ESSENTIAL OIL AND BIOACTIVE COMPOUNDS FROM *MIOGYNE* SP.

BY :

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ASMAH ALIAS

JUN 2012

Contents

1. Letter of Report Submission

Wed 28 December, 2011

Prof. Dr. Abu Bakar bin Abdul Majeed
Deputy Vice Chancellor (Research),
Research Management Institute (RMI),
Universiti Teknologi MARA,
40450 Shah Alam,
Selangor

Prof,

RE: SUBMISSION OF FINAL REPORT FOR FRGS PROJECT
PROJECT TITLE: ESSENTIAL OIL AND BIOACTIVE COMPOUNDS FROM *MIOGYNE* SP.
RMI FILE NUMBER: 600-RMI/ST/DANA 5/3/Dst (192/2009)

With regards to the above, we are pleased to submit the final report of our project entitled "Essential Oil and Bioactive Compounds from *Miogyne* sp.", which was funded under the Dana Kecemerlangan (Danakep).

Please find the full report of the project herewith for your attention.

Thank you.

Yours Sincerely,

PROF. DR. NOR HADIANI ISMAIL
Project Leader

2. Letter of Offer (Research Grant)

3. Acknowledgements

We wished to thank the following parties for assistance and supports towards the success of this research project;

Universiti Teknologi MARA
for financial support and facilities provided;

Research Management Institute of UiTM;

Faculty of Applied Sciences, UiTM; and,

Laboratory assistants and technicians
for technical support.

4. Enhanced Research Title and Objectives

Original Title as Proposed:

Essential Oil and Bioactive Compounds from *Miogyne* sp.

Improved/Enhanced Title:

No changes

Original Objectives as Proposed:

1) to extract the essential oils of *Meiogyne* sp. using hydrodistillation method.
2) to analyse the compounds present in the essential oils of *Meiogyne* sp. using GC-MS
3) to isolate and purify the chemical constituents using chromatographic techniques such as solid-phase extraction, column chromatography, medium pressure liquid chromatography, preparative thin layer chromatography and chromatotron.
4) to identify the structure of the compounds using spectroscopic techniques such as NMR, IR, UV and GC-MS.

Improved/Enhanced Objectives:

1) to extract the essential oils of *Meiogyne monosperma* using hydrodistillation method.
2) to analyse the compounds present in the essential oils of *Meiogyne monosperma* using GC-MS
3) to isolate and purify the chemical constituents of *Meiogyne virgata* using chromatographic techniques such as solid-phase extraction, column chromatography, medium pressure liquid chromatography, preparative thin layer chromatography and chromatotron.
4) to identify the structure of the isolated compounds of *Meiogyne virgata* using spectroscopic techniques such as NMR, IR, UV and GC-MS.

5. Report

5.1 Proposed Executive Summary

Natural products are chemical constituents or substances produced by living organisms that usually possess pharmacological activity useful as lead compound in drug discovery program. Therefore, since the 1990s, interest in natural products for pharmaceutical industries has increased rapidly (Sticher, 2008) resulting the discovery of many drugs. Many higher plants contain secondary metabolites with biological properties (Achmad *et al.*, 2005) provide opportunity for some medicines to be obtained directly from natural sources. Some plant extracts from natural sources contain biological active compounds that have special interest in the development of new medicinal agent to treat diseases. This resulted almost 80 per cent of all medicines were derived from herbs and plants by the middle of the nineteenth century (Gilani, 2005). These medicinal plants are very important to treat diseases especially as folk medicine. Although some active principles from medicinal plants have limited application in modern medicine, they are valuable as pharmacological tools for evaluating the mode of action of drugs.

Phytochemical investigations of bioactive plants are actively pursued in Malaysia. Alkaloids have been found especially in the family of Annonaceae, Lauraceae, Apocynaceae and Rubiaceae. For example, *Eurycoma longifolia* (*tongkat ali*), *Labisia pumila* (*kacip Fatimah*), *Andrographis paniculata* (*hempedu bumi*) and *Phyllanthusniruri* (*dukung anak*) have been studied for their alkaloidal content (Hendrickson, 1983).

In this investigation, *Meiogyne* species from the family Annonaceae will be studied for their bioactive compounds. Plants of the family Annonaceae are known as source of a variety of alkaloids, triterpenoids and saponins (Kamarudin, 1989). A large number of alkaloidal compounds have been isolated from some Annonaceae species. From the previous study, Annonaceae species were found to contain tertiary and quaternary isoquinoline and quinoline alkaloids which some of the compounds have strong antimicrobial activity (Abbasoglu *et al.*, 1991). For example, liriodenine alkaloid isolated from *Fissistigma glaucescens* (Lo *et al.*, 2000) demonstrates evidence of a wide range of antimicrobial activity (Cordell, 1981).

5.2 Enhanced Executive Summary

The genus *Meiogyne* (Annonaceae) consists of about 24 species, widely distributed in Indo-china, Thailand, Peninsular Malaysia, Sumatra, Java, Borneo and the Philippines. Several plants of the genus *Meiogyne* has been used as a folk medicine. In previous studies these plants were found to contain aporphines, oxoaporphines and azaanthracene alkaloids, lactones and sesquiterpenes (Tadic *et al.*, 1987; Alias *et al.*, 2011; Bousserouel *et al.*, 2011; Litaudon *et al.*, 2011). *Meiogyne* has a very similar appereance to *Cyathocalyx* species especially in their spreading petals and similarity of stamens (Sinclair, 1955).

Isolation and purification of alkaloids from the bark of *Meiogyne virgata* afforded nine alkaloids; four oxoaporphines, liriodenine **1**, lanuginosine **2**, asimilobine **3** and lysicamine **4**; four aporphines, anonaine **5**, N-methylanonaine **6**, nornuciferine **7** and norushinsunine **8**; and one azaanthracene alkaloid, cleistopholine **9**. This paper reports presence of alkaloids **2**, **4**, **6** and **7** for the first time from *Meiogyne virgata*. The structural elucidation was accomplished by spectroscopic methods such as ID-NMR (^{1}H, ^{13}C, DEPT), 2D-NMR (COSY, HMQC, HMBC), UV, IR and MS and comparison with published data.

To our knowledge, no chemical studies on *Meiogyne monosperma* have been reported. As part of continuing survey on chemical composition of *Meiogyne* species, we also report the chemical constituents of the leaves and stem of *Meiogyne monosperma*.

The chemical composition of the leaf and stem oils of *Meiogyne monosperma* was investigated by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Both leaf and stem oils of *M. monosperma* were rich in sesquiterpenoids. The oils were were found to possess similar chemical components but with quantitative differences in the concentration of each component. The major constituents of the leaf oil were humulene epoxide (19.4%), caryophyllene oxide (12.1%), veridiflorol (7.9%), pogostol (4.0%), epi-cyclocolorenone (1.8%) and β-selinene (1.2%) while the major constituents of the stem were caryophylene oxide (16.0%), veridiflorol (7.3%), β-selinene (4.4%), α-humulene (3.6%) and δ-cadinene (1.8%). About 29 and 11 sesquiterpenes were identified in the leaf and stem oils of *M.monosperma* respectively.

5.3 Introduction

The flora of Malaysia is generally considered as one of the richest flora in the world due to the constantly warm and uniformly humid climate. Considering that Malaysia has about 12 000 species of flowering plants of which is about 1300 have potential as medicinal agent (Burkill, 1935), the potential of drug discovery from Malaysian plants is very good. The huge diversity of Malaysian flora means that researchers can obtain various samples to analyze and evaluate in drug discovery screen or bioassays.

Annonaceae, known as *Mempisang* in Malaysia (Kamarudin, 1988) is a family of flowering plants consisiting of trees, shrubs or woody lianas. This family is the largest family in the Magnoliales consisting of more than 130 genera with about 2300 to 2500 species. Plants of the family Annonaceae are well known as source of a variety of alkaloids (Cordell, 1981). Many alkaloids have important physiological effects on human and exhibit marked pharmacological activity which is useful as medicine. For examples, atropine, the optically inactive from hyoscyamine is used widely as an antidote to cholinesterase inhibitors such as physostigmine. Morphine and codeine are narcotic analgesics and antitusive agent while caffeine, which occurs in coffee, tea and cocoa is a central nervous system, cardiac and respiratory stimulant (Parker, 1997). The first report on phytochemical studies of alkaloids from Malaysian Annonaceae plants was on the leaves of *Desmos dasymachalus* which has led to the isolation of new 7-hydroxyaporphine, dasymachaline (Chan & Toh, 1985).

The phytochemical investigation of Annoaceous plants for their alkoloidal content continue to flourish. Phytochemical survey of the flora of the Peninsula Malaysia and Sabah, with systematic screening for alkaloids resulted in reports on chemical constituents of several plants from Annonaceae illustrating great interest in this field (Teo, et al., 1990).

Meiogyne cylindrocarpa, *Meiogyne monosperma* and *Meiogyne virgata* are the only three *Meiogyne* species found in Malaysia. Only *Meiogyne virgata* was studied by Tadic et al. (1987). The sample collected from Mount Kinabalu, Sabah was reported to contain azafluorene alkaloid, kinabaline, together with liriodenine, cleistopholine and other aporphine alkaloids.

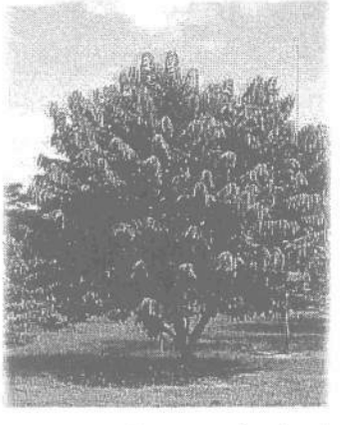
5.4 Brief Literature Review

Annonaceae

Annonaceae family commonly called custard apple family (Mabberley, 1987). Annonaceae is a family of flowering plants consisiting of trees, shrubs or rarely woody lianas. This family has about 2300 to 2500 species with more than 130 genera and it is the largest family in the Magnoliales. Although Annonaceae are well known as plants producing fruit, only four genera, *Annona*, *Rollinia*, *Uvaria* and *Asimina* produce edible fruits (Bridg, 2001). The family is concentrated in the tropics, with few species found in temperate regions. About 900 species are Neotropical, 450 are Afrotropical, and the others are Indomalayan.

Some species, such as *Annona cherimola* (cherimoya), *Annona muricata* (guanabana, soursop), *Annona squamosa* (sugar apple, sweet apple), and *Rollinia mucosa* (biriba) are found throughout the tropics and even the subtropics because of their edible fruits (Bridg, 2001). Annonaceous woods are also valued for firewood and used for poles, canoes, and bridges. Some species of the family have aromatic oils that are useful for perfumes or spices (Chatrou, 2005) while some species have been used as yellow and brown dyes.

Recently, Annonaceae plants have grow to be important in pharmaceutical research because of the antifungal, bacteriostatic, and especially cytostatic capability of some chemical constituents of the leaves and bark (Harborne and Baxter, 1993) that becomes special interest in the development of new medicinal agents. A large number of chemical compounds including flavonoids, alkaloids and acetogenins have been extracted from many parts of Annonaceae plants. (Liao, 2002).



Annona cherimola

Rollinia mucosa

Figure 5.1: Some Malaysian Annonaceous Plants

Medicinal Uses of Annonaceae Plants

Natural products from plants have been used in traditional medicine for several thousand years (Abu Rabia, 2005). During the last few decades there has been increasing interest in the study of phytochemistry and traditional use of plants in different parts of the world (Hegnauer, 1988). According to World Health Organization (WHO), about 80% of the world's people depend on conventional treatment from traditional medicine for their primary healthcare needs (Diallo, 1999).

Annonaceae plants are used in traditional folk medicine for liver protection, antitumor action, anti-inflammatory and anti-arthritic effects (Chia *et al.*, 1999). In China, *Fissistigma bracteolatum* from Annonaceae family is applied externally to stop bleeding or used to treat broken bones. In Vietnam, it is used with other ingredients to treat infections and also to enhance blood circulation in human body (*Chia et al.*, 1998). *Uvaria* species which is known as Langad-langad in Sabah is used for rheumatism and as tonic (Kulip, 1996). In Jamaica and Western India, the juice from fruits of *Annona muricata* or Graviola are used in order to diminish fever, treat diarrhea and combat parasites, as well as increase milk secretion of breast-feeding women (Taylor, 2002). Roots of some *Annona* species are used as sedative, antispasmodic, hipotensive and nervine. Other than that, the bark is very useful to treat heart diseases, cold, flu, childbirth, asthma and hypertension (Taylor, 2002).

The barks, leaves and roots of some Annonaceae species are use in folk medicine and pharmaceutical products have been developed for the international market (Harborne and Baxter, 1993).

Genus *Meiogyne*

The genus *Meiogyne* (Annonaceae) consists of about 24 species, widely distributed in Indo-china, Thailand, Peninsular Malaysia, Sumatra, Java, Borneo and the Philippines. Several plants of the genus *Meiogyne* has been used as a folk medicine. In previous studies these plants were found to contain aporphines and oxoaporphines alkaloids (Tadic *et al.*, 1987).

Meiogyne has a very similar appereance to *Cyathocalyx* species. But, the morphology of *Meiogyne* is different from *Cyathocalyx* in several ways. The leaf texture is different. Flowers are axillary and not extra-axillary or leaf-opposed. Arrangement of diverging from a broad base and not clawed and constricted and the base is not ad pressed over the stamens. The warted base of the inner petal is peculiar. The stamens and stigmas are similar to *Cyathocalyx*. *Meiogyne* is similar to *Polyalthia* in its spreading petals and similarity of stamens, but the large number of seeds and sessile, discoid stigma are distinguishing features (Sinclair, 1955).

The species from this genus have not been widely studied. Therefore, not much literature was found on plants of this genus.

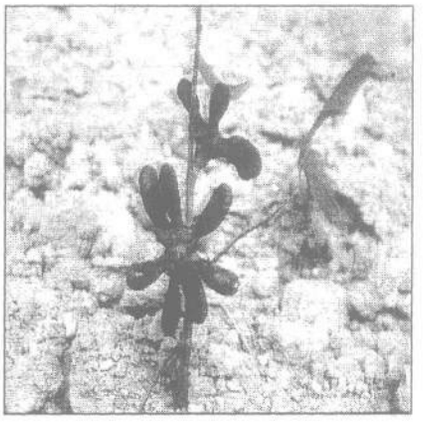


Figure 5.2: *Meiogyne virgata*

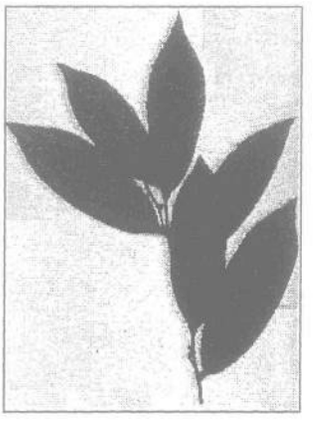


Figure 5.3: *Meiogyne monosperma*

5.5 Methodology

Plant material

Fresh leaves and stem of *Meiogyne monosperma* were collected from Kuala Keniam, Taman Negara Pahang while bark and leaves of *Meogyne virgata* were collected from Hulu Terengganu.

General

The ^{1}H-NMR and ^{13}C-NMR were recorded in $CDCl_3$ and CD_3OD on a Bruker 300 Ultrashield NMR spectrometer measured at 300 and 75 MHz. Chemical shifts are reported in ppm and δ scale and the coupling constants are given in Hz. Melting points were taken on a hot stage Gallen Kamp melting point apparatus with

microscope and were uncorrected. The infrared (IR) was recorded on a Perkin Elmer spectrum one FT-IR spectrometer using CH_2Cl_2 as solvent. Optical rotations were measured on a JASCO P1020 digital polarimeter. The ultraviolet (UV) spectra were obtained in ethanol on a Shimadzu UV-Vis 160i instrument.

Isolation of essential oil

Fresh leaves (300 g) and stem (300 g) of *Meiogyne monosperma* were separately hydrodistiled for 8 hours in a Clevenger's apparatus, using pentane as the collecting solvent. The solvent was carefully removed using a gentle stream of nitrogen gas, yielding yellow aromatic oil in each case. The oils obtained were dried over anhydrous sodium sulfate and stored at 4–6 °C before analysis. The oil yields (w/w) were 0.04% (leaves) and 0.03% (stem), all on a fresh weight-basis.

Gas chromatography (GC)

GC analysis were performed on a Shimadzu GC 14A gas chromatograph (FID), fitted with a 30 m × 0.25 mm i.d. ZB-1 capillary column coated with 100% methyl polysiloxane, using nitrogen as the carrier gas. The oven temperature was programmed from 60 °C (after 10 min) to 230 °C at 3 °C/min and the end temperature was held for 10 min.

Gas chromatography mass spectrometry (GC–MS)

Analyses were carried out on a 5971A mass selective detector (MSD), directly coupled to a HP 5980 Series II gas chromatograph with a 30 m × 0.25 mm i.d. ×0.25 μm FT DB-1 capillary column, using helium as the carrier gas. The oven temperature was programmed from 60 °C (after 10 min) to 230 °C at 3 °C/min and the end temperature was held for 10 min.

Identification of constituents of essential oil

The constituents of the oils were identified by matching their mass spectra with reference spectra in the computer library and also by comparing their Kovat's retention indices with reference libraries.

Extraction and isolation of the alkaloids

Dried and ground bark of *M.vigata* (1 kg) was defatted with petroleum ether (5 L) overnight before being extracted with dichloromethane (5 L) and methanol (10 L) for eight hours using a Soxhlet extractor. The methanol extract was subjected to acid-base extraction. The crude alkaloids (2.5 g) from the MeOH extract were subjected

to vacuum liquid chromatography (VLC) using a gradient elution system of hexane (hex):ethyl acetate (EA) and dichloromethane (DCM):methanol (MeOH) to give 30 fractions. The combined fractions 10–21 were subjected to PTLC using a 95:5 DCM:MeOH solvent system to yield a mixture of compounds **1**(15.3 mg), **2** (5.1 mg) and **3** (7.0 mg). These compounds were separated by repeated PTLC using the same solvent system. Fraction 23 from the VLC was subjected to PTLC using a 95:5 DCM:MeOH solvent system to obtain compounds **4** (5.2 mg) and **5** (5.7 mg). The combined fractions 26 and 27 from the VLC were subjected to column chromatography using a gradient elution system of hex:EA and DCM:MeOH to obtain 13 fractions. Fractions 7–10 were combined and subjected to PTLC using a 93:7 DCM:MeOH solvent system to yield compounds **6** (6.4 mg) and **7** (8.2 mg). The combined fractions 11–13 were subjected to PTLC using a 90:10 DCM:MeOH solvent system to obtain alkaloid **8** (5.1 mg). Fraction 29 from the VLC was subjected to PTLC using a 90:10 DCM:MeOH solvent system to yield compounds **9** (4.6 mg). Spectral data for compounds **1-9** were in agreement with published data.

Liriodenine 1, yellow needles

mp : 257 - 260 °C

MS m/z : 275, $C_{17}H_9O_3N$

UV λ_{max} nm EtOH : 215, 246, 268, 395, 412

IR u_{max} cm^{-1} : 3054, 1726, 1421, 1265

^1H NMR ($CDCl_3$, 300 MHz) δ ppm : 8.9 (1H, d, J = 5.1 Hz, H-5), 8.66 (1H, dd, J_o = 7.2 Hz; J_m = 1.2 Hz, H-11), 8.59 (1H, dd, J_o = 7.8 Hz; J_m = 1.2Hz, H-8), 7.79 (1H, d, J = 5.1 Hz, H-4), 7.76 (1H, td, J_o = 7.8 Hz; 7.2 Hz; J_m = 1.5 Hz, H-10), 7.59 (1H, td, J_o = 7.8 Hz;7.2; J_m = 1.2 Hz, H-9), 7.16 (1H, s, H-3), 6.40 (2H, s, O-CH_2-O).

^{13}C NMR ($CDCl_3$, 75 MHz) δppm : 151.7 (C-2), 147.9 (C-1), 146 (C-6a), 145.4 (C-3a), 144.9 (C-5), 135.7 (C-1a), 133.9 (C-10), 132.9 (C-7a), 131.3 (C-11a), 128.8(C-8), 128.6 (C-9), 127.4 (C-11), 124.2 (C-4), 108.2 (C-1b), 103.3 (C-3), 102.4 (O-CH_2-O), 182.4 (C-7).

Lanuginosine 2, yellow needles

mp : 315 – 317 °C

MS m/z : 305, $C_{18}H_{11}O_4N$

UV λ_{max} nm EtOH : 246, 271, 315, 258, 283, 334

IR u_{max} cm^{-1} : 3055, 2987, 2306, 1712, 1635, 1363, 1265, 1046, 896

^1H NMR ($CDCl_3$, 300MHz) δ ppm : 8.85 (1H, d, J = 5.4 Hz, H-5), 8.58 (1H, d, J_o = 9.0 Hz, H-11), 8.04 (1H, d, J = 3 Hz, H-8), 7.79 (1H, d, J = 5.4 Hz, H-4), 7.32 (1H, dd, J_o = 9.0 Hz; J_m = 3 Hz, H-10), 7.17 (1H, s, H-3), 6.47 (2H, s, O – CH_2 – O).

^{13}C NMR ($CDCl_3$, 75MHz) δ ppm : 158.0 (C-9), 151.0 (C-2), 146.0 (C-1), 144.9 (C-5), 144.0 (C-6a), 136.0 (C-3a), 133.0 (C-7a), 131.9 (C-1b), 129.1 (C-11), 126.2 (C-

11a), 124.3 (C-4), 122.6 (C-10), 110.2 (C-8), 109.0 (C-1a), 102.3 (C-3), 55.8 (OCH_3), 102.5 (O – CH_2 – O), 182.0 (C-7).

Asimilobine 3, brownish amorphous

mp : 170 – 176 °C

MS m/z : 267, $C_{17}H_{17}O_2N$

UV λ_{max} nm EtOH : 274, 308

IR u_{max} cm^{-1} : 3390, 1675, 1600, 1225

^1H NMR ($CDCl_3$, 300MHz) δ ppm : 8.30 (1H, d, J = 7.8 Hz, H-11), 7.36 – 7.25 (3H, m, H-8, H-9, H-10), 6.73 (1H, s, H-3), 3.92 (1H, m, H-6a), 3.50 (1H, m, H-5'), 3.08 (1H, d, H-4'), 3.04 (1H, d, H-5), 2.99 (1H, m, H7), 2.85 (1H, m, H7), 2.74 (1H, d, H-4), 3.61 (3H, s, OCH_3), 2.00 (1H, s, N-H).

^{13}C NMR ($CDCl_3$, 75MHz) δ ppm : 148.6 (C-2), 143.0 (C-1), 135.6 (C-7a), 131.7 (C-11a), 129.4 (C-16), 128.1 (C-3a), 127.7 (C-8), 127.4 (C-10), 127.3 (C-9), 127.2 (C-11), 125.5 (C-1a), 114.6 (C-3), 53.4 (C-6a), 42.8 (C-5), 36.7 (C-7), 28.2 (C-4), 60.4 (OCH_3).

Lysicamine 4, yellow amorphous

mp : 196 – 200 °C

MS m/z : 291, $C_{18}H_{13}O_3N$

UV λ_{max} nm EtOH : 214, 250, 255, 261, 319

IR u_{max} cm^{-1} : 1675, 1600, 1225

^1H NMR ($CDCl_3$, 300MHz) δ ppm : 9.10 (1H, d, J_o = 5.1 Hz, H-11), 8.70 (1H, d, J_o = 6.9 Hz, H-5) 8.48 (1H, dd, J_o = 7.5 Hz; J_m = 1.5 Hz, H-8), 7.76 (1H, td, J_o = 7.2 Hz; J_m = 1.5 Hz, H-10), 7.7 (1H, d, J_o = 6.9 Hz, H-4), 7.55 (1H, td, J_o = 7.2 Hz; J_m = 1.2 Hz, H-9), 7.24 (1H, s, H-3), 4.05 (3H, s, OCH_3), 3.97 (3H, s, OCH_3)

^{13}C NMR ($CDCl_3$, 75MHz) δppm : 156.7 (C-6a), 152.0 (C-2), 145.2 (C-1), 139.0 (C-5), 135.3 (C-3a), 134.7 (C-11a), 132.0 (C-7a), 130.9 (C-10), 125.7 (C-9), 122.0 (C-1b), 119.6 (C-1a), 108.7 (C-3), 65.1 (OCH_3), 56.8 (OCH_3), 182.5 (C=O)

Anonaine 5, yellow amorphous

mp : 120 – 123 °C

MS m/z : 265, $C_{17}H_{13}O_2N$

UV λ_{max} nm EtOH : 234, 272, 315

IR u_{max} cm^{-1} : 1040, 945

^1H NMR ($CDCl_3$, 300MHz) δ ppm : 8.09 (1H, d, J_o = 7.5 Hz, H=11), 7.36 – 7.19 (3H, m, H-8, H-9, H-10), 6.6 (1H, s, H-3), 4.04 (1H, dd, H-6a), 3.48 (1H, m, H-5'), 3.1 (1H, m, H-4'), 3.07 (1H, m, H-5), 3.02 (1H, m, H-7'), 6.12 (1H, d, J_m = 1.5, CH – O), 5.97 (1H, d, J_m = 1.5, CH – O).

^{13}C NMR ($CDCl_3$, 75MHz) δ ppm : 147.0 (C-2), 143.0 (C-1), 135.4 (C-7a), 131.4 (C-11a), 129.0 (C-1b), 128.0 (C-3a), 127.8 (C-8), 127.7 (C-9), 127.0 (C-10), 126.1 (C-11), 116.3 (C-1a), 53.6 (C-6a), 43.6 (C-5), 37.4 (C-7), 29.6 (C-4), 100.6 (O – CH_2 – O).

***N*-methylanonaine 6**, yellow amorphous

mp : 97 – 103°C

MS m/z : 279, $C_{18}H_{17}O_2N$

UV λ_{max} nm EtOH : 234, 264

IR υ_{max} cm^{-1} : 1401, 1361, 1053, 942

^1H NMR ($CDCl_3$, 300MHz) δ ppm : 8.09 (1H, d, J_o = 7.5 Hz, H-11), 7.34 – 7.24 (3H, m, H-8, H-9, H-10), 6.59 (1H, s, H-3), 4.000 (1H, m, H-6a), 3.4 (1H, m, H-5'), 3.10 (1H, m, H-4'), 3.00 (1H, m, H-5), 2.90 (1H, m, H-7'), 2.80 (2H, m, H-7, H-4), 6.11 (1H, d, J_m = 1.2 Hz, CH-O), 5.96 (1H, d, J_o = 1.2 Hz, CH-O), 2.62 (3H, s, CH_3).

^{13}C NMR ($CDCl_3$, 75MHz) δppm : 146.7 (C-2), 142.8 (C-1), 136.3 (C-7a), 128.1 (C-8), 128.0 (C-1b), 127.6 (C-9), 127.0 (C-10), 127.0 (C-11), 125.4 (C-3a), 126.5 (C-1a), 126.0 (C-11a), 125.4 (C-3a), 62.4 (C-6a), 53.3 (C-6a), 43 (C-5), 36.9 (C-7), 28 (C-4), 100.7 (O – CH_2 – O), 39.0 (CH_3).

Nornuciferine 7, Colourless crystalline solid
mp : 120 – 123 °C

MS m/z : 281 (M^+)

UV λ_{max} nm EtOH : 234, 272, 315

IR υ_{max} cm^{-1} : 1040, 945

^1H NMR ($CDCl_3$, 300 MHz) δ ppm : 8.39 (1H, *d*,J= 7.8 Hz, H-11), 7.33-7.20 (3H, *m*, H-8, H-9, H-10), 6.65 (1H, *s*, H-3), 3.98 (1H, *dd*, J= 13.4; 5.2 Hz, H-6a), 3.41 (1H, *dd*, J= 12.3; 6.3 Hz, H-5'), 3.90 (3H, *s*, OMe-2), 3.68 (3H, *s*, OMe-1), 3.08 (1H, *dd*, J=13.2 Hz, H-4), 3.04 (1H, *td*, J= 12.3; 5.1 Hz, H-5), 2.85 (1H, *dd*, J= 13.4 ; 5.2 Hz, H-7'), 2.68 (1H, *dd*, J= 13.2; 6.0 Hz, H-4'), 2.64 (1H, *t*, J= 13.4 Hz, H-7).
^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm : 152.3 (C-2), 145.2 (C-1), 135.0 (C-7a), 132.1 (C-1b), 132.1 (C-11a), 131.2 (C-3a), 128.4 (C-8), 127.8 (C-10), 127.4 (C-9), 127.1 (C-11), 126.6 (C-1a), 111.8 (C-3), 60.3 (OMe-1), 55.9 (OMe-2), 53.6 (C-6a), 43.0 (C-5), 37.2 (C-7), 29.7 (C-4).

Norushinsunine 8, Colourless crystalline solid

MS m/z : 281

UV λ_{max} nm EtOH : 217, 247, 252, 259, 273, 319

IR υ_{max} cm^{-1} : 3488, 3355, 1574, 1215

^1H NMR ($CDCl_3$, 300MHz) δ ppm : 8.16 (1H, dd, *J* = 7.2;1.2 Hz, H=11), 7.45 (1H, td, *J* = 8.7;1.2 Hz, H-10), 7.40 (1H, dd, *J* = 8.1;0.9 Hz, H-8), 7.34 (1H, td, *J* = 7.2;1.2 Hz, H-9), 6.59 (1H, s, H-3), 6.11 (1H, d, *J* =1.5 Hz, O – CH_2 – O), 5.95 (1H, d, *J* = 1.2 Hz, O – CH_2 – O), 4.61 (1H, d, *J* = 3.0 Hz, H-7), 4.06 (1H, d, *J* = 3.3 Hz, CH – O). 3.37 (1H, ddd, *J* = 5.0;3.9;1.2 Hz, H-4', 2.68 (1H,dd, J=16.2;3.9 Hz, H-4)

^{13}C NMR ($CDCl_3$, 75MHz) δ ppm : 147.1 (C-1), 142.6 (C-2), 135.6 (C-1a), 130.3 (C-7a), 129.4 (C-9), 129.1 (C-3a), 123.6 (C-1b), 115.6 (C-11a), 108.4 (O – CH_2 – O), 71.0 (C-7), 57.2 (C-6a), 43.1 (C-5), 29.2 (C-4).

Cleistopholine 9, yellow glassy solid

mp : 120 – 123 °C

MS m/z : 281 (M^+)

UV λ_{max} nm EtOH : 234, 272, 315

IR υ_{max} cm^{-1} : 1040, 945

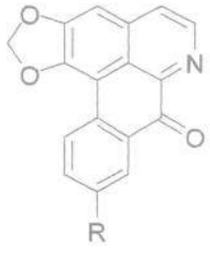
^1H NMR ($CDCl_3$, 400 MHz) δ ppm : 8.95 (1H, *d*,J= 4.8 Hz, H-2), 8.31 (1H, *dd*,J= 8.5;2.2 Hz, H-5), 8.21 (1H, *dd*,J= 8.5;2.2 Hz, H-8), 7.79 (1H, m, H-6), 7.79 (1H, m, H-7), 2.89 (1H, s, CH3)
^{13}C NMR ($CDCl_3$, 100.6 MHz) δ ppm : 184.7 (C-9), 181.9 (C-10), 153.4 (C-2), 151.6 (C-4), 150.1 (C-9a), 134.6 (C-7), 134.2 (C-6), 132.6 (C-10a), 131.2 (C-3), 129.1 (C-4a), 127.4 (C-5), 127.2 (C-8), 22.8 (CH3).

5.6 Results and Discussion

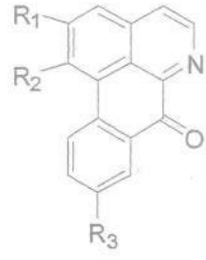
Alkaloids from *Meiogyne virgata*

In this work, we investigated the chemical constituents of the bark of *Meiogyne virgata*. The phytochemical procedures adopted were acid-base extraction followed by vacuum liquid chromatography, column chromatography and preparative thin layer chromatography. Isolation and purification of alkaloids from the bark of *Meiogyne virgata* afforded nine alkaloids; four oxoaporphines, liriodenine **1**, lanuginosine **2**, asimilobine **3** and lysicamine **4**; four aporphines, anonaine **5**, N-methylanonaine **6**, nornuciferine **7** and norushinsunine **8**; and one azaanthracene alkaloid, cleistopholine **9**. These compounds were characterized based on analysis of spectroscopic data and comparison with literature data [2-10]. Most of the compounds are yellowish or colorless hygroscopic liquid at room temperature while impure samples can appear brownish. They have low solubility in water but dissolve well in ethanol, chloroform, acetone, diethyl ether and other common organic solvent. It is also soluble in dilute acid as the protonated derivative. These melting point of these thype of compounds in range 100-300 °C. Compounds **1** - **8** which are oxo-aporphine and aporphine alkaloids showed the IR spectra are typified by the 7-oxo group for a band in the 1635-1660 cm^{-1} region. The UV spectra data for these type of compounds are quite characteristic for the skeletal type. There is indication that they may also be diagnostic for a particular oxygenation pattern. For example, 1, 2-methylenedioxy derivative in compound **1** gives increase to a bathochromic shift in the 235-250 nm bands on comparison with the corresponding compound **4.** The addition of acid will gives a substantial bathochromic shift of the longest-wavelength band. In oxoaporphine and aporphine, the oxygenated pattern is the most diverse

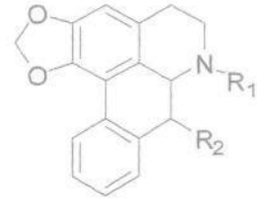
structural feature. Position 1 and 2 are constantly oxygenated. It is frequent to find further oxygen substituent at C-9, C-10 and C-11 and occasionally at C-8. Other than that, H-4 and H-5 will give a characteristic AB system with doublet of doublet at about 7.6 ppm and 8.7 ppm with a coupling constant about 5.4 Hz. The small J value is due to the adjacent of electronegative nitrogen atom. The methylenedioxyl group gives singlet peak at about 6.0 ppm due to the inductive effect cause by existence of the neighboring C-7 carbonyl. The C-11 proton usually the most deshielded and the C-3 protons appeared at a higher field then the aromatic hydrogen.



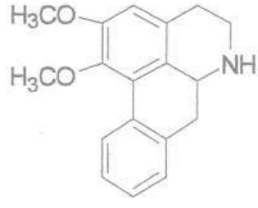
	R
1	H
2	OCH_3



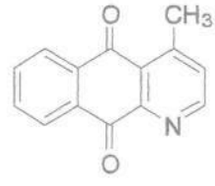
	R_1	R_2	R_3
3	OH	OCH_3	H
4	OCH_3	OCH_3	H



	R_1	R_2
5	H	H
6	CH_3	H
8	H	OH



7



9

Figure 5.4: Alkaloids from *Meiogyne virgata*

Essential oil of *Meiogyne monosperma*

The major constituents of the leaf oil were humulene epoxide (19.4%), caryophyllene oxide (12.1%), veridiflorol (7.9%), pogostol (4.0%), epi-cyclocolorenone (1.8%) and β-selinene (1.2%) while the major constituents of the stem were caryophylene oxide (16.0%), veridiflorol (7.3%), β-selinene (4.4%), α-humulene (3.6%) and δ-cadinene (1.8%). About 28 and 13 sesquiterpenes were identified in the leaf and stem oils of *M.monosperma* respectively.

Table 5.1: Sesquiterpenes in the leaf of *Meogyne monosperma*

No	Compound	GCMS (RT)	GC (RT)	%
1	α-cubebene	31.142	37.54	0.465
2	cyclosativene	31.908	37.726	0.176
3	α-ylagene	32.114	38.531	0.157
4	α-copaene	32.412	39.059	2.483
6	Germacrene D	32.978	39.414	0.167
7	β-elemene	33.167	39.761	0.887
8	(E-)caryophyllene	34.26	41.131	0.294
11	α-cis-Bergamotene	35.004	41.679	0.763
14	allo-aromadendrene	36.028	41.848	0.141
15	ar-curcumene	36.223	42.093	0.104
16	α-amorphene	36.892	43.855	1.636
17	β-selinene	37.516	46.1	1.237
18	viridiflorene	37.831	46.369	0.259
19	β-bisabolene	38.185	46.569	0.115
20	α-copaene	39.147	47.035	0.661
21	cis-calamenene	38.843	48.075	0.712
22	β-calacorene	39.644	48.462	0.172
23	caryophyllene oxide	41.493	49.306	12.101
24	viridiflorol	42.174	50.155	7.87
25	humulene epoxide II	42.729	50.717	19.371
26	pogostol	44.342	52.399	4.036
27	10-nor-calamen	46.007	54.199	1.022
28	epi-cyclocolorenone	47.672	56.086	1.846

Table 5.2: Sesquiterpenes in the stem of *Meogyne monosperma*

No	Compound	GCMS (RT)	GC (RT)	%
1	cyclosolongifolene	33.659	39.194	0.613
2	β-caryophyllen	34.237	39.87	1.338
4	α-humulene	35.754	41.519	3.558
5	γ-selinene	36.629	42.459	0.47
6	β-selinene	37.184	43.072	4.398
7	α-selinene	37.516	43.419	0.41
8	β-bisabolene	38.094	43.749	0.599
10	δ-cadinene	38.718	44.531	1.791
11	α-calacorene	39.559	45.609	1.107
12	caryophylene oxide	41.212	47.508	15.955
13	veridiflorol	41.779	48.167	7.349

5.7 Conclusion and Recommendation

The phytochemical study on the bark of *Meiogyne virgata* (Annonaceae) yielded nine known alkaloids liriodenine **1**, lanuginosine **2**, asimilobine **3** and lysicamine **4**; four aporphines, anonaine **5**, N-methylanonaine **6**, nornuciferine **7** and norushinsunine **8**; and one azaanthracene alkaloid, cleistopholine **9.** Alkaloids **2**, **4**, **6** and **7** have never been reported from *Meiogyne* species. The chemical composition of the leaf and stem oils of *Meiogyne monosperma* was investigated by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Both leaf and stem oils of *M. monosperma* were rich in sesquiterpenoids. The oils were were found to possess similar chemical components but with quantitative differences in the concentration of each component. Further phytochemical study will be conducted on this plant in order to discover the potential of *Meiogyne virgata and Meiogyne monosperma* as medicinal plant.

5.8 References/Bibliography

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6. Research Outcomes

Publications		
Number of articles/ manuscripts/ books *(Please attach the paper)*	**Indexed Journal**	***Non-Indexed Journal***
	1	1
Conference Proceeding *(Please attach the paper)*	**International**	***National***
	2	1
List of published journal/article/proceeding *(APA/IEE fromat)*	Alias, A; Awang, K; Li, A; Bihud, N; Kasim, N; Ismail, N. (2010). Coumarins from *Meiogyne virgata*. Malaysian journal of pharmaceutical sciences, supp 1, page 17.	
	Alias, A; Awang, K; Li, A; Bihud, N; Kasim, N; Ismail, N. (2011). Alkaloids from *Moiogyne virgata*. 59th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research. *Planta Medica*. volume 77.	
	Asmah Alias, Muhd Fauzi Safian, Nor Azah Mohd Ali, Noraini Kasim, Nur Vicky Vihud, Nor Hadiani Ismail. (2011). Characterisation of Aroma Volatiles in *Meiogyne monosperma* Essential Oils. Proceeding of the 24th Regional Symposium of Malaysian Analytical Sciences.	

Intelectual Property	
Patents *(Please attach the certificate)*	
Commercialised Products	

Human Capital Development					
Human Capital	**Number**				**Others** (please specify)
	On-going		**Graduated**		
Citizen	Malaysian	Non Malaysian	Malaysian	Non Malaysian	
PhD Student	1				
Master Student					
Undergraduate Student	4		4		
Total	5		4		

7. Appendix