After spraying, the plate was heated until the spots attained maximum color intensity. Terpenoid compounds exhibited purple pink or pink spots.

3.2.5 Removing solvents from samples

Solvents were removed under reduced pressure using the rotary evaporator (BUCHI) followed by evacuation using the vacuum pump to remove the last traces of solvents.

3.2.6 Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H-NMR spectra were recorded on a Varian EM306L spectrometer operating at 60 MHz, using deuterated solvent for NMR spectroscopy with tetramethylsilane as internal standard, at the department of Chemistry, Faculty of Science, Chiang Mai University.

The high resolution of one-dimensional ^{1}H-NMR (400 MHz), ^{13}C-NMR (100 MHz) and DEPT (135) spectra and two-dimensional NMR, including ^{1}H-^{1}H COSY, NOESY and proton-detected heteronuclear correlations in HMQC and HMBC spectra were recorded on the Bruker DRX 400, operating at 400 MHz for proton and 100 MHz for carbon, at the National Center for Genetic Engineering and Biotechnology (BIOTEC) /National Science and Technology Development Agency (NSTDA), Bangkok.

3.2.7 Mass Spectrometry

The electrospray-ion time of flight mass spectra were recorded on Perkin Elmer (Marinner) mass spectrometer at National Center for Genetic Engineering and Biotechnology (BIOTEC)/National Science and Technology Development Agency (NSTDA), Bangkok.

3.2.8 Infrared Spectroscopy (IR)

Infrared spectra were determined on a FT-IR Spectrometer (Nicolet 510) at the Department of Chemistry, Faculty of science, Chiang Mai University

3.2.9 Ultraviolet Spectroscopy (UV)

UV spectra were measured using UV-Vis spectrophotometer, Hewlett packard, HP8452 A, using absolute ethanol as a solvent in a quartz cell, path length 1 cm at Department of Chemistry, Faculty of science, Chiang Mai University.

3.2.10 Polarimeter

Optical rotations were measured in chloroform solution with sodium D Line (589 nm) on JASCO DIP-370 digital polarimeter at the Department of

Chemistry, Faculty of Science, Mahidol University. The measuring of optical rotation values were repeated for ten times.

3.2.11 Melting point

Melting points were determined by Electrothermal Melting Point apparatus. The temperatures were uncorrected.

3.2.12 Bioactivity Assays

In vitro antituberculous activity against *Mycobacterium tuberculosis* H37Ra of Microplate Alamar Blue Assay (MABA) method, cytotoxic activity of cancer cell lines, including KB, BC and NCI-H187 cell lines, antiplasmodial activity against *Plasmodium falciparum,* K1 strain of Microculture Radioisotope Technique and antifungal activity against *Candida albicans* were conducted by unit of bioassay lab at the National Center for Genetic Engineering and Biotechnology (BIOTEC)/National Science and Technology Development Agency (NSTDA), Bangkok.

3.3 Extraction, Isolation and Purification of *Caesalpinia pulcherrima*

3.3.1 Extraction

Air dried root of *Caesalpinia pulcherrima* (without rootbark) 561.99 g was ground and macerated in dichloromethane for 2 days at room temperature, followed by filtration. This process was repeated three times. The filtrates was combined and evaporated to dryness to give bright yellow viscous liquid as crude extract 17.43 g (3.10 % yield by weight from dried material). After that, the plant was macerated again in methanol followed by the same procedure giving dark brown viscous liquid 18.73 g (3.33 % yield by weight from dried material)

3.3.2 Isolation and Purification of RHCA

3.3 2.1 The dichloromethane crude extract (17.43 g) was subjected to gel filtration column chromatography (GFC).

Stationary phase : Sephadex LH-20 200 g

Mobile phase : MeOH

Diameter of column : 2 inches

The crude extract was dissolved in small volume of MeOH-CH_2Cl_2 (95:5,v/v) and then it was added to the column. Twenty fractions (code : C1-C20), approximately 40 ml./fraction were collected and evaporated to dryness.

3.3.2.2. Fraction C7 5.67 g was re-subjected to gel filtration column chromatography. Fifteen fractions (code : C7.1-C7.15), approximely 30 ml/fraction were obtained.

Stationary phase : Sephadex LH-20 200 g

Mobile phase : MeOH

Diameter of column : 2 inches

3.3.2.3. The separation of fraction C7.8 2.37 g was carried out using flash column chromatographic technique (FCC). It was triturated with silica gel.This mixture was dried and then loaded to the column.

Stationary phase : Silica gel 80 g

Mobile phase : gradient elution, first with CH_2Cl_2-EtOAc (9.5:0.5) and finally with CH_2Cl_2-EtOAc (1:1) by increasing 5% EtOAc

Diameter of column : 3 inches

Sixty-six fractions were collected, about 15 ml/fraction, and fractions having similar chromatographic pattern were combined to furnish 12 fractions. (code : C7.8.1-C7.8.12)

3.3.2.4. Fractions C7.8.4 (0.5666 g) and C7.8.5 (0.8033 g) were combined, then further purified by flash column chromatography.

Stationary phase : Silica gel 40 g

Mobile phase : three solvents system ;first with Hexane : CH_2Cl_2 : EtOAc = 1: 0.5 : 0.25 then Hexane : CH_2Cl_2 : EtOAc = 1: 0.75 : 0.25, finally with Hexane : CH_2Cl_2 : EtOAc = 1: 1 : 0.25

Diameter of column : 1.25 inches

After fractionation, Eighty-five fractions (Code : CC 1-CC85) were eluted from the column. The volume of each fractions were approximately 10 ml, except for fractions 20-23 (code : CC20-CC23) which were 3 ml each.

3.3.2.5. Fractions CC 20 (53.5 mg), CC 21-22 (77.1 mg) and CC 23 (96.1 mg) after drying , yielded pale yellow solid. Three fractions were further purified by dissolving in small volume of $CHCl_3$, 2-3 droplets of methanol were added. The solid obtained upon standing was washed with methanol. After drying, the coloress solid (code : RHCA) 0.182 g was obtained.

3.3.3 Isolation and Purification of RHCB

3.3.3.1. Fractions CC 40-CC 60 (from item 3.3.2.4) 467.2 mg and C7.8.6 (from item 3.3.2.) 465.5 mg were combined and purified by classical column chromatography (CCC). Fitty-two fractions were eluated , 20 ml of each, were grouped into 15 fractions. (code : CD1-CD15)

Stationary phase : Silica gel 110 g

Mobile phase : gradient elution; first with Hexane : CH_2Cl_2 : EtOAc = 1: 0.25 : 0.25 , finally with Hexane : CH_2Cl_2 : EtOAc = 1: 0.5 : 0.25

Diameter of column : 0.75 inches

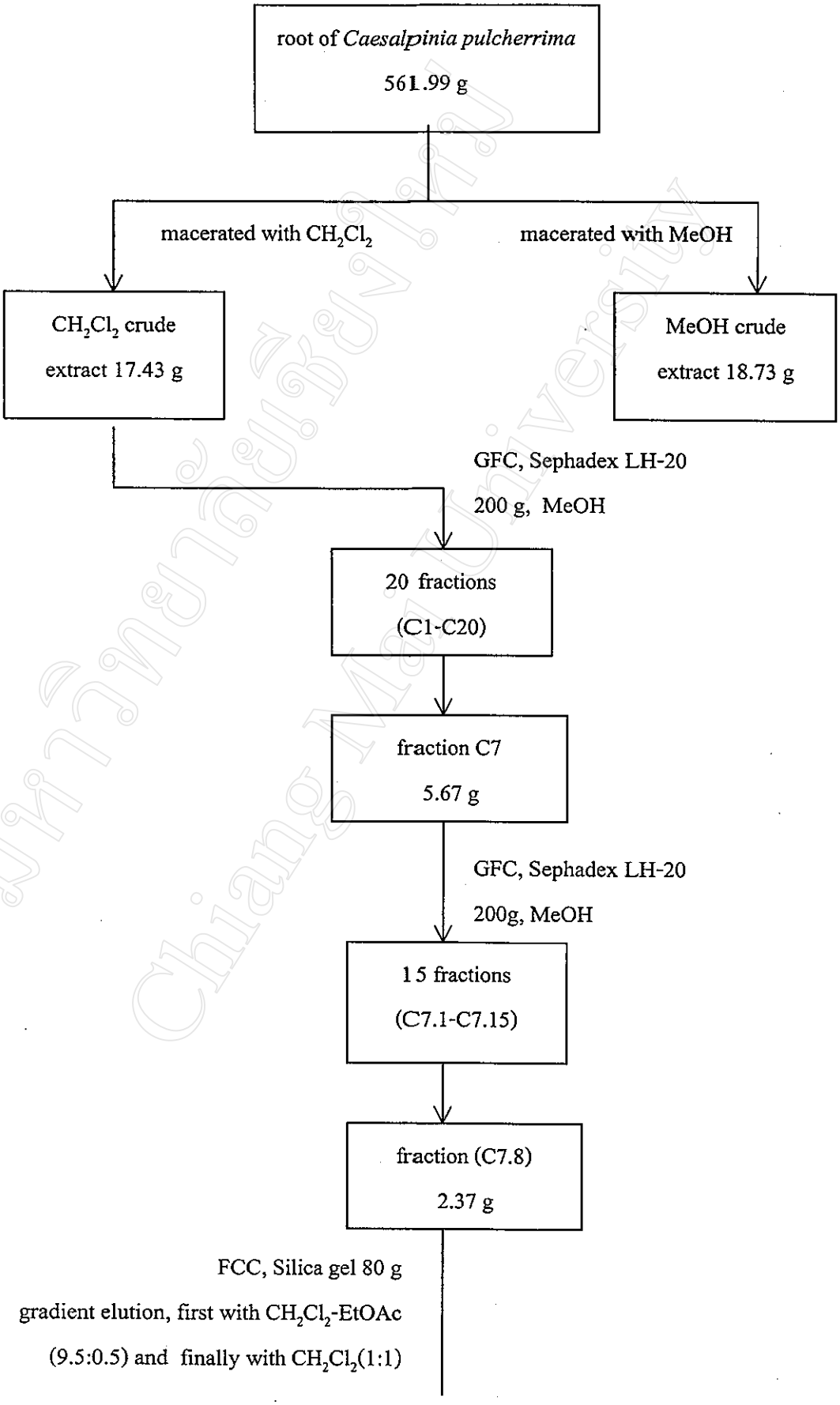
3.3.3.2. According to procedure in 3.3.3.1, fractions coded CD6 (70 mg), CD7 (30 mg), CD8 (26 mg) and CD9 (17 mg) were white solid. After dissolving with small volume of $CHCl_3$ and 2-3 droplets of methanol , finally washed the solid with methanol . The coloress solid (code : RHCB) 0.126 g was obtained.

We followed the fractions from each separation by using Thin-Layer Chromatography together with one-dimentional ^{1}H-NMR Spectroscopy. The TLC method was monitored using *p*-anisaldehyde reagent. These techniques helped to find some compounds having interesting structure. The process of extraction, isolation and purification of RHCA and RHCB were concluded as shown in **Fig. 3.1** and **Fig. 3.2,** respectively.

These compounds were subjected to structural elucidation using one- and two-dimentional NMR Spectroscopy, Infrared Spectroscopy, UV Spectroscopy and Mass Spectrometry.

RHCA : Colorless solid. m.p.193-195 °C, $[\alpha]^{30}_{D}$ +18.4 ($CHCl_3$, c 0.977 %). UV λ_{max} 223 and 276 nm. TOF-MS *m/z* 439.2486 (M^++H, 100%). IR ν_{max} 3520, 3050, 1710, 1600, 1500, 1460, 1290, 1130 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ_H: 8.05 (2H, *d*, *J* = 7.9 Hz), 7.59 (1H, *t*, *J* = 7.7 Hz), 7.46 (2H, *dd*, *J* = 7.5, 7.6 Hz), 7.25 (1H, *br s*), 6.20 (1H, *br s*), 5.82 (1H, *d*, *J* = 4.1 Hz), 4.43 (1H, *dd*, *J* = 4.1, 11.1 Hz), 3.04 (1H, *m*), 2.58 (2H, *d*, *J* = 8.5 Hz), 2.04 (1H, *ddd*, *J* = 5.1, 11.5, 11.9 Hz), 1.70 (2H, *br d*, *J* = 11.0 Hz), 1.55 (2H), 1.50 (2H), 1.52 (3H, *s*), 1.50 (1H), 1.19 (3H, *s*), 1.16 (1H), 1.12 (3H, *s*) and 1.08 (3H, *d*, *J* = 6.9 Hz). ^{13}C-NMR ($CDCl_3$) δc: 17.1, 17.6, 18.0, 21.8, 25.5, 27.4, 27.8, 35.0, 37.8, 38.9, 39.2, 41.0, 69.1, 72.9, 74.0, 109.8, 122.3, 128.8, 129.8, 130.0, 133.1, 140.5, 149.0, 167.5.

RHCB : Colorless solid. m.p. 213-215 °C (lit.[10] 218-221 °C), $[\alpha]^{30}_{D}$ +53.4 ($CHCl_3$, c 1.1.04 %). UV λ_{max} 220 and 278 nm. TOF-MS *m/z* 465.5 (M^++H, 100%). IR ν_{max} 3510, 3050, 1715, 1650, 1580, 1500, 1470, 1300, 1200 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ_H: 7.74 (1H, *d*, *J* = 16.1 Hz), 7.53 (2H, *m*), 7.40 (3H, *m*), 7.26 (1H, *br s*), 6.47 (1H, *d*, *J* = 16.1 Hz), 6.28 (1H, *d*, *J* = 1.3 Hz), 5.68 (1H, *d*, *J* = 4.1 Hz), 4.40 (1H, *dd*, *J* = 3.8, 11.0 Hz), 3.06 (1H, *m*), 2.56 (2H, *d*, *J* = 8.4 Hz), 2.45 (1H, *m*), 2.05 (1H, *ddd*, *J* = 5.0, 11.5, 11.6 Hz), 1.72 (2H, *br d*, *J* = 11.6 Hz), 1.69 (1H), 1.54 (1H), 1.51 (1H), 1.47 (3H, *s*), 1.24 (3H, *s*), 1.18 (1H), 1.11 (3H, *s*), 1.09 (3H, *d*, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$) δc: 17.1, 17.2, 18.1, 21.7, 25.5, 27.3, 27.7, 34.9, 37.1, 37.8, 37.9, 39.2, 41.1, 69.1, 73.5, 77.7, 109.7, 117.9, 121.9, 128.2, 128.9, 130.5, 134.1, 140.4, 145.9, 149.2, 167.4.



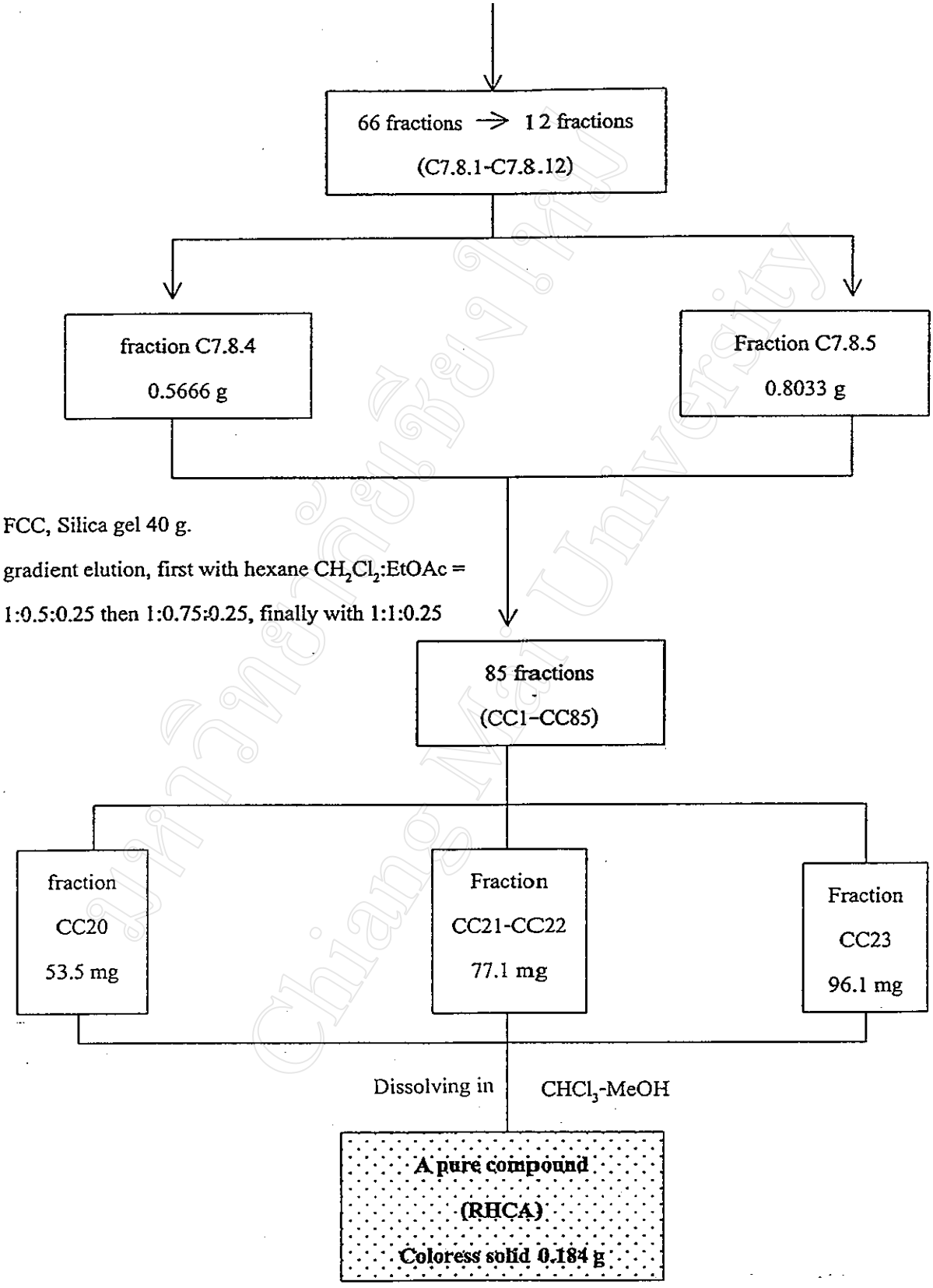
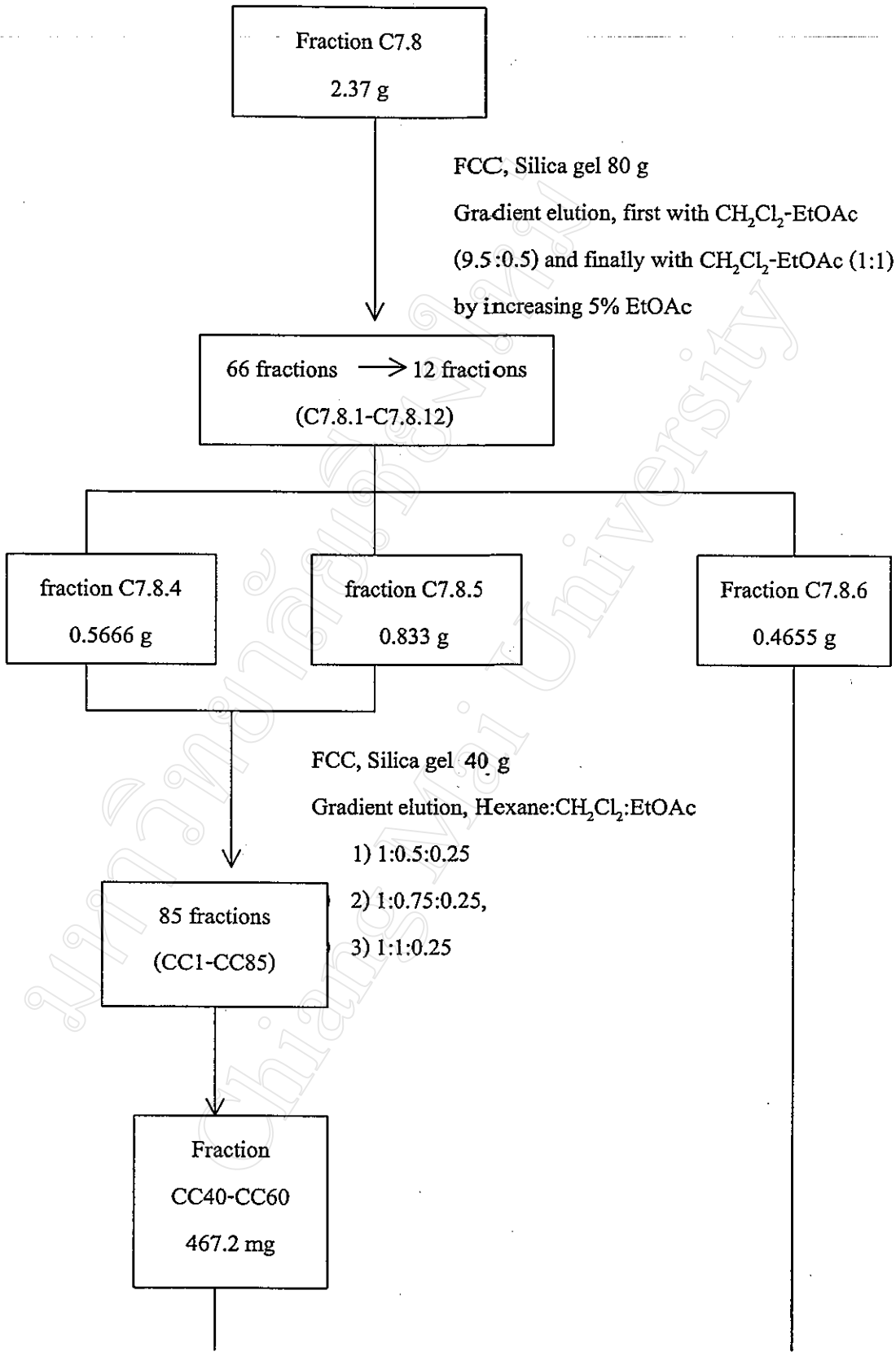


Figure 3.1 Isolation and purification of RHCA


Fraction C7.8
2.37 g
FCC, Silica gel 80 g
Gradient elution, first with CH_2Cl_2-EtOAc
(9.5:0.5) and finally with CH_2Cl_2-EtOAc (1:1)
by increasing 5% EtOAc
66 fractions → 12 fractions
(C7.8.1-C7.8.12)
fraction C7.8.4
0.5666 g
fraction C7.8.5
0.833 g
Fraction C7.8.6
0.4655 g
FCC, Silica gel 40 g
Gradient elution, Hexane:CH_2Cl_2:EtOAc
1) 1:0.5:0.25
2) 1:0.75:0.25,
3) 1:1:0.25
85 fractions
(CC1-CC85)
Fraction
CC40-CC60
467.2 mg

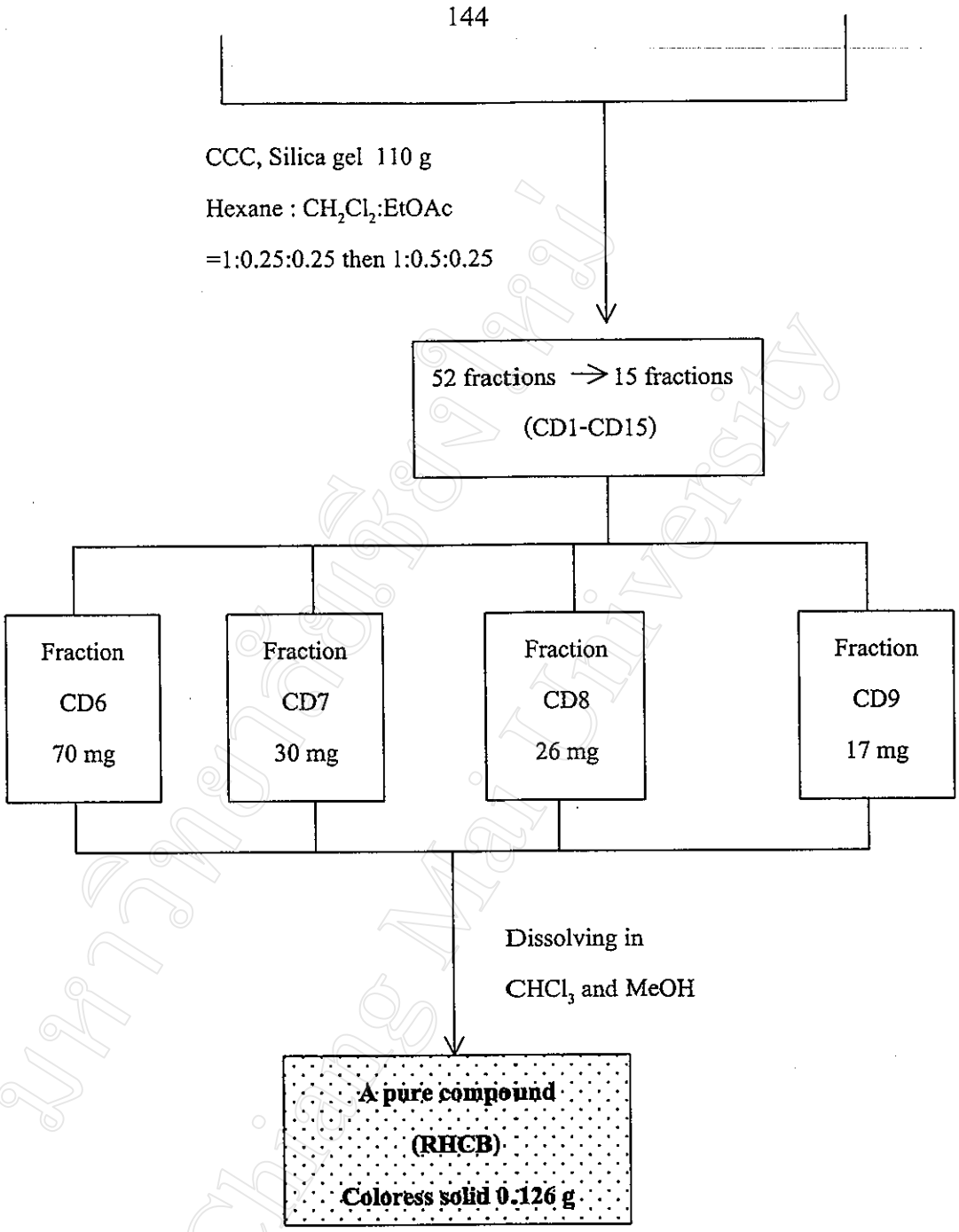


Figure 3.2 Isolation and purification of RHCB

3.4 Extraction, Isolation and Purification of *Acronychia pedunculata*

3.4.1 Extraction

Air dried root of *Acronychia pedunculata* 1016.29 g were ground and macerated in dichloromethane for 2 days at room temperature, followed by filtration. This process was repeated three times. After filtration and evaporation, dark brown viscous liquid 11.23 g were obtained (1.11 % yield by weight from dried material). And this plant was macerated again with MeOH to give dark red solid 15.06 g (1.48 % yield by weight from dired material).

The dichloromethane extract was partitioned. It was dissolved in 15% MeOH/H_2O and then extracted with hexane and EtOAc in separating funnel for 3-4 times. After evaporated the upper layer of hexane extract yielded hexane crude extract as yellow brown viscous liquid 1.52 g. Ethyl acetate extract was obtained as dark brown viscous liquid 5.45 g.

The extraction process was summarised as shown in **Fig. 3.3.**

3.4.2 Isolation and purification of SCHE1

3.4.2.1 The hexane extract (1.92 g) was subjected to gel filtration column chromatography. The extract was dissolved in small volume of MeOH-CH_2Cl_2 (97:3,v/v) and added to the top of column. Seventeen fractions, about 40 ml of each (code : A1-A17) were collected and evaporated to dryness.

Stationary phase : Sephadex LH-20 200 g

Mobile phase : MeOH

Diameter of column : 1.5 inches

3.4.2.2 Fraction A7 (621.8 mg) and fraction A8 (86.1 mg) were combined together and followed by gel filtration column chromatography to give 19 fractions (20 ml/fraction) which were coded A7.8.1-A7.8.19.

Stationary phase : Sephadex LH-20 100 g

Mobile phase : MeOH

Diameter of column : 1 inch

3.4.2.3 The EtOAc extract 3.20 g was isolated by flash column chrmatography. Forty-four fractions were collected, approximatedly 10 ml/fraction. After fractionation, 12 fractions (code : E1-E-12) were eluated from this column.

Stationary phase : Silica gel 90 g

Mobile phase : hexane:CH_2Cl_2 = 9:1 to 100 % CH_2Cl_2 by increasing 10% of CH_2Cl_2

Diameter of column : 2 inches

3.4.2.4 Fraction E3 (134.4 mg) was further purified by flash column chromatography. Forty-four fractions (5 ml/fraction) were eluated from the column and combined to eleven fractions (code : E3.1-E3.11).

Stationary phase : Silica gel 5 g

Mobile phase : hexane:CH_2Cl_2 = 9:1 to 100 % CH_2Cl_2 by increasing 10% of CH_2Cl_2

Diameter of column : 1 cm

3.4.2.5 Fraction E3.6 (from item 3.4.2.4) 5 mg was obtained as yellow viscous liquid, coded compound SCHE1.

3.4.2.6 Fraction A7.8.10 from item 3.4.2.2 (190 mg), fraction E3.2 from item 3.4.2.4 (526.7 mg) and fraction E3.4 from item 3.4.2.4 (411.4 mg) were combined and isolated again by classical column chromatography. The volume of eluate was

10 ml/fraction. After combination of fractions that showed similar chromatographic pattern, 12 fractions were obtained (code : AB1-AB12).

Stationary phase : Silica gel 110 g

Mobile phase : hexane:CH_2Cl_2 = 1:1 to 100 % CH_2Cl_2 by increasing 10% of dichloromathane

Diameter of column : about 1 inch

3.4.2.7 Fraction AB3 from item 3.4.2.6 (83.2 mg) was purified by flash column chromatography. The volume of each eluated fraction was about 5 ml. Forty-two fractions were grouped to 13 fractions (code : AB3.1-AB3.13).

Stationary phase : Silica gel 5 g

Mobile phase : hexane:CH_2Cl_2 = 9:1

Diameter of column : 1 cm

3.4.2.8 Fraction AB 3.7 (15 mg) was yellow viscous liquid. TLC and Spectroscopic data showed that it was the same SCHE1 as in item 3.4.2.5.

This compound was subjected to structural elucidation using one- and two-dimentional NMR Spectroscopy, Infrared Spectroscopy, UV Spectroscopy and Mass Spectrometry.

3.4.3 Isolation and purification of SCHE3

3.4.3.1 The ethyl acetate extract 2.25 g was isolated by gel filtration column chromatography. The volume of each eluate fractions was 40 ml. Nineteen fractions were collected from this column. (code : G1-G19)

Stationary phase : Sephadex LH-20 200 g

Mobile phase : MeOH

Diameter of column : 2 inches

3.4.3.2 Fraction G7 (659.9 mg) was purified by gel filtration chromatography. Ten ml/fraction were approximately collected. After evaporation of eluate fractions, 9 fractions were obtained. (code : G7.1-G7.9)

Stationary phase : Sephadex LH-20 100 g

Mobile phase : MeOH

Diameter of column : about 1 inch

3.4.3.3 Fraction G7.4 (391 mg) was further purified by gel filtration chromatography. The condition based were the same as in item 3.4.3.2. Ten fractions from this separation were obtained. (code : G7.4.1-G7.4.10)

3.4.3.4 Fraction G7.4.7 (339 mg) was partitioned into two parts, MeOH-insoluble fraction and MeOH-soluble fraction. The MeOH-insoluble fraction was dissolved in CH_2Cl_2 and 2-3 dropplets of MeOH were added to the solution. The white needles were obtained in only small amount, 7 mg This compound was coded SCHE3.

We followed the fractions from each separation by using Thin Layer Chromatography together with one-dimentional ^{1}H-NMR Spectroscopy. The TLC method was monitored using ultraviolet light. These techniques helped to find some compounds having interesting structure.

Isolation and Purification of SCHE1 and SCHE3 are shown in **Fig. 3.4** and **Fig. 3.5** , respectively.

SCHE1 was subjected to structural elucidation using one- and two-dimentional NMR Spectroscopy, Infrared Spectroscopy, UV Spectroscopy and Mass Spectrometry. SCHE3, the isolated yield was relatively low. Therefore, only some spectral data were observed *i.e.* IR, UV, ^{1}H-NMR, ^{13}C-NMR, and DEPT135 spectral data are shown in **Fig. 2.31-2.35.**

SCHE1 : Yellow viscous liquid. UV λ_{max} 242, 282 and 302 nm. TOF-MS *m/z* 317 (M^+-H, 100%). IR ν_{max} 3470, 3000, 3010, 1705, 1620, 1600 and 1450 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ_H: 5.22 (2H, *m*), 3.72 (3H, *s*), 3.38 (2H, *d*, *J* = 7.2 Hz), 3.35 (2H, *d*, *J* = 6.7 Hz), 2.70 (3H, *s*), 1.82 (6H, *s*), 1.78 (3H, *s*), 1.75 (3H, *s*). ^{13}C-NMR ($CDCl_3$) δ_C: 17.9, 21.8, 22.8, 25.7, 31.5, 62.7, 109.0, 111.0, 112.6, 121.7, 122.3, 134.5, 159.2, 160.7, 161.7, 203.6.

SCHE3 : White needles. UV λ_{max} 218 and 292 nm. IR ν_{max} 3340, 1600, 1490 and 1290 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ_H: 0.74 (3H, *s*), 0.87 (3H, *s*), 0.97 (3H, *s*), 0.98 (6H, *s*), 1.06 (3H, *s*), 1.07 (3H, *s*), 1.09 (3H, *s*), 1.75 (3H, *s*), 3.25 (1H, *dd*, *J* = 4.2, 8.1 Hz), 5.47 (1H, *br*). ^{13}C-NMR ($CDCl_3$) δ_C: 13.2, 14.8, 17.1, 24.1, 26.1, 27.0, 27.7, 28.2, 30.9, 31.6, 33.7, 33.9, 34.1, 34.6, 35.2, 36.0, 36.1, 36.6, 37.2, 38.8, 36.6, 41.6, 46.8, 48.8, 50.1, 79.3, 117.6, 147.5.

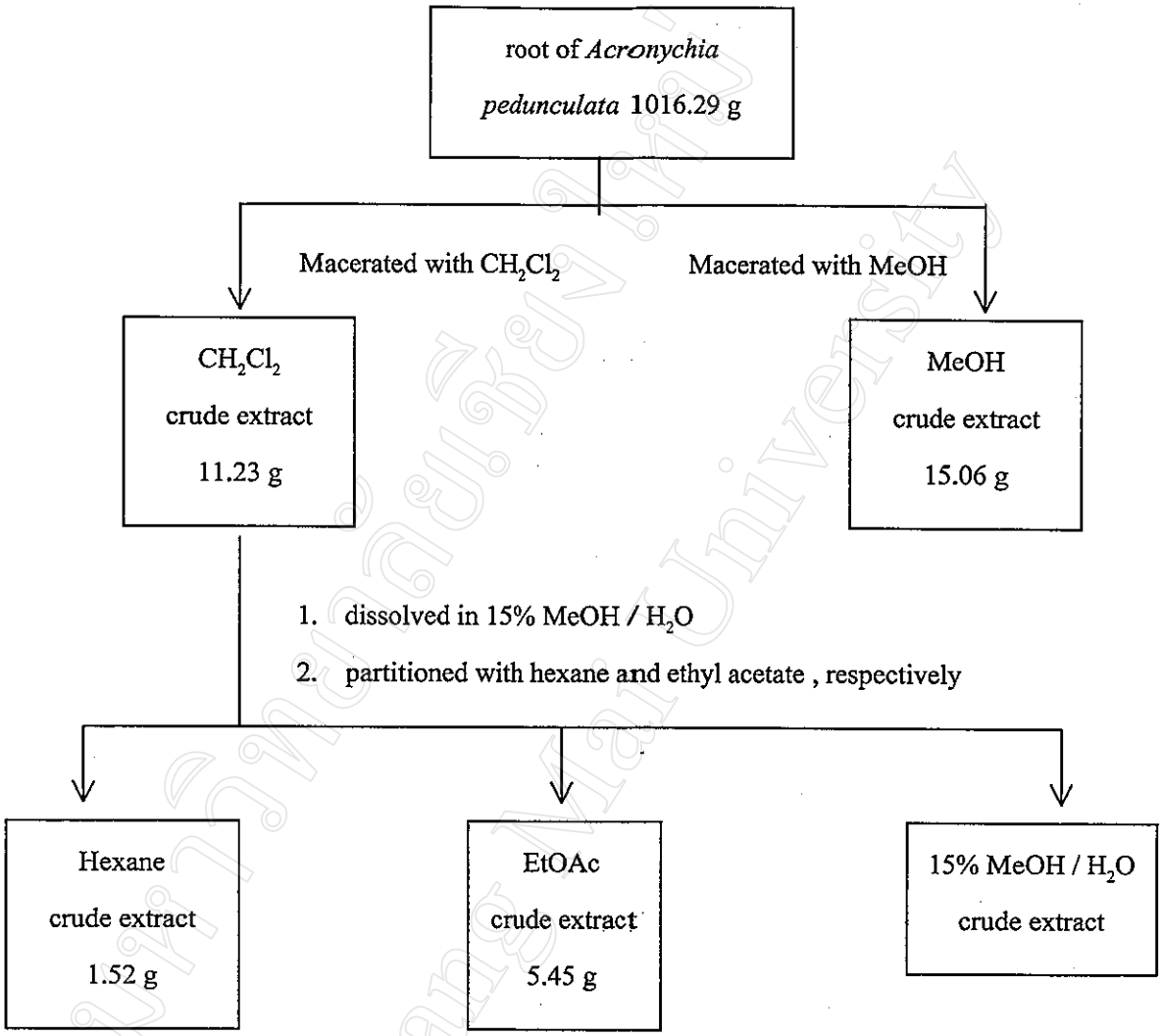
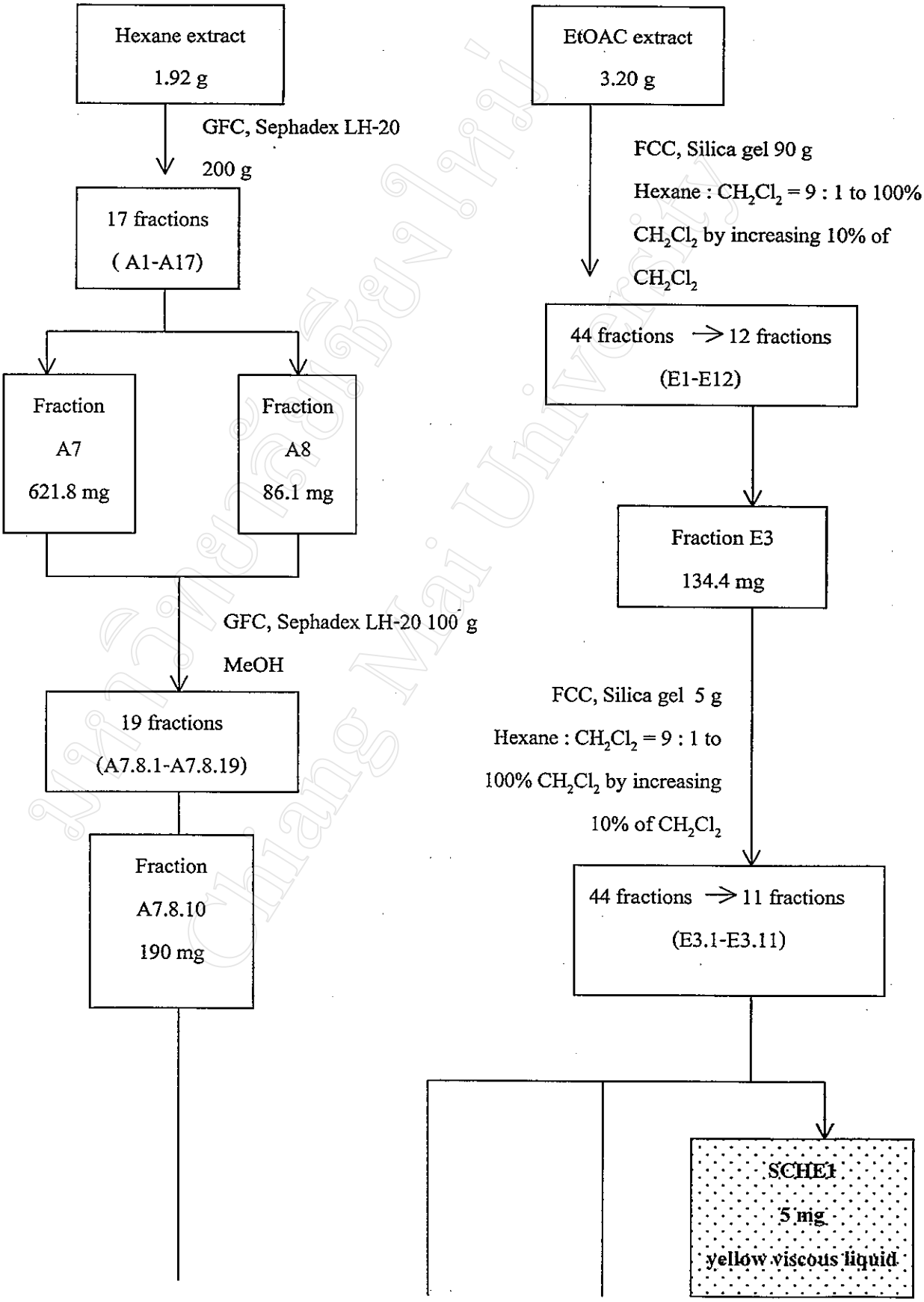


Figure 3.3 Extraction of root of *Acronychia pedunculata*


Hexane extract
1.92 g
GFC, Sephadex LH-20
200 g
17 fractions
(A1-A17)
Fraction
A7
621.8 mg
Fraction
A8
86.1 mg
GFC, Sephadex LH-20 100 g
MeOH
19 fractions
(A7.8.1-A7.8.19)
Fraction
A7.8.10
190 mg
EtOAC extract
3.20 g
FCC, Silica gel 90 g
Hexane : CH_2Cl_2 = 9 : 1 to 100%
CH_2Cl_2 by increasing 10% of
CH_2Cl_2
44 fractions → 12 fractions
(E1-E12)
Fraction E3
134.4 mg
FCC, Silica gel 5 g
Hexane : CH_2Cl_2 = 9 : 1 to
100% CH_2Cl_2 by increasing
10% of CH_2Cl_2
44 fractions → 11 fractions
(E3.1-E3.11)
SCHE1
5 mg
yellow viscous liquid

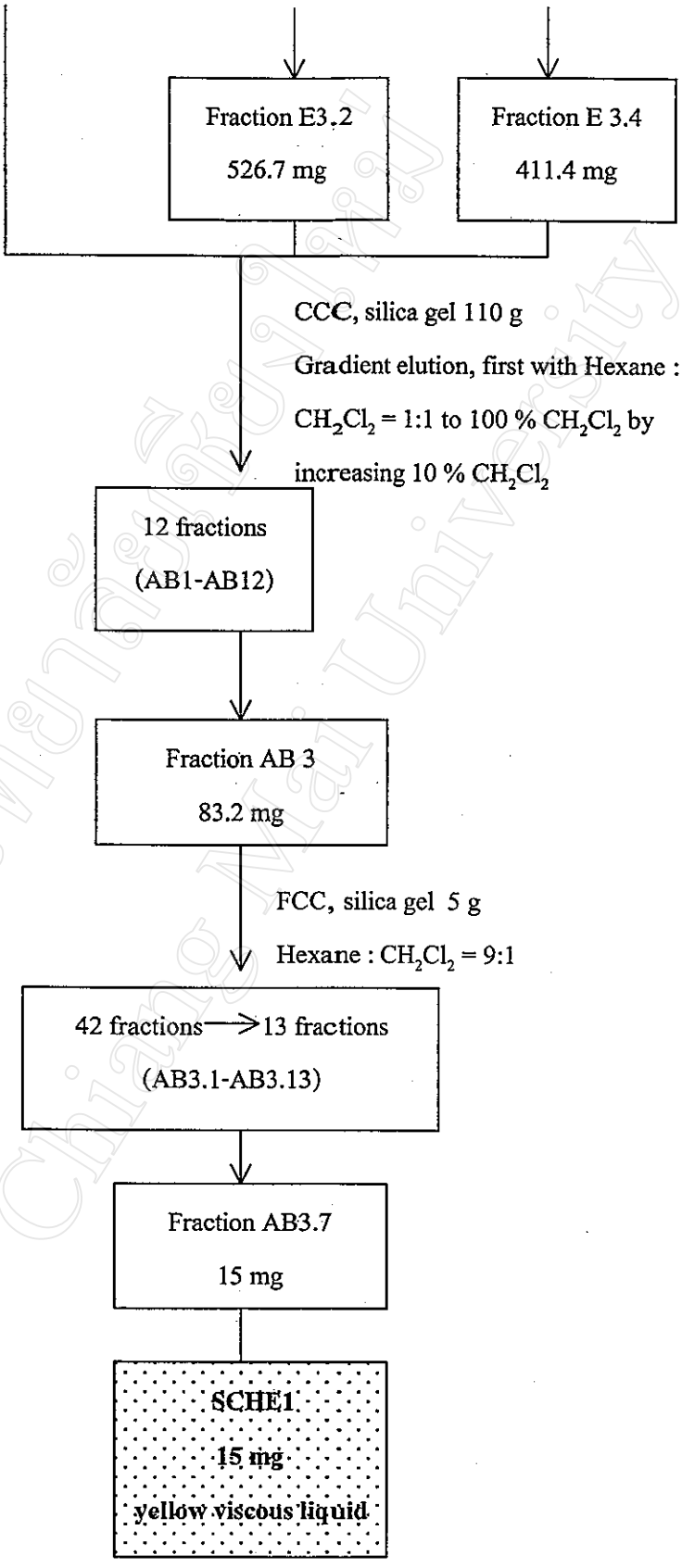


Figure 3.4 Isolation and purification of SCHE1

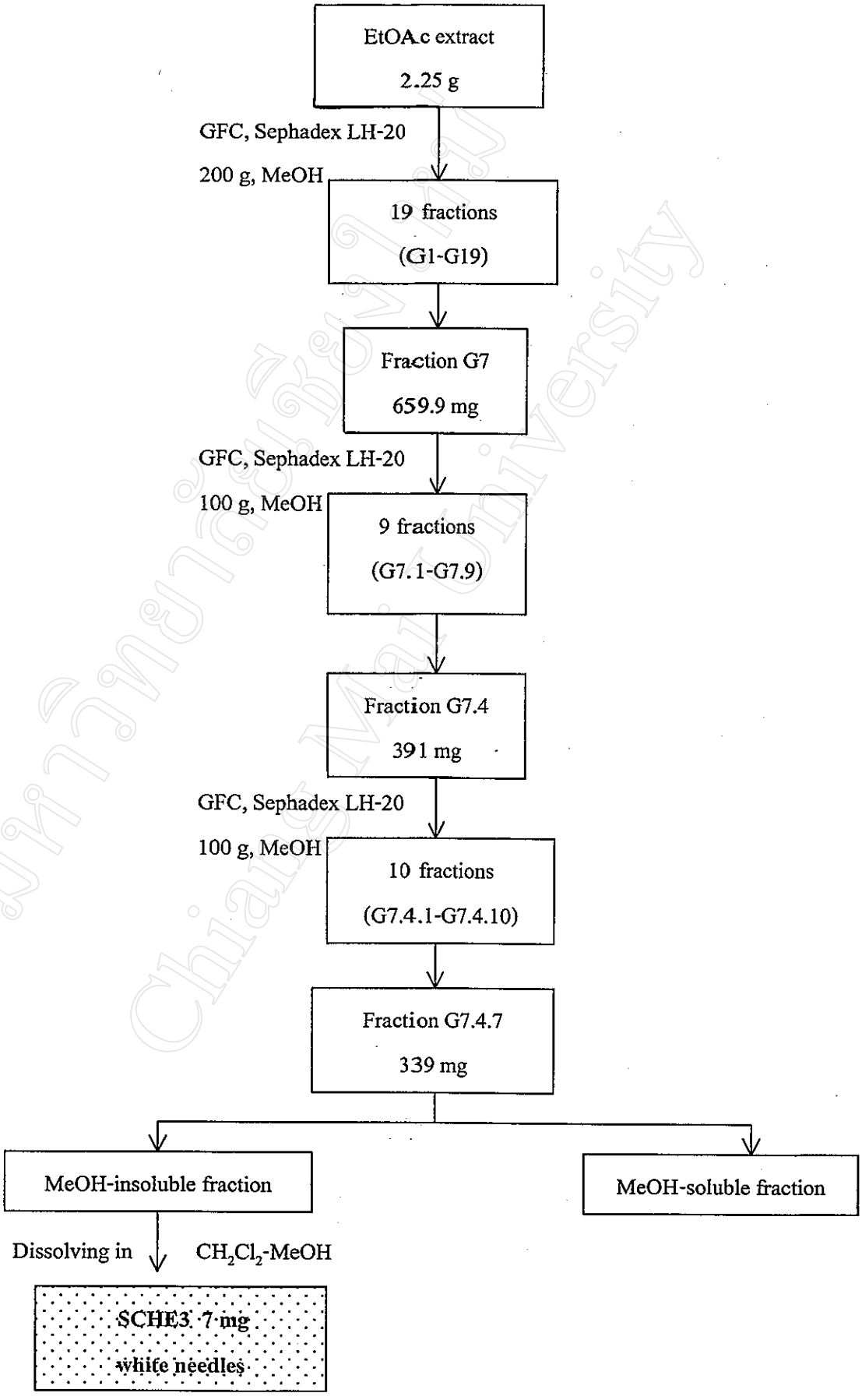


Figure 3.5 Isolation and purification of SCHE3