ISOLATION OF CHEMICAL COMPOUNDS AND ANTIBACTERIAL ACTIVITY FROM THE BARK OF *ALPHONSEA CYLINDRICA*

MUNIRAH BINTI ABDUL TALIP

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER IN SCIENCE (NATURAL PRODUCT)
(MASTER BY RESEARCH)

FACULTY OF SCIENCE AND MATHEMATIC
UNIVERSITI PENDIDIKAN SULTAN IDRIS

2017

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

ABSTRACT

The objectives of this study are to isolate and determine the chemical compounds from *Alphonsea cylindrica* bark and also to investigate their antibacterial activities. Samples were collected from Kechau Tui, Kuala Lipis, Pahang. The samples were oven dried, ground and serially extracted by maceration technique using hexane, dichloromethane and methanol. Acid base extraction was performed on the dichloromethane extract. Chemical compounds were isolated and purified by means of various chromatographic techniques. Their structures were elucidated with modern spectroscopic techniques including 1D and 2D NMR, MS, IR, UV as well as comparison with literature review. Antibacterial activity on crude extracts and pure compounds was completed using disc diffusion technique. The results of phytochemical study have led to the isolation of seven chemical compounds which were stigmasterol, kinabaline, muniranine, *O*-methylmoschatoline, lysicamine, atherospermidine and *N*-methylouregidione. Compound muniranine was successfully isolated as a new derivative of azafluorenone while stigmasterol, kinabaline, *O*-methylmoschatoline, lysicamine, atherospermidine and *N*-methylouregidione were isolated for the first time from *Alphonsea* species. Furthermore, the results of antibacterial activity showed that dichloromethane extract as well as compounds *O*-methylmoschatoline and lysicamine exhibited zone of inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Lysicamine gave minimum inhibition concentration (MIC) between 125 - 250 ug/mL compared with ampicillin, 300 ug/mL. As the conclusion, seven chemical compounds were successfully isolated and identified from *Alphonsea cylindrica* and two of them showed potential as antibacterial agent. For the implication, this study has potential in producing antibacterial agent from plant and supports the traditional use of *Alphonsea sp*. in the treatment of fever and diarrhoea.

PENGASINGAN SEBATIAN KIMIA DAN AKTIVITI ANTIBAKTERIA DARIPADA KULIT BATANG *ALPHONSEA CYLINDRICA*

ABSTRAK

Kajian ini bertujuan mengasing dan mengenal pasti sebatian kimia daripada kulit batang *Alphonsea cylindrica* serta mengkaji aktiviti antibakterianya. Sampel kajian dikumpul dari Kechau Tui, Kuala Lipis, Pahang. Sampel dikeringkan di dalam ketuhar, dikisar dan diekstrak secara bersiri dengan teknik rendaman pelarut heksana, diklorometana dan metanol. Pengekstrakan asid bes dijalankan ke atas ekstrak diklorometana. Sebatian kimia diasing dan ditulenkan melalui pelbagai teknik kromatografi. Struktur sebatian dikenalpasti menggunakan teknik spektroskopi moden iaitu 1D dan 2D RMN, SJ, IM, UL dan juga perbandingan dengan kajian lepas. Aktiviti antibakteria terhadap ekstrak mentah dan sebatian tulen telah dijalankan melalui teknik resapan cakera. Hasil kajian fitokimia telah membawa kepada pengasingan tujuh sebatian kimia iaitu stigmasterol, kinabalina, muniranina, *O*-metilmoskatolina, lisikamina, aterospermidina dan *N*-metiloregidiona. Sebatian muniranina telah berjaya diasingkan sebagai terbitan baharu azafluorinona manakala stigmasterol, kinabalina, *O*-metilmoskatolina, lisikamina, aterospermidina dan *N*-metiloregidiona telah diasingkan untuk pertama kali daripada spesis *Alphonsea*. Seterusnya, dapatan kajian aktiviti antibakteria menunjukkan bahawa ekstrak diklorometana serta sebatian *O*-metilmoskatolina dan lisikamina mempamerkan zon perencatan terhadap *Staphylococcus aureus* dan *Pseudomonas aeruginosa*. Lisikamina memberikan nilai kepekatan perencatan minimum (KPM) antara 125 - 250 ug/mL berbanding ampisillin, 300 ug/mL. Kesimpulannya, tujuh sebatian kimia telah berjaya diasing dan dikenalpasti daripada *Alphonsea cylindrica* dan dua daripadanya menunjukkan potensi sebagai agen antibakteria. Implikasinya, kajian ini berpotensi menghasilkan agen antibakteria daripada tumbuhan bagi menyokong penggunaan spesis *Alphonsea* secara tradisional dalam merawat demam dan cirit-birit.

CONTENTS

		Page
ACKNOWLEDGEMENT		iii
ABSTRACT		iv
ABSTRAK		v
CONTENTS		vi
LIST OF TABLES		x
LIST OF FIGURES		xi
LIST OF SCHEME		xiv
ABBREVIATIONS		xv
CHAPTER 1 INTRODUCTION		
1.1	Introduction	1
1.2	Problem statement	4
1.2.1	Significance of study	5
1.3	Objectives	5
CHAPTER 2 LITERATURE REVIEW		
2.1	Annonaceae	6
2.2	*Alphonsea* sp.	12
2.2.1	Botanical aspect and morphology of *Alphonsea* species	14
2.2.2	Traditional uses of *Alphonsea* species	15
2.2.3	Phytochemistry of *Alphonsea* species	15
2.2.4	Biological activities of *Alphonsea* species	22
2.3	*Alphonsea cylindrica* King.	23

2.4 General chemical aspects 24

2.4.1 Terpenoids 24

2.4.2 Alkaloids 28

2.5 Isolation and elucidation of chemical compounds 34

2.6 Antibacterial analysis 36

2.6.1 Antibiotic resistance and antibacterial agents 36

2.6.2 Natural product as antibacterial agents 37

CHAPTER 3 MATERIALS AND METHODS

3.1 Plant material 42

3.2 Phytochemical study *A. cylindrica* bark 43

3.2.1 Instruments and apparatus 43

3.2.2 Chemical reagents 43

3.2.3 Extraction method 46

3.2.4 Isolation and purification 49

3.2.4.1 Chemical compounds from hexane extract (HB) 51

3.2.4.2 Chemical compounds from alkaloid fraction (AB) 51

3.2.4.3 Chemical compounds from neutral fraction (NB) 53

3.3 Structure elucidation of the isolated compounds 54

3.3.1 Physicochemical and spectroscopic data of the isolated compounds 55

3.4 Antibacterial analysis 63

3.4.1 Instruments and apparatus 63

3.4.2 Chemical reagents 64

3.4.3 Preparation of microorganisms 64

3.4.4 Antibacterial disc diffusion assay 65

3.4.5 Determination of minimum inhibition concentration (MIC) 66

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Plant material 67

4.2 Phytochemical studies of *A. cylindrica* 68

4.2.1 Extraction 68

4.2.2 Yield of extraction 70

4.2.3 Isolation and purification 71

4.3 Structure elucidation of compounds from *A. cylindrica* bark 72

4.3.1 Terpenoid **ACB1**: Stigmasterol (**81**) 72

4.3.2 Alkaloid **ACB2**: Kinabaline (**82**) 82

4.3.3 Alkaloid **ACB3**: Muniranine (**83**) 93

4.3.4 Alkaloid **ACB4**: *O*-methylmoschatoline (**8**) 103

4.3.5 Alkaloid **ACB5**: Lysicamine (**84**) 114

4.3.6 Alkaloid **ACB6**: Atheropermidine (**85**) 119

4.3.7 Alkaloid **ACB7**: *N*-methylouregidione (**86**) 124

4.4 Antibacterial analysis 129

4.4.1 Determination of antibacterial activity by disc diffusion assay 129

4.4.2 Minimum inhibition concentration (MIC) 137

CHAPTER 5 CONCLUSIONS

Conclusions 142

Recommendations for future study 143

REFERENCES 145

APPENDICES 160

LIST OF TABLES

Table No.		Page
2.1	Chemical compounds from Annonaceae plants with their pharmacological activities	7
2.2	Chemical compounds isolated from *Alphonsea* species	16
2.3	The classification of terpenes based on the number of C_5 isoprene units in their structures	25
4.1	The yield of extraction *A. cylindrica* bark	70
4.2	The isolated compounds from *A. cylindrica* bark	71
4.3	Spectral data of **ACB1** ($CDCl_3$, 500 MHz) and Stigmasterol (**81**)	80
4.4	Spectral data of **ACB2** (500 MHz, $CDCl_3$), ^{1}H NMR data of kinabaline (**82**) and ^{13}C NMR data of 5,8-dimethoxy-7-hydroxy-1-methyl-4-azafluoren-9-one (**87**)	92
4.5	Spectral data of **ACB3** (500 MHz, $CDCl_3$) and ^{13}C NMR data of **ACB2**	102
4.6	NMR spectral data of **ACB4** (500 MHz, $CDCl_3$) and *O*-methylmoschatoline (**8**)	113
4.7	^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C ($CDCl_3$, 125MHz) of **ACB5** and Lysicamine (**84**)	118
4.8	^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C ($CDCl_3$, 125 MHz) of **ACB6** and Atherospermidine (**85**)	123
4.9	NMR spectral data ($CDCl_3$, 500 MHz) of **ACB7** and ^{1}H NMR ($CDCl_3$, 300 MHz) of *N*-methylouregidione (**86**) and ^{13}C NMR of Ouregidione (**88**)	128
4.10	Inhibition zones of hexane, dichloromethane and methanol extracts against microbial growth of *S. aureus* and *P. aeruginosa*	130
4.11	Diameter of inhibition zone of compounds from *A. cylindrica* barks and ampicillin against *S. aureus* and *P. aeruginosa*	134

LIST OF FIGURES

Figure No.		Page
2.1	Classification of Annonaceae	10
2.2	Classification of Annonaceae (Subfamily Annonaideae)	11
2.3	Distribution of *Alphonsea* and possible migratory path of *Alphonsea* in Southeast Asia	13
2.4	(a) Herbarium specimen of *Alphonsea borneensis* (b) Leaves and trunk of *Alphonsea maingayi*	14
2.5	(a) Herbarium specimen of *A. cylindrica* (b) Fruits of *A. cylindrica*	23
2.6	The general backbone skeleton of aporphine	30
2.7	The skeleton of oxoaporphine	31
4.1	GCMS spectrum of **ACB1**	73
4.2	ATR-FTIR of **ACB1**	74
4.3	^{1}H NMR spectrum of **ACB1**	75
4.4	^{13}C NMR spectrum of **ACB1**	76
4.5	COSY spectrum of **ACB1**	77
4.6	HMQC spectrum of **ACB1**	78
4.7	HMBC spectrum of **ACB1**	79
4.8	Selected HMBC and COSY correlations of **ACB1**	80
4.9	GCMS spectrum of **ACB2**	83
4.10	^{1}H NMR spectrum of **ACB2**	84
4.11	^{13}C NMR spectrum of **ACB2**	84
4.12	COSY spectrum of **ACB2**	87
4.13	HMQC spectrum of **ACB2**	88

4.14 HMBC spectrum of **ACB2** 89

4.15 Expansion HMBC spectrum of **ACB2** 90

4.16 COSY, ^{1}H and ^{13}C correlation observed in HMBC spectrum of **ACB2** 91

4.17 LCMS spectrum of **ACB3** 94

4.18 ATR-FTIR of **ACB3** 94

4.19 ^{1}H NMR spectrum of **ACB3** 95

4.20 ^{13}C NMR spectrum of **ACB3** 96

4.21 COSY spectrum of **ACB3** 98

4.22 NOESY spectrum of **ACB3** 99

4.23 HMQC spectrum of **ACB3** 100

4.24 HMBC spectrum of **ACB3** 101

4.25 COSY, NOESY, ^{1}H and ^{13}C correlation observed in HMBC spectrum of **ACB3** 102

4.26 GCMS spectrum of **ACB4** 104

4.27 ^{1}H NMR spectrum of **ACB4** 105

4.28 ^{13}C NMR spectrum of **ACB4** 106

4.29 COSY spectrum of **ACB4** 108

4.30 HMQC spectrum of **ACB4** 109

4.31 HMBC spectrum of **ACB4** 110

4.32 Expansion HMBC spectrum of **ACB4** 111

4.33 COSY, ^{1}H and ^{13}C correlation observed in HMBC spectrum of **ACB4** 112

4.34 GCMS spectrum of **ACB5** 115

4.35 ^{1}H NMR spectrum of **ACB5** 116

4.36 ^{13}C NMR spectrum of **ACB5** 117

4.37 LCMS spectrum of **ACB6** 120

4.38	^{1}H NMR spectrum of **ACB6**	121
4.39	^{13}C NMR spectrum of **ACB6**	122
4.40	LCMS of **ACB7**	125
4.41	^{1}H NMR spectrum of **ACB7**	126
4.42	^{13}C NMR spectrum of **ACB7**	127
4.43	Diagram of the cytoplasmic membrane of Gram positive bacteria (a) and the cytoplasmic membrane and the outer membrane of Gram negative bacteria (b).	133
4.44	Structure of compounds **8**, **84** and **85**	136
4.45	Minimum inhibition concentration of dichloromethane extract from *A. cylindrica* bark against *S. aureus* and *P. aeruginosa*	138
4.46	Minimum inhibition concentration of compound **8** from *A. cylindrica* bark against *S. aureus* and *P. aeruginosa*	139
4.47	Minimum inhibition concentration of compound **84** from *A. cylindrica* bark against *S. aureus* and *P. aeruginosa*	140
4.48	Minimum inhibition concentration of ampicillin against *S. aureus* and *P. aeruginosa*	141

LIST OF SCHEMES

Scheme No.		Page
2.1	The biogenetic pathway to an oxoaporphine	32
2.2	The derivation of onychine	33
3.1	Extraction process of *Alphonsea cylindrica* King. bark	48
3.2	Isolation and purification of chemical compounds from *Alphonsea cylindrica* bark	50

ABBREVIATIONS

^{1}H	Proton
^{13}C	13 Carbon
br s	Broad singlet
J	Coupling constant
δ	Delta
°C	Degree celsius
d	Doublet
dd	Doublet of doublet
d_c	Inner diameter of column
g	Gram
λ_{max}	Lambda maximum
m/z	Mass per charge
mg	Miligram
µL	Microlitre
mL	Mililitre
MHz	Megahertz
m	Multiplet
µg/mL	Microgram per millilitre
mg/mL	Miligram per millilitre
nm	Nanometre
±	Plus minus
cm^{-1}	Per centimetre
ppm	Part per million
s	Singlet

t	Triplet
td	Triplet of doublet
CC	Column Chromatography
$CHCl_3$	Chloroform
$CDCl_3$	Deuterated Chloroform
CH_2Cl_2	Dichloromethane
COSY	^1H-^1H Correlation Spectroscopy
DCM	Dichloromethane
DMSO	Dimethylsulphoxide
EIMS	Electron Ionization Mass Spectrum
FDA	Food Drug Administration
FTIR	Fourier Transformation Infra Red
FRIM	Forestry Research Institute Malaysia
GC-MS	Gas Chromatography-Mass Spectrometry
HMQC	Heteronuclear Multiple Quantum Correlation
HMBC	Heteronuclear Multiple Bond Correlation
HREIMS	High Resolution Elecrotrospray Ionization Mass Spectrum
IR	Infrared
LC-MS	Liquid Chromatography-Mass Spectrometry
MS	Mass Spectrometry
MeOH	Methanol
MIC	Minimum Inhibition Concentration
Na_2SO_4	Sodium sulphate
NH_4OH	Ammonium hydroxide solution
NMR	Nuclear Magnetic Resonance
NA	Nutrient Agar
NB	Nutrient Broth

OD	Optical Density
PTLC	Preparative Thin Layer Chromatography
TMS	Tetramethylsilane
TLC	Thin Layer Chromatography
UV	Ultraviolet
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

1.1 Introduction

In the eyes of the world, Malaysia is a developing country with tropical rain forests which are rich in the biodiversity of flora and fauna. The forests are also biologically and chemically source of excellent medicine since the plants can synthesize various chemical compounds as defense agents for their survival and growth. According to Jantan (2004), the earliest report on medicinal plant in Malaysia was studied on 205 plants in Sabah. The research reports were mostly on the phytochemical studies, which leading to the discovery of bioactive compounds and are important for drug development.

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

Most of the world populations use traditional herbal medicine as primary health care (Irchhaiya et al., 2014). Subsequently, studies on the bioactive compounds from plants or phytochemical studies have called lot of attentions globally. Research in Malaysia had focused on medicinal plant such as *Labisia pumila* (kacip fatimah), *Eurycoma longifolia* (tongkat ali), *Clinacanthus nutans* (belalai gajah), *Ficus deltoidea* (mas cotek), *Casia alata* (gelenggang), *Morinda citrifolia* (mengkudu), *Zingiber officinale* (halia), *Melastoma malabatricum* (senduduk), *Phyllanthus niruri* (dukung anak), *Orthosiphon stamineus* (misai kucing) and *Phaleria macrocarpa* (mahkota dewa). They were selected based on the traditional uses and the chemical compounds of the plant (Farizah et al., 2015).

Annonaceae is a flowering plant family and comprises of 130 genera with more than 2000 species (Wiart, 2006). Species from this family are trees, shrubs and climbers that distributed in the tropical and subtropical regions. These species have long been used as traditional medicines to treat diarrhoea, dysentery, fever and rheumatism (Bele at al., 2011; Moghadamtousi et al., 2015). Besides that, Annonaceae plants also used to treat snakebite, respiratory infections, malaria and pneumonia (Okhale et al., 2016; Mustapha, 2013). Scientific studies demonstrated that several species of this family exhibited antiplasmodial (Boyom et al., 2011), antioxidant, antidiabetic (Florence et al., 2014), antinociceptive, anti-inflammatory (Silva et al., 2015), cytotoxic activity (Thuy et al., 2012), insecticidal, antimicrobial (Tan et al., 2015) and anticancer (Piemi et al., 2014). The chemistry of family Annonaceae showed a various group of chemical constituents dominated by alkaloids including aporphinoids, oxoaporphines, phenanthrenes, isoquinolines,

benzylisoquinolines, bisbenzyltetrahydroisoquinolines, protoberberines and tetrahydroprotoberberines (Laboeuf et al., 1982).

Alphonsea cylindrica is a small tree belongs to the family Annonaceae and distributed in lowland forest but not widespread (Hanum et al., 2001). Based on author extensive searches, the chemistry and biological activity of this species has yet to be established and remain to be investigated. In addition, the fruits of *Alphonsea* species were traditionally used as emmanogogue and for diarrhoea and fever treatment (Batugal, 2004). Previous studies reported that *Alphonsea* species have antifungal (Indrani et al., 2015), antioxidant (Narendra, 2009), anticancer, cytotoxic activity (Horgen et al., 2001), anti-inflammatory (Johnson et al. 2013) and antitrypanosomal (Norhayati et al., 2013).

Antibiotic resistance has become a global health issue because of its impact on human death (WHO, 2000). Antibiotic resistance means that the pathogenic bacteria cannot be killed and withstand the effects of antibiotics. When the bacteria become resistant, it has the ability to pass their drug resistant genes to other strains and also to other bacteria. These phenomena showed that this problem can spread easily. In the nut of shell, US Food and Drug Administration (FDA) have encouraged the development of new antibiotics to reduce the bacterial resistance problem. In our study, natural products, particularly from plants, are the most preferred source used to develop new antibiotics.

1.2 Problem statement

Species of *Alphonsea* have been used as traditional medicines to treat bacterial-related diseases such as diarrhoea and fever, as well as emmenagogue. Current phytochemical studies of these species resulted in isolation of alkaloids as a dominant chemical constituent. Moreover, they were demonstrated pronounced biological activities including antimicrobial, anticancer, anti-inflammatory and antioxidant. High content of alkaloids has been indicated in the stem bark of *A. cylindrica* (Teo et al., 1990). However, investigation on chemical constituents including alkaloids of this plant has yet to be established and thus requiring more research.

In current situation, multi-drug resistance bacteria have spread widely and cause the treatment for the infectious disease becomes limited and difficult to solve. Normally the bacterial infection can be treating effectively by using antibiotics. However, when the bacteria become resistance, the antibiotics are no longer effective for the treatment. Antibiotics are sometime also associated with adverse side effects such as hypersensitivity, immuno suppressent and allergic reactions (Hussain et al., 2011).

Therefore, there is a need to study the chemical compounds from natural source such as plant that have antibacterial properties in order to discover the new antibacterial drug. Moreover, natural sources were reported to have fewer side effects, affordable, better patience tolerance and being renewable in nature (Hema et al., 2013).

1.2.1 Significance of study

This is the first phytochemical and biological studies of species *A. cylindrica*. Antibacterial activity of crude extract and chemical compounds isolated from this plant may serve as future references for development of new antibacterial drugs from natural resources.

1.3 Objectives

The objectives of this study were:

1) To extract, isolate and purify chemical compounds from the crude extracts of *A. cylindrica* barks using chromatographic techniques.

2) To identify and elucidate the molecular structures of the isolated compounds using modern spectroscopic techniques.

3) To investigate antibacterial activity of the crude extracts and isolated compounds from barks of *A. cylindrica* against Gram negative and Gram positive bacteria.

CHAPTER 2

LITERATURE REVIEW

2.1 Annonaceae

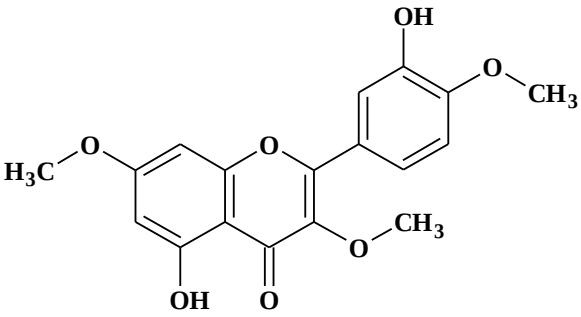
Annonaceae family is a family of flowering plant of trees, shrubs and climbers. This family is called as the custard apple family and locally known as 'Mempisang' in Malaysia (Burkill, 1966). This family is also the largest family in Magnoliales that consisted of 130 genera with more than 2000 species. Of these species, as many as 60 species have been used in traditional medicine in Asia and the Pacific (Wiart, 2006). These species are mostly found in tropics region and some species found in temperate regions. According to Sinclair (1955), there are 17 varieties of Annonaceae species in Malaysia. They grow in the lowland forest at below 2000 ft.

Annonaceae plants contain a large number of chemical compounds such as alkaloid, flavonoid and acetogenins (Lúcio et al., 2015; Esquinca et al., 2014). These compounds had shown several pharmacological activities that useful as medicine. Some examples of chemical compounds from Annonaceae and their pharmacological activities are listed in Table 2.1.

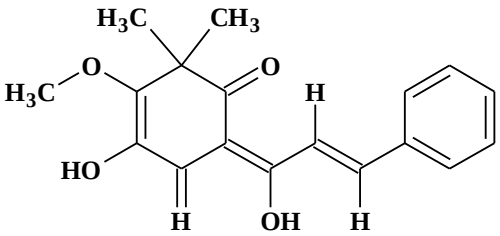
Table 2.1

Chemical compounds from Annonaceae plants with their pharmacological activities

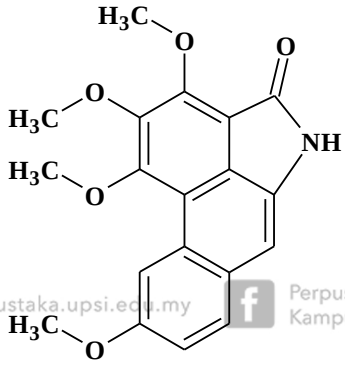
No. of compounds	Chemical compounds	Pharmacological activities	Plant source	References
1	5,3′-dihydroxy-3,7,4′-trimethoxyflavone	Anticancer ; [murine lymphocytic leukemia (P388), human colon cancer (Col-2), human breast cancer (MCF-7)]	*Miliussa smithiae*	Naphong et al., 2013
2	Desmosdumotin C	Antioxidant, anti-inflammatory, and anti-*Hpylori*.	*Mitrella kentia*	Sidahmed et al., 2013
3 **4**	Enterocarpam-III Stigmalactam	Cytotoxicity ; [human colon adenocarcinoma (HCT15) cell line.]	*Orophea enterocarpa*	Nayyatip et al., 2012
5 **6**	Pseuduvarine A Pseuduvarine B	Cytotoxicity; [breast cancer cells (MC7) human promyelocyctic leukemia (HL-60) cell lines]	*Pseuduvaria rugosa*	Taha et al., 2011
7 **8**	2′,4′-Dihydroxy-4,6′-dimethoxychalcone *O*-methylmoschatoline	Anti-bacterial; [*B.subtilis, E.aerogenes, E.coli, B.subtilis* and *S.aureus*]	*Ellipeia cuneifolia*	Yusof et al., 2015
9	Anonaine	Vasorelaxant, antibacterial, antifungal, antioxidative, anticancer and antidepressant.	*Michelia alba*	Li et al., 2013



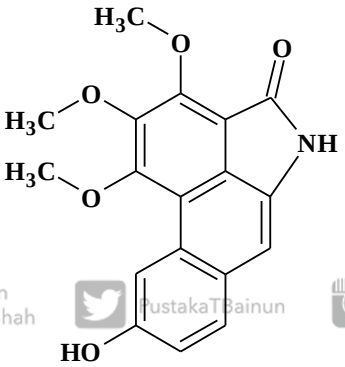
1



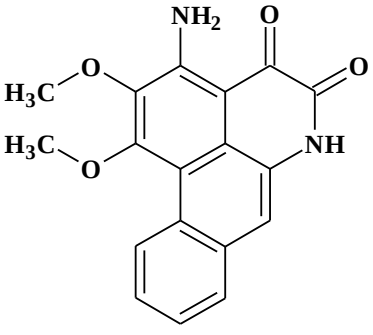
2



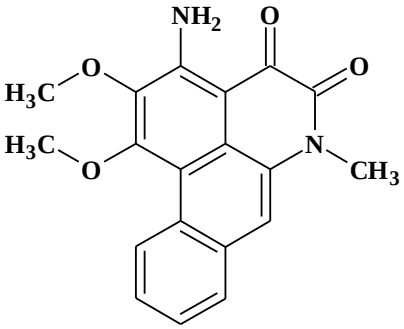
3



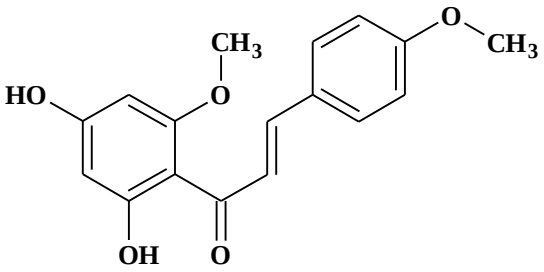
4



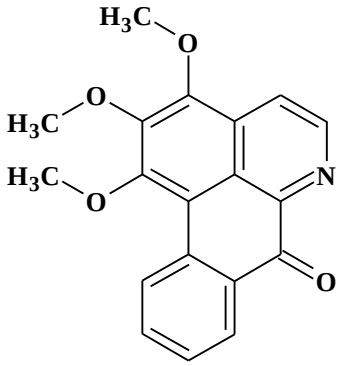
5



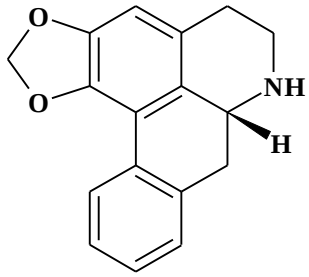
6



7



8



9