CHAPTER III
EXPERIMENTAL

3.1 General Experiment Procedures

Melting points were recorded in °C and determined on a Gallenkamp melting point apparatus. Infrared spectra were recorded as KBr disks, using a BRUKER TENSOR 27 FT-IR Spectrophotometer and major bands (ν) were recorded in wave number (cm^{-1}). Ultraviolet (UV) absorption spectra were measured on an Agilent 8453 UV-visible spectrophotometer and principle band (λ_{max}) were recorded as wavelengths (nm). Optical rotation was obtained using a JASCO DIP-1000 digital polarimeter with sodium D line (590 nm). Nuclear magnetic resonance (NMR) spectra were obtained from a Varian Mercury Plus 400 spectrometer (400 MHz). Chemical shifts were recorded on δ (ppm) scale using $CDCl_3$, CD_3OD, and D_2O as the 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), quintet (quint) and multiplet (m)] and coupling constants (*J*) in Hz assigned for nuclei concerned. Complete protons and carbons assignments were based on 1D (^1H, ^{13}C and DEPT) and 2D NMR experiments (COSY, HSQC, HMBC and NOESY). HRESITOFMS spectra were obtained using a Micromass LCT mass spectrometer, and the lock mass calibration was applied for the determination of accurate masses. Column chromatography (CC) and flash column chromatography (FCC) were carried out over MERCK silica gel 60 (less than 0.063 nm, 0.040-0.063 nm and 0.063-0.200 nm). The fractions obtained from CC and FCC 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. PLC 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 Fungi Material

The fungi were collected from the soil in Yala Province, Thailand and identified by Assoc. Prof. Dr. Kasem Soytong as *C. elatum* and *C. lucknowense*. A voucher specimens were deposited at the Department of Plant Pest Management, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The fungi were cultivated on Potato Dextrose Broth (PDB) at 25-28 °C for 4 weeks and filtered out to yield mycelial mat. Then, the dried mycelial mat of *C. elatum* and *C. lucknowense* were sent to our laboratory.

3.3 Extraction and Isolation

Air-dried mycelial mat of *C. elatum* (210 g) and *C. lucknowense* (282 g) were ground into power and then extracted successively with hexane (1.5L x 3), EtOAc (1.5L x 3), and MeOH (1.5L x 3) at room temperature. The filtrated samples were combined and the solvents were evaporated *in vacuo* to yield six crude extracts. The extracts obtained from *C. elatum* were crude hexane 8.4 g (4.00%), crude EtOAc 21.7 g (10.33%) and crude MeOH 28.5 g (13.57%), while the *C. lucknowense* extracts yielded crude hexane 8.4 g (2.98%), crude EtOAc 18.0 g (6.38%), and crude MeOH 45.3 g (16.06%). The solvent extraction schemes of *C. elatum* and *C. lucknowense* are shown in Figures 3.1 and 3.2, respectively.

3.3.1 Crude hexane (*C. elatum*)

The crude hexane extract of *C. elatum* (8.4 g) was first separated by FCC on silica gel with gradient system elution of hexane:EtOAc (0-100%) and EtOAc:MeOH (0-100%). The 100 ml of eluent was collected for each fraction to give a total of 76 fractions. Based on TLC patterns, overall 76 fractions were combined to give seven major fractions, FH_1-FH_7 as shown in Table 3.1. Fraction FH_4 (261.5 mg) was further subjected to silica gel FCC, eluted with a gradient system hexane-EtOAc to afford a white solid of compounds **I** (129.0 mg) and **II** (23.6 mg). The isolation scheme of crude hexane extract of *C. elatum* is shown in Figure 3.3.

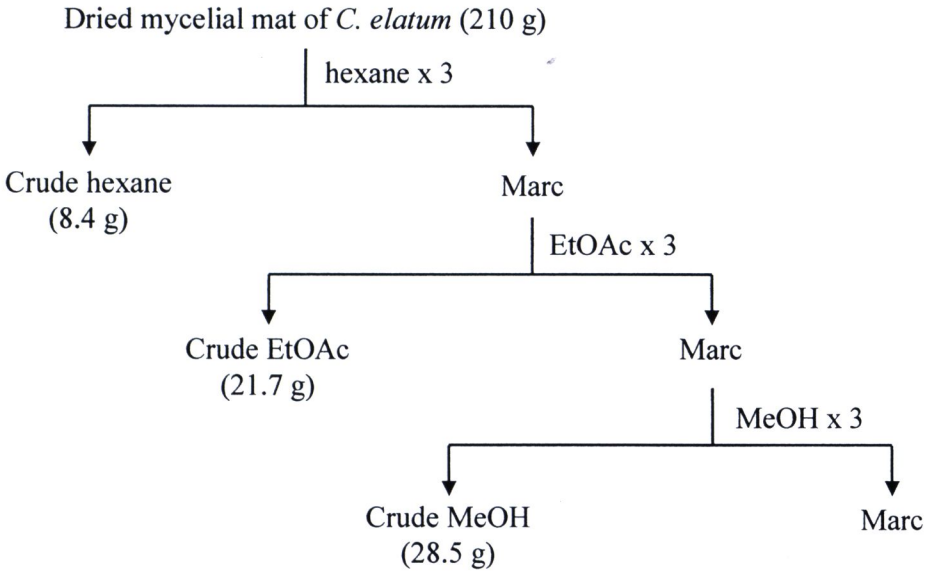

Figure 3.1 Extraction scheme of dried mycelium mat of *C. elatum*.

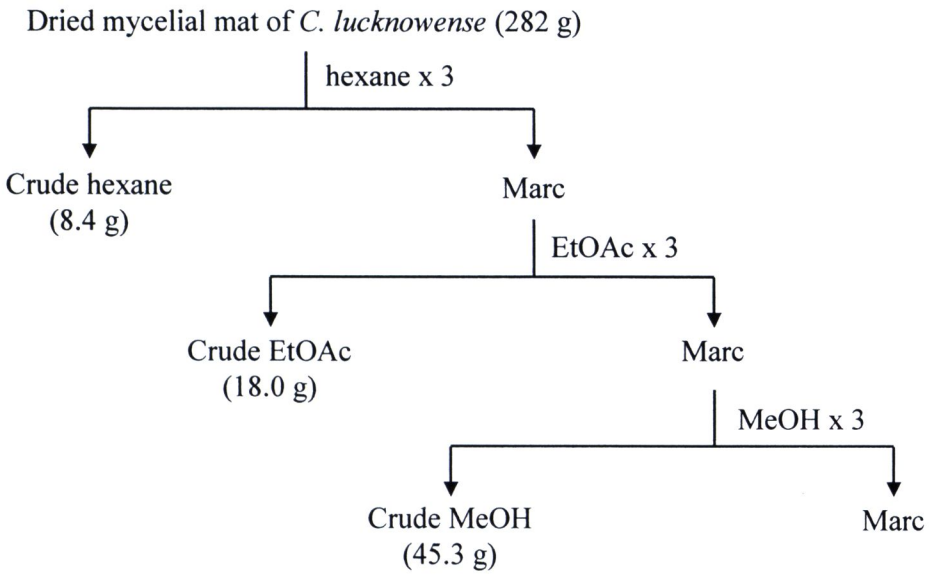

Figure 3.2 Extraction scheme of mycelial mat of *C. lucknowense*.

Table 3.1 Combined fractions from FCC of crude hexane extract of *C. elatum*

Fractions	Eluents (%v/v)	Weight (g)	Evaporated Residue
FH_1 (f_{1-22})	hexane, 15% EtOAc:hexane	5.2472	yellow liquid
FH_2 (f_{23-29})	15-20% EtOAc:hexane	0.9850	yellow liquid
FH_3 (f_{30-38})	30-40% EtOAc:hexane	0.8439	brown liquid
FH_4 (f_{39-42})	40-50% EtOAc:hexane	0.2615	brown viscous liquid
FH_5 (f_{43-60})	50-80% EtOAc:hexane	0.3799	brown viscous liquid
FH_6 (f_{61-69})	90% EtOAc:hexane, EtOAc, 10-30% MeOH:EtOAc	0.1050	brown viscous liquid
FH_7 (f_{70-76})	40% MeOH:EtOAc, MeOH	0.6585	brown viscous liquid

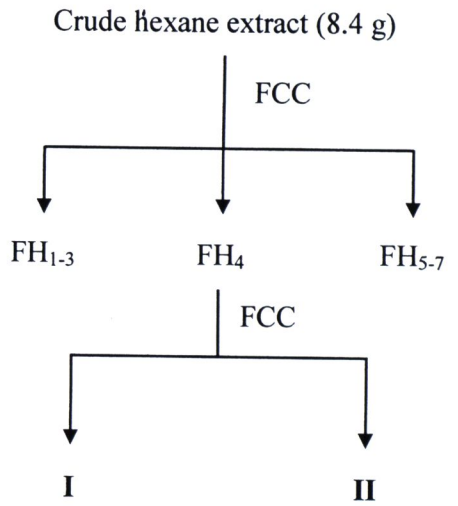

Figure 3.3 Isolation scheme of crude hexane extract of *C. elatum*.

3.3.2 Crude EtOAc (*C. elatum*)

The white precipitate in crude EtOAc extract of *C. elatum* (21.7 g) was filtered out and washed with a cool EtOAc to give a white solid of compound **III** (2.4174 g). The filtrate was evaporated to dryness and then (19.0 g) separated over silica gel FCC, eluted with a gradient system of EtOAc:hexane (0-100%) and MeOH:EtOAc (0-100%). A 100 ml of eluent was collected for each fraction to give a total of 76 fractions. Based on TLC patterns, overall 76 fractions were combined to give seven major fractions, FE_1-FE_7 as shown in Table 3.2.

Table 3.2 Combined fractions from FCC of crude EtOAc extract of *C. elatum*

Fractions	Eluents (%v/v)	Weight (g)	Evaporated Residue
FE_1 (f_{1-16})	hexane, 10% EtOAc:hexane	1.806	yellow liquid
FE_2 (f_{17-21})	10-20% EtOAc:hexane	0.502	yellow liquid
FE_3 (f_{22-30})	20-40% EtOAc:hexane	0.687	brown liquid
FE_4 (f_{31-40})	40-60% EtOAc:hexane	3.391	brown viscous liquid
FE_5 (f_{41-50})	60-80% EtOAc:hexane	4.557	brown viscous liquid
FE_6 (f_{51-65})	80% EtOAc:hexane, EtOAc	1.837	brown viscous liquid
FE_7 (f_{66-76})	10% MeOH:EtOAc, MeOH	2.296	black viscous liquid

The isolation scheme of crude EtOAc extract of *C. elatum* is shown in Figure 3.4 and the result of the separation is summarized as below.

The fraction FE_2 (502 mg) was further subjected to FCC, eluted with a gradient system of EtOAc:hexane (0-100%) to afford a white solid of an additional amount of compound **I** (212.1 mg) and the overall filtrate of fraction FE_2 (206.5 mg) was then separated over silica gel FCC with gradient elution of EtOAc:hexane (0-100%) followed by MeOH:EtOAc (0-50%) to gave an additional amount of **I** (25.1 mg) and **II** (28.5 mg).

The fraction FH_4 (3.391 g) was then separated over silica gel FCC with gradient elution of EtOAc:hexane (0-100%) followed by MeOH:EtOAc (0-100%) to give three combined subfractions, $FE_{4.1}$-$FE_{4.3}$. Subfraction $FE_{4.1}$ was precipitate with

CH_2Cl_2-MeOH to give a pale yellow solid of compound **X** (43.4 mg). The precipitate in fraction $FE_{4.2}$ was filtered out and recrystallized from the combination solvent of CH_2Cl_2 and MeOH to give a white solid of compound **III** (37.2 mg). The fraction $FE_{4.3}$ (1.0557 g) was separated over silica gel FCC with gradient elution of EtOAc:hexane (10-100%) followed by MeOH:EtOAc (0-50%) to gave seven combined subfractions, $FE_{4.3.1}$-$FE_{4.3.7}$. Subfraction $FE_{4.3.5}$ (275.0 mg) was further separated by PLC using 10%MeOH:CH_2Cl_2 as eluent (developed x 2) to give compounds **IX** (21.0 mg) and **XI** (10.9 mg).

Separation of fraction FE_5 (4.557 g) by FCC with gradient elution of EtOAc:hexane (50-100%) followed by MeOH:EtOAc (0-100%) to give eight subfractions, $FE_{5.1}$-$FE_{5.8}$. The white precipitate in subfraction $FE_{5.1}$ was filtered out and recrystallized from CH_2Cl_2 and MeOH to give an additional amount of compound **III** (14.2 mg). The isolated subfraction $FE_{5.2}$ was recrystallized from CH_2Cl_2 and MeOH to give a yellow solid of compound **VII** (204.2 mg). The white precipitate in subfraction $FE_{5.4}$ was filtered out and recrystallized from the combination solvent of CH_2Cl_2 and MeOH to give compound **IV** (17.0 mg). The subfraction $FE_{5.7}$ was purified by CC to give a white precipitate which was recrystallized from CH_2Cl_2 and MeOH to give a white solid of compound **V** (19.6 mg). The fraction $FE_{5.5}$ (552.5 mg) was then separated over silica gel FCC with gradient elution of MeOH:CH_2Cl_2 (0-100%) to give four combined subfractions, $FE_{5.5.1}$-$FE_{5.5.4}$. The isolated subfraction $FE_{5.5.1}$ was recrystallized from CH_2Cl_2 and MeOH to give an additional amount of compound **III** (16.8 mg). The white precipitate in subfraction $FE_{5.5.2}$ was filtered out and was recrystallized from the combination solvent of CH_2Cl_2 and MeOH to give compound **VI** (9.3 mg). The precipitate in subfraction $FE_{5.5.3}$ was filtered out and was recrystallized from the combination solvent of CH_2Cl_2 and MeOH to give a white solid of compound **IV** (50.1 mg). The isolated fraction $FE_{5.5.4}$ was recrystallized from CH_2Cl_2 and MeOH to give pale yellow crystals of compound **VIII** (43.8 mg).

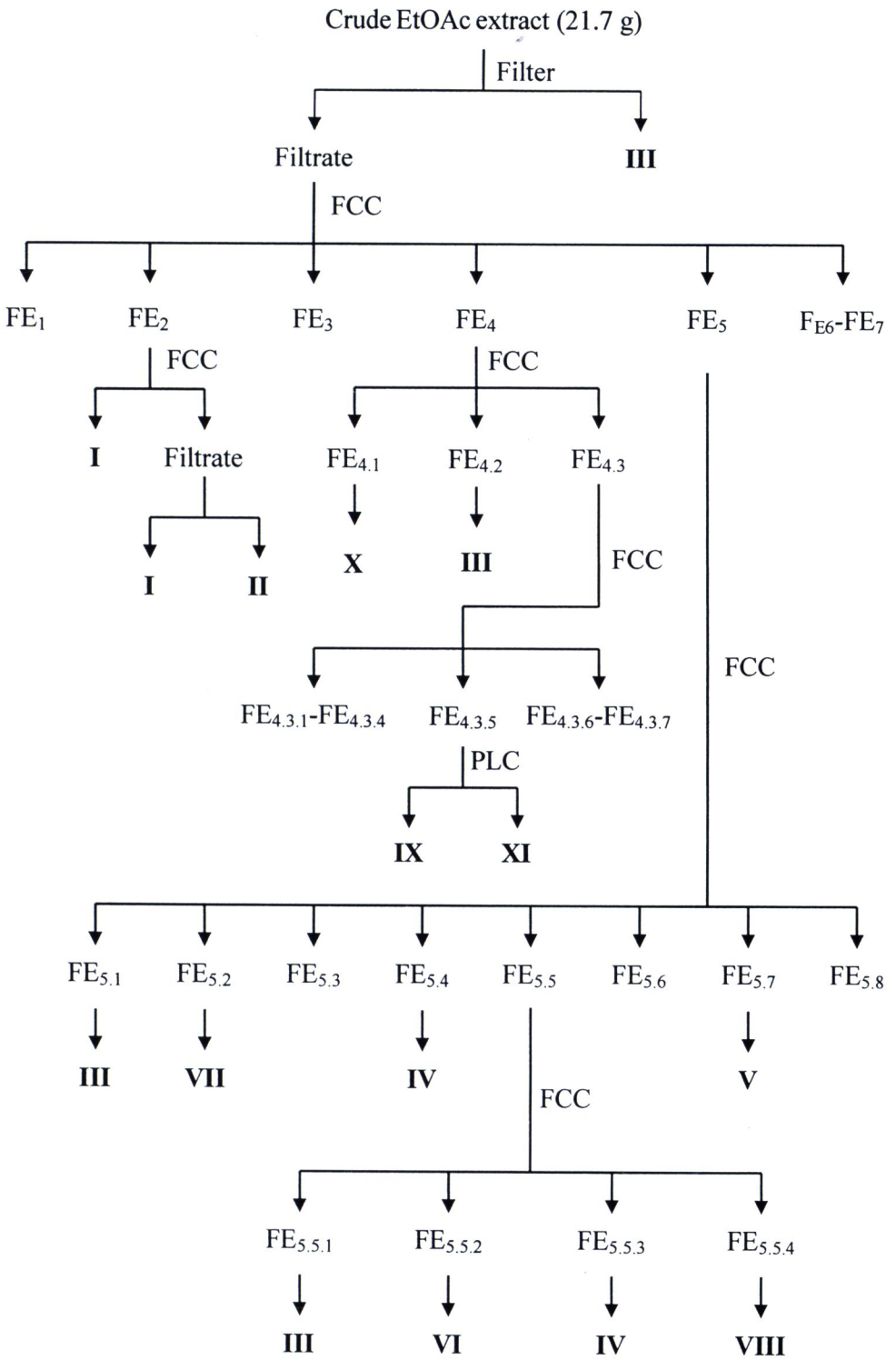

Figure 3.4 Isolation scheme of crude EtOAc extract of *C. elatum*.

3.3.3 Crude MeOH (*C. elatum*)

The MeOH extract of *C. elatum* (28.5) was chromatographed on a silica gel FCC with gradient elution of MeOH:CH_2Cl_2 (0-100%). The 100 ml of eluent was collected for each fraction to give a total of 51 fractions. Based on TLC patterns, overall 51 fractions were combined to give five major fractions, FM_1-FM_5 as shown in Table 3.3. The fraction FM_2 was recrystallized from CH_2Cl_2-hexane to afford a white solid of compound **I** (3.9 mg). The precipitate in fraction FM_3 was filtered out and was recrystallized from the combination solvent of CH_2Cl_2 and MeOH to give an additional amount of compound **III** (1.6378 g). The white precipitate in FM_5 (16.0579 g) was filtered out and washed with MeOH to give a white solid of compound **XIX** (1.2473 g). The isolation scheme of crude MeOH extract of *C. elatum* is shown in Figure 3.5.

Table 3.3 Combined fractions from FCC of crude MeOH extract of *C. elatum*

Fractions	Eluents (%v/v)	Weight (g)	Evaporated Residue
FM_1 (f_{1-5})	CH_2Cl_2, 1% MeOH:CH_2Cl_2	2.4293	yellow liquid
FM_2 (f_{6-10})	1-3% MeOH:CH_2Cl_2	0.2354	yellow liquid
FM_3 (f_{11-15})	3-5% MeOH:CH_2Cl_2	3.0157	brown viscous liquid
FM_4 (f_{16-20})	5-10% MeOH:CH_2Cl_2	1.3238	brown viscous liquid
FM_5 (f_{21-51})	10% MeOH:CH_2Cl_2, MeOH	16.0579	brown viscous liquid

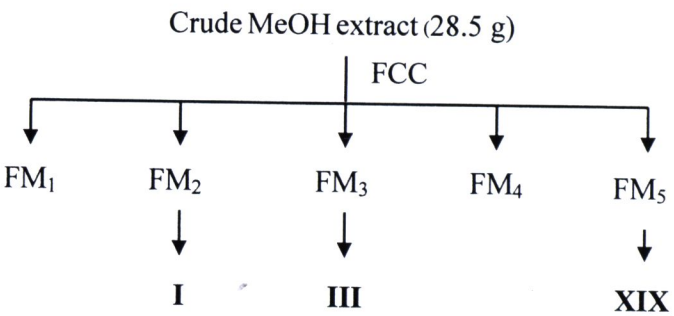

Figure 3.5 Isolation scheme of crude MeOH extract of *C. elatum*.

3.3.4 Crude hexane (*C. lucknowense*)

The crude hexane extract of *C. lucknowense* (8.4 g) was separated over silica gel FCC eluting with gradient of EtOAc:hexane (0-100%) and MeOH:CH_2Cl_2 (10-100%). The 100 ml of eluent was collected for each fraction to give a total of 51 fractions. Based on TLC patterns, overall 51 fractions were combined to 8 major fractions, FH_1-FH_8 as shown in Table 3.4

Table 3.4 Combined fractions from FCC of crude hexane extract of *C. lucknowense*

Fractions	Eluents (%v/v)	Weight (g)	Evaporated Residue
FH_1 (f_{1-9})	hexane, 20% EtOAc:hexane	3.086	yellow liquid
FH_2 (f_{10-12})	30% EtOAc:hexane	0.967	yellow liquid
FH_3 (f_{13-15})	30% EtOAc:hexane	0.371	yellow liquid
FH_4 (f_{16-18})	40% EtOAc:hexane	0.749	red viscous liquid
FH_5 (f_{19-21})	50% EtOAc:hexane	0.641	red viscous liquid
FH_6 (f_{22-24})	60% EtOAc:hexane	0.587	red viscous liquid
FH_7 (f_{25-39})	70% EtOAc:hexane, EtOAc	0.594	brown viscous liquid
FH_8 (f_{40-51})	10% MeOH:CH_2Cl_2, MeOH	0.223	brown viscous liquid

The isolation scheme of crude hexane extract of *C. lucknowense* is shown in Figure 3.6 and the result of the separation is summarized as follows.

The isolated fraction FH_1 (3.086 g) was further rechromatographed on silica gel FCC with gradient elution of EtOAc:hexane (0-100%) followed by MeOH:CH_2Cl_2 (10-100%) to give six combined subfractions, $FH_{1.1}$-$FH_{1.6}$. The orange-yellow precipitate in subfraction $FH_{1.4}$ was filtered out and was recrystallized from the combination solvent of CH_2Cl_2 and hexane to afford an orange-yellow needles of compound **XII** (10.0 mg).

Fraction FH_3 (371.0 mg) was further rechromatographed on silica gel FCC, eluted with 10% EtOAc:hexane, to give a white solid which was recrystallized from CH_2Cl_2 and hexane to yield colorless needles of compound **I** (17.8 mg) and a white solid of compound **II** (19.4 mg).

Solid of fraction FH_4 (749.0 mg) was recrystallized from the combination solvent of CH_2Cl_2 and hexane to give red-orange needles of compound **XIV** (137.0 mg).

Fraction FH_5 (641.0 mg) was further purified by FCC, eluted with 30% EtOAc:hexane, to afford a white solid which was recrystallized from CH_2Cl_2 and hexane to give an additional amount of compound **II** (50.3 mg).

Fraction FH_6 (587.0 mg) was further subjected to silica gel FCC, eluted with gradient system hexane:EtOAc to afford a white solid of compound **XV** (188.3 mg)

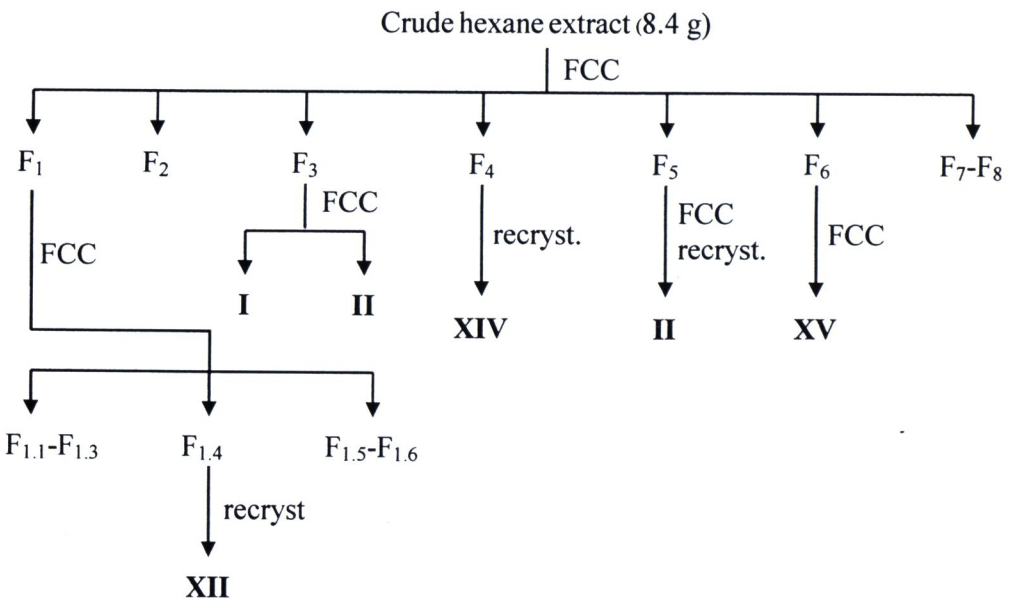

Figure 3.6 Isolation scheme of crude hexane extract of *C. lucknowense*.

3.3.5 Crude EtOAc (*C. lucknowense*)

The crude EtOAc extract of *C. lucknowense* (18.0 g) was separated over silica gel FCC with gradient elution of EtOAc:hexane (0-100%) and MeOH:EtOAc (0-100%). A 100 ml of eluent was collected for each fraction to give a total of 27 fractions. Based on TLC patterns, overall 27 fractions were combined to give five major fractions, FE_1-FE_5 as shown in Table 3.5.

Table 3.5 Combined fractions from FCC of crude EtOAc extract of *C. lucknowense*

Fractions	Eluents (%v/v)	Weight (g)	Evaporated Residue
FE_1 (f_{1-4})	hexane, 20% EtOAc:hexane	3.802	yellow liquid
FE_2 (f_{5-9})	20-50% EtOAc:hexane	1.749	red wax
FE_3 (f_{10-12})	50-70% EtOAc:hexane	3.574	red wax
FE_4 (f_{13-18})	80% EtOAc:hexane, EtOAc 10-30% MeOH:EtOAc	3.948	brown viscous liquid
FE_5 (f_{19-27})	40% MeOH:EtOAc, MeOH	3.745	brown viscous liquid

The isolation scheme of the crude EtOAc extract of *C. lucknowense* is shown in Figure 3.7 and the result of the separation is summarized as follows.

Fraction FE_1 (3.802 g) was further subjected to FCC, eluted with a gradient system of 10% EtOAc:hexane to afford an additional amount of compound **XII** (28.8 mg).

Fraction FE_2 (1.749 g) was separated by FCC with gradient elution of EtOAc:hexane (0-100%) followed by MeOH:EtOAc (10-100%) to give ten subfractions, $FE_{2.1}$-$FE_{2.10}$. The orange-yellow precipitate in subfraction $FE_{2.1}$ was filtered out and recrystallized from CH_2Cl_2 and hexane to give an additional amount of compound **XII** (42.7 mg). The isolated subfraction $FE_{2.3}$ was recrystallized from CH_2Cl_2 and hexane to give an additional amount of compound **I** (29.8 mg). The orange-yellow precipitate in subfraction $FE_{2.5}$ was filtered out and was recrystallized from the combination solvent of EtOAc and hexane to give compound **XIII** (25.7 mg). The subfraction $FE_{2.7}$ was purified by recrystallized from CH_2Cl_2 and hexane to give a white solid of compound **II** (16.3 mg).

Fraction FE_3 (3.574 g) was then separated over silica gel FCC with gradient elution of EtOAc:hexane (40-100%) followed by MeOH:EtOAc (10-100%) to give 7 combined subfractions, $FE_{3.1}$-$FE_{3.7}$. The subfraction $FE_{3.3}$ was purified by FCC to give yellow precipitate which was recrystallized from CH_2Cl_2 and hexane to give a yellow solid of compound **XVI** (705.0 mg).

The fraction FE_4 (3.948 g) was recrystallized from CH_2Cl_2-EtOAc to yield a yellow crystals of compound **XVIII** (289.5 mg).

The fraction FE_5 (3.745 g) was chromatographed on silica gel FCC, gradually eluted with MeOH and CH_2Cl_2 by increasing polarity of solvents to obtain 6 subfractions designed as $FE_{5.1}$ to $FE_{5.6}$. The subfraction $FE_{5.1}$ recrystallized from CH_2Cl_2 and EtOAc to yield an additional amount of compound **XVIII** (116.2 mg). Subfraction $FE_{5.4}$ was further purified by FCC to yield a yellow solid of compound **XVII** (30.0 mg).

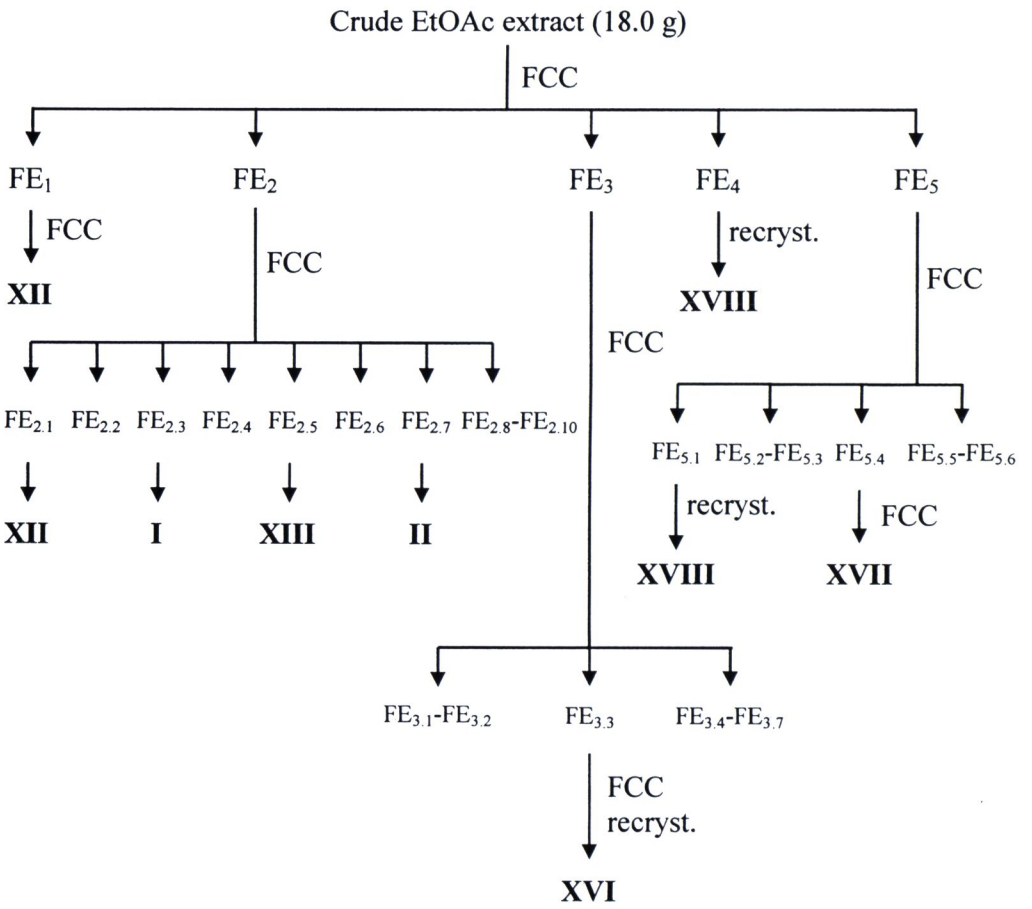

Figure 3.7 Isolation scheme of crude EtOAc extract of *C. lucknowense*.

3.3.6 Crude MeOH (*C. lucknowense*)

The MeOH extract of *C. lucknowense* (45.3 g) was chromatographed on a silica gel FCC with gradient elution of MeOH:CH_2Cl_2 (0-100%). The fraction of eluted (100 ml) was collected for each fraction to give a total of twenty fractions.

Based on TLC patterns, overall twenty fractions were combined to give six major fractions, FM_1-FM_6 as shown in Table 3.6.

Table 3.6 Combined fractions from FCC of crude MeOH extract of *C. lucknowense*

Fractions	Eluents (%v/v)	Weight (g)	Evaporated Residue
FM_1 (f_{1-3})	CH_2Cl_2, 10% MeOH:CH_2Cl_2	2.919	red liquid
FM_2 (f_{4-6})	20-30% MeOH:CH_2Cl_2	7.787	red viscous liquid
FM_3 (f_{7-9})	40-50% MeOH:CH_2Cl_2	4.512	brown viscous liquid
FM_4 (f_{10-12})	60-70% MeOH:CH_2Cl_2	6.152	brown viscous liquid
FM_5 (f_{13-15})	80-90% MeOH:CH_2Cl_2	7.929	brown viscous liquid
FM_6 (f_{16-20})	MeOH	7.923	brown viscous liquid

The isolation scheme of the crude MeOH extract of *C. lucknowense* is shown in Figure 3.8 and the result of the separation is summarized as follows.

The fraction FM_1 (2.919 g) was further purified by FCC, eluted with a gradient system of EtOAc:hexane (0-100%) to yield an additional amount of compound **XII** (98.7 mg).

Separation of fraction FM_2 (7.787 g) by FCC with gradient elution of EtOAc:hexane (0-100%) followed by MeOH:CH_2Cl_2 (10-100%) to give five subfractions, $FM_{2.1}$-$FM_{2.5}$. The orange-yellow precipitate in subfraction $FM_{2.1}$ was filtered out and was recrystallized from the combination solvent of CH_2Cl_2 and hexane to give compound **XII** (13.7 mg). The orange-yellow precipitate in subfraction $FM_{2.2}$ was filtered out and was recrystallized from EtOAc-hexane to give compound **XIII** (29.0 mg). The subfraction $FM_{2.3}$ was recrystallized from CH_2Cl_2 and EtOAc to yield an additional amount of compound **XVIII** (73.8 mg).

The white precipitate in FM_3 (4.512 g) was filtered out and washed with MeOH to give a white solid of compound **XIX** (729.3 mg).

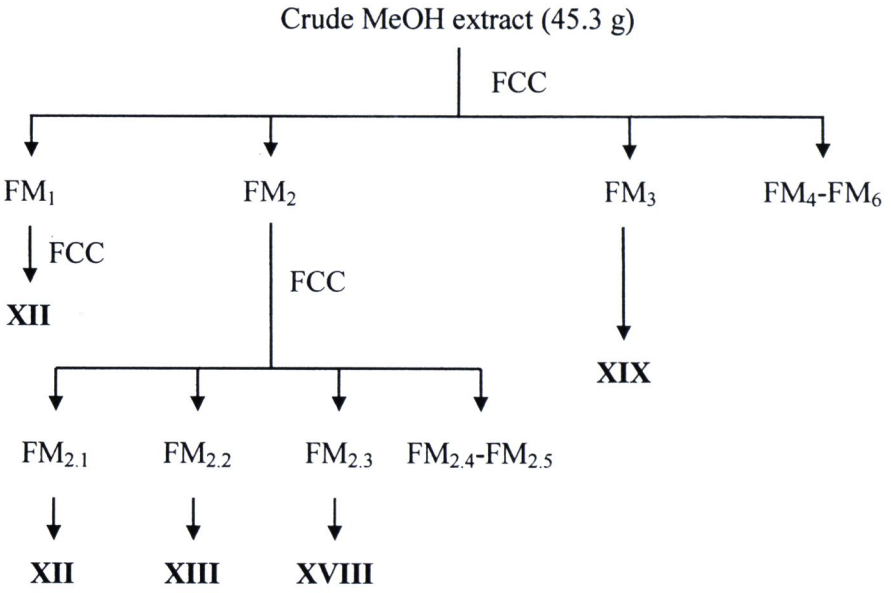

Figure 3.8 Isolation scheme of crude MeOH extract of *C. lucknowense*.

3.4 Bioassay Experiments

The bioassay experiments were carried out at the National Center for Genetic Engineering and Biotechnology (BIOTEC) and Department of Pharmacology Faculty of Medicine Khon Kaen University. The crude extract and the isolated compounds were tested for antimalarial activity against *Plasmodium falciparum*, antituberculosis activity against *Mycobacterium tuberculosis* and cytotoxicity towards human epidermoid carcinoma in the mouth (KB), human breast cancer cell (BC1), small cell lung cancer (NCI-H187), and cholangiocarcinoma (CCA).

3.4.1 Antimalarial Assay

Antimalarial activity was evaluated against the parasite *Pasmodium falciparum* (K1, multidrug resistant strain), using the method of Trager and Jensen.[71] 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.[72] 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, artemisinin, exhibited an IC_{50} value of 1 ng/mL.

3.4.2 Antimycobacterial Assay

Antimycobacterial activity was assessed againt *Mycobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA).[73] The standard drugs, isoniazid and kanamycin sulfate, showed respective MIC values of 0.04–0.09 and 2.0–5.0 μg/mL.

3.4.3 Cytotoxicity Assay

Cytotoxicity assays against human epidermoid carcinoma (KB), human breast cancer (BC1) and human small cell lung cancer (NCI-H187) cell lines were performed employing the colorimetric method as described by Skehan and coworkers.[74] The reference substance was ellipticine, showed IC_{50} values of 0.36, 0.32, and 0.26 μg/mL, respectively.

3.4.4 Cholangiocarcinoma Assay

Cholangiocarcinoma (CCA) cultured cell lines established in the Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, including KKU-100 and KKU-OCA17, derived from human intrahepatic cholangiocarcinoma tissues with histological types of poorly differentiated and well differentiated adenocarcinoma, respectively.[75,76] The CCA cells were routinely cultured in Ham's F12 media supplemented with 4 mM L-glutamine, 1 mM sodium pyruvate, 12.5 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES; pH 7.3), 100 U/mL penicillin, 100 μg/mL streptomycin sulfate, and 10% fetal calf serum. The media was renewed every 3 days, trypsinized with 0.25% trypsin-EDTA, and subcultured in the same media. The assay against cholangiocarcinoma cell line was performed employing the method described by Voigt.[77] The reference substance was 5-fluorouracil.

Ergosterol (I) was obtained as a white solid 370.1 mg (*C. elatum*) 0.176% and 47.6 mg (*C. lucknowense*) 0.017%; R_f = 0.70 (50% EtOAc/hexane); mp 156-158 °C (lit.[57] 157 °C); $[\alpha]_D^{28}$: -55 (*c* 0.145, $CHCl_3$).

IR (KBr) ν_{max} cm^{-1} : 3423 (O-H, br), 3044 (=C-H, vw), 2954, 2871 (C-H, s), 1654, 1605 (C=C, vw), 1460, 1383, 1368 (C-H, w), 1056, 1037 (C-O, w).

^1H and ^{13}C NMR data were given in Table 2.1 (Page 35).

24(*R*)-5α,8α-epidioxyergosta-6-22-diene-3β-ol (II) was obtained as a white solid 52.1 mg (*C. elatum*) 0.025% and 86.0 mg (*C. lucknowense*) 0.030%; R_f = 0.57 (50% EtOAc/hexane); mp 180-181 °C (lit.[58] 182-184 °C); $[\alpha]_D^{30}$: +104 (*c* 0.10, $CHCl_3$).

IR (KBr) ν_{max} cm^{-1} : 3521, 3300 (O-H, br), 3065 (=C-H, vw), 2955, 2869 (C-H, s), 1653 (C=C, vw), 1459, 1438, 1381, 1368 (C-H, m), 1074, 1047 (C-O, m).

^1H and ^{13}C NMR data were given in Table 2.2 (Page 37).

Chaetoglobosin C (III) was obtained as a white solid 4.1234 g (*C. elatum*) 1.96%; R_f = 0.55 (100% EtOAc); mp 258-259 °C (lit.[22] 260-263 °C); $[\alpha]_D^{25}$: -80 (*c* = 0.10, MeOH).

UV (MeOH) λ_{max} nm (log ε) : 221 (4.57), 274 (4.25), 280 (3.82), 290 (3.74).

IR (KBr) ν_{max} cm^{-1} : 3462, 3316 (N-H, m), 3122, 3085, 3059 (H-C=C,w), 2976, 2961, 2938, 2923, 2906, 2869, 2844 (C-H, m), 1714, 1703, 1688, 1647 (C=O, s), 1624 (C=C, m), 1458, 1389 (C-H, m), 1355, 1330, 1263, 1231, 1102 (C-O, m), 986 (m), 743 (m).

HRESITOFMS *m/z* 551.2522 $[M+Na]^+$ (calcd for $C_{32}H_{36}N_2O_5$ + Na, 551.2522).

^1H and ^{13}C NMR data were given in Table 2.3 (Page 41).

Isochaetoglobosin D (IV) was obtained as a white solid 67.1 mg (*C. elatum*) 0.032%; R_f = 0.60 (100% EtOAc); mp 261-262 °C (lit.[35] 260-263 °C); $[\alpha]_D^{25}$: +47 (*c* = 0.13, MeOH).

UV (MeOH) λ_{max} nm (log ε) : 220 (4.41), 252 (3.80), 282 (3.70), 290 (3.59).

IR (KBr) ν_{max} cm^{-1} : 3542 (O-H, w), 3454, 3329 (N-H, m), 3083, 3061 (H-C=C, vw), 2963, 2926, 2867, 2843 (C-H, m), 1713, 1703, 1685 (C=O, s), 1648, 1623 (C=C, s),

1459, 1446, 1425, 1390, 1356 (C-H, m), 1330, 1265, 1103, 1012 (C-O, m), 983 (m), 744 (m).

HRESITOFMS *m/z* 551.2522 $[M+Na]^+$ (calcd for $C_{32}H_{36}N_2O_5$ + Na, 551.2522).

^1H and ^{13}C NMR data were given in Table 2.4 (Page 45).

Chaetoglobosin F (V) was obtained as a white solid 19.6 mg (*C. elatum*) 0.009%; R_f = 0.42 (5% MeOH:CH_2Cl_2); mp 173-175 °C (lit.[30] 177-178 °C); $[\alpha]_D^{25}$: -125 (*c* = 0.14, MeOH).

UV (MeOH) λ_{max} nm (log ε) : 221 (4.60), 274 (3.85), 280 (3.85), 290 (3.77).

IR (KBr) ν_{max} cm^{-1} : 3421, 3361 (O-H, br), 3421, 3361 (N-H, br, m), 2961, 2929, 2851, (C-H, w), 1695, 1677 (C=O, s), 1619 (C=C, w), 1455, 1426, 1385 (C-H, w), 1261, 1232, 1111, 1054 (C-O, w), 979 (w), 742 (w).

HRESITOFMS *m/z* 553.2678 $[M+Na]^+$ (calcd for $C_{32}H_{38}N_2O_5$ + Na, 553.2678).

^1H and ^{13}C NMR data were given in Table 2.5 (Page 49).

Chaetoglobosin G (VI) was obtained as a white solid 9.3 mg (*C. elatum*) 0.004%; R_f = 0.35 (5% MeOH:CH_2Cl_2); mp 249-251 °C (lit.[23] 251-253 °C); $[\alpha]_D^{25}$: +148 (*c* = 0.13, MeOH).

UV (MeOH) λ_{max} nm (log ε) : 221 (4.63), 274 (3.88), 281 (3.88), 290 (3.80).

IR (KBr) ν_{max} cm^{-1} : 3508 (O-H, br), 3508, 3436 (N-H, s), 2927, 2896, 2840, (C-H, w), 1725, 1713, 1691 (C=O, s), 1651, 1624 (C=C, m), 1457, 1436, 1355 (C-H, w), 1257, 1228, 1125, 1024 (C-O, w), 987 (w), 742 (w).

HRESITOFMS *m/z* 551.2522 $[M+Na]^+$ (calcd for $C_{32}H_{36}N_2O_5$ + Na, 551.2522).

^1H and ^{13}C NMR data were given in Table 2.6 (Page 53).

Chaetoglobosin B (VII) was obtained as a yellow solid 204.2 mg (*C. elatum*) 0.097%; R_f = 0.62 (10% MeOH/CH_2Cl_2); mp 187-189 °C (lit.[30] 186-187 °C); $[\alpha]_D^{25}$: -242 (*c* = 0.10, MeOH).

UV (MeOH) λ_{max} nm (log ε) : 221 (4.74), 274 (3.99), 280 (4.00), 290 (3.91).

IR (KBr) ν_{max} cm^{-1} : 3416 (N-H, m), 3185, 3117 (H-C=C,vw), 2952, 2920, 2895 (C-H, w), 1694, 1675 (C=O, s), 1619 (C=C, w), 1457, 1385 (C-H, w), 1270, 1250, 1230, 1098, 1080 (C-O, w), 972 (m), 748 (m).

HRESITOFMS *m/z* 551.2522 $[M+Na]^+$ (calcd for $C_{32}H_{36}N_2O_5$ + Na, 551.2522).

^1H and ^{13}C NMR data were given in Table 2.7 (Page 57).

Chaetoglobosin D (VIII) was obtained as pale yellow crystals 43.8 mg (*C. elatum*) 0.023%; R_f = 0.49 (5% MeOH:CH_2Cl_2); mp 215-216 °C (lit.[22] 216 °C); $[\alpha]_D^{25}$: -346 (*c* = 0.10, MeOH).

UV (MeOH) λ_{max} nm (log ε) : 221 (4.63), 274 (3.88), 280 (3.88), 290 (3.80).

IR (KBr) ν_{max} cm^{-1} : 3548 (O-H, w), 3461, 3297 (N-H, m), 3101, 3061 (H-C=C, vw), 2956, 2923, 2879, (C-H, m), 1695, 1664 (C=O, s), 1606 (C=C, m), 1454, 1433, 1377 (C-H, m), 1298, 1251, 1232, 1088, 1021 (C-O, m), 984 (m), 749 (m).

HRESITOFMS *m/z* 551.2522 $[M+Na]^+$ (calcd for $C_{32}H_{36}N_2O_5$ + Na, 551.2522).

^1H and ^{13}C NMR data were given in Table 2.8 (Page 61).

Prochaetoglobosin III (IX) was obtained as a yellow solid 21.0 mg (*C. elatum*) 0.010%; R_f = 0.44 (5% MeOH:CH_2Cl_2); mp 137-138 °C (lit.[63] 137-139 °C); $[\alpha]_D^{25}$: -283 (*c* = 0.10, MeOH).

UV (MeOH) λ_{max} nm (log ε) : 222 (4.58), 280 (3.81), 290 (3.71).

IR (KBr) ν_{max} cm^{-1} : 3420 (O-H, br), 2967, 2925 (C-H, w), 1694, 1680 (C=O, s), 1659, 1632, 1610 (C=C, m), 1457, 1431 (C-H, w), 1384 (C-H, s), 1251, 1091, 1053 (C-O, w), 972 (w), 743 (w).

HRESITOFMS *m/z* 535.2573 $[M+Na]^+$ (calcd for $C_{32}H_{36}N_2O_4$ + Na, 535.2573).

^1H and ^{13}C NMR data were given in Table 2.9 (Page 65).

Prochaetoglobosin IIIed (X) was obtained as a pale yellow solid 43.4 mg (*C. elatum*) 0.021%; R_f = 0.73 (100% EtOAc); mp 154-155 °C (lit.[63] 153-155 °C); $[\alpha]_D^{25}$: -45 (*c* = 0.10, MeOH).

UV (MeOH) λ_{max} nm (log ε) : 222 (4.57), 280 (3.81), 290 (3.71).

IR (KBr) ν_{max} cm^{-1} : 3500-3300 (O-H, br), 3410 (N-H, m), 3113 (H-C=C, vw), 2952, 2922 (C-H, w), 1693 (C=O, s), 1617 (C=C, m), 1457, 1434, 1387 (C-H, w), 1251, 1095, 1036 (C-O, w), 972 (w), 744 (w).

HRESITOFMS *m/z* 535.2573 $[M+Na]^+$ (calcd for $C_{32}H_{36}N_2O_4$ + Na, 535.2573).

^1H and ^{13}C NMR data were given in Table 2.10 (Page 69).

Chaetoglobosin V (XI) was obtained as a white solid 10.9 mg (*C. elatum*) 0.005%; R_f = 0.40 (60% EtOAc:Hexane); mp 240-241 °C; $[\alpha]_D^{25}$: -28 (*c* = 0.12, MeOH).

UV (MeOH) λ_{max} nm (log ε) : 222 (4.48), 280 (3.72), 290 (3.62).

IR (KBr) ν_{max} cm^{-1} : 3525-3264 (O-H, br), 3522, 3329 (N-H, m), 2951, 2917 (C-H, w), 1745, 1704 (C=O, s), 1676, 1657, 1616 (C=C, s), 1456, 1440, 1384 (C-H, w), 1259, 1233, 1046 (C-O, w), 977 (w), 744 (w).

HRESITOFMS *m/z* 521.2780 $[M+Na]^+$ (calcd for $C_{32}H_{38}N_2O_3$ + Na, 521.2780).

^1H and ^{13}C NMR data were given in Table 2.11 (Page 74).

Chrysophanol (XII) was obtained as a orange needles 193.9 mg (*C. lucknowense*) 0.069%; R_f = 0.65 (50% EtOAc:Haxane); mp 197-198 °C (lit.[35] 197-198 °C).

UV (CH_2Cl_2) λ_{max} nm (log ε) : 229 (4.34), 259 (4.31), 290 (4.05), 432 (4.04).

IR (KBr) ν_{max} cm^{-1} : 3064 (=C-H, vw), 2922 (C-H, vw), 1672 (C=O, m), 1630 (C=O, s), 1607, 1593, 1573 (C=C, m), 1477, 1458, 1386 (C-H, m), 1272 (C=O, s), 1214 (C-O, m), 1089, 1027 (C-O, w), 753, 726 (m).

^1H and ^{13}C NMR data were given in Table 2.12 (Page 77).

Emodin (XIII) was obtained as an orange solid 54.6 mg (*C. lucknowense*) 0.019%; R_f = 0.51 (40% EtOAc:hexane); mp 261-262 °C (lit.[65] 255-256 °C).

UV (CH_2Cl_2) λ_{max} nm (log ε) : 253 (4.51), 266 (4.49), 289 (4.54), 436 (4.29).

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

^1H and ^{13}C NMR data were given in Table 2.13 (Page 77).

Chaetoviridin A (XIV) was obtained as a red-orange needles 137.0 mg (*C. lucknowense*) 0.048%; R_f = 0.40 (40% EtOAc:hexane); mp 157-158 °C (lit.[31] 121-124 °C); $[\alpha]_D^{27}$: +97 (*c* = 0.05, $CHCl_3$).

UV (CH_2Cl_2) λ_{max} nm (log ε) : 229 (3.95), 306 (4.32), 366 (4.15)

IR (KBr) ν_{max} cm^{-1} : 3421 (O-H, br), 2971, 2957 (=C-H, w), 2922, 2874 (C-H, w), 1773, 1680, 1649 (C=O, s), 1623, 1517 (C=C, s), 1454, 1402, 1375, 1359 (C-H, m), 1255, 1240 (C=O, m), 1119, 1039 (C-O, m), 983 (w), 733 (w).

^1H and ^{13}C NMR data were given in Table 2.14 (Page 81).

Cochliodone D (XV) was obtained as pale yellow solids 188.3 mg (*C. lucknowense*) 0.067%; R_f = 0.40 (50% EtOAc/hexane); mp 158-160 °C (lit.[66] 158-160 °C); $[\alpha]_D^{31}$: -276 (*c* 0.22, $CHCl_3$).

UV (CH_2Cl_2) λ_{max} nm (log ε) : 230 (3.81), 299 (4.32), 334 (4.26).

IR (KBr) ν_{max} cm^{-1} : 3059 (=C-H, w), 2975, 2940, 2877, 2850 (C-H, m), 1764, 1713 (C=O, s), 1676 (C=O, s), 1604, 1582 (C=C, s), 1446, 1411, 1372, 1345 (C-H, m), 1243, 1219 (C=O, s), 1084 (C-O, s).

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

^1H and ^{13}C NMR data were given in Table 2.15 (Page 85).

Xanthoquinodin A1 (XVI) was obtained as a yellow solid 705.0 mg (*C. lucknowense*) 0.250%; R_f = 0.49 (100% EtOAc); mp 298-300 °C (decomp); $[\alpha]_D^{25}$: +804 (*c* = 0.10, $CHCl_3$).

UV (CH_2Cl_2) λ_{max} nm (log ε) : 339 (4.51), 379 (4.38)

IR (KBr) ν_{max} cm^{-1} : 3436 (O-H, br), 2952 (=C-H, w), 1741, 1687 (C=O, m), 1610 (C=O, s), 1584 (C=C, s), 1483, 1435, 1362 (C-H, m), 1295, 1256 (C=O, m), 1148, 1086 (C-O, m), 966 (w), 796 (w).

HRESITOFMS *m/z* 595.1215 $[M+Na]^+$ (calcd for $C_{31}H_{24}O_{11}$ + Na, 595.1216).

^1H and ^{13}C NMR data were given in Table 2.16 (Page 89).

Xanthoquinodin B2 (XVII) was obtained as a yellow solid 30.0 mg (*C. lucknowense*) 0.011%; R_f = 0.44 (10% MeOH/EtOAc); mp 304-306 °C (decomp); $[\alpha]_D^{25}$: +67 (*c* = 0.10, MeOH).

UV (CH_2Cl_2) λ_{max} nm (log ε) : 237 (4.11), 274 (3.85), 362 (4.01)

IR (KBr) ν_{max} cm^{-1} : 3430 (O-H, br), 1623 (C=O, s), 1596 (C=C, s), 1455, 1386, 1362(C-H, m), 1322, 1271 (C=O, m), 1097 (C-O, m), 801 (w), 721 (w).

HRESITOFMS *m/z* 595.1220 $[M+Na]^+$ (calcd for $C_{31}H_{24}O_{11}$ + Na, 595.1216).

^1H and ^{13}C NMR data were given in Table 2.17 (Page 93).

XVIII was obtained as yellow crystals 479.5 mg (*C. lucknowense*) 0.170%; R_f = 0.24 (100% CH_2Cl_2); mp 317-318 °C (decomp); $[\alpha]_D^{31}$: +304 (*c* = 0.20, $CHCl_3$).

UV (CH_2Cl_2) λ_{max} nm (log ε) : 239 (4.25), 281 (4.29), 282 (4.13)

IR (KBr) ν_{max} cm^{-1} : 3421 (O-H, br), 2993, 2958 (=C-H, w), 2920, 2895 (C-H, w), 1797, 1784, 1749, 1685 (C=O, m), 1648, 1630 (C=O, s), 1600, 1564 (C=C, s), 1484, 1428, 1362(C-H, m), 1294, 1277 (C=O, m), 1217, 1137 (C-O, m), 962 (w), 746 (w).

HRESITOFMS *m/z* 595.1226 $[M+Na]^+$ (calcd for $C_{31}H_{24}O_{11}$ + Na, 595.1216).

^1H and ^{13}C NMR data were given in Table 2.18 (page 98).

D-mannitol (XIX) was obtained as a white solid 1.2473 g (*C. elatum*) 0.594% and 729.3 mg (*C. lucknowense*) 0.259%; mp 167-168 °C(lit.[69] 169 °C).

IR (KBr) ν_{max} cm^{-1} : 3389, 3289 (O-H, br), 2970, 2954, 2939, 2917 (C-H, m), 1458, 1429, 1388, 1317 (C-H, m), 1080, 1019 (C-O of 2°, 1° OH, s).

^1H NMR (D_2O, 400 MHz) : δ 3.77 (1H, dd, *J* = 12, 2.3 Hz, H-1b), 3.71-3.64 (2H, m, H-2, H-3), 3.57 (1H, dd, *J* = 12, 6, H-1a).

^{13}C NMR (D_2O, 100 MHz) : δ 70.8, 69.2, 63.2.