# CHAPTER III

# EXPERIMENTAL

## 1. General Techniques

### 1.1 Thin Layer Chromatography (tlc)

| | | |
|---|---|---|
| Technique | : | one way, ascending |
| Adsorbent | : | Silica gel G (E. Merck), 13% calcium sulfate binder, 30 g/60 ml of distilled water |
| Plate size | : | 10 cm x 20 cm |
| Layer thickness | : | 0.25 mm |
| Activation | : | air dried for 15 minutes/heat at 110°C for 1 hour. |
| Solvents | : | a) ethyl acetate : benzene (2:8)<br>b) chloroform : ethyl acetate (9:1)<br>c) ethyl acetate : cyclohexane (2:8)<br>d) benzene<br>e) chloroform |
| Distance | : | 15 cm |
| Laboratory temperature | : | 25-30°C |

Detection : *1 Liebermann-Burchard spray reagent

**2 Anisaldehyde - sulfuric acid spray reagent (89).

1.2 Column Chromatography (cc)

Column : flat bottom glass column of 10 cm in diameter

Adsorbent : Silica gel 0.040 - 0.063 mm (E. Merck)

Packing method : wet packing

Solvents : a) benzene : ethyl acetate (9:1)

b) methanol

1.3 Melting Point (mp)

Melting point was determined on Melting point Apparatus (Gallenkamp) and was uncorrected.

1.4 Infrared (ir) Absorption Spectrophotometry

Infrared absorption spectrum was obtained on a Hitachi 260 spectrophotometer, absorption bands were reported in wave number ($cm^{-1}$).

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*1 5 ml acetic anhydride mixed under cooling with 5 ml conc. sulfuric acid; this mixture is added cautiously to some ethanol.

**2 1 ml conc. sulfuric acid is added to a solution of 0.5 ml anisaldehyde in 50 ml acetic acid.

1.5 Nuclear Magnetic Resonance (nmr) Spectrometry

$^{1}H$ nmr spectrum was recorded at 270 MHz on Jeol nuclear magnetic resonance spectrometer Model Fx 270. Tetramethylsilane was used as an internal standard and chemical shifts were reported on the ppm scale.

1.6 Mass Spectrometry (ms)

The low resolution mass spectrum was obtained on a Hitachi RMU-60 mass spectrometer. The high resolution mass spectral study was performed on a Hitachi RMU-7M mass spectrometer.

2. Phytochemical Screening

Powdered stem bark (100 g) was macerated with methanol (200 ml) over night. After filtration, the filtrate was used for the screening procedure.

2.1 Screening for Sterols and Triterpenoids

The methanolic extract (24 ml) was concentrated to a syrupy mass on a steam bath. It was diluted with equal volume of distilled water. The aqueous methanolic extract was partitioned three times with petroleum ether. The petroleum ether extract was evaporated to dryness on a steam bath. After the contents were cooled to room temperature, it was used for the Liebermann-Burchard's test. The result showed the progression of color from purple to blue.

2.2 Screening for Alkaloids

The methanolic extract (120 ml) was evaporated to dryness on a steam bath. After the contents were cooled to room temperature, 10 ml of dil. HCl was added to dissolve the residue. After filtration,

the filtrate was used for the precipitation test with alkaloidal reagents. The results were shown negative with *Dragendorff's and **Mayer's reagents.

2.3 Screening for Flavonoids

The methanolic extract (5 ml) was evaporated to dryness on a steam bath. After the dried residue was cooled to room temperature, it was defatted several times with 15 ml portion of petroleum ether until the last volume of petroleum ether was colorless. The defatted residue was dissolved in 30 ml of 80% ethanol and filtered. The filtrate was submitted to the cyanidin test. The result was shown negative.

## 3. Isolation of Chemical Substances from *Aglaia pirifera* Hance Stem Bark

### 3.1 Extraction

Dried powdered stem bark (11 kg) was macerated three times for 7-day-periods with methanol (36 L, 27 L, 21 L). The methanolic extract was evaporated under reduced pressure to give a syrupy mass and was fractionated according to Fig. 1 (Page 46). The syrupy mass

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*Solution a 0.85 g basic bismuth nitrate in 10 ml acetic acid and 40 ml water.

Solution b 8 g potassium iodide in 20 ml water.

Reagent 0.5 ml of solution a and b is mixed with 2 ml acetic acid and 10 ml water.

**1.36 g of mercuric chloride in 60 ml water and 5 g of potassium iodide in water are mixed and diluted to 100 ml with water.

was partitioned with *n*-pentane (4 L) in a continuous liquid-liquid extractor until the pentane layer became colorless. After the two layers were separated, the pentane layer was evaporated to give 50.49 g of pentane soluble residue. Thin layer chromatography of pentane extract showed 3 spots with Liebermann-Burchard spray reagent and anisaldehyde-sulfuric acid spray reagent. (Fig. 8, Page 76).

The crude pentane extract (50.49 g) was divided into 5 portions, each portion was subjected to silica gel column chromatography in the same manner. Each portion (approx.10 g) was dissolved in 20 ml of eluting solvent (benzene : ethyl acetate,9:1) and placed on the top of a 10 cm diameter column of silica gel (200 g). Fifty fractions (50 ml each) were collected and the column was washed with methanol. Monitoring of all the fractions was routinely carried out by thin layer chromatography. Those of similar pattern on thin layer chromatography were combined and evaporated. Fractions 1-4 showed no spot on the tlc plate and was discarded. Fractions 5-50 gave one spot on five tlc systems. They are:

1) Silica gel G/ethyl acetate : benzene,(2:8)
2) Silica gel G/ethyl acetate : chloroform,(1:9)
3) Silica gel G/ethyl acetate : cyclohexane,(2:8)
4) Silica gel G/benzene
5) Silica gel G/chloroform

## 3.2 Purification of Isolated Compound

The combined fractions from 5 columns yielded 6.38 g of residue. It was crystallized in diethyl ether to give colorless crystals (764.9 mg) and was designated as Ap.

4. Examination of Ap

Ap was obtained as colorless crystals. It was soluble in diethyl ether, chloroform, petroleum ether, benzene and methanol.

The following data were obtained on sample of Ap :

hRf Values a) 52 Fig. 4 Page 72

b) 57 Fig. 5 Page 73

c) 35 Fig. 6 Page 74

d) 12 Fig. 7 Page 75

e) 51 Fig. 8 Page 76

Melting point 124.5 - 125.5°C

Infrared Absorption Spectrum (Fig. 9, Page 77)

$\nu_{max}$ ($CHCl_3$) 1600, 1510, 1470, 1420, 1330, 1130, 1000, 830 $cm^{-1}$

Nuclear Magnetic Resonance Spectrum (Fig. 10, Page 78)

$^1H$ nmr (270 MHz, $CDCl_3$) δ 1.10 (d, $J$ 9, 6H), 1.80 (m, 2H), 3.84 (s, 6H), 3.89 (s, 12H), 4.65 (d, $J$ 9, 2H), 6.63 (s, 4H)

Mass Spectrum (Fig. 11, Page 79)

$m/z$ 432 ($M^+$ 34.2% $C_{24}H_{32}O_7$), 237 (9.6%), 236 (54.1%), 224 (72.5%), 221 (49.2%), 209 (22.1%), 208 (100%), 205 (43.3%), 196 (4.4%), 190 (9.4%), 168 (10.9%), 146 (5%), 91 (4.5%)

Molecular Weight 432

Empirical Formula $C_{24}H_{32}O_7$

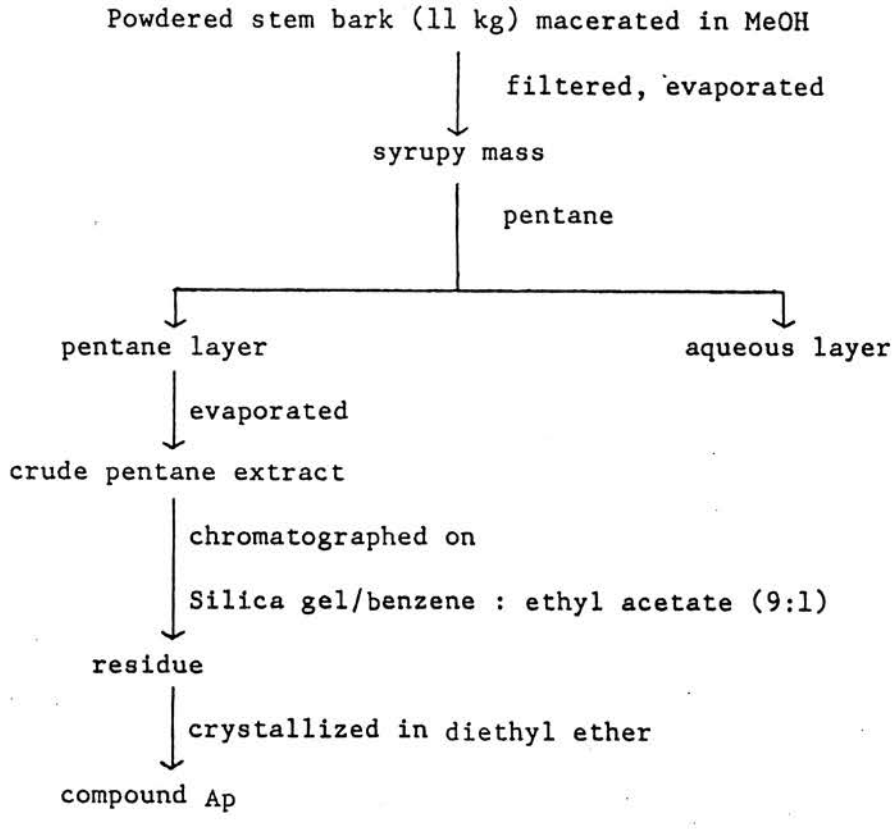


Fig. 1

(Extraction procedure of *Aglaia pirifera* stem bark)