CHAPTER III
EXPERIMENTAL

3.1 General Experiment Procedures

Melting points were determined on a Gallenkamp melting point apparatus and uncorrected. IR spectra were recorded as KBr disks, using a Perkin Elmer Spectrum One spectrophotometer. NMR spectra were obtained from a Varian Mercury Plus 400 spectrometer. Chemical shifts were recorded on δ (ppm) scale using $CDCl_3$, CD_3OD, DMSO-d_6, and D_2O as the solvents. The internal standard was referenced from the residue of those solvents. ^1H NMR data were listed in order of the number of protons, multiplicity [singlet (s), broad singlet (brs), doublet (d), triplet (t), quartet (q), and multiplet (m)] and coupling constants (J) in Hz assigned for nuclei concerned. Complete protons and carbons assignments were based on 1D NMR data (^1H, ^{13}C, and DEPT) and 2D NMR experiments (COSY, HSQC, HMBC, and NOESY). HRESITOF mass spectra were obtained using a Micromass LCT mass spectrometer, and the lock mass calibration was applied for the determination of accurate masses. Opitical rotation was obtained using a JASCO DIP-1000 digital polarimeter. UV spectra were measured on an Agilent 8453 UV-visible spectrophotometer. Column chromatography was carried out over MERCK silica gel 60 (less than 0.063 nm and 0.063-0.200 nm). Fractions obtained from CC were monitored by TLC on silica gel 60 F_{254} aluminium sheets, the spots were visualized under UV light (254 nm and 366 nm) and further by spraying with anisaldehyde and ceric sulfate reagents and then heated until charred. Preparative TLC was carried out on silica gel 60 PF_{254} (0.5 mm, Merck) plates. Commercial grade solvents were distilled at their boiling point ranges prior to use for extraction and chromatographic separation (CC, FCC, and PLC), whereas AR grade solvents were used for crystallization.

3.2 Fungal Material

The three species of *Chaetomium* were isolated from the soil in Chiangmai and Rayong provinces, Thailand and were identified by Assoc. Prof. Kasem Soytong as

Chaetomium brasiliense, *C. bostrychodes* and *C. siamense*, respectively. Voucher specimens were deposited at the Department of Plant Pest Management, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. There were cultivated on Potato Dextrose Broth (PDB) at 25-28 °C for 4 weeks and filtered out to yield mycelial mats. Then, all dried mycelial mats were proceeded to our laboratory.

3.3 Extraction and Isolation

3.3.1 *Chaetomium brasiliense*

Air-dried mycelial mats of *C. brasiliense* (300 g) was ground into powder and then extracted successively with hexane (700 mL x 3), EtOAc (700 mL x 3), and MeOH (700 mL x 3) at room temperature. The filtrated samples were combined, and the solvents were evaporated *in vacuo* to yield three crude extracts, hexane 6.8 g (2.27%), EtOAc 17.8 g (5.93%), and MeOH extracts 20.6 g (6.87%), respectively. The extraction scheme of *C. brasiliense* is shown in Figures 3.1.

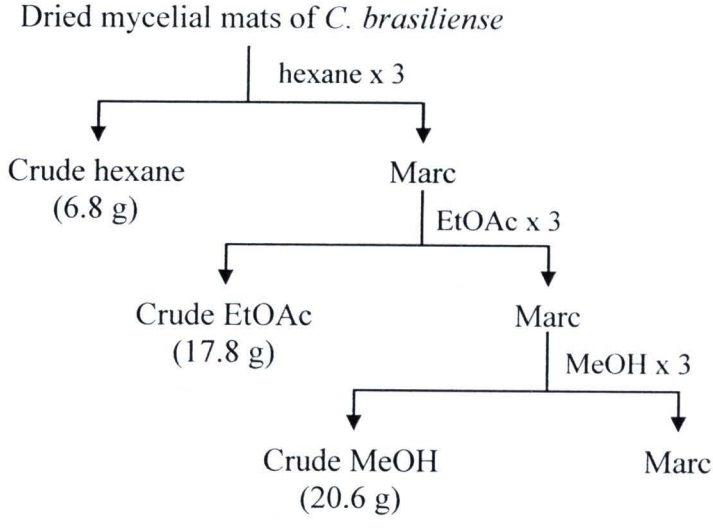

Figure 3.1 Solvents extraction scheme of *C. brasiliense*.

3.3.1.1 Crude hexane extract

CH_2Cl_2 (35 mL):hexane (300 mL) was added to the hexane extract 6.8 g to give a solid (95 mg), which was recrystallized from EtOAc:hexane to give compound **1.8** (34 mg). The filtrate was evaporated to yield a residue, which was subjected to silica gel FCC, eluted with EtOAc:hexane (0-100%) followed by

MeOH:EtOAc (0-50%). A 75 ml of eluent was collected for each fraction to give a total of 86 fractions. The fractions were combined on the basis of TLC profiles to yield 6 major fractions designed as F_1 to F_6 as shown in Table 3.1.

Table 3.1 Fractions combined from FCC of crude hexane of *C. brasiliense*

Fractions	Eluents (%v:v)	Weight (g)	Evaporated Residue
F_1 (f_{1-20})	hexane, 10% EtOAc:hexane	0.489	Yellow liquid
F_2 (f_{21-37})	10% EtOAc:hexane	0.762	Yellow liquid
F_3 (f_{38-50})	10-40% EtOAc:hexane	0.973	Brown liquid
F_4 (f_{51-67})	50-70% EtOAc:hexane	1.911	Brown viscous liquid
F_5 (f_{68-75})	80% EtOAc:hexane	2.119	Brown viscous liquid
F_6 (f_{76-86})	90% EtOAc:hexane, EtOAc, 10-50% MeOH:EtOAc	1.276	Brown viscous liquid

The separation scheme of the crude hexane extract is shown in Figure 3.2 and the result of separation is summarized as follows :-

Fraction F_2 was recrystallized from EtOAc:hexane to give compound **1.2** (74.0 mg). The filtrate was evaporated to yield a residue, which was further subjected to silica gel FCC eluted with a gradient system of hexane:EtOAc to give compound **1.1** (104.2 mg).

Fraction F_3 was purified by preparative TLC using 20% EtOAc:hexane to give compound **1.10** (24.1 mg).

Fraction F_4 was rechromatographed by FCC, eluted with 20% EtOAc:hexane, to afford an additional compounds **1.8** (26.3 mg) and **1.5** (43.1 mg).

Fraction F_5 was purifed by silica gel FCC, eluted with gradient of Hexane:EtOAc to give four subfractions, $F_{5.1}$-$F_{5.4}$ Subfraction $F_{5.2}$ was subjected to silica gel FCC, eluted with a gradient of hexane:EtOAc, to give compound **1.6** (18.9 mg).

Fraction F_6 was purified by silica gel FCC, eluted with a gradient of hexane:EtOAc, to give subfractions $F_{6.1}$-$F_{6.4}$ Subfraction $F_{6.1}$ was rechromatographed by FCC, eluted with 20% EtOAc:hexane, to yield additional amounts of compounds **1.8**

(26.3 mg) and **1.5** (45.0 mg). Fraction$_{6.3}$ was rechromatographed by FCC, eluted with 40% EtOAc:hexane to give additional compound **1.10** (31.1 mg).

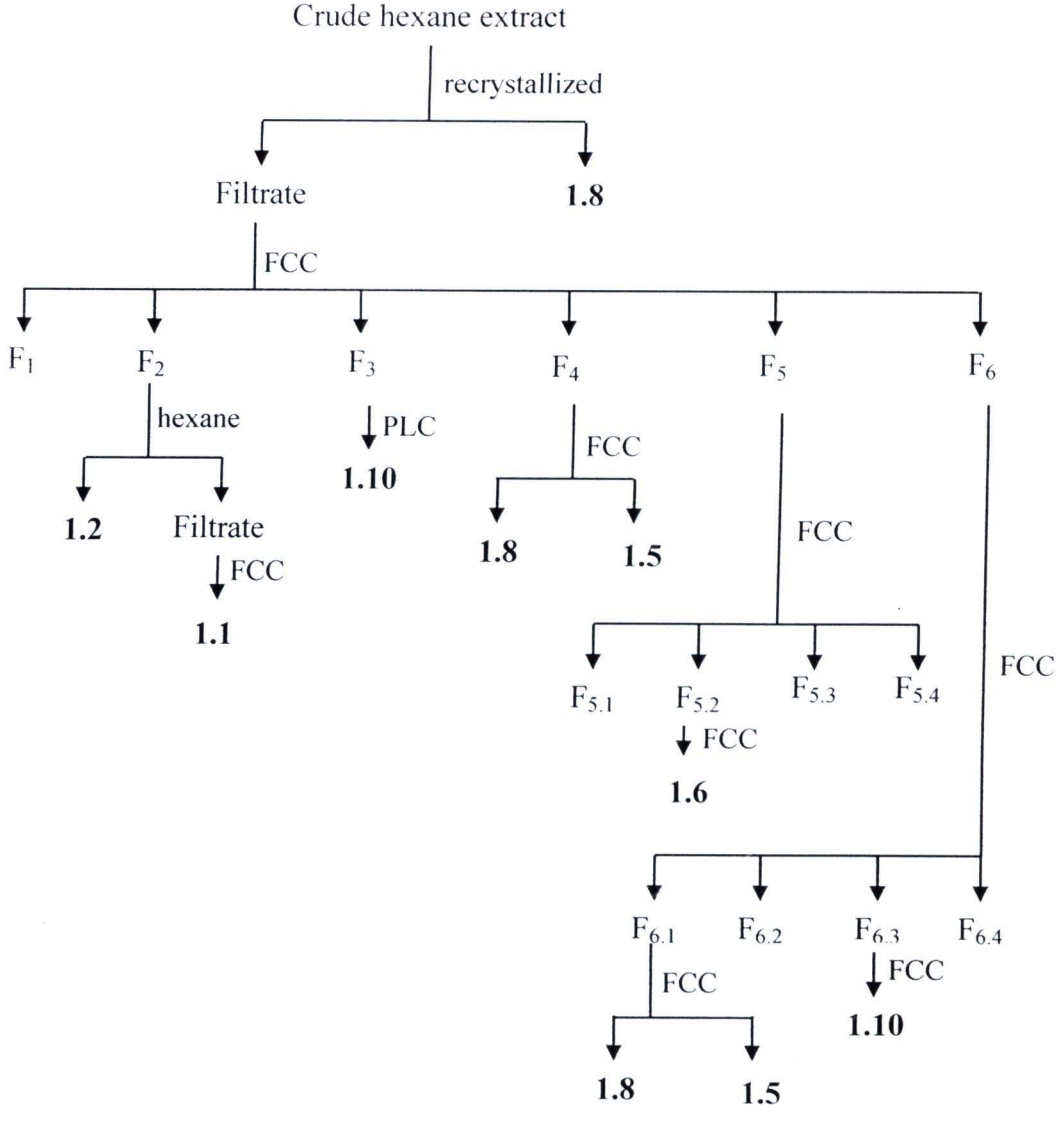

Figure 3.2 Isolation of crude hexane extract of *C. brasiliense*.

3.3.1.2 Crude EtOAc extract

The crude EtOAc extract 17.8 g was initially subjected to silica gel FCC, eluted with the same gradient system as the hexane extract. A 75 ml of eluent was collected for each fraction to give a total 95 fractions. Then, fractions were combined on the basis of TLC profiles to yield 10 major fractions designated as F_1 to F_{10} as shown in Table 3.2.

Table 3.2 Fractions combined from FCC of EtOAc extract of *C. brasiliense*

Fractions	Eluents (%v:v)	Weight (g)	Evaporated Residue
F_1 (f_{1-6})	10% EtOAc:hexane	3.581	Yellow liquid
F_2 (f_{7-10})	20-30% EtOAc:hexane	0.722	Yellow liquid
F_3 (f_{11-15})	30% EtOAc:hexane	1.911	Brown liquid
F_4 (f_{16-25})	30% EtOAc:hexane	2.089	Brown viscous liquid
F_5 (f_{26-30})	30% EtOAc:hexane	1.043	Brown viscous liquid
F_6 (f_{31-43})	40-50% EtOAc:hexane	2.172	Black viscous liquid
F_7 (f_{44-52})	50-60% EtOAc:hexane	2.187	Black viscous liquid
F_8 (f_{53-60})	60-90% EtOAc:hexane	1.076	Black viscous liquid
F_9 (f_{61-78})	EtOAc, 10-20% MeOH:EtOAc	0.877	Black plate
F_{10} (f_{79-95})	20-50% MeOH:EtOAc	1.438	Black plate

The separation scheme of the crude EtOAc extract is shown in Figure 3.3 and the result of the separation is summarized as follows :-

Fraction F_1 was subjected to silica gel FCC, eluted with a gradient of hexane:EtOAc, to give compound **1.4** (54.0 mg), an additional amount of compound **1.10** (15.3 mg), and compound **1.11** (6.0 mg).

Fraction F_4 was separated by FCC, eluted with a gradient of hexane:EtOAc, to yield additional compound **1.5** (67.0 mg) and compound **1.8** (30.0 mg).

Fraction F_5 was subjected to FCC, eluted with a gradient of hexane:EtOAc, to give compound **1.9** (13.2 mg), compound **1.8** (10.0 mg), compound **1.7** (7.8 mg), and compound **1.11** (16.0 mg).

Fraction F_6 was separated by silica gel FCC, eluted with a gradient of Hexane:EtOAc, to give three subfrations, $F_{6.1}$-$F_{6.3}$. Subfraction $F_{6.1}$ was rechromatographed by FCC, eluted with 40% EtOAc:hexane, to yield compound **1.6** (6.3 mg). Subfraction $F_{6.2}$ was rechromatographed by FCC, eluted with 50% EtOAc:hexane, to yield compound **1.3** (14 mg).

Fraction F_7 was further purified by silica gel FCC, eluted with a gradient of hexane:EtOAc, to give three subfractions, $F_{7.1}$- $F_{7.3}$. Subfraction $F_{7.1}$ was

further purified by preparative TLC using 20% EtOAc:hexane as eluent to yield additional compound **1.8** (15.0 mg). Subfraction $F_{7.2}$ was rechromatographed by FCC, eluted with 50% EtOAc:hexane, to give compound **1.12** (21.3 mg).

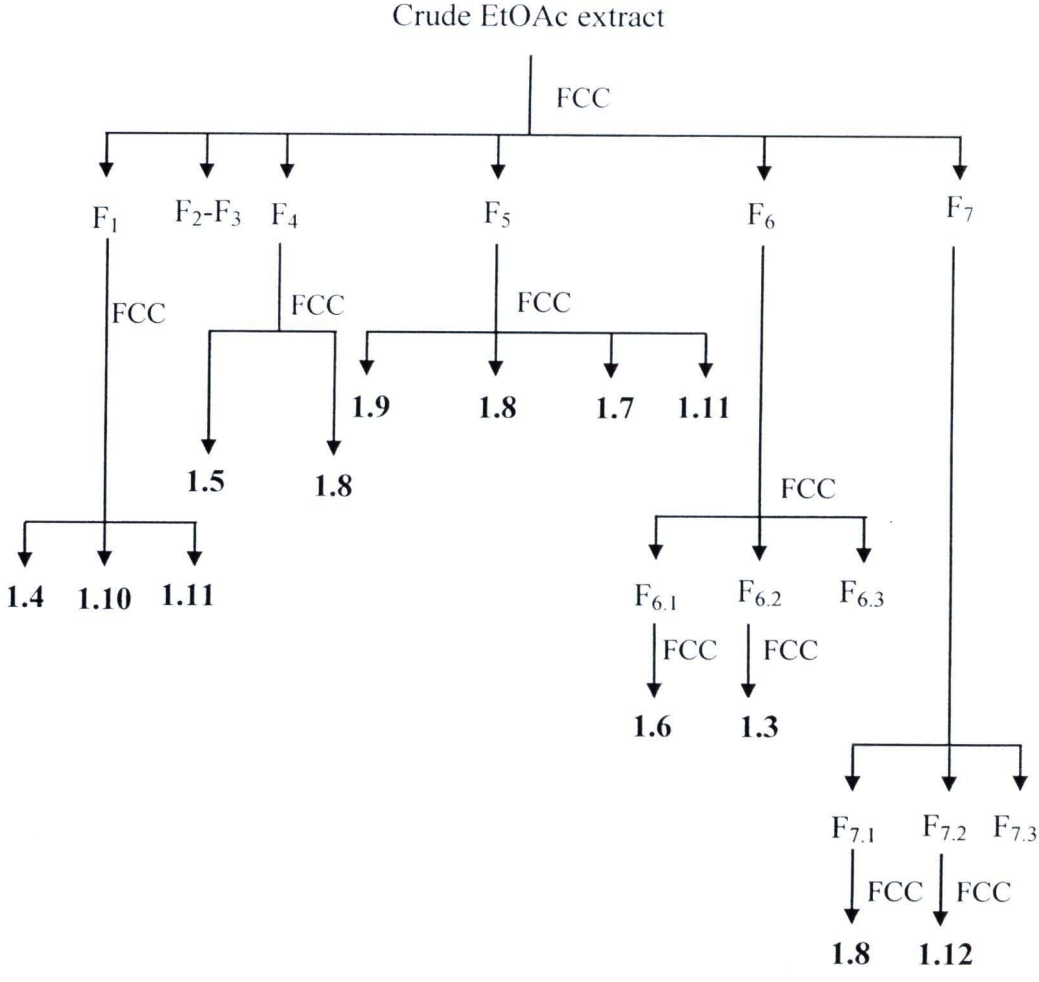

Figure 3.3 Isolation of crude EtOAc extract of *C. brasiliense*.

3.3.1.3 Crude MeOH extract

The MeOH extract 20.6 g was subjected to silica gel column chromatography, eluted with a gradient of hexane:EtOAc and EtOAc:MeOH. A 100 ml of eluent was collected for each fraction to give a total of 60 fractions. The fractions were combined on the basis of TLC profiles to yield 4 major fractions designated as F_1 to F_4 as shown in Table 3.3.

Table 3.3 Fractions combined from FCC of crude MeOH extract of *C. brasiliense*

Fractions	Eluents (%v:v)	Weight (g)	Evaporated Residue
F_1 (f_{1-25})	10%-50%EtOAc:hexane	2.160	Brown viscous liquid
F_2 (f_{26-34})	60-90%EtOc:hexane	2.005	Brown viscous liquid
F_3 (f_{35-55})	EtOAc,10%-20%MeOH:EtOAc	2.556	Brown viscous liquid
F_4 (f_{56-60})	30%- -90%MeOH:EtOAc,MeOH	7.512	Brown viscous liquid

The separation scheme of crude MeOH extract is shown in Figure 3.4 and the result of the separation of the principle fractions is summarized as follows :-

Fraction F_2 yielded additional compound **1.9** (7.2 mg). Fraction F_3 affored additional compound **1.10** (11.4 mg).

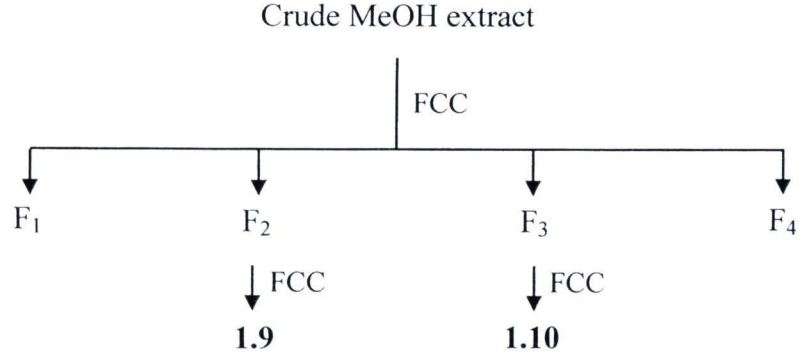

Figure 3.4 Isolation of crude MeOH extract of *C. brasiliense*.

3.3.2 *Chaetomium bostrychodes*

Air-dried mycelial mats of *C. bostrychodes* (119 g) was ground into powder and then extracted successively with hexane (700 mL x 3), EtOAc (700 mL x 3), and MeOH (700 mL x 3) at room temperature. The filtrated samples were combined, and the solvents were evaporated *in vacuo* to yield three crude extracts, crude hexane 1.5 g (1.26%), crude EtOAc 2.1 g (1.76%), and crude MeOH 4.5 g (3.78%), respectively. The extraction scheme of *C. bostrychodes* is shown in Figures 3.5.

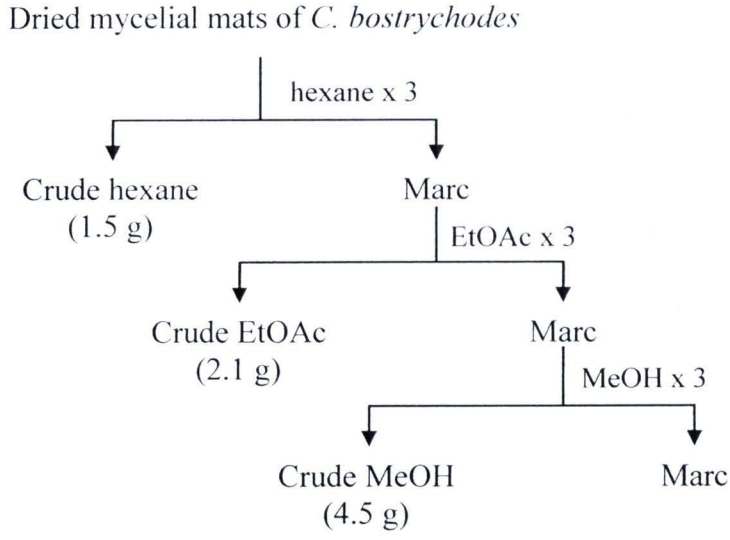

Figure 3.5 Solvents extraction scheme of *C. bostrychodes*.

3.3.2.1 Crude hexane extract

The crude hexane extract was redissolved with EtOAc and fitered. The filtrate was evaporated *in vacuo*, and the residue was chromatographed on a silica gel column eluted with a gradient of hexane:EtOAc and EtOAc:MeOH. A 50 ml of eluent was collected for each fraction to give a total of 85 fractions. The fractions were combined on the basis of TLC profiles to yield 7 major fractions designed as F_1 to F_7 as shown in Table 3.4.

Table 3.4 Fractions combined from FCC of crude hexane of *C. bostrychodes*

Fractions	Eluents (%v:v)	Weight (g)	Evaporated Residue
F_1 (f_{1-6})	hexane-50% EtOAc:hexane	0.423	Yellow liquid
F_2 (f_{7-24})	60% EtOAc:hexane	0.338	Orange liquid
F_3 (f_{25-28})	70-80% EtOAc:hexane	0.028	Orange wax liquid
F_4 (f_{29-53})	90% EtOAc:hexane, EtOAc	0.177	Orange wax liquid
F_5 (f_{54-72})	EtOAc	0.095	Orange wax liquid
F_6 (f_{73-78})	10-30% MeOH:EtOAc	0.139	Orange wax liquid
F_7 (f_{79-85})	40-90% MeOH:EtOAc, MeOH	0.176	Brown viscous liquid

The separation scheme of the crude hexane extract is shown in Figure 3.6 and the result of separation is summarized as follows:-

Fraction F_2 was subjected to silica gel FCC, eluted with a gradient of hexane:EtOAc, to give compound **2.1** (12.1 mg) and compound **2.2** (30.2 mg).

Fraction F_3 was rechromatographed by FCC, eluted with 20% EtOAc:hexane to afford compound **2.6** (8.5 mg).

Fraction F_6 was rechromatographed by FCC, eluted with gradient of hexane:EtOAc to give compound **2.7** (9.5 mg) and additional compound **2.3** (8.7 mg).

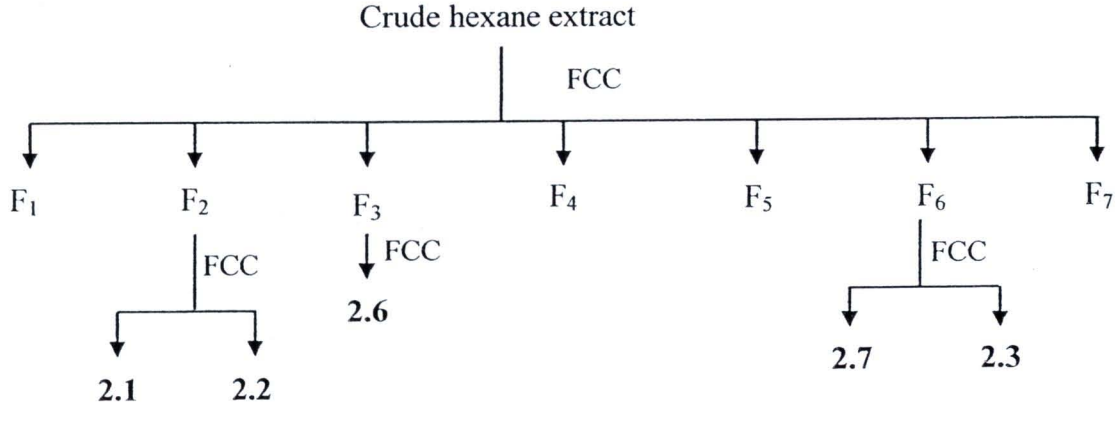

Figure 3.6 Isolation of crude hexane extract of *C. bostrychodes*.

3.3.2.2 Crude EtOAc extract

The crude EtOAc extract 4.5 g was initially subjected to silica gel FCC, eluted with the same gradient system as the hexane extract. A 50 ml of eluent was collected for each fraction to give a total 80 fractions. The fractions were combined on the basis of TLC profiles to yield 6 major fractions designated as F_1 to F_6 as shown in Table 3.5.

Table 3.5 Fractions combined from FCC of EtOAc extract of *C.bostrychodes*

Fractions	Eluents (%v:v)	Weight (g)	Evaporated Residue
F_1 (f_{1-13})	10-40% EtOAc:hexane	0.233	Orange liquid
F_2 (f_{14-20})	40% EtOAc:hexane	0.261	Orange liquid
F_3 (f_{21-32})	50% EtOAc:hexane	0.245	Orange viscous liquid
F_4 (f_{33-40})	60-80% EtOAc:hexane	0.185	Brown viscous liquid
F_5 (f_{41-60})	90%EtOAc:hexane, EtOAc	0.777	Brown viscous liquid
F_6 (f_{61-80})	10-90%MeOH:EtOAc, MeOH	0.532	Brown viscous liquid

The separation scheme of the crude EtOAc extract is shown in Figure 3.7 and the result of the separation is summarized as follows:-

Fraction F_1 was subjected to silica gel FCC, eluted with a gradient of hexane:EtOAc, to give addtional compound **2.1** (32.5 mg).

Fraction F_2 was rechromatographed by FCC, eluted with gradient of hexane:EtOAc to give compound **2.5** (9.5 mg).

Fraction F_3 was separated by FCC, eluted with a gradient of hexane:EtOAc, to yield compound **2.2** (17.8 mg) and compound **2.5** (76.8 mg).

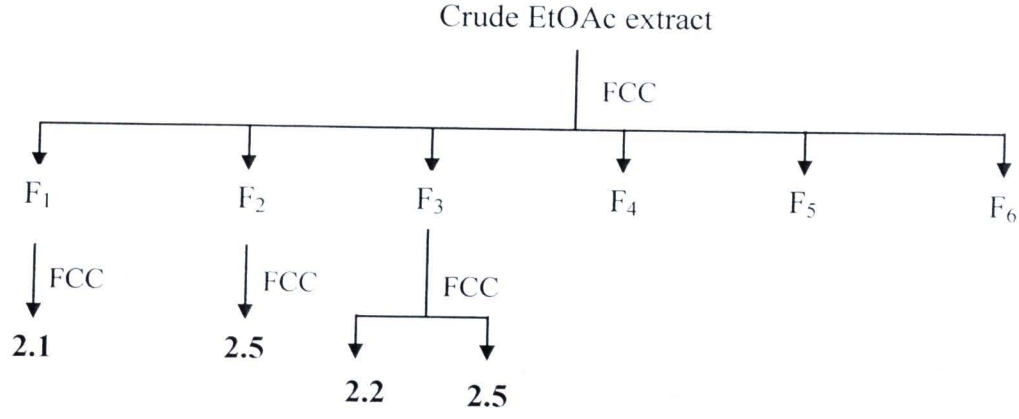

Figure 3.7 Isolation of crude EtOAc extract of *C. bostrychodes*.

3.3.2.3 Crude MeOH extract

The MeOH extract 7.6 g was subjected to silica gel FCC, eluted with a gradient of hexane:EtOAc and EtOAc:MeOH. A 75 ml of eluent was collected for each fraction to give a total of 90 fractions. The fractions were combined on the basis of TLC profiles to yield 6 major fractions designated as F_1 to F_6 as shown in Table 3.6.

Table 3.6 Fractions combined from FCC of crude MeOH extract of *C. bostrychodes*

Fractions	Eluents (%v:v)	Weight (g)	Evaporated Residue
F_1 (f_{1-15})	10-20% EtOAc:hexane	0.679	Orange viscous liquid
F_2 (f_{16-27})	20-30% EtOAc:hexane	0.479	Brown viscous liquid
F_3 (f_{28-30})	40% EtOAc:hexane	0.774	Brown viscous liquid
F_4 (f_{31-48})	40-700% EtOAc:hexane	1.174	Brown viscous liquid
F_5 (f_{49-72})	80-90% EtOAc:hexane,EtOAc	0.682	Brown viscous liquid
F_6 (f_{73-90})	EtOAC,20-90% MeOH:EtOAC	0.930	Brown viscous liquid

The separation scheme of crude MeOH extract is shown in Figure 3.8 and the result of the separation of the principle fractions is summarized as follows:-

Fraction F_1 subjected to silica gel FCC, eluted with 30% EtOAc:hexane to give additional compound **2.4** (45.2 mg).

Fraction F_2 subjected to silica gel FCC, eluted with 30% EtOAc:hexane to give compound **2.2** (4.0 mg) and additional compound **2.6** (2.1 mg).

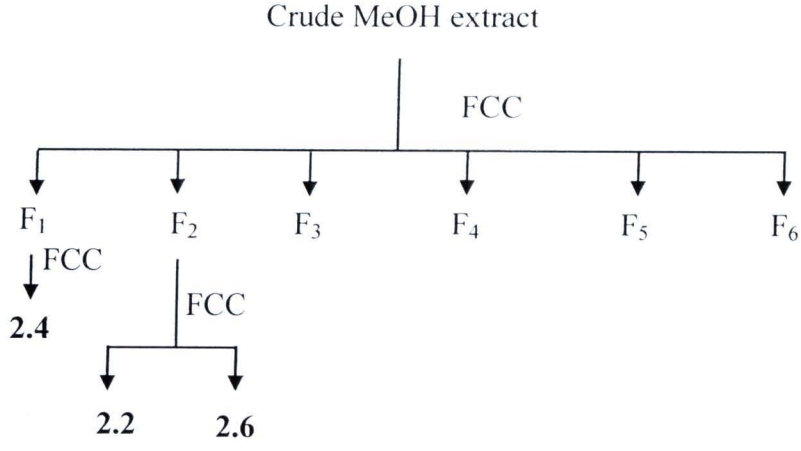

Figure 3.8 Isolation of crude MeOH extract of *C. bostrychodes.*

3.3.3 *Chaetomium siamense*

Air-dried mycelial mats of *C. siamense* (135 g) was ground into powder and then extracted successively with hexane (700 mL x 3), EtOAc (700 mL x 3), and MeOH (700 mL x 3) at room temperature. The filtrated samples were combined, and the solvents were evaporated *in vacuo* to yield three crude extracts, crude hexane 3.6 g (2.67%), crude EtOAc 4.9 g (3.63%), and crude MeOH 7.6 g (5.63%), respectively. The extraction scheme of *C. siamense* is shown in Figures 3.9.

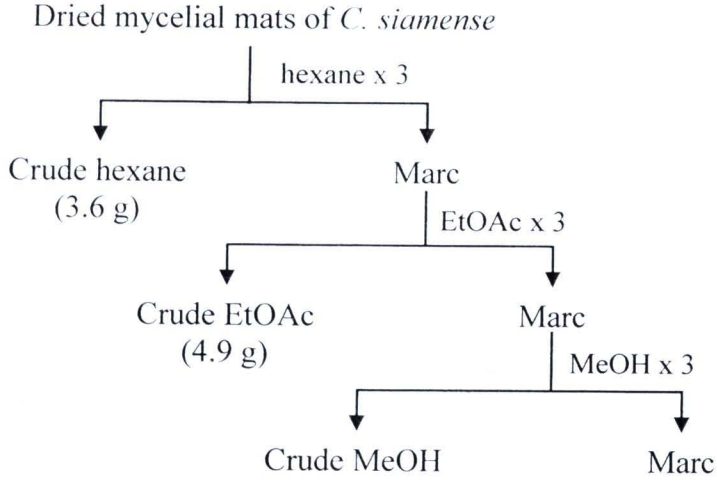

Figure 3.9 Solvents extraction scheme of *C. siamense*.

3.3.3.1 Crude hexane extract

The crude hexane extract was redissolved with EtOAc and fitered. The filtrate was evaporated *in vacuo*, and the residue was chromatographed on a silica gel column eluted with a gradient of hexane:EtOAc and EtOAc:MeOH. A 100 ml of eluent was collected for each fraction to give a total of 65 fractions. The fractions were combined on the basis of TLC profiles to yield 7 major fractions designed as F_1 to F_7 as shown in Table 3.7.

Table 3.7 Fractions combined from FCC of crude hexane of *C. siamense*

Fractions	Eluents (%v:v)	Weight (g)	Evaporated Residue
F_1 (f_{1-7})	hexane, 20% EtOAc:hexane	0.227	Yellow liquid
F_2 (f_{8-18})	30% EtOAc:hexane	0.350	Orange wax liquid
F_3 (f_{19-28})	40-60% EtOAc:hexane	1.702	Orange wax liquid
F_4 (f_{29-34})	60-70% EtOAc:hexane	0.540	Orange liquid
F_5 (f_{35-47})	80-90% EtOAc:hexane	0.539	Orange liquid
F_6 (f_{48-54})	90% EtOAc:hexane	0.539	Orange liquid
F_7 (f_{55-65})	90% EtOAc:hexane, EtOAc, 10-30% MeOH:EtOAc	0.776	Brown viscous liquid

The separation scheme of the crude hexane extract is shown in Figure 3.10 and the result of separation is summarized as follows:-

Fraction F_2 was filtered out to yield orange needles of compound **3.1** (22.5 mg).

Fraction F_3 was rechromatographed by FCC, eluted with 10% EtOAc:hexane to afford additional compound **3.1** (4.1 mg) and compound **3.2** (35.6 mg).

Fraction F_4 was rechromatographed by FCC, eluted with gradient of hexane:EtOAc to give additional compound **3.2** (32.5 mg) and compound **3.3** (24.2. mg).

Fraction F_5 was rechromatographed by FCC, eluted with gradient of hexane:EtOAc to afford compound **3.5** (50.4 mg).

Fraction F_6 was purifed by silica gel FCC, eluted with gradient of hexane:EtOAc to give four subfractions, $F_{6.1}$-$F_{6.4}$. Subfraction $F_{6.3}$ was subjected to silica gel FCC, eluted with a gradient of hexane:EtOAc, to give compound **3.2** (18.9 mg).

Fraction F_7 was purified by silica gel FCC, eluted with a gradient of hexane:EtOAc, to give subfractions $F_{7.1}$ -$F_{7.5}$. Subfraction $F_{7.1}$ was rechromatographed by FCC, eluted with 20% EtOAc:hexane, to yield additional amount of compound **3.6** (15.5 mg). Subfraction $F_{7.3}$ was rechromatographed by FCC, eluted with 20% EtOAc:hexane to give compound **3.4** (13.3 mg).

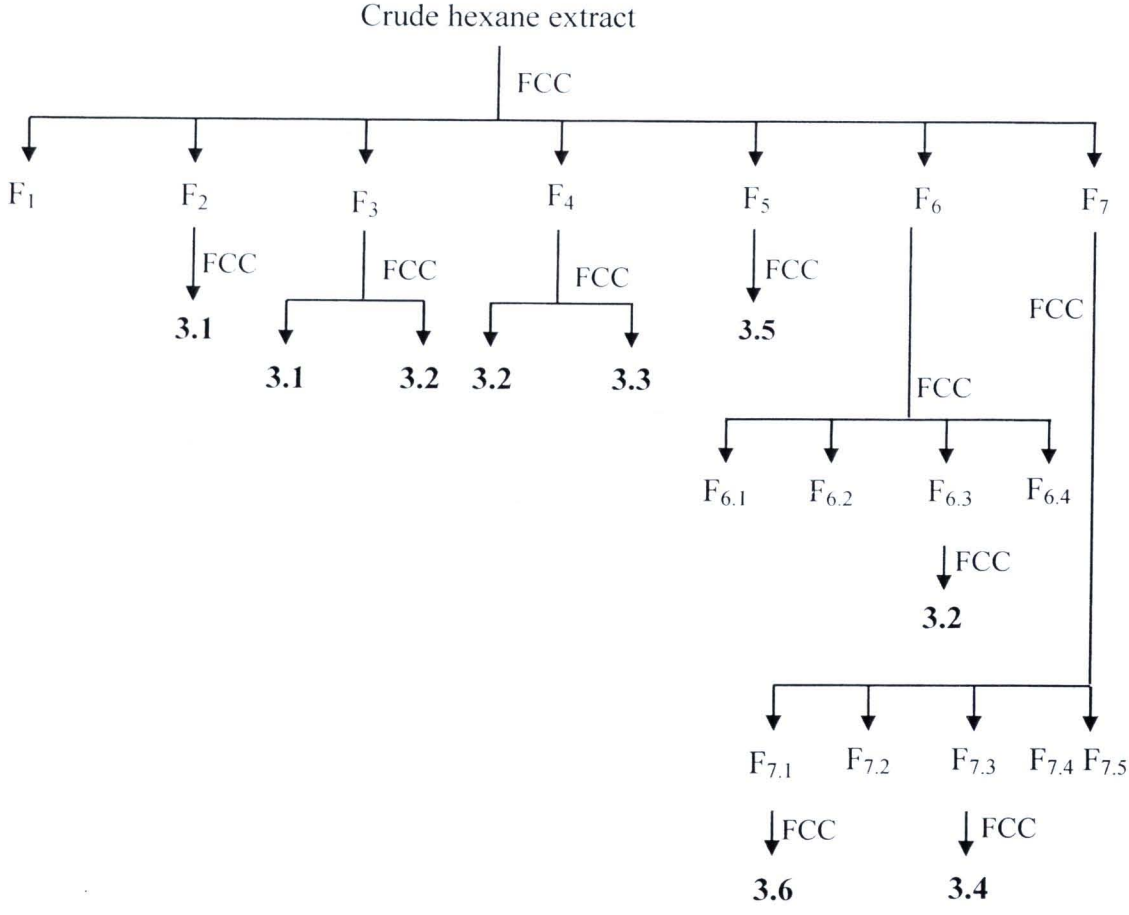

Figure 3.10 Isolation of crude hexane extract of *C. siamense*.

3.3.3.2 Crude EtOAc extract

The crude EtOAc extract 4.9 g was initially subjected to silica gel FCC, eluted with the same gradient system as the hexane extract. A 75 ml of eluent was collected for each fraction to give a total 90 fractions. The fractions were combined on the basis of TLC profiles to yield 7 major fractions designated as F_1 to F_7 as shown in Table 3.8.

Table 3.8 Fractions combined from FCC of EtOAc extract of *C.siamense*

Fractions	Eluents (%v:v)	Weight (g)	Evaporated Residue
F_1 (f_{1-11})	10- 30% EtOAc:hexane	1.341	Yellow liquid
F_2 (f_{12-27})	40-50% EtOAc:hexane	1.261	Orange liquid
F_3 (f_{28-42})	50% EtOAc:hexane	0.311	Orange liquid
F_4 (f_{43-50})	60% EtOAc:hexane	0.267	Orange viscous liquid
F_5 (f_{51-62})	70-80% EtOAc:hexane	0.277	Orange viscous liquid
F_6 (f_{63-73})	80-90% EtOAc:hexane,EtOAc	0.432	Orange viscous liquid
F_7 (f_{74-90})	EtOAc,10%-30% MeOH-EtOAc	1.676	Brown viscous liquid

The separation scheme of the crude EtOAc extract is shown in Figure 3.11 and the result of the separation is summarized as follows:-

Fraction F_1 was subjected to silica gel FCC, eluted with a gradient of hexane:EtOAc, to give compound **3.5** (866.1 mg).

Fraction F_5 was separated by FCC, eluted with a gradient of hexane:EtOAc, to yield additional compounds **3.5** (67.0 mg) and **3.3** (116.8 mg).

Fraction F_6 was separated by silica gel FCC, eluted with 10% hexane:EtOAc, to give compound **3.4** (74.0 mg).

Fraction F_7 was further purified by silica gel FCC, eluted with a gradient of hexane:EtOAc, to give three subfractions, $F_{7.1}$-$F_{7.4}$. Subfraction $F_{7.2}$ was further purified by FCC using 10% EtOAc:hexane as eluent to yield additional compound **3.4** (11.0 mg). Subfraction $F_{7.4}$ was rechromatographed by FCC, eluted with 20% EtOAc:hexane, to give compound **3.1** (11.3 mg) and compound **3.2** (14.5 mg).

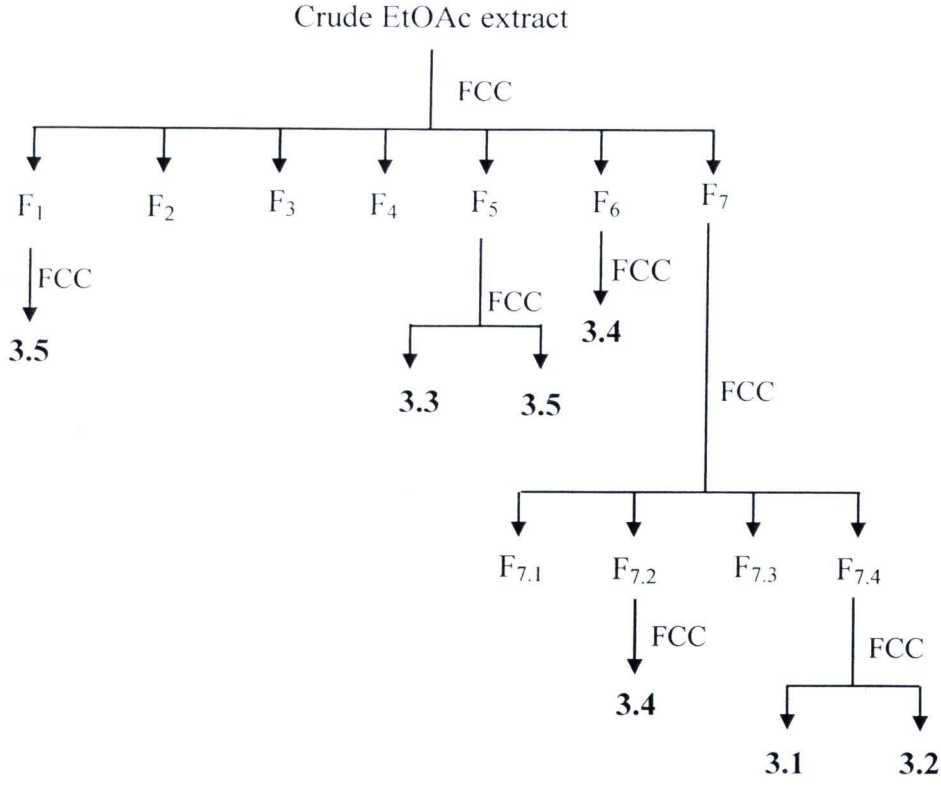

Figure 3.11 Isolation of crude EtOAc extract of *C. siamense*.

3.3.3.3 Crude MeOH extract

The MeOH extract 7.6 g was subjected to silica gel FCC, eluted with a gradient of hexane:EtOAc and EtOAc:MeOH. A 100 ml of eluent was collected for each fraction to give a total of 60 fractions. The fractions were combined on the basis of TLC profiles to yield 6 major fractions designated as F_1 to F_6 as shown in Table 3.9.

Table 3.9 Fractions combined from FCC of crude MeOH extract of *C. saimense*

Fractions	Eluents (%v/v)	Weight (g)	Evaporated Residue
F_1 (f_{1-14})	10- 30% EtOAc:hexane	0.892	Orange viscous liquid
F_2 (f_{15-23})	30-50% EtOAc:hexane	1.134	Orange viscous liquid
F_3 (f_{24-30})	50% EtOAc:hexane	1.267	Brown viscous liquid
F_4 (f_{31-34})	60-90% EtOAc:hexane	0.986	Brown viscous liquid
F_5 (f_{35-49})	90% EtOAc:hexane,EtOAc	1.349	Brown viscous liquid
F_6 (f_{50-60})	EtOAc,20-90% MeOH:EtOAc	2.435	Brown viscous liquid

The separation scheme of crude MeOH extract is shown in Figure 3.12 and the result of the separation from principle fractions are summarized as follows:-

Fraction F_2 was subjected to silica gel FCC, eluted with 30% EtOAc:hexane to give compound **3.7** (7.5mg).

Fraction F_3 was subjected to silica gel FCC, eluted with 30% EtOAc:hexane to give compound **3.8** (2.0 mg) and affored additional compound **3.7** (3.4 mg).

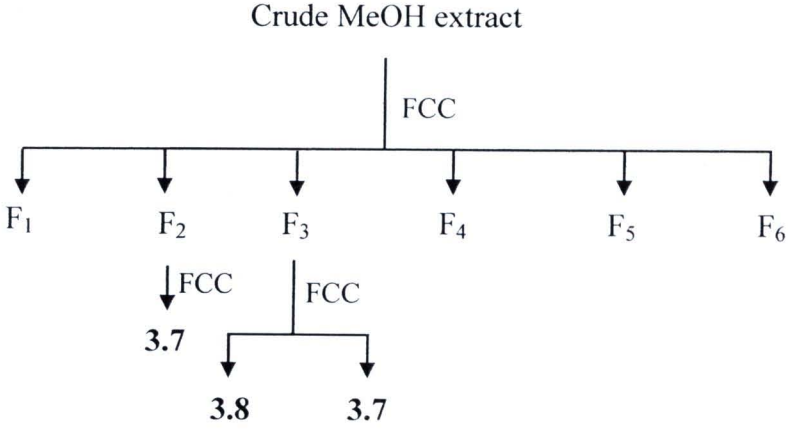

Figure 3.12 Isolation of crude MeOH extract of *C. siamense*.

3.4 Cyclization of compound 1.5

A solution of compound **1.5** (32.1 mg) in MeOH (5 mL) was added *p*-toluensulfonic acid (9.4 mg), and the solution was strirred at 50°C for 4 h. Cooled water was added to the reaction mixture, and then was extracted with EtOAc (10 mL x 3). The organic layer was combined, washed with water and brine, and dried over anhydrous Na_2SO_4. The filtrate was evaporated to dryness, and the residue was separated by preparative TLC (10% EtOAc:hexane) to give compound **1.8** (29.2 mg, 91%); mp 202-204 °C; IR and NMR spectra were identical to those of compound **1.8.**

3.5 Bioassay Experiments

Antimalarial activity against *Plasmodium falciparum*, antituberculosis activity against *Mycobacterium tuberculosis*, antifungal activity againt *Candida albicans* and cytotoxicity against human epidermoid carcinoma (KB), human breast cancer (BC1) and human small cell lung cancer (NCI-H187) were sent to evaluated at the National Center for Genetic Engineering and Biotechnology (BIOTEC), NSTDA, Bangkok, Thailand. Cytotoxicity against cholangiocarcinoma cell lines was sent to test at the Liver Fluke and Cholangiocarcinoma research center, Faculty of Medicine, Khon Kean University, Thailand. In addition, platelet aggregation and platelet activating factor antagonistic activities were carry out at Faculty of Pharmacy, University Kebangsaan Malaysia, Malaysia.

3.5.1 Antimalarial Assay

Antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug resistant strain), using the method of Trager and Jensen.[76] Quantitative assessment of malarial activity *in vitro* was determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al.[77] The inhibitory concentration (IC_{50}) represents the concentration which causes 50% reduction in parasite growth as indicated by the *in vitro* uptake [^{3}H]-hypoxanthine by *P. falciparum*. The standard compound was artemisinin.

3.5.2 Antimycobacterial Assay

Antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA).[78] The standard drugs, isoniazid and kanamycin sulfate were used as the reference compounds.

3.5.3 Antifungal Assay

An antifungal assay was performed against clinical isolated *Candida albicans* using a method modified from the soluble formazan assay described by Scudiero and co- workgers.[79] The number of living cells was determined by measuring the absorbance of XTT formazan at 450 nm. The reference substance was amphotericin B.

3.5.4 Cytotoxicity Assay

Cytotoxicity assays against human epidermoid carcinoma (KB), human breast cancer (BC1) and human small cell lung cancer (NCI-H187), and cholangiocarcinoma cell lines were performed employing the colorimetric method as described by Skehan and coworkers.[80] The reference substance was ellipticine.

3.5.5 Platelet aggregation

The use of human whole blood in this study was approved by the Ethics Committee of the University Kebangsaan Malaysia. Blood was collected from volunteers based on the criteria that they were healthy, non-smokers, had not taken any medications, including aspirin, within the last 2 weeks and had not taken any food within the last 8 h. Whole blood (20 mL) was withdrawn from the right arm of a subject into a vacutainer containing 3.8% sodium citrate. The blood and the anticoagulant were thoroughly mixed by inverting the vacutainer several times. The blood sample was diluted with normal saline in the ratio of 1:1. The dried methanol extracts and each isolated compound was dissolved in DMSO to obtain stock solutions of 20, 10, 5 and 2.5 mg/mL. Five microliters of the stock solutions was added to a cuvette containing the diluted whole blood and the mixture was allowed to incubate at 37 °C for 4 min prior to the addition of AA (0.5 mM), ADP (10 mM) or collagen

(2 mg/mL). The total volume of the mixture was 1 mL. The final concentrations of the sample in the mixture were 100, 50, 25 and 12.5 mg/mL.

Platelet aggregation was measured by a Whole Blood Lumi-Aggregometer (Chrono-Log Corp., Havertown, PA) using an electrical impedance method.[81] The mean platelet aggregation in whole blood was measured as a change in impedance over 6 min after the addition of inducers by comparison to that of a control group impedance.[82] A mixture containing 0.5% DMSO in the diluted whole blood was used as control. Aspirin was used as the positive control. The final concentration of DMSO in the whole blood was 0.5% to eliminate the effect of the solvent on the aggregation.[83]

Each sample was measured in triplicate and the data are represented as means ± SE. A one-way analysis of variance was used for multiple comparisons and if significant variation occurred between treatment groups, the mean values for inhibitors were compared with those for controls by Student's t test. $p<0.05$ was considered to be statistically significant. The IC_{50} values of the compounds were obtained from at least three determinations.

3.5.6 Platelet Activating Factor (PAF) Antagonistic Activities

Dried methanol extracts were dissolved in DMSO and diluted with normal saline to give final concentrations of 200, 100, 50, 20 and 10 μg/mL. A reaction mixture containing 0.2% DMSO in saline was used as control. The final concentration of DMSO in reaction mixtures was 0.2% to avoid interference with the receptor-binding studies.

Tris-tyrode buffer (10 mM, pH 7.0) was used as media forbinding studies. CD solution (0.15M trisodium citrate, 0.075M citric acid, pH 5.2) was used as anticoagulant. Buffer A (20% ACD solution, 60% K_2HPO_4 buffer, 20% sodium citrate, pH 6.8) and buffer B (50 ml K_2HPO_4, 0.1 gm bovine serum albumin, pH 7.0) were used for washing of platelets. Bovine serum albumin was purchased from Boehringer Mannheim Co. (Germany). Radiol abeled PAF (1-O-^{3}H-octadecyl-2-acetyl-sn-glycero-3-phosphocholine, 125 Ci m/mol) was purchased from Amersham (UK). Unlabeled PAF was obtained from Sigma Chemical Co. (USA). Six volumes of blood were collected from the rabbit (New Zealand White) marginal ear vein and introduced directly into one volume of ACD solution. The blood was centrifuged at 270 g for 10

min at room temperature, and the top platelet-rich plasma removed carefully. The latter was further centrifuged at 500 g for15 min. The platelet pellets were washed twice by centrifugation at 500 g (15 min) in buffer A, followed once at 150 g (10 min) in buffer B. The top whitish layer was removed and centrifuged at 500 g (15 min) to obtain the platelets. The final platelet concentration was adjusted to $3x10^8$ platelets mL.

The PAF receptor-binding inhibitory assay was carried out using washed rabbit platelets according to the modified method of Valone et al. (1982). The reaction mixture consisted of 200 µl of washed rabbit platelet suspension, 25 µl of ^{3}H-PAF (2.0µM) with or without unlabeled PAF (2.0 µM) and 25 µl of sample or control solution. The final concentrations of sample in the reaction mixtures were 18.2, 9.1, 4.5, 1.8 or 0.9 µg mL. Cedrol, a known PAF receptor antagonist, was used as a standard in the bioassay. The reaction mixture was incubated at room temperature for 1 h. The free and bound ligands were separated by filtration technique using a Whatman GF/C glass fiber filter . The radioactivity was measured by scintillation counting. The difference between total radioactivity of bound ^{3}H-PAF in the absence and the presence of excess unlabeled PAF is defined as specific binding of the radiolabeled ligand. Percentage inhibition of the sample was obtained according to the following equation:

$$\% \text{ Inhibition} = [(Sc-Ss)/Sc]x100$$
$$= \{[(Tc-Nc)-(Ts-Ns)]/(Tc-Nc)\}x100$$

Sc = the specific binding of control;

Ss = specificbinding of sample;

Tc = the total binding of control;

Ts = the total binding of sample;

Nc = the nonspecific binding of control

Ns = the nonspecific binding of sample.

Ergosterol was obtained as a white solid, **(1.1)** 104 mg (0.0347%) from *C. brasiliense*, **(2.1)** 44.6 mg (0.0387%) from *C. bostrychodes*, and **(3.1)** 37.9 mg (0.0274%) from *C. siamense.*

R_f = 0.67 (40% EtOAc:hexane); mp 156-158 °C (lit.[54] 157 °C);

IR (KBr) ν_{max} cm^{-1}: 3429 (O-H, br), 3044 (=C-H, w), 2953, 2870 (C-H, s), 1655 (C=C, w), 1458, 1382, 1368 (C-H, m), 1055, 1032 (C-O, m);

^1H NMR ($CDCl_3$, 400 MHz): δ 5.58 (1H, dd, J=5.6, 2.4 Hz), 5.38 (1H, dd, J=5.6, 2.4 Hz), 5.22 (1H, dd, J=15.6, 7.0 Hz), 5.18 (1H, dd, J=15.6, 7.0 Hz), 3.66 (1H, m, CH-O), 2.50-1.10 (20H, m, CH, CH_2), 1.03 (3H, d, J=6.4 Hz), 0.94 (3H, s), 0.91 (3H, d, J=6.8 Hz), 0.84 (3H, d, J=6.4 Hz), 0.82 (3H, d, J=6.4 Hz), 0.63 (3H, s);

^{13}C NMR ($CDCl_3$, 100 MHz): δ 141.4, 139.8 (C=C), 135.6, 132.0 (C=C), 119.5, 116.7 (C=C), 70.5 (C-O), 55.8, 54.6, 46.3, 42.9, 40.8, 40.4, 39.1, 38.4, 37.1, 33.1, 32.0, 28.8, 23.0, 21.1, 20.0, 19.7, 17.6, 16.3, 12.1 (C, CH, CH_2, CH_3).

24(*R*)-5α,8α-Epidioxyergosta-6-22-diene-3β-ol was obtained as a white solid, **(1.2)** 7.4 mg (0.0025%) from *C. brasiliense*, **(2.2)** 52 mg (0.0437%) from *C. bostrychodes*, and **(3.2)** 136.2 mg (0.0454%) from *C. siamense*.

R_f=0.32 (40% EtOAc:hexane); mp 180-181 °C (lit.[54] 182-184 °C);

IR (KBr) ν_{max} cm^{-1}: 3301 (O-H, br), 3080 (=C-H, w), 2955, 2871 (C-H, s), 1655 (C=C, w), 1458, 1377 (C-H, m), 1044 (C-O, m);

^1H NMR ($CDCl_3$, 400 MHz): δ 6.49 (1H, d, J=8.7 Hz), 6.23 (1H, d, J=8.7 Hz), 5.20 (1H, dd, J=15.3, 7.2 Hz), 5.18 (1H, dd, J=15.3, 8.1 Hz), 3.96 (1H, m, CH-O), 2.11-1.18 (20H, m, CH, CH_2), 1.00-0.78 (18H, $6CH_3$);

^{13}C NMR ($CDCl_3$, 100 MHz): δ 135.4, 135.2, 132.3, 130.8 (C=C), 82.2, 79.4, 66.5 (C-O), 56.2, 51.7, 51.1, 44.6, 42.8, 39.7, 39.4, 37.0, 36.9, 34.7, 33.1, 30.1, 28.6, 23.4, 20.9, 20.6, 19.9, 19.6, 18.2, 17.6, 12.9 (C, CH, CH_2, CH_3).

Mollicellin H (1.3) was obtained as a white solid, 14 mg (0.0047%) from *C.brasiliense*.

R_f=0.27 (50% EtOAc:hexane); mp 137-138 °C;

IR (KBr) ν_{max} cm^{-1} : 3207(O-H, br), 2977, 2897 (=C-H, w), 1731 (C=O, s), 1655, 1638, 1593, 1573 (C=C, m);

^1H and ^{13}C NMR data were given in Table 2.2 (Page 33)

Mollicellin J (1.4) was obtained as a white solid, 54 mg (0.0018%) from *C.brasiliense*.

R_f = 0.25 (50% EtOAc:hexane); mp 178-179 °C;

IR (KBr) ν_{max} cm^{-1}: 3410 (O-H, br), 2959, 2923, 2855(=C-H, w), 1713 (C=O, s), 1637, 1562 (C=C, w);

^{1}H and ^{13}C NMR data were given in Table 2.4 (Page 36).

Mollicellin K (1.5) was obtained as a white solid, 155.1 mg (0.0517%) from *C.brasiliense*.

R_f = 0.34 (CH_2Cl_2); mp 178-181 °C;

UV (MeOH) λ_{max} nm (log ε): 203 (4.47), 264 (4.52);

IR (KBr) ν_{max} cm^{-1} : 3407(O-H, w), 2977(C-H, w), 2360 (C-H, w), 1731(C=O, s), 1656, 1638 (C=C, m), 1594, 1573(C=C, s);

HRESITOFMS m/z 383.1161 $[M+H]^+$ (calcd for $C_{21}H_{18}O_7$+H, 383.1131);

^{1}H and ^{13}C NMR data were given in Table 2.5 (Page 39).

Mollicellin L (1.6) was obtained as a white solid, 25.2 mg (0.0083%) from *C.brasiliense*.

R_f = 0.45 (50% EtOAc:hexane); mp 215-219 °C;

UV (MeOH) λ_{max} nm (log ε): 203 (4.27), 264 (4.47);

IR (KBr) ν_{max} cm^{-1} : 3439 (O-H, w), 3028, 2983, 2931, 1729 (C=O, s), 1677 (C=O, w), 1644 (C=C, s), 1608, 1568 (C=C, w);

HRESITOFMS m/z 397.1286 $[M+H]^+$ (calcd for $C_{22}H_{20}O_7$+H, 397.1287);

^{1}H and ^{13}C NMR data were given in Table 2.7 (Page 42).

Mollicellin M (1.7) was obtained as a white solid, 7.8 mg (0.0026%) from *C.brasiliense*.

R_f = 0.49 (50% EtOAc:hexane); mp 247-250 °C;

UV (MeOH) λ_{max} nm (log ε): 201 (4.01), 261 (3.95);

IR (KBr) ν_{max} cm^{-1}: 3454 (O-H, w), 2979, 2917, 1736 (C=O, s), 1688 (C=O, w), 1652(C=C, s), 1600, 1562 (C=C, w);

HRESITOFMS m/z 439.0561 $[M+Na]^+$ (calcd for $C_{21}H_{17}ClO_7$+H, 439.0561);

^{1}H and ^{13}C NMR data were given in Table 2.8 (Page 45).

Mollicellin B (1.8) was obtained as a white solid, 67.6 mg (0.023%) from *C. brasiliense*.

R_f = 0.45 (CH_2Cl_2); mp 203-205 °C;

UV (MeOH) λ_{max} nm (log ε): 201 (4.05), 261 (3.95);

IR (KBr) ν_{max} cm^{-1} : 3461 (O-H, w), 3089 (=C-H, w), 2979, 2929 (C-H, m), 2854 (C-H, w), 1739 (C=O, s), 1686 (C=O-H, s), 1651 (C-C=O), 1602, 1574 (C=C, m);

HRESITOFMS m/z 405.0953 $[M+Na]^+$(calcd for $C_{21}H_{18}O_7$+Na, 405.0905);

^1H and ^{13}C NMR data were given in Table 2.9 (Page 48).

Mollicellin C (1.9) was obtained as a white solid, 13.2 mg (0.0045%) from *C. brasiliense*.

R_f = 0.45 (50% EtOAc:hexane); mp 200-202 °C;

UV (MeOH) λ_{max} nm (log ε): 206 (4.21), 267 (3.97);

IR (KBr) ν_{max} cm^{-1} : 3389 (O-H, br), 2928 (=C-H, w), 1734 (C=O, s), 1645 (C=O-H, s), 1559 (C=C, m);

HRESITOFMS m/z 435.0648 $[M+Na]^+$(calcd for $C_{22}H_{20}O_8$+Na, 435.1055);

^1H and ^{13}C NMR data were given in Table 2.11 (Page 52).

Mollicellin E (1.10) was obtained as a white solid, 66.6 mg (0.022%) from *C.brasiliense*.

R_f = 0.37 (40% EtOAc:hexane); mp 167-170 °C;

UV (MeOH) λ_{max} nm (log ε): 205 (4.18), 267 (3.80);

IR (KBr) ν_{max} cm^{-1}: 3382 (O-H, br), 2921, 2850 (=C-H, w), 1741 (C=O, s), 1651 (C=O-H, s), 1566 (C=C, w);

HRESITOFMS m/z 469.0427 $[M+Na]^+$(calcd for $C_{22}H_{19}ClO_8$+Na, 469.0665);

^1H and ^{13}C NMR data were given in Table 2.13 (Page 55).

Mollicellin N (1.11) was obtained as a white solid, 16.2mg (0.0054%) from *C.brasiliense*.

R_f = 0.50 (CH_2Cl_2); mp 251-253 °C;

IR (KBr) ν_{max} cm^{-1} : 3355(O-H, w); 2979, 2932, 1738 (C=O, s), 1688 (C=O, w), 1644, 1574 (C=C, w);

HRESITOFMS m/z 421.0897 $[M+Na]^+$ (calcd for $C_{21}H_{18}O_8$+Na, 421.0899);

^1H and ^{13}C NMR data were given in Table 2.14 (Page 58).

Mollicellin F (1.12) was obtained as a white solid, 21.3 mg (0.007%) from *C.brasiliense*.

R_f = 0.40 (30% EtOAc:hexane); mp 256-257 °C;

UV (MeOH) λ_{max} nm (log ε): 201 (3.71), 267 (3.86), 224 (4.05);

IR (KBr) ν_{max} cm^{-1} : 3400 (O-H, br), 2980, 2924 (=C-H, w), 1741 (C=O, s), 1688, 1644, 1565 (C=C, w);

HRESITOFMS m/z 455.0318 $[M+Na]^+$(calcd for $C_{21}H_{17}ClO_8$+Na, 455.0406);

^1H and ^{13}C NMR data were given in Table 2.16 (Page 62).

Ergosterylpalmitate was obtained as amorphous solid, (**2.3**) 8.7 mg (0.0073%) from *C. bostrychodes* and **(3.3)** 116.7 mg (0.0087%) from *C. siamense*.

R_f = 0.72 (50% CH_2Cl_2:hexane); mp 100-101 °C (lit.[11] 102-103°C);

IR (KBr) ν_{max} cm^{-1}: 3340 (H-C=C, b), 2955, 2929, 2870 (C-H, s), 1738 (C=O, s), 1457, 1377 (C-H, m), 1173 (C-O, w);

^1H NMR ($CDCl_3$, 400 MHz): δ 5.58 (1H, dd, *J*=5.6, 2.4 Hz), 5.38 (1H, dd, *J*=5.6, 2.4 Hz), 5.22 (1H, dd, *J*=15.6, 7.0 Hz), 5.18 (1H, dd, *J*=15.6, 7.0 Hz), 3.66 (1H, m, CH-O), 2.50-1.10 (20H, m, CH, CH_2), 1.03 (3H, d, *J*=6.4 Hz), 0.94 (3H, s), 0.91 (3H, d, *J*= 6.8 Hz), 0.84 (3H, d, *J*=6.4 Hz), 0.82 (3H, d, *J*=6.4 Hz), 0.63 (3H, s);

^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.3 (C-O), 141.4, 133.6 (C=C), 135.6, 131.9 (C=C), 120.1, 116.2 (C=C), 72.4 (C-O), 55.7, 54.5, 46.0, 42.7, 40.3, 39.0, 37.9, 37.0, 36.6, 34.6, 33.0, 28.1, 25.0, 22.6, 21.0, 19.6, 17.5, 16.1, 14.0, 12.0 (C, CH, CH_2, CH_3).

Chaetoviridin A was obtained as orange crystals, **(2.4)** 145.2 mg (0.0.122%) from *C. bostrychodes* and (**3.5**) 983.5 mg (0.729%) from *C. siamense*.

R_f = 0.40 (40% EtOAc:hexane); mp 155-156 °C (lit.[27] 121-124 °C);

IR (KBr) ν_{max} cm^{-1}: 3436 (O-H, br), 3108 (C-H, w), 2968, 2930, 2875 (C-H, w), 1774 (C=O, s), 1678, 1648, 1619 (C=O, s), 1516 (C=C, s), 1453, 1401, 1375, 1358 (C-H, w), 1248, 1016 (C-O, m);

^{1}H and ^{13}C NMR data were given in Table 2.17 (Page 66).

Chaetoviridin F was obtained as a red solid, **(2.5)** 9.5 mg (0.079%) from *C. bostrychodes*, and **(3.6)** 15.5 mg (0.0115%) from *C. siamense*.

R_f = 0.62 (40% EtOAc:hexane); mp 84-86 °C, (lit.[27] 121-124 °C);

IR (KBr) ν_{max} cm^{-1}: 3107 (=C-H, w), 2962, 2927, 2873, (C-H, m), 1769, 1682, 1647 (C=O, s), 1620, 1510 (C=C, s), 1458, 1402, 1348, 1325 (C-H, m), 1163, 1014 (C-O, m);

^{1}H and ^{13}C NMR data were given in Table 2.17 (Page 66).

Chrysophanol was obtained as orange needles, **(2.6)** 3.6 mg (0.0302%) from *C. bostrychodes* and **(3.8)** 2.0 mg (0.0014%) from *C. siamense*.

R_f = 0.60 (20% EtOAc:hexane); mp 197-198 °C (lit.[11] 195-196 °C);

IR (KBr) ν_{max} cm^{-1}: 3433 (O-H, br), 3036 (=C-H, w), 2925, 2854 (C-H, m), 1676, 1627 (C=O, s), 1605, 1567 (C=C, m), 1474, 1452, 1369 (C-H, m), 1269, 1158 (C-O, s);

^{1}H and ^{13}C NMR data were given in Table 2.18 (Page 69).

Emodin (**2.7**) was obtained as an orange solid, 9.5 mg (0.0079%) from *C. bostrychodes*.

R_f = 0.51 (40% EtOAc:hexane); mp 248-249 °C (lit.[11] 254-256 °C);

IR (KBr) ν_{max} cm^{-1}: 3367 (O-H, br), 3050 (=C-H, w), 2920 (C-H, w), 1630 (C=O, s), 1588, 1559 (C=C, m), 1480, 1372, 1338 (C-H, m), 1295, 1163 (C-O, s), 758 (s);

^{1}H and ^{13}C NMR data were given in Table 2.18 (Page 69).

Cochliodone D (**3.4**) was obtained as a pale yellow solid, 13.3 mg (0.0096%) from *C. siamense*.

R_f = 0.40 (50% EtOAc/hexane); mp 150-151 °C (lit.[27] 158-160 °C);

IR (KBr) ν_{max} cm^{-1}: 3059 (=C-H, w), 2975, 2940, 2877, 2850 (C-H, m), 1764, 1746, 1713 (C=O, s), 1676 (C=O, s), 1603, 1582 (C=C, s), 1446, 1411, 1372, 1345 (C-H, m), 1243, 1219, 1084 (C-O, s);

HRESITOFMS *m/z* 777.2371 $[M+Na]^+$ (calcd for $C_{38}H_{42}O_{16}$+Na, 777.2292).

Chaetoviridin G (**3.7**) was obtained as a yellow solid, 10.9 mg (0.0014%) from *C. siamense*.

R_f = 0.72 (20% EtOAc:CH_2Cl_2); mp 178-179 °C (decomp);

IR (KBr) ν_{max} cm^{-1}: 3460 (O-H, br), 3108 (=C-H, w), 2958, 2922, 2872, 2861 (C-H, s), 1788, 1649, 1632 (C=O, m), 1570 (C=C, m), 1462, 1374 (C-H, m), 1201, 1118, 1065 (C-O, m);

^1H and ^{13}C NMR data were given in Table 2.19 (Page 72).