

## CHAPTER III

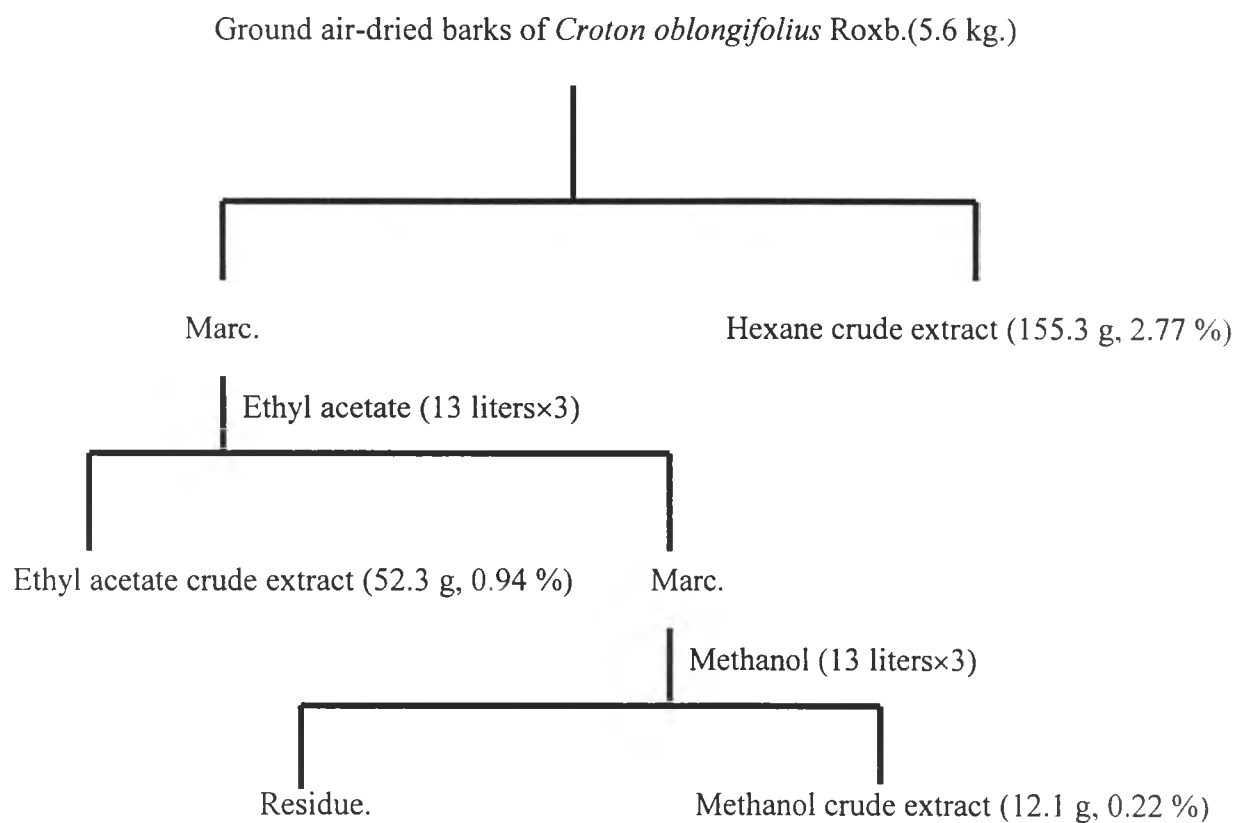
### EXPERIMENTAL

#### 3.1 Plant materials

The plant material of *Croton oblongifolius* Roxb. used in this study was collected from Amphoe Phurua, Loei Province, Thailand in March 2001.

#### 3.2 Extraction

The sun-dried and ground stem bark (5.6 kg) was extracted with hexane (13 liters×3) for 1 month at room temperature. The solvent was filtered and evaporated under reduced pressure, the plant material was subsequently extracted with hexane (13 liters×3), ethyl acetate (13 liters×3), and methanol (13 liters×3), respectively. The solvents were removed to obtain crude hexane extract (155.3 g), crude ethyl acetate extract (52.3 g) and crude methanol extract (12.1 g), respectively. The procedure and results of extraction are shown in Scheme 1.



**Scheme 1** The solvent extraction of *Croton oblongifolius* Roxb.

### 3.3 Separation

#### Separation Techniques

##### 1) Column chromatography<sup>41</sup>

Glass columns with appropriate size and length were used. Ratio of crude to adsorbent is 1:20 by weight. Elution was come out under medium pressure (flash column) or gravimetrically using a suitable solvents system.

##### 2) Thin-layer chromatography<sup>42</sup>

Merck's precoated aluminium TLC sheets, 20x20 cm<sup>2</sup>, layer 0.2 mm., silica gel 60 F<sub>254</sub> sheets, layer thickness 0.2 mm was used and was developed using a suitable solvent system.

### 3.4 Isolation of crude extract of *Croton oblongifolius* Roxb.

#### 3.4.1. Separation of crude hexane extract

The dark-yellow oil crude hexane extract (155.3 g.) was fractionated by silica gel column chromatography using Merck's silica gel Art 1.09385.1000 (230-400 mesh ATMS) as absorbent. The column was eluted with hexane, hexane-ethyl acetate gradient in a stepwise fashion. The volume of eluting solvents used for each fraction was approximately 200 cm<sup>3</sup>. The similar fractions were combined and the solvent was removed by rotary evaporator to obtain Compounds **1,2,3,4**, and **5** respectively. Each fraction was evaporated to about 30 cm<sup>3</sup> and was transferred to a small flask, then analysed by TLC. Fractions with similar TLC were combined together. The results from separation of hexane crude extract are summarized in table 4. The fractions were further purified by column chromatography or crystallization. The results of purified compounds are shown in table 28.

#### 3.4.2. Separation of crude ethyl acetate extract

The crude ethyl acetate extract (105.3 g.) was separated by column chromatography on Merck's silica gel Art 1.09385.1000 (230-400 mesh ATMS). The column was eluted with hexane, hexane-ethyl acetate, ethyl acetate and ethyl acetate-methanol, respectively. Each fraction was collected and then checked by TLC to

combine the fraction which had the same components. The compounds in ethyl acetate and hexane extract were found to be the same.

### **3.4.3. Separation of crude methanol extract**

The crude methanol extract was a gummy residue that was insoluble in methanol, and not separated by column chromatography.

## **3.5 Instruments and equipments**

### **1. Nuclear Magnetic Resonance Spectrometer (NMR)**

The  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectra were recorded at 200.13 and 50.32 MHz, respectively, on a Bruker Model AC-F200 spectrometer, and at 500.13 and 125.65 MHz on a JEOL JNM-A500 spectrometer in  $\text{CDCl}_3$ . Chemical shifts are given in parts per million using residual protonated solvent as a reference. HMQC, HMBC, COSY and NOSEY experiments were performed on the JEOL JNM-A500 spectrometer.

### **2. X-ray Diffractometer**

Results from the x-ray diffractometer were obtained on a SIEMEN SMART diffractometer at Department of Physics, Faculty of Science and Technology, Thammasart University.

### **3. Fourier Transform-Infrared Spectrophotometer (FT-IR)**

IR spectra were recorded on a Nicolet Impact 410 Spectrophotometer. Spectra of Solid samples were recorded as KBr pellets. Liquid samples were recorded as thin films on NaCl cell.

### **4. Mass Spectrometer (MS)**

Low resolution mass spectra were obtained with a Fisons Instruments Mass spectrometer model Trio 2000 at 70 eV.

### **5. Optical Rotation**

The optical rotation spectra were recorded on a Perkin-Elmener 341 polarimeter.

## 6. Rotary vacuum evaporator

The Büchi rotary vacuum evaporator was used for the rapid removal of large amounts of volatile solvents.

## 7. UV Visible spectra

UV Visible spectra were recorded on a Hewlet Packard 8452A diode array spectrophotometer in  $\text{CHCl}_3$ .

### 3.6 Chemicals

3.6.1 Solvent used in this research such as hexane, dichloromethane, ethyl acetate and methanol were of commercial grade and purified prior to use by distillation.

#### 3.6.2 Other chemicals

1. Merck's silica gel Art 1.09385.1000 (230-400 mesh ATMS) was used as absorbent for column chromatography.
2. Merck's TLC aluminium sheet, silica gel 60 F<sub>254</sub> precoated 25 sheets, 20×20 cm<sup>2</sup>, layer thickness 0.2 mm. was used to identical fraction and was developed using a suitable solvent system.