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ISOLATION OF TERPENOID FROM *ALSTONIA SPATHULATA* AND *KOPSIA SINGAPURENSIS* (APOCYNACEAE)

TEE CHUAN THING

UNIVERSITI PENDIDIKAN SULTAN IDRIS

2014

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ISOLATION OF TERPENOID FROM *ALSTONIA SPATHULATA* AND *KOPSIA SINGAPURENSIS* (APOCYNACEAE)

TEE CHUAN THING

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ABSTRACT

The aim of this study was to extract and identify the chemical compounds isolated from *Alstonia spathulata* and *Kopsia singapurensis*. The isolated compounds were tested for their biological activities include cytotoxicity (MCF-7 cell line), antibacterial (*Bacillus cereus*) and antioxidant (DPPH). The separation of the chemical components from both species was carried out using different chromatographic techniques (column chromatography and thin layer chromatography). The structures of isolated compounds have been elucidated through spectral analysis including 1D-NMR (^{1}H, ^{13}C and DEPT), 2D-NMR (^{1}H-^{1}H COSY, HSQC/HMQC and HMBC), IR, UV, MS (GCMS) and also by comparison with previous literature data. Studies on the chemical constituents of the bark of *Alstonia spathulata* has led to the isolation of five triterpenes; β-amyrin **113**, β-amyrin acetate **114**, β-sitosterol **115**, lupeol acetate **116**, stigmasterol **117** and a mixture of three triterpenes (lupeol **118**, α-amyrin **119** and β-amyrin **113)**. Four known tritepenes were also successfully isolated from the root of *Kopsia singapurensis*; β-amyrin **113**, β-amyrin acetate **114**, lupeol acetate **116** and stigmasterol **117**. These compounds were isolated for the first time from both species. Compound **113**, **114**, **116** and **117** exhibited cytotoxic effects against MCF-7 cell line with IC_{50} values of 15.5, 22.5, 26.0 and 14.5 μg/mL, respectively. All triterpenes showed weak antioxidant activity with IC_{50} > 500 μg/mL and were not active against the gram positive bacteria, *Bacillus cereus*. Both species have been traditionally used to cure diseases however there are not many publications on triterpenes extracted from this plants species. Isolation of chemical constituents from these two species has potential for producing new drugs.

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PEMENCILAN TERPENOID DARIPADA POKOK *ALSTONIA SPATHULATA* DAN *KOPSIA SINGAPURENSIS* (APOCYNACEAE)

ABSTRAK

Kajian ini bertujuan mengekstrak dan mengenalpasti sebatian kimia yang dipencil dari pokok *Alstonia spathulata* dan *Kopsia singapurensis*. Sebatian kimia yang dipencil diuji aktiviti biologi termasuk sitotoksisiti (MCF-7 *cell line*), antibakteria (*Bacillus cereus*) dan antioksidan (DPPH). Pemisahan komponen kimia dari kedua-dua spesis ini telah dijalankan menggunakan teknik kromatografi yang berbeza (kromatografi turus dan kromatografi lapisan nipis). Struktur sebatian kimia dikenalpasti melalui analisis spektrum termasuk 1D-NMR (^{1}H, ^{13}C dan DEPT), 2D-NMR (^{1}H-^{1}H COSY, HSQC / HMQC dan HMBC), IR, UV, MS (GCMS) dan juga dibandingkan dengan data daripada kajian lepas. Kajian ke atas kandungan sebatian kimia kulit batang *Alstonia spathulata* telah membawa kepada pengasingan lima triterpena; β-amirin **113**, β-amirin asetat **114**, β-sitosterol **115**, lupeol asetat **116**, stigmasterol **117** dan campuran tiga triterpena (lupeol **118**, α-amirin-**119** dan β-amirin **113)**. Empat tritepena juga berjaya dipencil dari akar *Kopsia singapurensis*; β-amirin **113**, β-amyrin asetat **114**, lupeol asetat **116** dan stigmasterol **117**. Semua sebatian kimia ini merupakan pertama kali dipencil dari kedua-dua spesis ini. Sebatian **113**, **114**, **116** dan **117** masing-masing menunjukkan kesan sitotoksik terhadap MCF-7 dengan nilai IC_{50}; 15.5, 22.5, 26.0 dan 14.5 μg/mL. Semua triterpena menunjukkan aktiviti antioksidan yang lemah dengan IC_{50}> 500 μg/mL dan tidak aktif terhadap bakteria gram positif, *Bacillus cereus*. Kedua-dua spesis telah digunakan secara tradisional untuk menyembuhkan pelbagai penyakit tetapi tidak banyak penerbitan mengenai triterpena yang diekstrak daripada kedua-dua spesis tumbuhan ini. Pengasingan sebatian kimia daripada kedua-dua spesis ini berpotensi bagi penghasilan ubat-ubatan baru.

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LIST OF SYMBOLS AND ABBREVIATIONS

α	Alpha
β	Beta
λ	Maximum wave length
δ	Chemical shift
g	Gram
kg	Kilogram
M	Molar
mM	Milimolar
ml	Mililitre
m	Meter
MHz	Mega Hertz
Hz	Hertz
UV	Ultraviolet
IR	Infrared
ppm	Part per million
eV	Electron Volt
MeOH	Methanol
$CHCl_3$	Chloroform
CH_2Cl_2	Dichloromethane
DMSO	Dimethylsulphoxide
EA	Ethyl Acetate
OCH_2O	Methylenedioxy
CH_3	Methyl group

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OCH_3	Methoxyl group
OH	Hydroxyl group
NH_3	Ammonia
pH	Power of Hydrogen
HCl	Hydrogen chloride
TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
CC	Column Chromatography
NMR	Nuclear Magnetic Resonance
FT-NMR	Fourier Transform Nuclear Magnetic Resonance
cm^{-1}	Per centimeter
J	Coupling constant
d	Doublet
dd	Doublet of doublet
t	Triplet
dt	Doublet of triplet
s	Singlet
m	Multiplet
q	Quartet
dbh	Diameter at Breast Height
$^{\circ}C$	Degree Celsius
1D-NMR	One Dimension Nuclear Magnetic Resonance
2D-NMR	Two Dimension Nuclear Magnetic Resonance
^{1}H	Proton NMR
^{13}C	13-Carbon NMR

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COSY	1H-1H Correlation Spectroscopy
DEPT	Distortioness Enhancement by Polarization Transfer
HMQC	Heteronuclear Multiple Quantum Correlation
HSQC	Heteronuclear Single Quantum Correlation
HMBC	Heteronuclear Multiple Bond Correlation
NOESY	Nuclear Overhauser Effect Spectroscopy
GC-MS	Gas Chromatography Mass Spectrometry
LC-MS	Liquid Chromatography-Mass Spectrometry
MS	Mass Spectrometry
HRESIMS	High Resolution Electrospray Ionization Mass Spectrometry
m/z	Mass per charge
$CDCl_3$	Deuterated chloroform
CD_3OD	Deuterated methanol
$FeCl_3$	Ferric chloride
DMF	Dimethylformamide

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Nature especially flora furnishes interesting and diverse types of molecular structures some of which provide the chemist and pharmacologist with model compounds to forms the design or prototype for synthetic or semi synthetic medicines, pesticides and other valuable compounds. Mention may be made of previous discoveries of important drugs from plant such as ephedrine **1**, quinine **2**, reserpine **3** and vincristine **4** which are used directly or have been modeled for modern pharmaceuticals. Furthermore, the most common insecticide currently used in households depends on the discovery of pyrethrins **5** of Pyrethrum flowers which kill a variety of insects but remain relatively harmless to mammalian life (U.S Department of health and human services, 2003). In recent times, viral diseases including AIDs and many resistant bacterial strains have appeared and the search for more natural products to be used as

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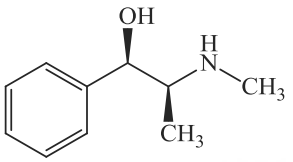
new drug models has become more urgent. The number and variety of tropical plants provide immense opportunities for the discovery of new chemically and pharmacologically active principles. However, about 15000 species of the medicinal plants are globally threatened (Hamilton, 2008). Less than 1% of all tropical plants had been screened for possible pharmaceutical use and that habitats are being destroyed faster than scientists can research the plants. At current extinction rates, experts estimate that the Earth is losing at least one potential major drug every two years (Groombridge & Jenkins, 2002).

The Malaysian flora is among the world's richest containing some of the primitive species originating from the earliest forms of angiosperms and gymnosperms of some 200 million years ago. The lack of major geologic upheavals in this region has allowed many of the floras maintain some of their primitive characteristic. It is therefore possible to find plants of great botanical interest apart from their potential as producers of useful natural products.

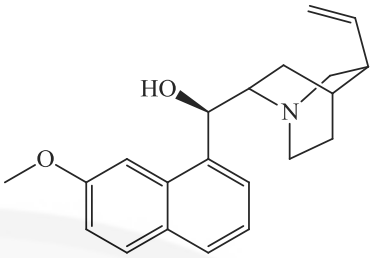
Malaysia has about 14500 species of flowering plants of which about 2000 have been reported to contain medicinal properties and many have been scientifically proven (Jaganath & Ng, 2000). The huge diversity of the Malaysian flora means that we can expect well diverse chemical structures from their secondary metabolites, and chemical diversity is one of the plus factors that make natural product excellent candidates for any screening program.

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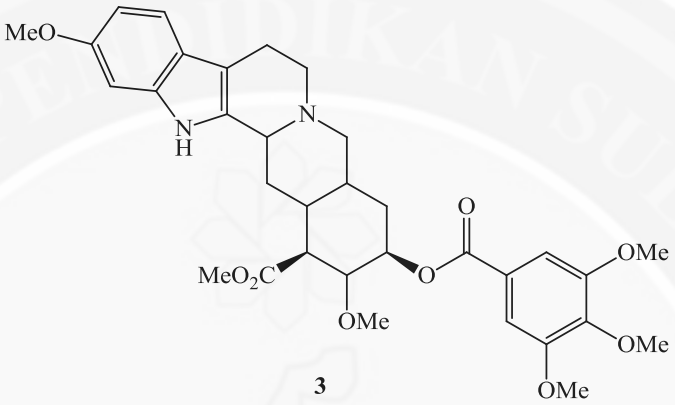
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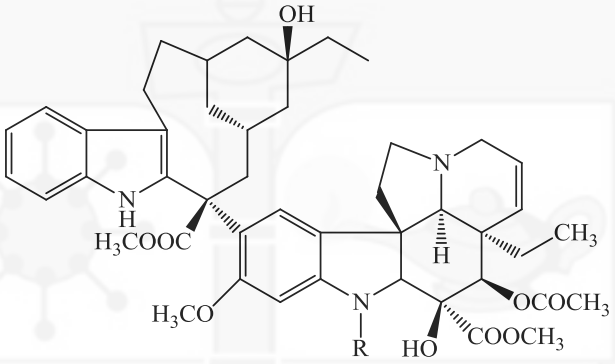
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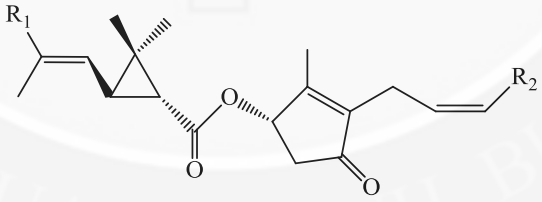
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3



4 R= CHO
6 R= CH_3



5
Pyrethrin I $R_1 = CH_3$ $R_2 = CH{=}CH_2$
Pyrethrin II $R_1 = CH_3O_2C$ $R_2 = CH{=}CH_2$

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1.2 Objectives

This study aimed on investigating the chemical constituents of *Alstonia spathulata* Bl. and *Kopsia singapurensis* Ridl. The objectives guiding this inquiry were to:

1. Extract, isolate and purify the chemical constituents of the *Alstonia spathulata and Kopsia singapurensis*.
2. Elucidate and identify the structure of the isolated compounds using modern spectroscopic methods such as NMR, UV, IR and MS.
3. Determine the bioactivities of the compounds.

1.3 Significant of research

In this research study, isolate and purify the chemical constituents of the *Alstonia spathulata* and *Kopsia singapurensis* are determined. This is because plant natural products have long been and will continue to be important sources and models of spices, flavors and fragrances, vegetable oils, soaps, natural rubber, gums, resins, drugs, insecticides, and other industrial, medicinal, and agricultural raw materials. For example, many *Alstonia* species are commercial timbers. Trees from the section *Alstonia* produce light timber. However, since most plant species have never been described much less surveyed for chemical or biologically active constituents, it is reasonable to expect that new sources of valuable materials remain to be discovered. Furthermore, if the current trend of destruction of tropical forests continues at its present rate. Phytochemists and other plant scientists may have only a few decades

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remaining in which to investigate much of the plant kingdom for useful chemicals (Balandrin, Klocke, Wurtele, & Bollinger, 1985). According to IUCN Red List of Threatened Species (Chua, 1998), *Kopsia singapurensis* was one of the threatened species and traditional used to poultice ulcerated nose in tertiary syphilis (Perry & Metzger, 1980). Thus, it is important to isolate and purify the chemical constituents of the plant species as well as from *Alstonia spathulata* and *Kopsia singapurensis.*

1.4 Apocynaceae

1.4.1 Botanical aspect of Apocynaceae

The Apocynaceae were first described by Jussieu (1789) as "Apocinae". It is a large family which currently recognized has 424 genera and 1500 species (Endress & Bruyns, 2000); cosmopolitan in distribution, but more common in the tropics (Bhattacharyya & Johri, 1998). In Malaysia, only 10 genera and 38 species are trees and shrubs; the remaining are climbers or scrambling shrubs except *Vallariopsis lancifolia* which is an epiphytic shrub (Whitmore, 1973). Plants of Apocynaceae are adapted to various habitats, from sea level to mountain tops, mainly on dry soils, but also on rocks or in flood areas, and sometimes river margins (Smith et al., 2004). Members of Apocynaceae are usually herbs, shrubs, vines or trees. Some of the members are large, stout and woody climbers (Bhattacharyya & Johri, 1998). The cardinal botanical features of Apocynaceae are an exudation of abundant milky latex (Wiart, 2006).

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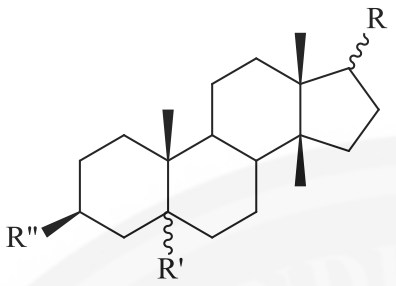
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Plants in the Apocynaceae usually have simple, opposite or whorled leaves. However, some genera of Apocynaceae are stem succulents with vestigial or no leaves (Hodgkiss, 2011). Flowers of Apocynaceae are bisexual and in clusters. They are usually showy, often pure white, salver-shaped and slightly fragrant flowers with five contorted lobes (Wiart, 2006). Fruits and seed pods are usually produced in pairs of follicle that split open at maturity. Seeds are flat and winged or have a tuft of hairs at one end.

Economically, the family has limited importance. Some species are valuable sources of medicine, insecticides and timber. Plants of the Apocynaceae are often poisonous and are rich in indole alkaloids or glycosides, especially in the seeds and latex (Tao, Leeuwenberg & Middleton, 1995). Because of the profusion of secondary metabolites, the family is the important source of bioactive compounds. This family is known for plants that have a very high biological activity and medical properties like treatments of disorders of skin, liver diseases, leprosy, dysentery, ulcers, ear aches, tumors, malaria, fevers and hypertension (Perry & Metzger, 1980; Holdsworth, 1986; Ambasta, Ramchandran, Kashyapa & Chand, 1992). It has medical importance owing to the presence of these bioactive compounds such as vinblastine **6**, vincristine **4** and cardiac glycosides **7** (Smith et al., 2004). Vincristine **4** found in *Catharanthus roseus* is the source of anticancer drugs. *Rauwolfia serpentine* roots are used medicinally as a cure for epilepsy, high blood pressure, insanity and cardiac diseases. Besides, some of the members of Apocynaceae also grown as ornamentals such as *Nerium odorum* and *Thevetia peruviana*. The wood of *Wrightia tinctoria* and *Alstonia scholaris* which is soft are used for wood carving. The members of Apocynaceae also contribute to rubber and fibre yielding (Bhattacharyya & Johri, 1998).

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R = unsaturated lactone or pyrone ring system
R' = H or OH
R" = sugar moiety

7

1.4.2 Classification of Apocynaceae

The Apocynaceae belong to the order Gentianales and have traditionally been divided into two subfamilies, the Rauvolfioideae (Plumerioideae) and the Apocynoideae (Smith, Mori, Henderson, Stevenson, & Heald, 2004). However, this family recently are divided into 5 subfamilies which are Rauvolfioideae (Plumerioideae), Apocynoideae, Periplocoideae, Secamonoideae and Asclepiadoideae (Sennblad & Bremer, 2002; Endress, 2004; Middleton, 2009). There are 17 newly recircumscribed tribes were also recognized in the family of Apocynaceae. Rauvolfioideae is further divided into nine tribes which are Alstonieae, Vinceae (Rauvolfieae), Willughbeieae, Tabernaemontaneae, Melodineae, Hunterieae, Plumerieae, Alyxieae and Carisseae. Wrightieae, Malouetieae, Apocyneae, Mesechiteaeand Echiteae are five tribes that classified in the Apocynoideae. While subfamilies of Periplocoideae and Secamonoideae do not consists of tribe (Sennblad & Bremer, 2002). Each tribe comprises of several genera. Figure 1.1 shows the subfamilies, tribes and examples of genus of the Apocynaceae.

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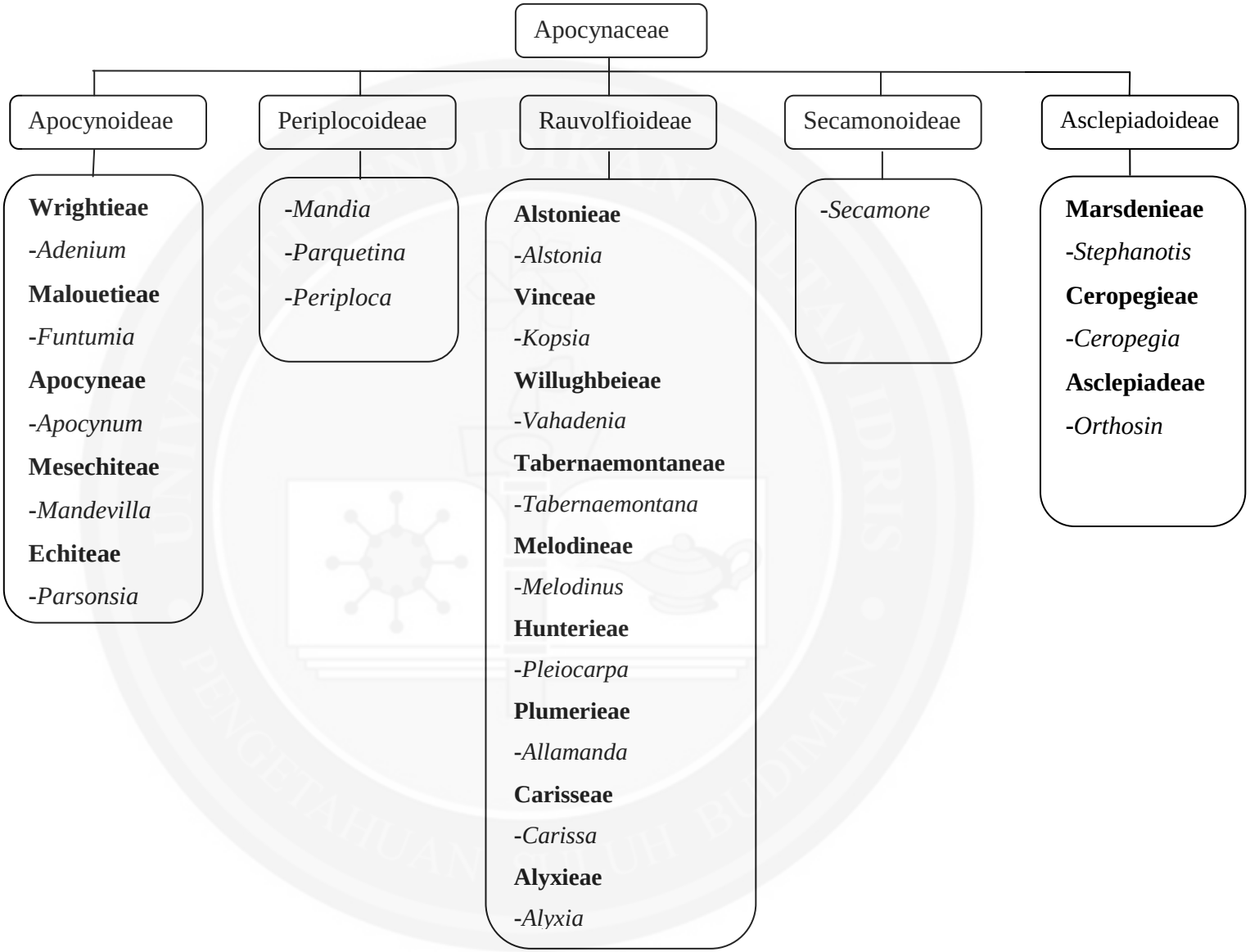


Figure 1.1. Classification of the Apocynaceae and sampling of taxa. Classifications are according to Endress and Bruyns (2000)

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1.5 The Genus *Alstonia*

1.5.1 Botanical aspect of *Alstonia*

Alstonia is genus that under tribe of Alstonieae in Rauvolfioideae subfamily of Apocynaceae. It is the largest and most widespread genus of trees and shrub (Sidiyasa & Baas, 1998). The natural range of *Alstonia* is an immense, almost continuous belt in the tropical and subtropical Africa, Central America, Southeast Asia, Polynesia and Australia, with most species in the Malesian region (Webster Online Dictionary, 1913). Species grow at altitudes and under conditions varying from sea level to very great heights, in deep swamps and rain forests to rather dry ground and areas of moderate rainfall, in soils of rich humus to clay or limestone (Monachino, 1949).

Leaves of *Alstonia* are verticillate which is forming one or more whorls of leaves around the stem. Shape of the leaf is ovate, leathery and sessile. The leaf blade is dorsiventral, while the leaf venation is pinnate, with numerous veins ending in a marginal vein. The inflorescence is terminal or axillary, frequently in whorls or umbel-like. Flower is funnel-shaped and in white, yellow, pink or green colour. They consist of five petals and five sepals which arranged in four whorls. The fertile flowers are hermaphrodite. Sepals are without colleters inside and ovate. Corolla lobes are overlapping to the left (such as *A. rostrata*) or to the right in bud (such as *A. macrophylla*). Corolla lobes will spread at maturity. Ovary of two separate camels is united into a common style with glabrous or ciliate. Ovules are numerous. Seeds are oblong, end rounded or acuminate (Monachino, 1949; Middleton, 2002; Webster Online Dictionary, 1913). The form of ciliation in the seeds of *Alstonia* is almost unique in the Apocynaceae. The seeds of *Alstonia* are invested with cilia at both ends,

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the distinct hairs being in some species sparsely distributed and in others rather densely set (Monachino, 1949).

Alstonia is the genus of laticiferous shrubs or trees. The plants contain a milky sap which rich in poisonous alkaloids. While *Alstonia* alkaloids occupy an important place in chemical literature, much less is known about the value of the latices. The widely distributed *A. scholaris* is immense trees bearing copious latex of good quality for use as chewing gum (Monachino, 1949). Many of its species provide important timbers of commerce, and several species are used in traditional local medicine. Among the timber producing genera of *Alstonia*, there are two distinct groups which are lightweight woods traded as 'Pulai' with thin-walled fibres (including *A. pneumatophora*, *A. scholaris*, and *A. spathulata*) and medium heavy woods traded as 'Hard Alstonia ' with thick-walled fibres (including *A. macrophylla* and *A. spectabilisy*) (Sidiyasa & Baas, 1998).

Alstonia trees also are used in traditional medicine. The different ethnic communities in India have used different species of *Alstonia* in the treatment of various human ailments (Sutha, Devi, Tresina, Maruthupandian, & Mohan, 2011). Roots of *A. Venenata* are useful in skin diseases, leprosy, cobra bit and other venomous bites, fatigue, fever and otalgia (Orient Longman, 1994).

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1.5.2 Species of genus *Alstonia*

Based on the Plant List (2010), there are total 42 species are recognized. Those plants are listed below:

1. *Alstonia actinophylla* (A.Cunn.) K.Schum.
2. *Alstonia angustifolia* Wall. ex A.DC.
3. *Alstonia angustiloba* Miq.
4. *Alstonia annamensis* (Monach.) Sidiyasa
5. *Alstonia balansae* Guillaumin
6. *Alstonia beatricis* Sidiyasa
7. *Alstonia boonei* De Wild.
8. *Alstonia boulindaensis* Boiteau
9. *Alstonia breviloba* Sidiyasa
10. *Alstonia congensis* Engl.
11. *Alstonia constricta* F.Muell.
12. *Alstonia coriacea* Pancher ex S.Moore
13. *Alstonia costata* (G.Forst.) R.Br.
14. *Alstonia curtisii* King & Gamble
15. *Alstonia deplanchei* Van Heurck & Müll.Arg.
16. *Alstonia glaucescens* (K.Schum.) Monach.
17. *Alstonia guanxiensis* D.Fang & X.X.Chen
18. *Alstonia iwahigensis* Elmer
19. *Alstonia lanceolata* Van Heurck & Müll.Arg.
20. *Alstonia lanceolifera* S.Moore
21. *Alstonia legouixiae* Van Heurck & Müll.Arg.

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22. *Alstonia lenormandii* Van Heurck & Müll.Arg.

23. *Alstonia macrophylla* Wall. ex G.Don

24. *Alstonia mairei* H.Lév.

25. *Alstonia muelleriana* Domin

26. *Alstonia neriifolia* D.Don

27. *Alstonia odontophora* Boiteau

28. *Alstonia parvifolia* Merr.

29. *Alstonia penangiana* Sidiyasa

30. *Alstonia pneumatophora* Baker ex Den Berger

31. *Alstonia quaternata* Van Heurck & Müll.Arg.

32. *Alstonia rubiginosa* Sidiyasa

33. *Alstonia rupestris* Kerr

34. *Alstonia scholaris* (L.) R. Br.

35. *Alstonia sebusi* (Van Heurck & Müll.Arg.) Monach.

36. *Alstonia spathulata* Blume

37. *Alstonia spectabilis* R.Br.

38. *Alstonia sphaerocapitata* Boiteau

39. *Alstonia venenata* R.Br.

40. *Alstonia vieillardii* Van Heurck & Müll.Arg.

41. *Alstonia vietnamensis*D.J.Middleton

42. *Alstonia yunnanensis* Diels

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1.5.3 Botanical aspect of *Alstonia spathulata* Blume

In Malaysia, *Alstonia spathulata* Bl. is locally known as *pulai basong*, *pulai paya* or marsh pulai. *A. spathulata* Bl. is a tree that grows to a height of 12–24m with a girth of 1.2–1.6m. They are swamp dweller. However, they also distribute in peat forest and fringes of ex-tin mining ponds. The tree is easily recognized by its pagoda-like crown formed by a unique layered-branching habit. The bark is smooth and grayish (Figure 1.2). The inner bark is yellow and shows abundant milky latex. The sapwood is pale orange. All parts of plant produce copious white latex when injured. Leaves are simple, sessile, whorled, and estipulate. Leaf blade is dark green above, yellowish beneath and often notched at tip (Figure 1.3). Besides, *A. spathulata* also is deciduous tree. They are shedding leaves at regular intervals. The flowers are salver-shaped, fragrant and white in colour. The corolla lobes are 0.7cm to 1.2cm long and 1.3cm to 1.9cm wide, produced in lose terminal clusters (Figure 1.4). Fruits are occurs as a pairs of green–blue long hanging pods typical of Apocynaceae family. Fruit is also hairless, about 20cm long, glabrous and producing seeds with rounded ends (Lim, Kho, Tay & Low, 1998; Wiart, 2006). The wood of *A. spathulata* is among the lightest known (Monachino, 1949). The two key features that distinguish *A. spathulata* from the other *Alstonia* species are the low deeply fluted buttress and the leaf whorls containing on average only four leaves arranged at right angles to each other.

In Cambodia, Laos, Vietnam, and Malaysia, the latex from *Alstonia spathulata* Bl. is applied externally to sores and diseased skin. The bark is used to lower fever and to expel worms from the intestine (Wiart, 2006). The light wood is

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used to make pith-helmets, setting board for insects and float for nets (Lim, Kho, Tay, & Low, 1998).



Figure 1.2. Bark of *Alstonia spathulata*



Figure 1.3. Leaves of *Alstonia spathulata*



Figure 1.4. Flower of *Alstonia spathulata*

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1.6.1 Botanical aspect of Genus *Kopsia*

In the most recent subclassification of the Apocynaceae, *Kopsia* is placed in the tribe Vinceae, subfamily Rauvofioideae (Endress & Bruyns, 2000). The plant can be found at South and South East Asia and distributed throughout Malesia, Solomon Island, mainly Malaya and Borneo. Most of the species are found mainly lowland and few species grow on limestone (Whitmore, 1973).

All species of *Kopsia* are shrubs or small trees as part of the understory vegetation in forests, at forest edges, or in the open. The largest of any species recorded is 14 m tall for *K. arborea*. The leaves are always opposite. They may or may not be sessile, a character which is somewhat subjective as many species have very short petioles. However, in only two species, *K. teoi* and *K. pauciflora,* are there cases where the petioles do appear to be absent. The flowers are usually white, cream or pink in colour. The fruit of all species is a small drupe, usually paired (Middleton, 2004).

Kopsia species were one of the most popular plants that have been widely investigated by several workers for their alkaloidal contents since the early nineties (Awang et al., 1991; Sevenet et al., 1994; Uzir et al. 1997; Kam et al., 1993, 1994, 1996, 1999a, 1999b; Kam & Choo 2003). Various *Kopsia* species are used in folk medicine. In Thailand, root and the stem of *K. macrophylla* have been used to relief fever and toxicemia (Pongbunrod, 1979; Wuthammawech, 1997). Root of *K. fructicosa* has been used to poultice ulcerated nose in tertiary syphilis in Myanmar (Perry & Metzger, 1980).

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1.6.2 Species of genus *Kopsia*

Based on the Plant List (2010), there are total 24 species are recognized. Those plants are listed below:

1. *Kopsia angustipetala* Kerr
2. *Kopsia arborea* Blume
3. *Kopsia dasyrachis* Ridl.
4. *Kopsia deverrei* L.Allorge
5. *Kopsia flavida* Blume
6. *Kopsia fruticosa* (Roxb.) A.DC.
7. *Kopsia grandifolia* D.J.Middleton
8. *Kopsia griffithii* King & Gamble
9. *Kopsia hainanensis* Tsiang
10. *Kopsia harmandiana* Pierre ex Pit.
11. *Kopsia lapidilecta* Sleesen
12. *Kopsia larutensis* King & Gamble
13. *Kopsia macrophylla* Hook.f.
14. *Kopsia pauciflora* Hook.f.
15. *Kopsia profunda* Markgr.
16. *Kopsia rajangensis* D.J.Middleton
17. *Kopsia rosea* D.J.Middleton
18. *Kopsia singapurensis* Ridl.
19. *Kopsia sleeseniana* Markgr.
20. *Kopsia sumatrana* D.J.Middleton

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21. *Kopsia tenuis* Leenh. & Steems

22. *Kopsia teoi* L.Allorge

23. *Kopsia tonkinensis* Pit.

24. *Kopsia vidalii* D.J.Middleton

1.6.3 Botanical aspect of *Kopsia singapurensis* Ridl.

Kopsia singapurensis (white flower) or locally known as 'selada' is a small tree or shrub that found in swampy areas or lowland rainforest. The tree can grow up to 12 m tall, 24 cm diameter at breast height with a conical crown (Figure 1.5). It has a five petal white propeller flower with red eye (Figure 1.6). Shiny dark green opposite leaves with alternating orientation (Figure 1.7). Fruits are oblique glabrous and ellipsoid with either a simple angle or a spur on one side (Middleton, 2004). This species is endemic only to Peninsular Malaysia and Singapore. In Malaysia, this plant distributed from Negeri Sembilan southward to Singapore and common in lowland swampy forest. This species was a threatened species which state in the IUCN Red List of Threatened Species (Chua, 1998). This plant usually plant grown as an ornamental tree or shrub in the Singapore Botanic Gardens (Davison, Ng & Chew, 2008). This species are traditional used to poultice ulcerated nose in tertiary syphilis (Perry & Metzger, 1980).

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Figure 1.5. Bark of *Kopsia singapurensis*



Figure 1.6. Flower of *Kopsia singapurensis*



Figure 1.7. Leaves of *Kopsia singapurensis*

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CHAPTER 2

GENERAL CHEMICAL ASPECTS

2.1 Introduction

Plants produce an enormous variety of natural products with highly diverse structures. These products are commonly termed secondary metabolites in contrast to the primary metabolites which are essential for plant growth and development. Secondary metabolites were formerly regarded as waste products without physiological function for the plant. However, these natural products fulfill important functions in the interaction between plants and their biotic and abiotic environment. They can serve as defense compounds against herbivores and pathogens, as flower pigments that attract pollinators, or as hormones or signal molecules. In addition to their physiological function in plants, natural products also have a strong impact on human culture and have been used throughout human history as condiments, pigments, and pharmaceuticals (Springob & Kutchan, 2009). Plant secondary metabolites can be

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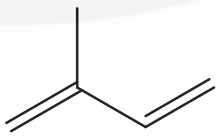
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classified into three different groups according to their biosynthetic origin: alkaloids, phenolics, and terpenoids. Plants in the Apocynaceae family are a rich source of triterpenoids and alkaloid. General chemical characteristic and survey of the literature of the two species will be discussed in this chapter.

2.2 Plant secondary metabolites

2.2.1 Terpenes

The terpenes or isoprenoids are one of the most diverse groups of more than 36,000 lipid-soluble compounds (Buckingham, 2007). Terpenes are derived biosynthetically from units of isoprene (2-methybutadiene), which has the molecular formula C_5H_8 (Paduch, Kandefer-Szerszen, Trytek, & Fiedurek, 2007) and ubiquitously synthesized by all organisms through two potential pathways, the mevalonate and more recently identified methylerythritol phosphate (MEP) pathway (Ashour, Wink & Gershenzon, 2010). Terpenes decompose at high temperature to give isoprene **8**. Terpenes can exist as hydrocarbons or have oxygen-containing compounds such as alcohols, aldehydes or ketones are also found. These derivatives are frequently named terpenoids (Paduch et al., 2007). Terpenoids can be described as modified terpenes, where methyl groups are moved or removed, or oxygen atoms added. Inversely, some authors use the term "terpenes" more broadly, to include the terpenoids.



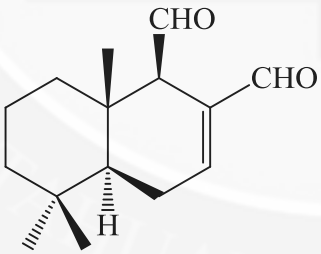
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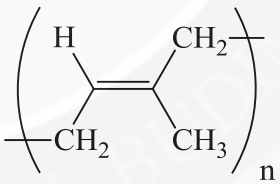
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Terpenes occur widely in nature. They are a large and varied class of hydrocarbons which are produced by a wide variety of plants and by some animals. In plants, it functions as infochemicals, attractants or repellents, as terpene are responsible for the typical fragrance of many plants. One of the best-known examples of a terpene involved in plant defense is polygodial **9**, a drimane type sesquiterpene dialdehyde (Ashour, Wink & Gershenzon, 2010). On the other hand, high concentrations of terpenes can be toxic and are thus an important weapon against herbivores and pathogens (Paduch et al., 2007).

There have been many applications of terpenes in human societies. Pharmaceutical and food industries have exploited them for their potentials and effectiveness as medicines and flavor enhancers. Perhaps the most widely known terpene is rubber **10** (polyterpenes), which has been used extensively by humans (Zwenger & Basu, 2008).



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2.2.2 Classification of terpenes

The classification of terpene is based on the number of isoprenoid units present in their structure. The basic formula of all terpenes is $(C_5H_8)_n$, where *n* is the number of linked isoprene units (Gao & Singh, 1998). In 1887, Wallach proposed the 'isoprene rule' which was later extended to the 'biogenetic isoprene rule' by Ruzicka, who suggested that all isoprenoids are synthesized from a biological 'active isoprene' (Ruzicka, Eschenmoser, & Heusser, 1953; Ruzicka, 1959). It stated that each class of isoprenoid was derived from a single parent compound unique to that class. The largest categories are those made up of compounds with one isoprenoid is classified as hemiterpene; two isoprenoid units is classified as monoterpenes; sequiterpenes incorporate three units of isoprenoid; diterpenes comprise four units of isoprenoid; five isoprenoids are known as sesterterpenes; triterpenes incorporate six units of isoprenoid; and eight units of isoprenoid is classified as tetraterpenes (Ashour et al., 2010). Terpenoid with 25 carbons are rarely found. Monoterpenes, sesquiterpenes, diterpenes and sesterpenes contain the isoprene units linked in a head to tail fashion. While the triterpenes and tetraterpenes (carotenoids) which contain two C_{15} and C_{20} units respectively linked head to head. The classification of terpenes is summarized in Table 2.1.

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Table 2.1

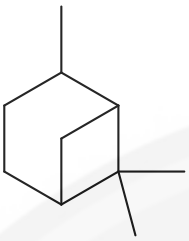
The Classification of Terpenes is Based on The Number of C_5 Isoprenoid Units in Their Structures.

Isoprene units, *n*	Carbon atoms, *n*	Name	Parent isoprene	Example
1	5	Hemiterpenes	-	Isoprene **8**
2	10	Monoterpenes	GPP	Pinane **11**
3	15	Sesquiterpenes	FPP	Arteannuic acid **12**
4	20	Diterpenes	GGPP	Taxadiene **13**
5	25	Sesterterpenes	GFPP	Cericerane **14**
6	30	Triterpenes	Squalene	β-Amyrin **15**
8	40	Tetraterpenes	Phytoene	β-Carotene **16**
9-30000	>40	Polyterpenes	GGPP + $(C_5)_n$	Rubber **10**

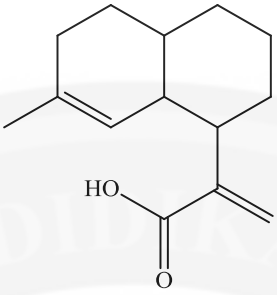
Note. Adapted from "Annual plant Reviews," by M. Ashour, M. Wink, & J. Gershenzon, 2010, United Kingdom: Blackwell Publishing Ltd.

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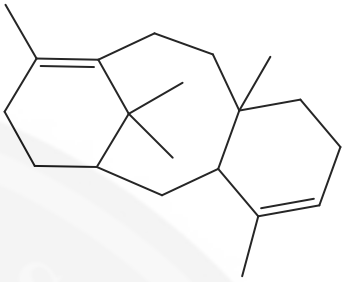
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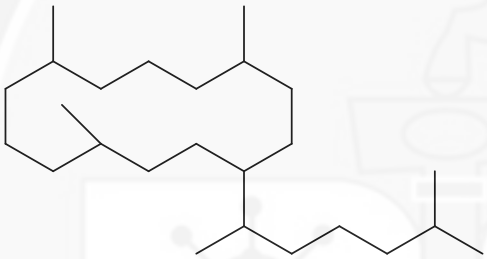
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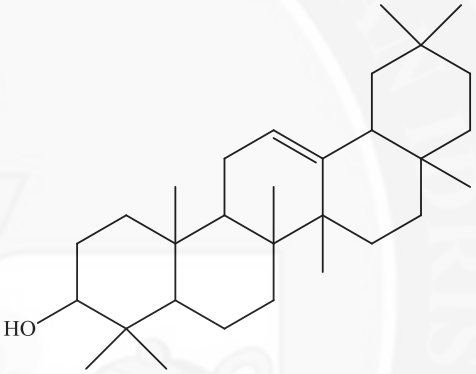
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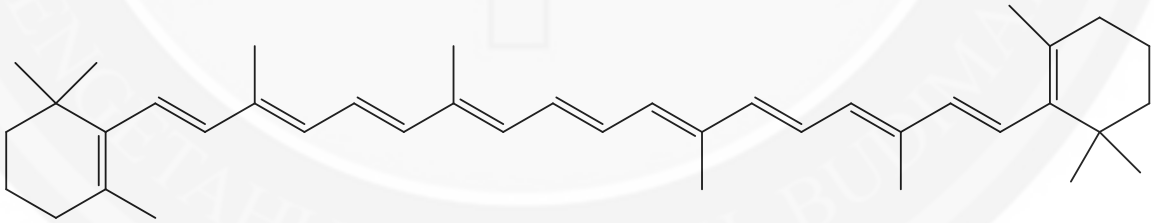
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2.2.3 Biosynthesis of terpenes

According to Ashour et al. (2010), the biosynthetic pathway to terpenoids is conveniently treated as comprising four stages, the first of which involves the formation of isopentenyl diphosphate (IPP), the biological C_5 isoprene unit. Plants synthesis IPP and its allylic isomer, dimethylallyl diphospahte (DMAPP), by one of two routes: the well known mevalonic acid pathway (MVA) or newly discovered methylerythritol phosphate (MEP) pathway. In the second stage, terpene biosynthesis involves the fusion of the basic C_5 building blocks to yield larger metabolic intermediates. IPP and its more reactive allylic isomer, DMAPP, condense in a head-to-tail orientation to form three larger prenyl diphosphate which are geranyl diphospahte (GPP, C_{10}), farnesyl diphosphate (FPP, C_{15}) and geranylgeranyl diphosphate (GGPP, C_{20}). In the third stage, the C_{10}-C_{20} diphosphate undergo a wide range of cyclization and rearrangements to produce the parent carbon skeleton of each terpene class. GPP is converted to monoterpenes, FPP is converted to the sesquiterpenes and GGPP is converted to the diterpenes. FPP and GGPP can also dimerize in a head-to-head fashion to form the precursors of the C_{30} and the C_{40} terpenoids respectively. The fourth and final stage encompasses a variety of oxidations, reduction isomerizations, conjugations and other transformations by which the parent skeletons of each terpene class are converted to thousands of distinct terpene metabolites. Overview of terpene biosynthesis is shown in Figure 2.1. Although MVA and MEP pathway are located in different compartments, there is an exchange between the two biosynthetic routes, especially from the plastidial to the cytosolic pathway (Laule et al., 2003 ; Schuhr et al., 2003). Several sesquiterpenes,

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which are synthesized from DMAPP and IPP units are provided by the MEP pathway, but not from MVA (Dudareva et al., 2005 ; Piel et al., 1998) .

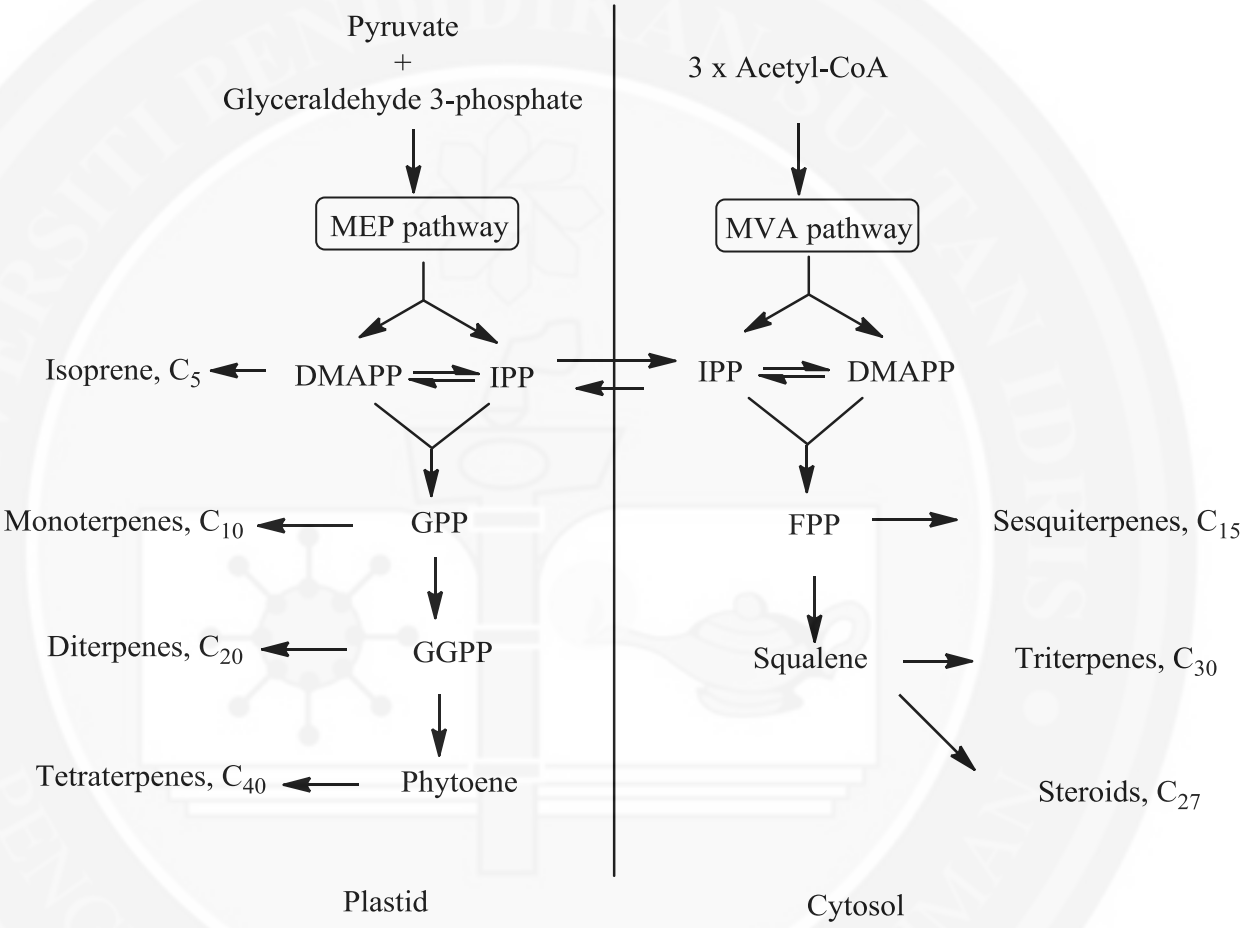


Figure 2.1. Schematic overview of terpene biosynthesis in plants. DMAPP, dimethylallyl diphosphate; DXP, desoxyxylulose phosphate; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; GPP, geranyl diphosphate; IPP, isopentenyl diphosphate; MVA, mevalonate

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2.2.4 Triterpenes

Triterpenes consist of six isoprene units (squalene) and have the molecular formula $C_{30}H_{48}$ (James & Dubery, 2009). It is a large group of lipid substances which comprise a broad chemical group of active principles, are implicated in the mechanisms of action and pharmacological effects of many medicinal plants used in folk medicine against diseases in which the immune system is implicated. They have been described as anti-inflammatory, antiviral, antimicrobial, and antitumoral agents, as well as being immunomodulator compounds (Rios, 2010). Besides, many of these molecules show important and interesting biological activities such as antifungal, anti-HIV and antioxidant (Ebizuka, Katsube, Tsutsumi, Kushiro, & Shibuya, 2003).

Triterpenes are C_{30} compounds arising from the cyclization of squalene **17** and subsequent skeletal rearrangements. Apart from the linear squalene itself and some bicyclic, highly substituted skeletons, most triterpenes are either tetracyclic or pentacyclic compounds. The various structural classes are designated by the names of representative members. They comprised of a variety of structurally diversed compounds, which include steroids and sterols. These important compounds are classed as nortriterpenes to indicate that they lack some of the 30 carbons of the triterpenes skeleton where they only have 27 to 29 carbons (Hudlicky, 2008).

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2.2.5 Biosynthesis of triterpenes

Fundamental triterpenes are derived via cyclization of squalene. Squalene derived from two moleculs of FPP, which is the precursor of sesquiterpenes. The unique tail-to-tail condensation of FPP is catalyzed by an enzyme squalene synthase. Squalene is oxidized into (3*S*)-2,3-oxidosqualene (OS) **18** by squalene epoxidase. OS is cyclized into cyclic triterpenes by enzyme oxidosqualene cyclase (OSC). Because of the present of an epoxide in oxidosqualene, all of the cyclic triterpene derived from this precursor possess oxygen functionality at the C-3 position. The cyclization can be divided into the following four parts (Mander & Liu, 2010) (Figure 2.2).

1. Initiation of a reaction. A proton attack on the epoxide moiety generates an initial tertiary carbocation. The resulting OH group resides on every triterpene.
2. Propagation of a reaction through cation-π cyclization. Once formed, the carbocation undergoes attack by nearby π electron onto an empty 2p orbital of a carbocationic center to form a C-C σ bond.
3. Further propagation through 1,2-shifts (Wagner-Meerwein rearrangements) which include hydride, methyl, and alkyl group migration that take places suprafacially. Ring expansion is part of this process.
4. Termination of a reaction. Deprotonation from a carbon adjacent to a carbocationic center will generate an olefin. On the other hand, a water attack onto a carbocationic center will produce a hydroxyl group.

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In addition, each reaction pathway leading to a particular product is determined by the number of cyclization, which dictates the number of rings formed, and by the extent to which hydride and methyl migration takes places. The path by which these rearrangements take place on the triterpene skeleton is called backbone rearrangement. As depicted in Figure 2.3, in each skeletal type, rearrangements take place along the carbon atoms show in bold lines, which give only the tertiary carbocation during this process. The initial conformation of the OS will determine the orientation of the biosynthesis towards steroids or triterpene. Cyclization of the chair-boat-chair-boat conformation gives the protostane cation and cyclization of the chair-chair-chair-boat conformation leads to the dammarane cation. Thus, hundreds of different triterpene are produced from cyclization of a single precursor which is oxidosqualene (Figure 2.4). In typical triterpene cyclization, twelve to twenty covalent bonds are broken or formed; seven to nine chiral centers are established and four to five rings are built.

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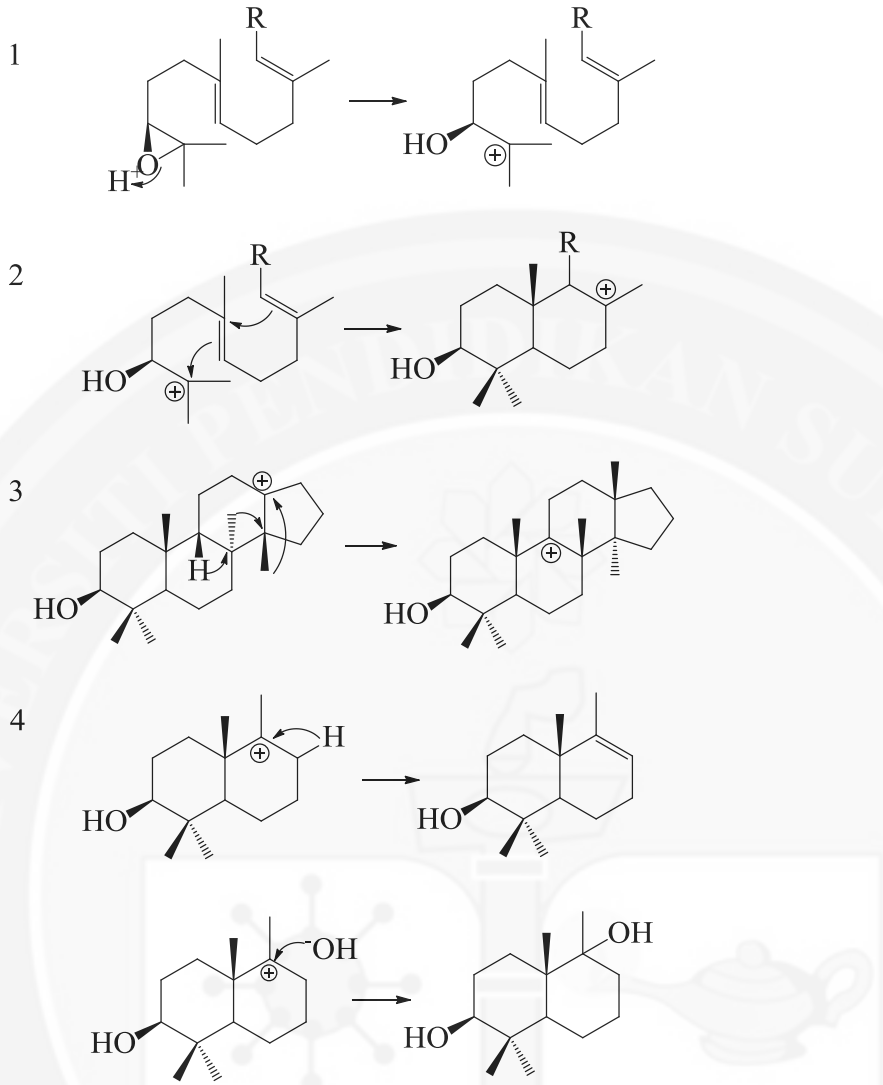


Figure 2.2. Four basic reactions in cyclization reaction.

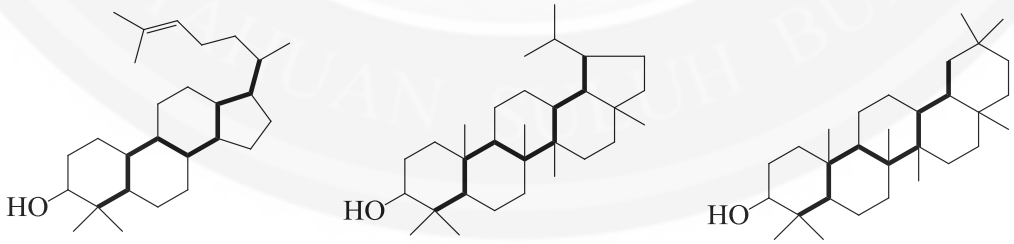


Figure 2.3. The path by which backbone rearrangement take place.

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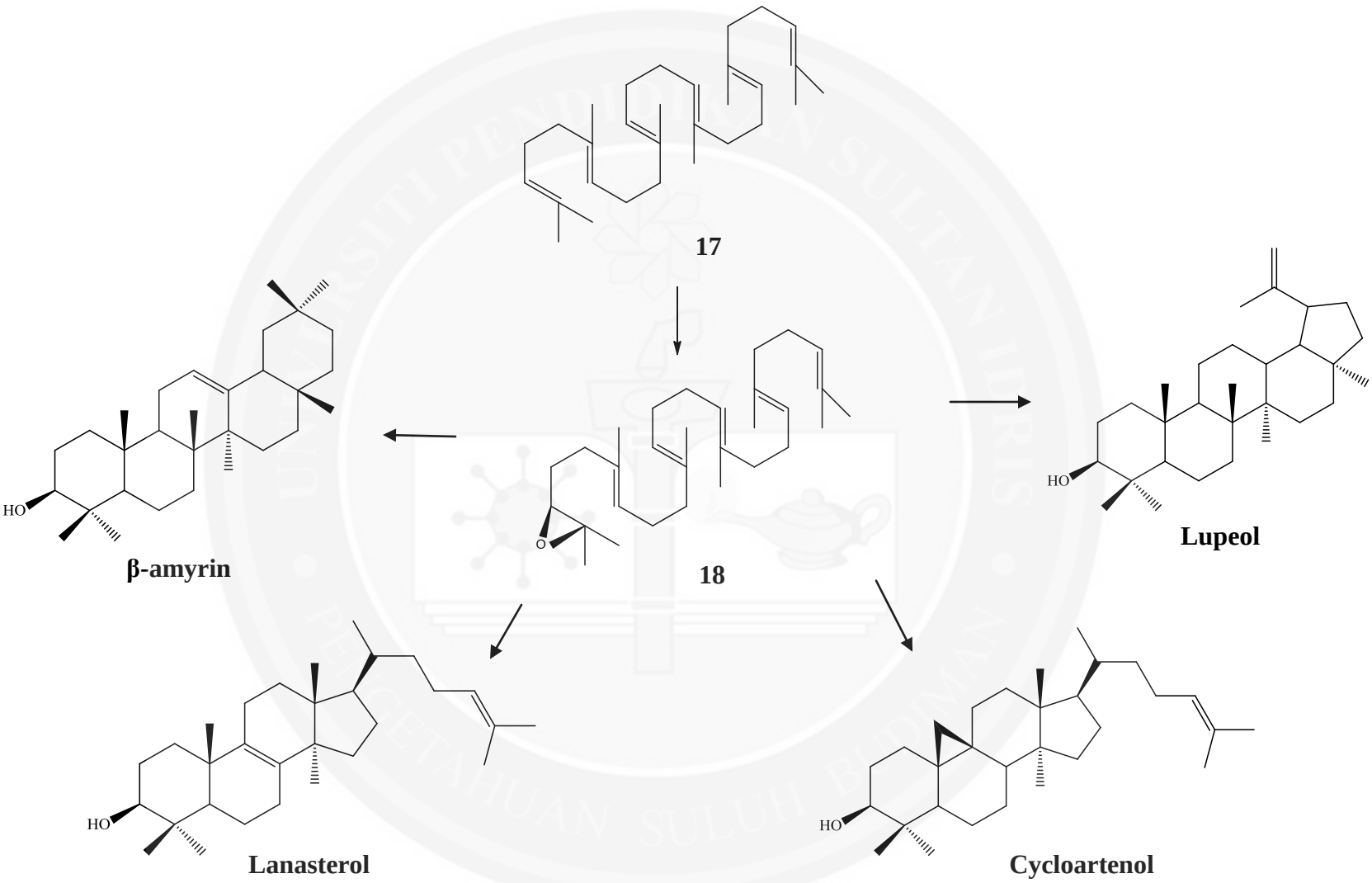


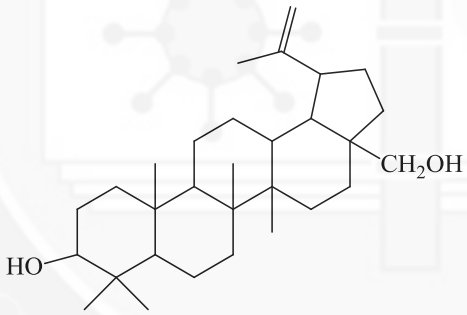
Figure 2.4. Products diversity from the cyclization of squalene or (3*S*)-2,3-oxidosqualene.

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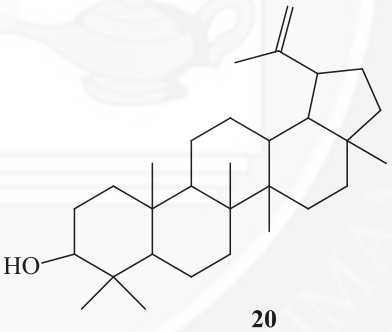
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2.3 Types of triterpenes found in Apocynaceae

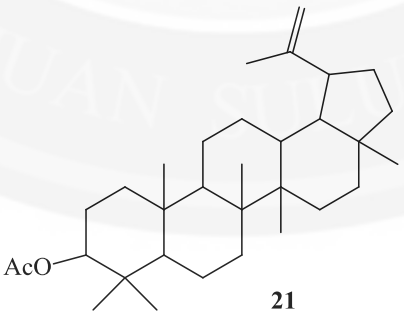
Plants of the family Apocynaceae are most commonly found in tropical and subtropical regions and have ornamental value. These plants are also well known for the alkaloids they contain, many of which are poisonous and a number of which have since found their way into modern medicine (Joselin, Brintha, Florence, & Jeeva, 2012). Besides consists of alkaloids, it also consists of triterpenes. Most of the triterpenes found in the plant of family Apocynaceae are dammarane, lupane, oleanane, ursane type of triterpenes. A known compound, betulin (lup-20(29)-ene-3,28-diol) **19** which belong to lupane type of triterpene was isolated from the stem-bark of *Adenium obesum* (Tijjani, Ndukwe & Ayo, 2012). Two lupane type triterpenes, lupeol **20** and lupeol acetate **21** is found from *Aspidosperma illustre* (Barbosa, Mathias, Braz-Filho, & Vieira, 2010).



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2.3.1 Literature review of *A. spathulata*

The occurrence of chemical constituents of this species has been summarized in Table 2.2. Since this species have never been described much for chemical compound isolated, therefore no report on the isolation of triterpene on species *A. spathulata* in the literature to date.

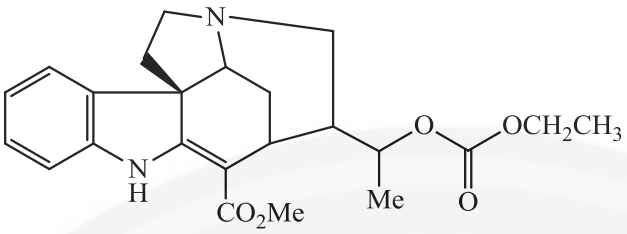
Table 2.2

Chemical Constituents Isolated from A. spathulata

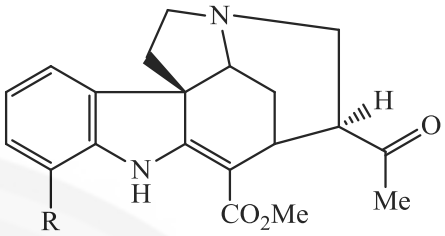
Part of the plant	Chemical compounds	References
Leaves Stem bark	- Alstolucine A **22** - Alstolucine B **23** - Alstolucine C **24** - Alstolucine D **25** - Alstolucine E **26** - Alstolucine F **27** - Alstolobine A **28** - Nor-6,7-secoangustilobine A **29** - Scholaricine **30** - *N*(4)-demethylalstogustine **31** - *N*(4)-demethyl-1,2-methoxyalstogustine **32** - 19-epi-*N*(4)-demethylalstogustine **33**	- (Tan et al., 2010)

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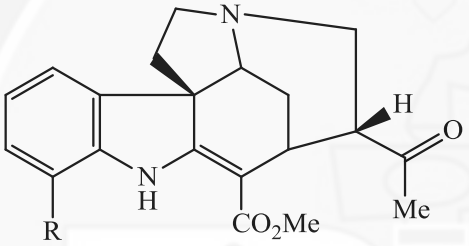
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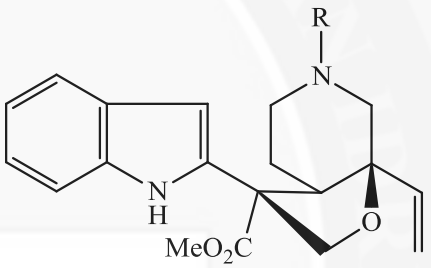
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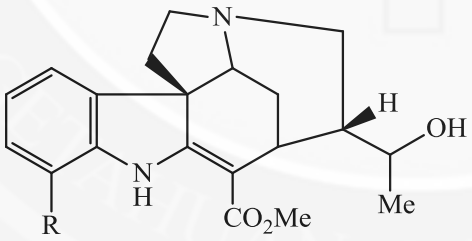
23 R = H
24 R = H, N(4) → O
25 R = OH



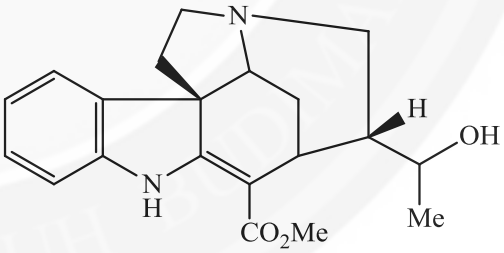
26 R = OH
27 R = H



28 R = CO_2Et
29 R = H



30 R = OH
31 R = H
32 R = OMe



33

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2.3.2 Literature review of *K. singapurensis*

The chemical constituents isolated from this species are summarized in Table 2.3.

Table 2.3

Chemical Constituents Isolated from K. singapurensis

Part of the plant	Chemical compounds	References
Leaves	- Mersiphyllines A **34** - Mersiphyllines B **35**	- (Low et al., 2009)
	- Singaporentine A **36** - Kopsifoline A **37** - Kopsininic acid **38**	- (Awang et al., 2008)
	- Akuammidine **39** - 16-*Epi*-akuammiline **40** - 16-*Epi*-decetylakuammiline 41 - Kopsidarine **42** - Kopsidine A **43** - Kopsidine C **44** - Kopsidine C *N*-oxide **45** - Kopsimaline F **46** - Kopsinganol **47** - Kopsingine **48** - Kopsinitarine A **49** - Kopsinitarine B **50** - Mersingine A **51**	- (Subramaniam & Kam, 2008)
	- Mersinine A **52** - Mersinine B **53** - Mersinine C **54** - Mersiloscine **55** - Mersiloscine A **56** - Mersiloscine B **57** - Mersifoline A **58** - Mersifoline B **59** - Mersifoline C **60**	- (Subramaniam, Choo, Hiruku, Komiyama, & Kam, 2008)

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Table 2.3

Chemical Constituents Isolated from K. singapurensis (cont.)

Part of the plant	Chemical compounds	references
	- Mersidasine A **61** - Mersidasine B **62** - Mersidasine C **63** - Mersidasine D **64** - Mersidasine E **65** - Mersidasine F **66** - Mersidasine G **67**	
	- 16-*Epi*-akuammiline **40** - 16-*Epi*-decetylakuammiline **41** - 16-*Epi*-kopsinine **68** - Kopsidine D **69** - Kopsilongine **70** - Kopsilongine *N*-oxide **71** - Kopsiloscine A **72** - Kopsiloscine B **73** - Kopsiloscine C **74** - Kopsiloscine D **75** - Kopsiloscine E **76** - Kopsiloscine F **77** - Kopsingine **48** - Vincophylline **78**	- (Subramaniam et al., 2007)
	- 15α-Hydroxykopsinine **79** - Kopsifoline A **37** - Kopsifoline B **80** - Kopsifoline C **81** - Kopsifoline D **82** - Kopsifoline E **83** - Kopsifoline F **84**	- (Kam & Choo, 2004b)
	- 5,21-Dihydrorhazinilam **85** - 11,12-Methylenedioxykopsaporine **86** - Rhazinilam **87**	- (Awang, Thoison, Hadi, Pais, & Sevenet, 1993)
Bark	- Akuammidine **29** - 16-*Epi*-akuammiline **40** - 16-*Epi*-decetylakuammiline **41** - Aspidodasycarpine **88** - Aspidophylline B **89**	- (Subramaniam & Kam, 2008)

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Table 2.3

Chemical Constituents Isolated from K. singapurensis (cont.)

Part of the plant	Chemical compounds	References
	- 5,21-Dihydrorhazinilam **85** - Kopsiloscine C **74** - Kopsiloscine G **90** - Kopsinganol **47** - Kopsingine **48** - Kopsinine **91** - Lonicerine **92** - Rhazinilam **87**	
	- Akuammidine **39** - Aspidodasycarpine **88** - Aspidophylline A **93** - 16-*Epi*-akuammiline **40** - 16-*Epi*-decetylakuammiline **41** - Kopsaporine **94** - Kopsinganol **47** - Kopsingine **48** - Kopsinine **91** - Leuconolam **95** - Lonicerine **92** - Rhazinal **96** - Rhazinilam **87** - Tetrahydroalstonine **97**	- (Subramaniam et al., 2007)
	- Akuammigine **98** - 16-*Epi*-decetylakuammiline **40** - 16-*Epi*-kopsinine **68** - 15α-Hydroxykopsinine **79** - 16-Hydroxymethylpleiocarpamine **99** - Kopsanone **100** - Kopsinine **91** - Lonicerine **92** - Picramicine **101** - Pleiocarpamine **102** - Venalstonine **103**	- (Kam & Choo, 2004b)
	- 11,12-Methylenedioxykopsaporine **86** - Singapurensine A **104** - Singapurensine B **105** - Singapurensine C **106** - Singapurensine D **107**	- (Awang, Thoison, Hadi, Pais, & Sevenet, 1993)

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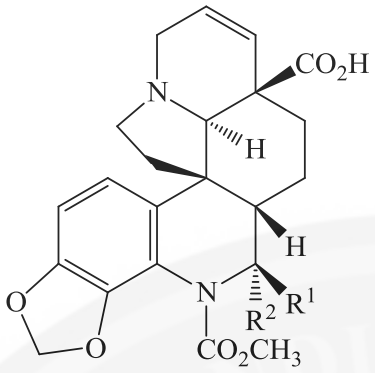
Table 2.3

Chemical Constituents Isolated from K. singapurensis (cont.)

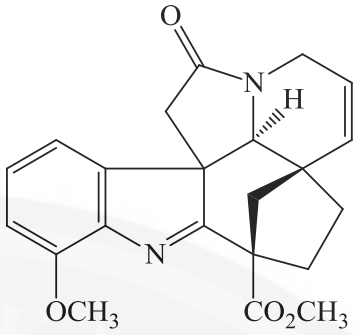
Part of the plant	Chemical compounds	References
Root	- *N*(1)-formylkopsininic acid **108** - *N*(1)-formylkopsininic acid-*N*(4)-oxide **109** - 15-hydroxykopsamine **110** - 14α-hydroxy-*N*(4)-methylcondylocarpine **111** - Singaporentinidine **112**	- (Ahmad, Hirasawa, Nugroho, Hadi & Morita, 2012)

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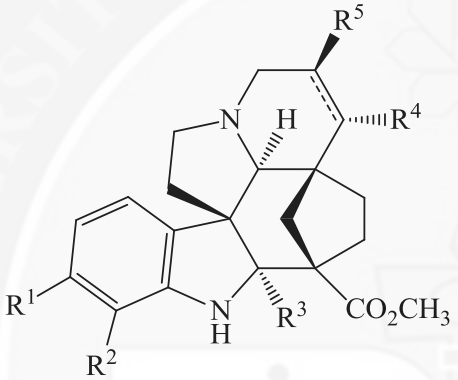
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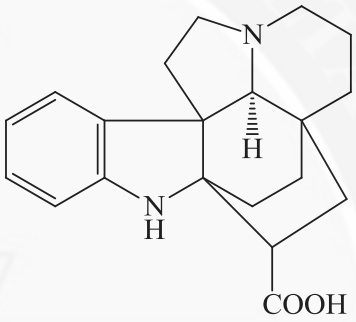


34 $R^1 = CO_2CH_3$, $R^2 = OH$
35 $R^1 = H$, $R^2 = CO_2CH_3$



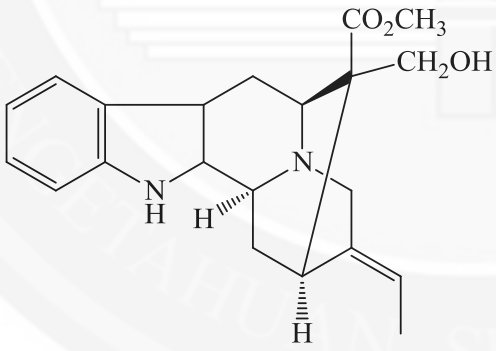
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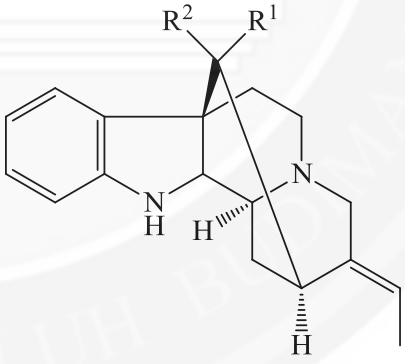




37 $R^1 = R^4 = R^5 = H$, $R^2 = OCH_3$, $R^3 = OH$, $r^{14,15}$
80 $R^1 = R^5 = H$, $R^2 = OCH_3$, $R^3 = R^4 = OH$
81 $R^1 = H$, $R^2 = OCH_3$, $R^3 = R^4 = R^5 = OH$
84 $R^1 = OCH_3$, $R^2 = R^3 = R^4 = R^5 = H$

38



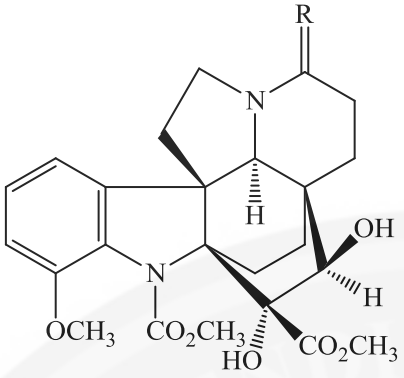


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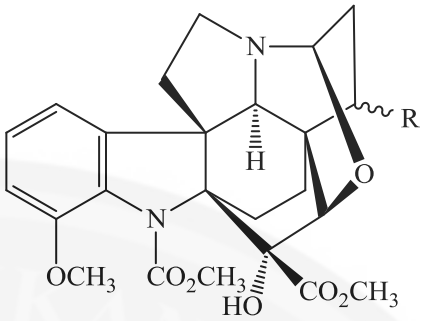
40 $R^1 = CH_2OAc$, $R^2 = CO_2CH_3$
41 $R^1 = CH_2OH$, $R^2 = CO_2CH_3$

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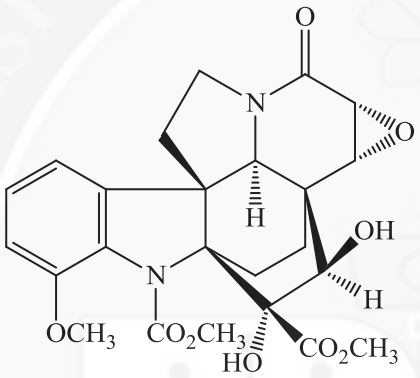
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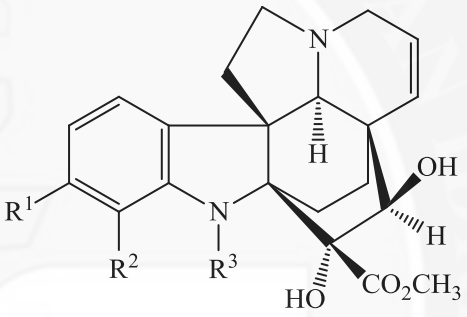
42 R = O, 15-aOH
47 R = H,H. $r^{14,15}$



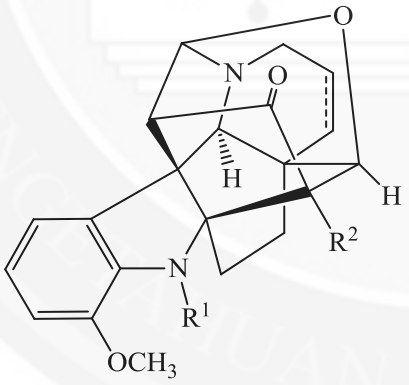
43 R = a-OCH_3
44 R = a-OH
45 R = a-OH, N(4)→ O
69 R = b-OH



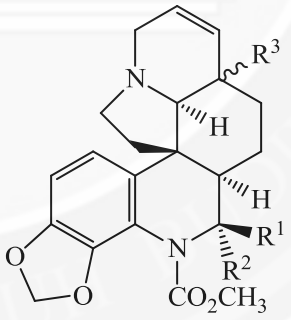
46



48 R^1 = H, R^2 = OCH_3, R^3 = CO_2CH_3
86 R^1, R^2 = OCH_2O, R^3 = CO_2CH_3
94 R^1 = R^2 = H, R^3 = CO_2CH_3



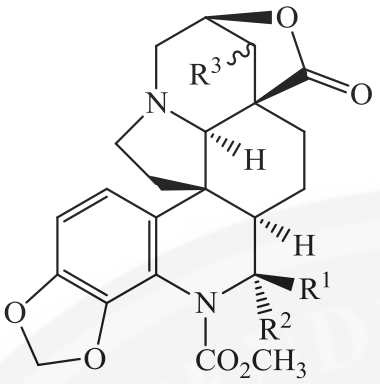
49 R^1 = CO_2CH_3, R^2 = OH, $r^{14,15}$
50 R^1 = H, R^2 = OH, $r^{14,15}$
51 R^1 = H, R^2 = NH_2, $r^{14,15}$



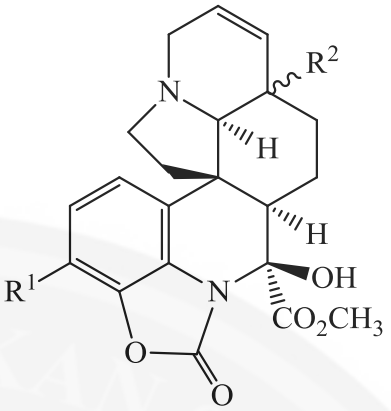
52 R^1 = CO_2CH_3, R^2 = OH, R^3 = b-CO_2CH_3
53 R^1 = OH, R^2 = CO_2CH_3, R^3 = b-CO_2CH_3
54 R^1 = OH, R^2 = CO_2CH_3, R^3 = a-CO_2CH_3

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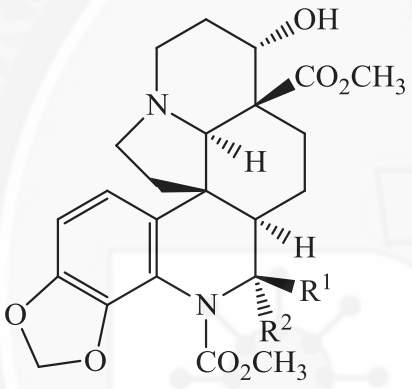
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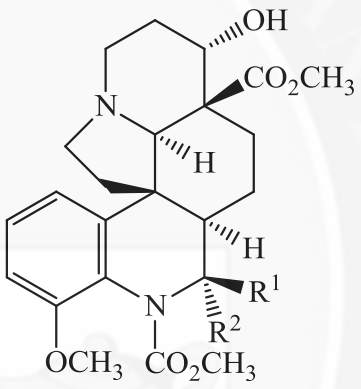
55 R^1 = OH, R^2 = CO_2CH_3, R^3 = a-OH
56 R^1 =CO_2CH_3, R^2 = OH, R^3 = a-OH
57 R^1 = OH, R^2 = CO_2CH_3, R^3 = b-OH



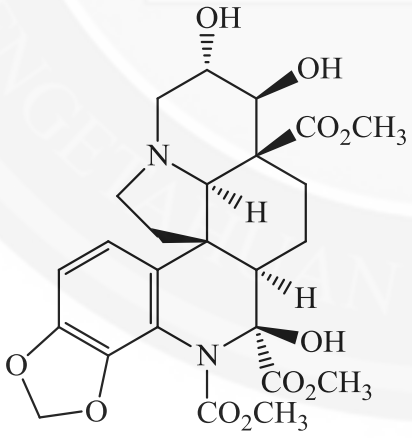
58 R^1 =H, R^2 = b-CO_2CH_3
59 R^1 = OCH_3, R^2 = b-CO_2CH_3
60 R^1 = H, R^2 = a-CO_2CH_3



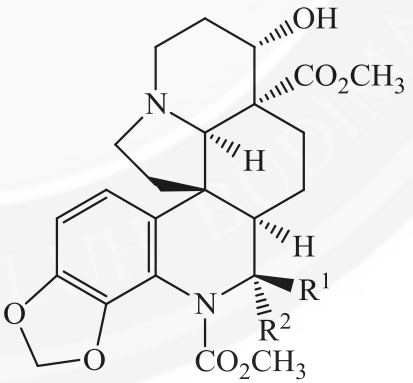
61 R^1 = CO_2CH_3, R^2 = OH
62 R^1 = OH, R^2 = CO_2CH_3



63 R^1 = CO_2CH_3, R^2 = OH
64 R^1 = OH, R^2 = CO_2CH_3



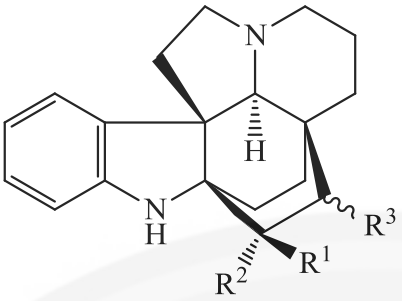
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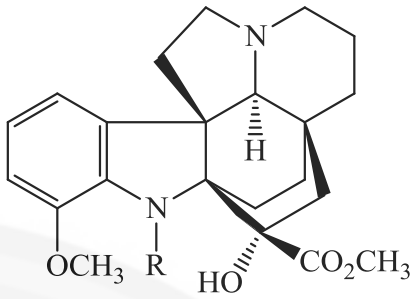
66 R^1 = CO_2CH_3, R^2 = OH
67 R^1 = OH, R^2 = CO_2CH_3

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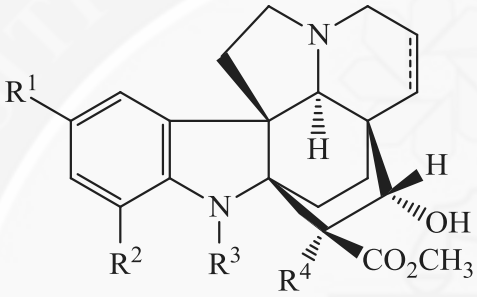
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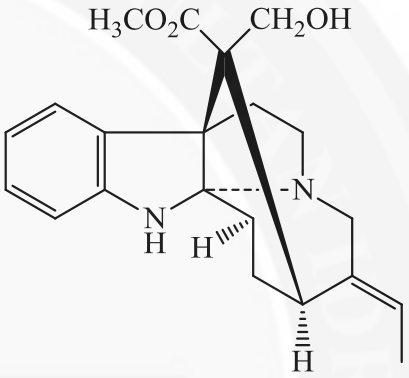
68 R^1 = H, R^2 = CO_2CH_3, R^3 = H
91 R^1 = CO_2CH_3, R^2 = R^3 = H



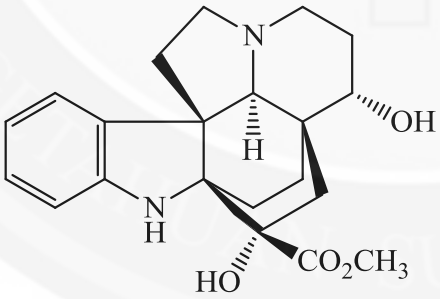
70 R = CO_2CH_3
71 R = CO_2CH_3, N(4)gO



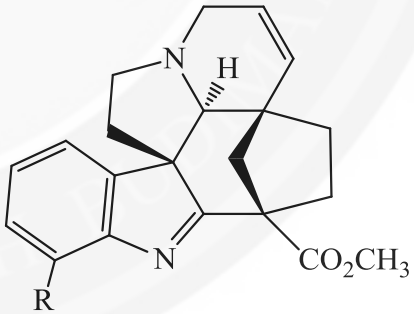
72 R^1 = R^2 = H, R^3 = CO_2CH_3, R^4 = OH, $r^{14,15}$
73 R^1 = R^2 = H, R^3 = CO_2CH_3, R^4 = OH
74 R^1 = R^2 = H, R^3 = CO_2CH_3, R^4 = OH, 15-a-OH
75 R^1 = OCH_3, R^2 = H, R^3 = CO_2CH_3, R^4 = OH
76 R^1 = OCH_3, R^2 = H, R^3 = CO_2CH_3, R^4 = OH, 15-a-OH
77 R^1 = H, R^2 = OCH_3, R^3 = CO_2CH_3, R^4 = OH, 15-a-OH
90 R^1 = R^2 = R^3 = R^4 = H



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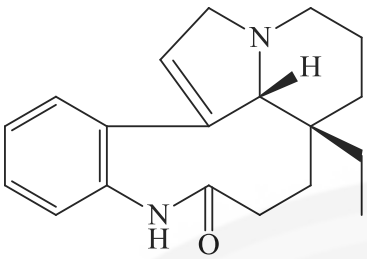
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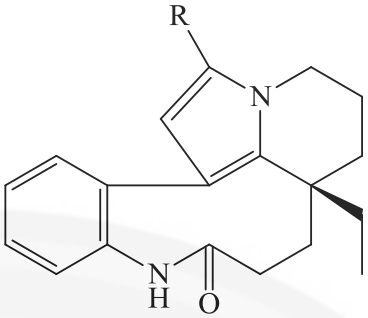
82 R = H
83 R = OCH_3

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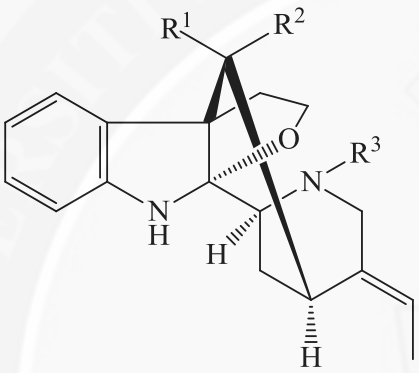
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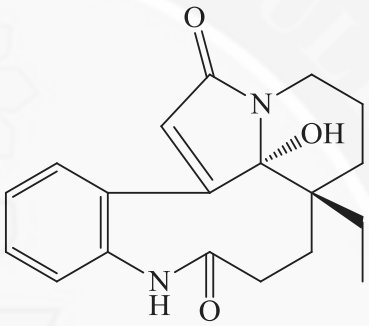


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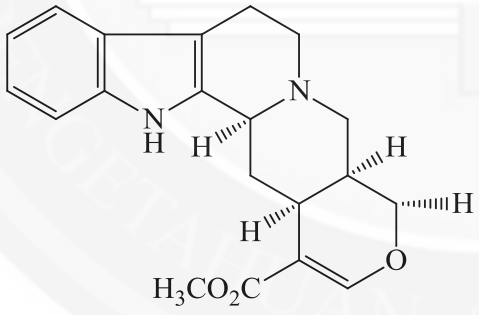
87 R = H
96 R = CHO



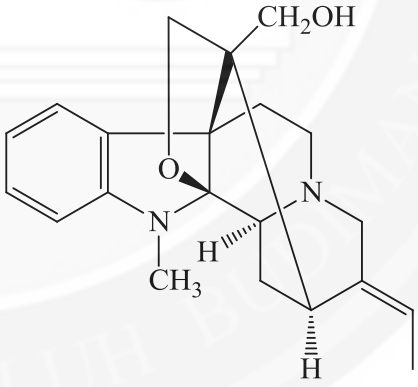


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88 $R^1 = CH_2OH$, $R^2 = CO_2CH_3$, $R^3 = H$
89 $R^1 = CH_2OAc$, $R^2 = CH_2OH$, $R^3 = H$
92 $R^1 = CO_2CH_3$, $R^2 = CH_2OH$, $R^3 = H$
93 $R^1 = H$, $R^2 = CO_2CH_3$, $R^3 = CHO$



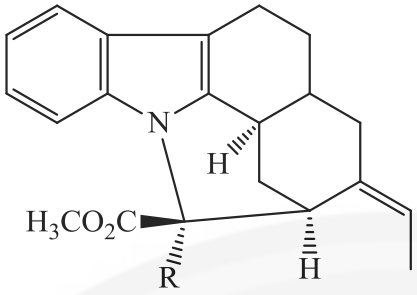
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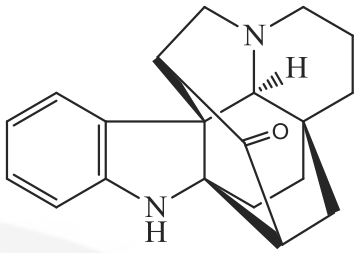
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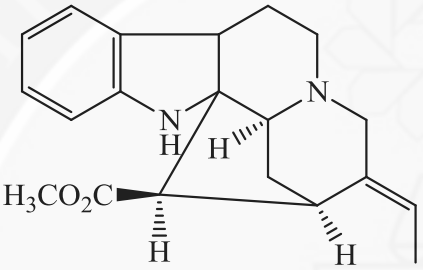
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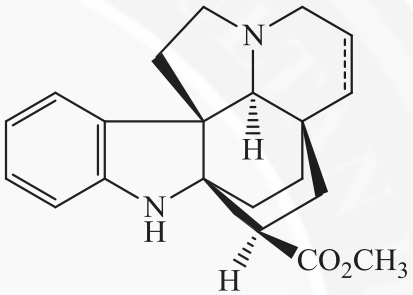
99 R = CH_2OH
102 R = H



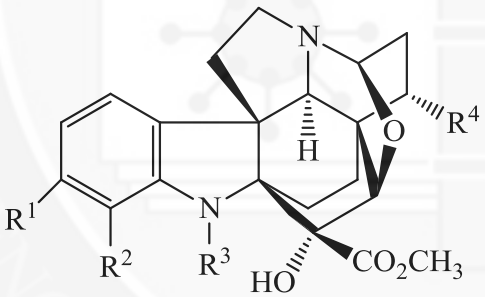
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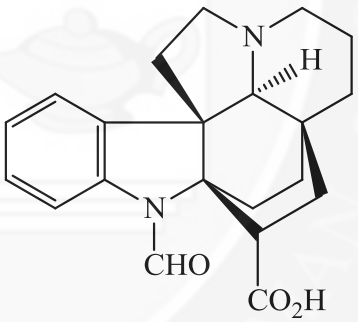
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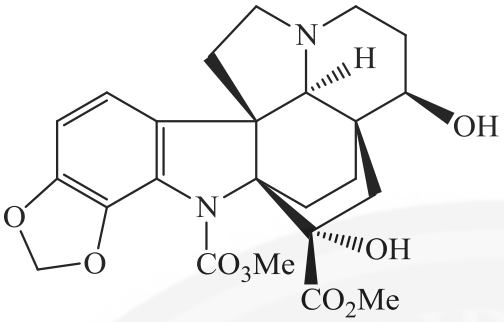
104 $R^1 = R^2 = H$, $R^3 = CO_2CH_3$, $R^4 = OH$
105 $R^1 = R^2 = H$, $R^3 = CO_2CH_3$, $R^4 = OCH_3$
106 $R^1, R^2 = OCH_2O$, $R^3 = CO_2CH_3$, $R^4 = OH$
107 $R^1, R^2 = OCH_2O$, $R^3 = CO_2CH_3$, $R^4 = OCH_3$



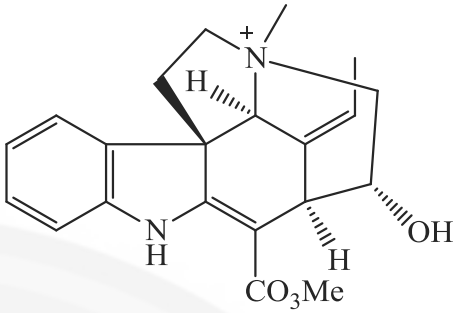
108
109 $N_4 \rightarrow O$

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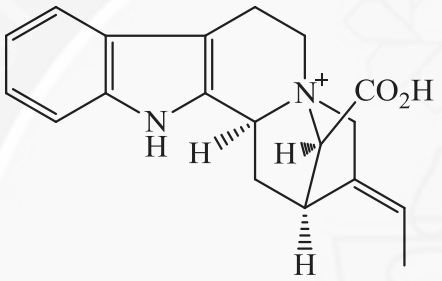
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CHAPTER 3

EXPERIMENTAL

3.1 Introduction

A systematic research procedure will help the execution of this study becoming more smoothly. Nowadays, with modern instrumentation the identification task may require only a minute, a situation vastly difference from the state of affair in the 19th and early 20th centuries. Even very small amount of samples also can be comprehensive structurally elucidated. Thus, this chapter basically is all about the research method that is discussed in detail.

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3.2 General Methods

Spectra were recorded on the following instruments as follows:

Ultra Violet Spectra (UV)

The UV spectra were measured by Perkin Elmer UV- visible spectrophotometer and the wavelength of the spectrum was recorded in the range of 200 to 400nm, with dichloromethane (CH_2Cl_2) as solvent.

Infrared Spectra (IR)

The infrared spectra (IR) were recorded by Nicolet 6700 FTIR spectrophotometer, with CH_2Cl_2 as solvent**.**

Mass Spectra (MS)

The mass spectra were measured using the GC-MS (GC-MS Agilent 5975C Series). Chromatographic separation was performed on Agilent HP-5MS capillary column where the dimension of column was 30m ×250 µm and 0.25 µm film thickness. Helium was used as the carrier gas with a constant linear velocity of 40cms^{-1}. The oven temperature program was: 0.8 min at 105 ℃, from 105 ℃ to 220 ℃ at 15 ℃ min^{-1}, from 220 ℃ to 300 ℃ at 40 ℃ min^{-1} and 20min at 300 ℃. Injector temperature was set at 290 ℃. Samples were injected in split mode (split mode 1:75). The injection volume was 2 µL. The transfer line temperature was held at 290 ℃. The mass spectrometer was operated in the electron positive-mode ionization, with electron energy at 70eV.

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Ion source temperature was 235 ℃. The MS data were obtained in full scan mode (total ion current-TIC, mass range 50-750amu) (Razborsek, Voncina, Dolecek, & Voncinab, 2007).

Nuclear Magnetic Resonance (NMR) Spectra

NMR spectra were recorded using deuterated chloroform ($CDCl_3$) as NMR solvent on JEOL ECA (500MHz) and BRUKER (600 MHz). Chemical shifts (δ) were reported in ppm and the coupling constants (*J*) were given in Hz.

Column Chromatography (CC) and Thin Layer Chromatography (TLC)

Colum chromatography were prepared by using Silica Gel 60F, 70-230 mesh ASTM (Merck 1.07734.1000); Silica Gel 60F, 230-400 mesh ASTM (Merck 1.09385.1000) and silica Gel 60 containing Gypsum F_{254} (Merck 1.07749.1000) as stationary phase. The ratio of silica gel to the sample was approximately 30:1 for crude sample and 100:1 for fractions that need further purification. The silica gel was slurred with hexane before it was packed into the column.

Analytical Thin Layer Chromatography (TLC) was performed on commercially precoated aluminium supported silica gel $60F_{254}$ TLC sheets (Merck 1.05554.0001) while preparative TLC was prepared by using glass supported silica gel $60F_{254}$ TLC plates (Merck 1.05715.0001).

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Solvent used

The industrial grade solvents (hexane, dichloromethane and methanol) were used or the bulk extraction. These solvent were distilled before used in the chromatographic separation of the compounds.

Chemical reagents

Sulphuric acid was used as reagents to determine the presence of terpenes. A 5.0 % solution of sulphuric acid was spray on the TLC plates, present of terpenes on TLC plate is indicated by the formation of purple spot on the TLC plates once the plate is heated to 110^{o}C for minutes.

3.3 Plant Material

The species selected for the current study are *Alstonia spathulata* Bl. (KL5472) which was collected from Mersing, Johor and *Kopsia singapurensis* Ridl. (KL5334) collected from Kluang, Johor. Species were identified by the phytochemical group, Chemistry Department, Faculty Science, University of Malaya. The Voucher specimens were deposited at the Chemistry Department, Faculty Science, University of Malaya, Kuala Lumpur, Malaysia and the Herbarium of the Forest Research Institute, Kepong, Malaysia.

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3.4 Extraction and Isolation

3.4.1 *Alstonia spathulata* Bl.

Plant extraction was carried out by cold percolation. Dried grounded bark of the *Alstonia spathulata* was first defatted with hexane for three days at room temperature and the hexane extract was filter and dried by using rotary evaporator. Dried plant were wetted with 25% of ammonia solution and left to soak two hours, this was to aggregate the nitrogen containing compound in the plant. They were then re-extracted with dichloromethane (CH_2Cl_2) for another three days, yielded dichloromethane crude.

The dichloromethane crude extract was dissolved in 500 ml CH_2Cl_2 and re-extracted with 5 % hydrochloric acid (HCl) until a negative result was formed with Mayer's reagent. The combined extract then were basified with 25 % NH_3 solution (pH 11) and re-extracted with CH_2Cl_2 until a negative Mayer's test was obtained and later washed with distilled water and sodium chloride solution and dried with sodium sulfate anhydrous. Finally it was evaporated to dryness to give alkaloid crude extract. Dichloromethane residue extract (water layer) will also obtain in this acid-base extraction. The extraction procedure was shown in Figure 3.1.

For preliminary fractionation, the hexane crude and DCM crude (residue) extract were each subjected separately to column chromatography over silica gel and eluted with different combinations solvent systems of hexane, dichloromethane and methanol mixture by increasing its polarity. Eluents were collected in fractions and were concentrated using rotary-evaporator. Each concentrated fractions was test on aluminium supported TLC plate with a suitable solvent system and test with 5%

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sulphuric acid. The fractions having spots with same retention factor (R_f) value were grouped together. Each grouped of fractions were subjected to repeated CC or preparative TLC until a single spot on the TLC was obtained. Figure 3.2 showed the overview of extraction and isolation of triterpenes from *Alstonia spathulata.*

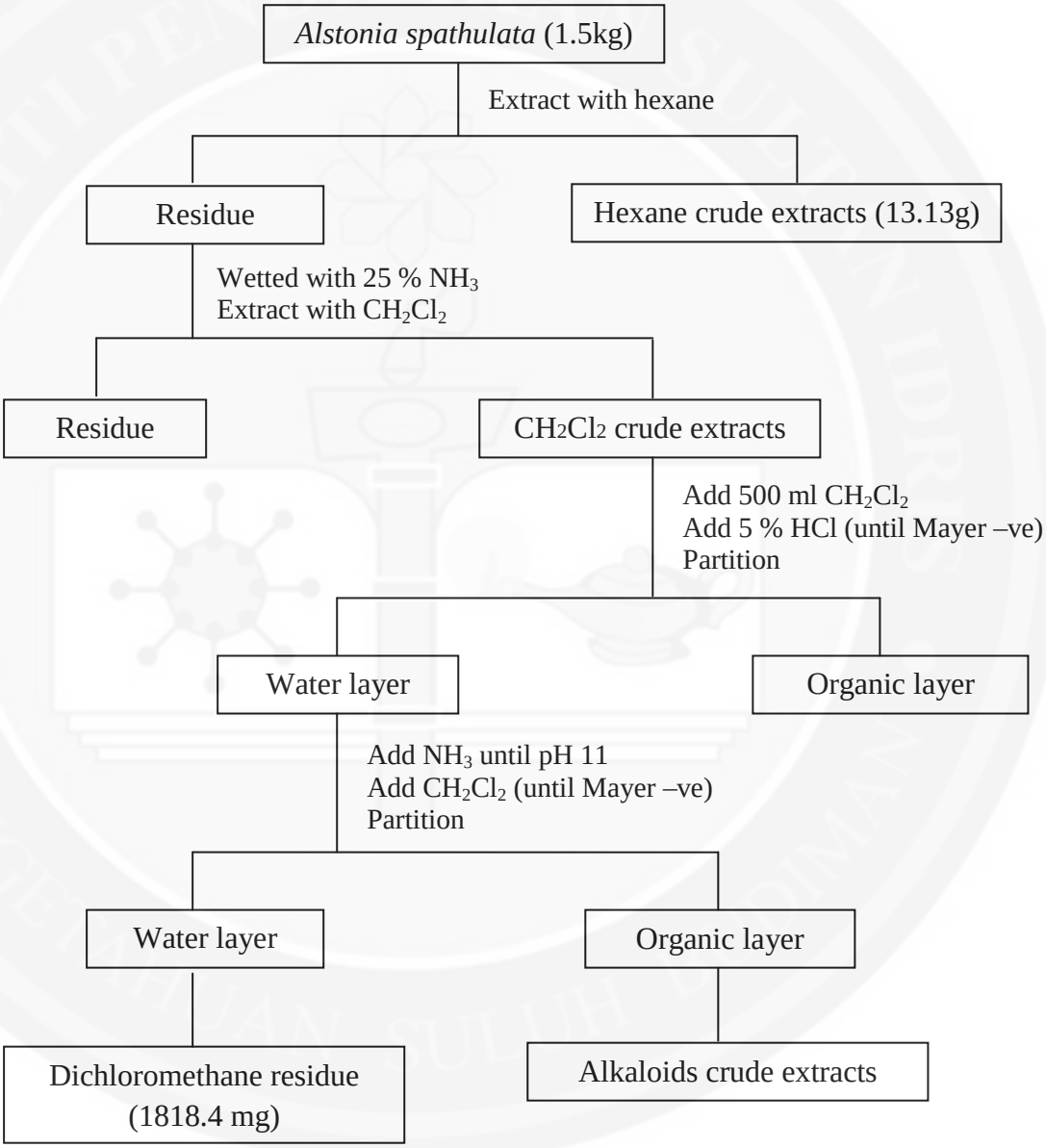


Figure 3.1. Extraction of *Alstonia spathulata.*

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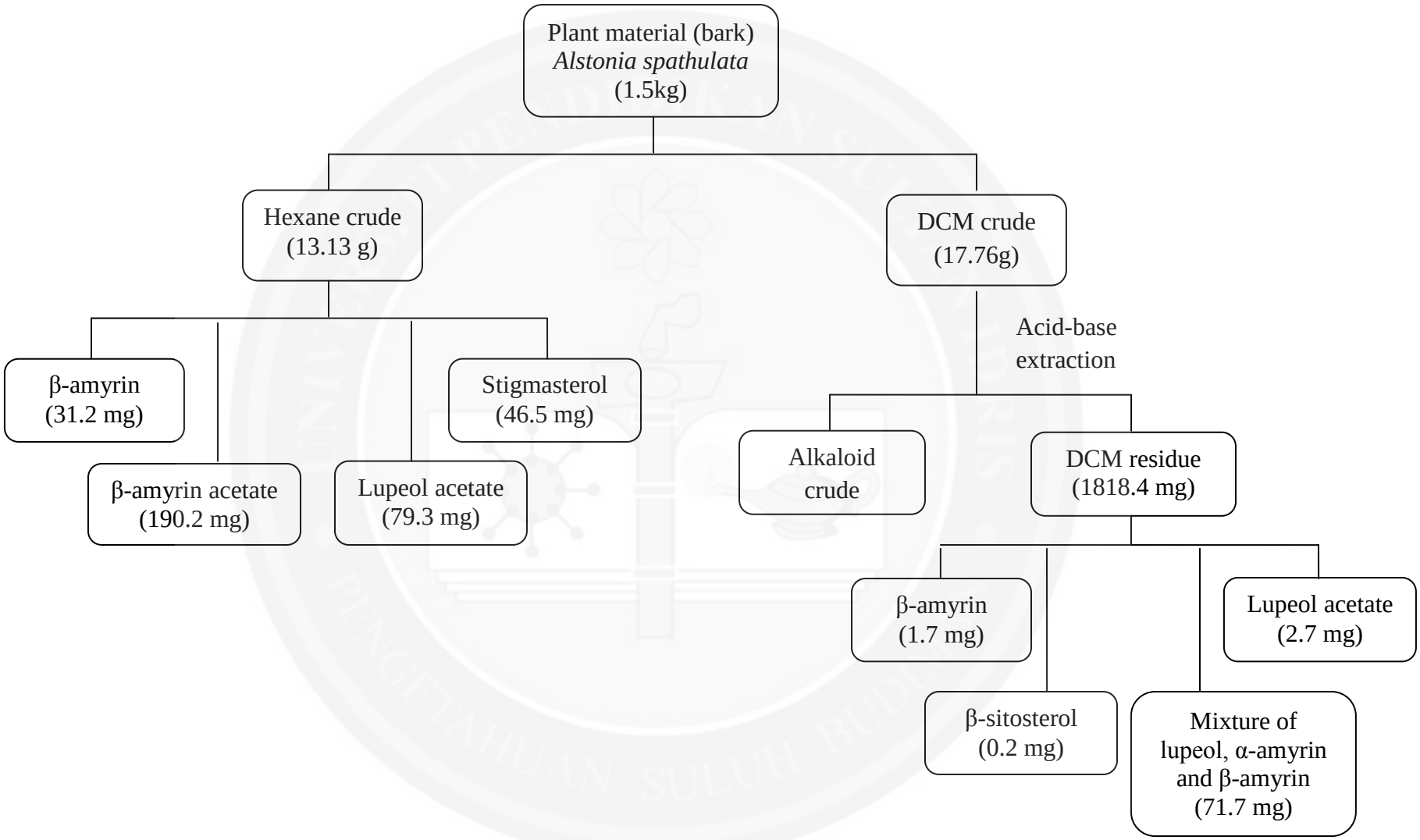


Figure 3.2. Overview of extraction and isolation of triterpenes from *Alstonia spathulata.*

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3.4.2 *Kopsia singapurensis* Ridl.

Extraction was carried out by cold percolation method. Dried grounded root was defatted with hexane for three days at room temperature and the hexane extract was filter and dried by using rotary evaporator.

Same as previous work of isolation of *Alstonia spathulata* Bl.**,** crude hexane (2.47g) was subjected to column chromatography over silica gel and isolation step was based on gradient elution method. Further purification was subjected to repeated CC or preparative TLC pure compound was obtained. Overview of extraction and isolation of triterpenes from *Kopsia singapurensis* was shown in Figure 3.3.

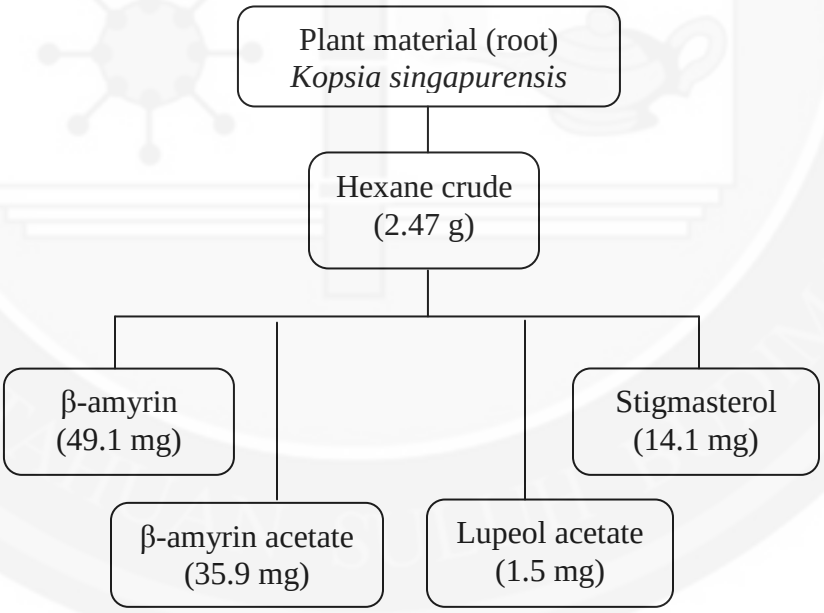


Figure 3.3. Overview of extraction and isolation of triterpenes from *Kopsia singapurensis.*

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3.5 Physical and spectral data of isolated compounds

β-amyrin 113 : White amorphous

Molecular formula : $C_{30}H_{50}O$

UV $_{\lambda \max}$ (CH_2CI_2) : 273.4 , 229.0

IR $_{v \max}$ (CH_2CI_2), cm^{-1} : 3360, 1709, 1294

Mass spectrum, *m/z* : 449 $[M+Na]^+$

^{1}H NMR ($CDCl_3$), δ ppm :

1.61 (*m*, H-1a, 1H), 0.98 (*s*, H-1b, 1H), 1.58 (*m*, H-2a, 1H), 3.20 (*dd*, *J*=11.0, 5.0 Hz, H-3, 1H), 0.74 (*m*, H-5, 1H), 1.53 (*m*, H-6a, 1H), 1.37 (*m*, H-6b, 1H), 1.50 (*m*, H-7a, 1H), 1.31 (*m*, H-7b, 1H), 1.54 (*m*, H-9, 1H), 1.85 (*m*, H-11, 1H), 5.l6 (*t*, *J* = 6.8 Hz, H-12, 1H), 1.74 (*m*, H-15a, 1H), 0.95 (*m*, H-15b, 1H), 1.97 (*m*, H-16, 1H), 1.94 (*m*, H-18, 1H), 1.64 (*m*, H-19a, 1H), 1.00 (*m*, H-19b, 1H), 1.31 (*m*, H-21a, 1H), 1.10 (*m*, H-21b, 1H), 1.38 (*m*, H-22a, 1H), 1.21 (*m*, H-22b, 1H), 0.98 (*s*, H-23, 3H), 0.91 (*s*, H-24, 3H), 0.77 (*s*, H-25, 3H), 0.95 (*s*, H-26, 3H), 1.12 (*s*, H-27, 3H), 0.81 (*s*, H-28, 3H), 0.85 (*s*, H-29, 3H), 0.85 (*s*, H-30, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

38.7 (C-1), 27.3 (C-2), 79.1 (C-3), 38.9 (C-4), 55.3 (C-5), 18.5 (C-6), 32.7 (C-7), 39.9 (C-8), 47.7 (C-9), 37.0 (C-10), 23.6 (C-11), 121.8 (C-12), δ 145.3 (C-13), 41.8 (C-14), 26.2 (C-15), 27.0 (C-16), 32.8 (C-17), 47.2 (C-18), 46.9 (C-19), 31.2 (C-20), 34.8 (C-21), 37.2 (C-22), 28.2 (C-23), 15.6 (C-24), 15.7 (C-25), 16.9 (C 26), 26.1 (C-27), 28.5 (C-28), 33.5 (C-29), 23.8 (C-30).

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β-amyrin acetate 114 : Colourless needles

Molecular formula : $C_{32}H_{52}O_2$

UV λ_{max} (CH_2CI_2) : 277.8, 229.7

IR $_{v\ max}$ (CH_2CI_2), cm^{-1} : 3054, 1712, 1264

Mass spectrum, *m/z* : 468 $[M]^+$

Melting point, ^oC : 239.0 - 240.0 (lit. m.p.: 238-245; Extrasythese, 2007)

^1H NMR ($CDCl_3$), δ ppm :

1.63 (*m*, H-1a, 1H), 1.06 (*m*, H-1b, 1H), 1.88 (*m*, H-2a, 1H), 1.60 (*m*, H-2b, 1H), 4.50 (*t*, *J*=8.0 Hz, H-3, 1H), 0.84 (*m*, H-5, 1H), 1.53 (*m*, H-6a, 1H), 1.40 (*m*, H-6b, 1H), 1.52 (*m*, H-7a, 1H), 1.33 (*m*, H-7b, 1H), 1.57 (*m*, H-9, 1H), 1.80 (*m*, H-11a, 1H), 1.63 (*m*, H-11b, 1H), 5.l8 (*t*, *J* = 3.5 Hz, H-12, 1H), 1.76 (*m*, H-15a, 1H), 0.85 (*m*, H-15b, 1H), 1.99 (*m*, H-16a, 1H), 0.79 (*m*, H-16b, 1H), 1.93 (*d*, *J* = 4.2 Hz, H-18, 1H), 1.66 (*m*, H-19a, 1H), 1.02 (*m*, H-19b, 1H), 1.08 (*m*, H-21a, 1H), 1.33 (*m*, H-21b, 1H), 1.42 (*m*, H-22a, 1H), 1.20 (*m*, H-22b, 1H), 0.88 (*s*, H-23, 3H), 0.86 (*s*, H-24, 3H), 0.96 (*s*, H-25, 3H), 0.97 (*s*, H-26, 3H), 1.13 (*s*, H-27, 3H), 0.83 (*s*, H-28, 3H), 0.87 (*s*, H-29, 3H), 0.87 (*s*, H-30, 3H), 2.05 (*s*, H-32, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

38.3 (C-1), 23.5 (C-2), 81.0 (C-3), 37.7 (C-4), 55.2 (C-5), 18.3 (C-6), 32.7 (C-7), 39.8 (C-8), 47.6 (C-9), 36.8 (C-10), 23.6 (C-11), 121.6 (C-12), 145.2 (C-13), 41.7 (C-14), 26.1 (C-15), 26.9 (C-16), 32.5 (C-17), 47.2 (C-18), 46.8 (C-19), 31.0 (C-20), 34.7 (C-21), 37.1 (C-22), 28.0 (C-23), 16.7 (C-24), 15.6 (C-25), 16.8 (C-26), 26.0 (C-27), 28.4 (C-28), 33.4 (C-29), 23.7 (C-30), 171.1 (C-31), 21.4 (C-32).

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β-sitosterol 115 : White amorphous

Molecular formula : $C_{29}H_{50}O$

UV λ_{max} (CH_2CI_2) : 225.0, 210.0

IR $_{v\ max}$ (CH_2CI_2), cm^{-1} : 3347.0, 1642.0, 1032.3

Mass spectrum, *m/z* : 414 [M^+]

^{1}H NMR ($CDCl_3$), δ ppm :

1.08 (*m*, H-1a, 1H), 1.86 (*m*, H-1b, 1H), 1.53 (*m*, H-2a, 1H), 1.86 (*m*, H-2b, 1H), 3.55 (*m*, H-3, 1H), 2.29 (*m*, H-4a, 1H), 2.23 (*m*, H-4b, 1H), 5.35 (*m*, H-6, 1H), 1.49 (*m*, H-7a, 1H), 1.95 (*m*, H-7b, 1H), 2.01 (*m*, H-8, 1H), 0.91 (*m*, H-9, 1H), 1.44 (*m*, H-11a, 1H), 1.50 (*m*, H-11b, 1H), 1.15 (*m*, H-12, 1H), 2.02 (*m*, H-12b, 1H), 1.00 (*m*, H-14, 1H), 1.59 (*m*, H-15, 1H), 1.26 (*m*, H-16a, 1H), 1.83 (*m*, H-16b, 1H), 1.08 (*m*, H-17, 1H), 0.68 (*s*, H-18, 3H), 1.01 (*s*, H-19, 3H), 1.35 (*m*, H-20, 1H), 0.92 (*d*, *J* = 7.0 Hz, H-21, 1H), 1.03 (*m*, H-22a, 1H), 1.33 (*m*, H-22b, 1H), 1.16 (*m*, H-23, 1H), 0.91 (*m*, H-24, 1H), 1.67 (*m*, H-25, 1H), 0.84 (*d*, *J* = 6.9 Hz, H-26, 3H), 0.81 (*d*, *J* = 6.85 Hz, H-27, 3H), 1.25 (*m*, H-28, 1H), 0.85 (*t*, *J* = 7.45 Hz, H-29, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

37.3 (C-1), 31.7 (C-2), 71.9 (C-3), 42.4 (C-4), 140.8 (C-5), 121.8 (C-6), 32.0 (C-7), 32.0 (C-8), 50.2 (C-9), 36.6 (C-10), 21.2 (C-11), 39.9 (C-12), 42.4 (C-13), 56.8 (C-14), 24.4 (C-15), 28.3 (C-16), 56.1 (C-17), 12.0 (C-18), 19.5 (C-19), 36.2 (C-20), 18.9 (C-21), 34.0 (C-22), 26.1 (C-23), 45.9 (C-24), 29.2 (C-25), 19.9 (C-26), 19.0 (C-27), 23.1 (C-28), 12.1 (C-29).

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Lupeol acetate 116 : Colourless amorphous

Molecular formula : $C_{32}H_{52}O_2$

UV λ_{max} (CH_2CI_2) : 277.6

IR $_{v\ max}$ (CH_2CI_2), cm^{-1} : 3054, 1712, 1263

Mass spectrum, *m/z* : 468 $[M]^+$

^{1}H NMR ($CDCl_3$), δ ppm :

0.99 (*m*, H-1, 1H), 1.62 (*m*, H-2, 1H), 4.47 (*dd*, *J*=10.7 Hz and 5.5 Hz, H-3, 1H), 0.79 (*m*, H-5, 1H), 1.50 (*m*, H-6a, 1H), 1.38 (*m*, H-6b, 1H), 1.49 (*m*, H-7a, 1H), 1.38 (*m*, H-7b, 1H), 1.28 (*m*, H-9, 1H), 1.39 (*m*, H-11a, 1H), 1.21 (*m*, H-11b, 1H), 1.66 (*m*, H-12a, 1H), 1.05 (*m*, H-12b, 1H), 1.65 (*m*, H-13, 1H), 1.67 (*m*, H-15a, 1H), 1.00 (*m*, H-15b, 1H), 1.47 (*m*, H-16a, 1H), 1.36 (*m*, H-16b, 1H), 1.35 (*m*, H-18, 1H), 2.40 (*m*, H-19, 1H), 1.91 (*m*, H-21a, 1H), 1.33 (*m*, H-21b, 1H), 1.38 (*m*, H-22a, 1H), 1.18 (*m*, H-22b, 1H), 0.84 (*s*, H-23, 3H), 0.83 (*s*, H-24, 3H), 0.85 (*s*, H-25, 3H), 1.03 (*s*, H-26, 3H), 0.94 (*s*, H-27, 3H), 0.78 (*s*, H-28, 3H), 4.69 (*s*, H-29a, 1H), 4.57 (*s*, H-29b, 1H), 1.68 (*s*, H-30, 3H), 2.05 (*s*, H-32, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

38.4 (C-1), 23.7 (C-2), 81.0 (C-3), 37.8 (C-4), 55.4 (C-5), 18.0 (C-6), 34.2 (C-7), 40.9 (C-8), 50.3 (C-9), 37.1 (C-10), 20.9 (C-11), 25.1 (C-12), 38.0 (C-13), 42.8 (C-14), 27.4 (C-15), 35.6 (C-16), 43.0 (C-17), 48.3 (C-18), 48.0 (C-19), 151.0 (C-20), 29.8 (C-21), 40.0 (C-22), 28.0 (C-23), 16.5 (C-24), 16.2 (C-25), 16.0 (C-26), 14.5 (C-27), 18.2 (C-28), 109.4 (C-29), 19.3 (C-30), 171.1 (C-31), 21.3 (C-32).

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Stigmasterol 117 : Colourless needles

Molecular formula : $C_{29}H_{48}O$

UV λ_{max} (CH_2CI_2) : 276.8

IR $_{v\ max}$ (CH_2CI_2), cm^{-1} : 3298, 1640, 1019

Mass spectrum, *m/z* : 413 $[M+H]^+$

Melting point, ^oC : 151.0 - 152.0 (lit. m.p.: 169-171; Koay, Wong, Osman, Eldeen, & Asmawi, 2013)

^1H NMR ($CDCl_3$), δ ppm :

1.07 (*m*, H-1a, 1H), 1.83 (*m*, H-1b, 1H), 1.52 (*m*, H-2a, 1H), 1.83 (*m*, H-2b, 1H), 3.52 (*m*, H-3, 1H), 2.23 (*m*, H-4a, 1H), 2.28 (*dd*, *J*=4.6 Hz and 1.7 Hz, H-4b, 1H), 5.35 (*s*, H-6, 1H), 1.46 (*m*, H-7a, 1H), 1.99 (*m*, H-7b, 1H), 1.52 (*m*, H-8, 1H), 0.92 (*m*, H-9, 1H), 1.52 (*m*, H-11, 1H), 1.15 (*m*, H-12a, 1H), 2.02 (*m*, H-12b, 1H), 0.99 (*m*, H-14, 1H), 1.02 (*m*, H-15a, 1H), 1.58 (*m*, H-15b, 1H), 1.25 (*m*, H-16a, 1H), 1.68 (*m*, H-16b, 1H), 1.07 (*m*, H-17, 1H), 0.68 (*s*, H-18, 3H), 1.00 (*s*, H-19, 3H), 2.02 (*m*, H-20, 1H), 0.92 (*d*, *J* = 6.3 Hz, H-21, 1H), 5.14 (*dd*, *J* = 15.5 Hz and 8.6 Hz, H-22, 1H), 5.00 (*dd*, *J* = 15.5 Hz and 9.2 Hz, H-23, 1H), 1.54 (*m*, H-24, 1H), 0.92 (*m*, H-25, 1H), 0.85 (*d*, *J* = 6.9 Hz, H-26, 3H), 0.79 (*d*, *J* = 6.8 Hz, H-27, 3H), 1.25 (*m*, H-28, 1H), 0.80 (*t*, *J* = 6.9 Hz, H-29, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

37.3 (C-1), 31.7 (C-2), 71.9 (C-3), 42.4 (C-4), 140.8 (C-5), 121.8 (C-6), 31.9 (C-7), 31.9 (C-8), 50.2 (C-9), 36.6 (C-10), 21.2 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 24.4 (C-15), 29.0 (C-16), 56.0 (C-17), 12.0 (C-18), 19.5 (C-19), 40.6 (C-20), 21.2 (C-21), 138.4 (C-22), 129.3 (C-23), 51.3 (C-24), 45.9 (C-25), 21.2 (C-26), 19.1 (C-27), 29.8 (C-28), 12.1 (C-29).

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Mixture of Lupeol 118, α-Amyrin 119 and β-Amyrin 113

Appearance : White amorphous

Molecular formula : $C_{30}H_{50}O$

IR $_{v\ max}$ (CH_2CI_2), cm^{-1} : 3278, 1709, 1096

Mass spectrum, *m/z* : 426 $[M]^+$

^{13}C NMR ($CDCl_3$), δ ppm :

Lupeol 118: 38.8 (C-1), 27.5 (C-2), 79.1 (C-3), 38.9 (C-4), 55.3 (C-5), 18.4 (C-6), 34.4 (C-7), 40.9 (C-8), 50.5 (C-9), 37.3 (C-10), 21.0 (C-11), 25.3 (C-12), 38.2 (C-13), 42.9 (C-14), 27.5 (C-15), 35.7 (C-16), 43.1 (C-17), 48.4 (C-18), 48.1 (C-19), 151.1 (C-20), 29.9 (C-21), 40.1 (C-22), 28.1 (C-23), 15.5 (C-24), 16.2 (C-25), 16.1 (C-26), 14.6 (C-27), 18.1 (C-28), 109.9 (C-29), 19.4 (C-30).

α-Amyrin 119: 38.8 (C-1), 27.5 (C-2), 79.1 (C-3), 38.9 (C-4), 55.2 (C-5), 18.4 (C-6), 33.0 (C-7), 40.1 (C-8), 47.8 (C-9), 37.0 (C-10), 23.5 (C-11), 124.5 (C-12), 139.7 (C-13), 42.2 (C-14), 28.1 (C-15), 26.7 (C-16), 33.8 (C-17), 59.1 (C-18), 39.8 (C-19), 39.7 (C-20), 31.3 (C-21), 41.6 (C-22), 28.8 (C-23), 15.7 (C-24), 15.8 (C-25), 16.9 (C-26), 23.4 (C-27), 28.2 (C-28), 17.6 (C-29), 21.5 (C-30).

β-Amyrin 113: 38.7 (C-1), 27.4 (C-2), 79.1 (C-3), 39.8 (C-4), 55.3 (C-5), 19.4 (C-6), 32.7 (C-7), 40.1 (C-8), 47.7 (C-9), 37.0 (C-10), 23.8 (C-11), 121.8 (C-12), 145.3 (C-13), 41.8 (C-14), 28.2 (C-15), 27.0 (C-16), 32.6 (C-17), 47.3 (C-18), 46.9 (C-19), 31.2 (C-20), 34.8 (C-21), 36.7 (C-22), 29.7 (C-23), 15.6 (C-24), 15.7 (C-25), 16.9 (C-26), 26.2 (C-27), 28.5 (C-28), 33.4 (C-29), 23.6 (C-30).

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3.6 Biological Activity Assay

3.6.1 Introduction

Chemical and biological studies have shown that the terpenoids posses a variety of chemical, physical and biological activities. Almost all terpenes have some biological activities in animals including man and also play a meaningful role in human medicine. In recent years, a considerable number of studies conducted in many scientific centres have been devoted to triterpenes, triterpene glycosides, (also known as saponines), and other terpenoids. There is a growing interest in natural triterpenoids caused as much by the scientific aspects of extraction and structural analysis of these compounds, as by the fact of their wide spectrum of biological activities; they are bactericidal, fungicidal, antiviral, cytotoxic, analgetic, anticancer, spermicidal, cardiovascular, antiallergic and so on.

Hundreds of publications have highlighted the broad spectrum of biological activities of lupane, oleanane and ursane triterpenes. For example, betulin **19** exhibit the activity against *Streptpcpccus pyogenes* and *Fusarium oxysporum* (Cota, De Oliveira, De Souza-Filho, & Braga, 2003; Prachayasittikul, Saraban, Cherdtrakulkiat, Ruchirawat, & Prachayasittikul, 2010). According to Saleem et al. (2005), lupeol **20** was shown to have various pharmacological properties, inducing anti-inflammatory and anti-arthritic responses and inhibition of tumor growth in animal cells. In light of the considerable interest recently generated in the chemistry and pharmacological properties of triterpenes and their analogs, therefore more biological investigation has been embarked.

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3.6.2 Bioactivity

Triterpenes are particularly interesting substances because of their wide spectrum of biological activities and pharmacological activities. In addition to our systematic chemical investigations, triterpenes isolated from the present study were screened for their biological activity, in particular for their cytotoxic effects, antibacterial and antioxidant activity. This part of the work was carried out by at Department of Pharmacy, University of Malaya, Kuala Lumpur.

3.6.3 Preparation of the Bioactivity

3.6.3.1 Cell Culture and MTT Cytotoxicity Assay

A preliminary screening against cancer cells was performed using the MTT method (Mosmann, 1983) against MCF-7 cell line. The MCF-7 used in this study was obtained from the American Type Cell Collection (ATCC). The cell was cultured using RPMI 1640 culturing media (PAA, Leverkusen, Germany). Besides, cell was trypsinized and counted using hemocytometer and plated in a microtiter plate of 96-wells. After an overnight incubation to allow cell attachment, the medium was changed and 0.2 mL of new supplemented medium was added into each well. Cells were then treated with the different drug concentrations and incubated at 37℃, 5% CO_2 for 24 hours. Each concentration of the samples was assayed in triplicate. The colorimetric assay was performed at an absorbance of 570 nm. Results were expressed as a percentage of control

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giving a certain percentage of cell viability after 24 hour exposure to the test agent. The potency of cell growth inhibition for test agent was expressed as an IC_{50} value (Omar et al., 2013).

3.6.3.2 Antibacterial Activity

A gram positive bacterium, *Bacillus cereus* was selected for the *in vitro* antibacterial activity. The strain was stored in the appropriate medium before use. Antibacterial activity was determined by the disc diffusion method (Hashim, et al., 2012) with slight modifications in terms of sample concentration, volume of sample loaded and use of paper discs. The bacteria were cultured at 37 ℃ for overnight in nutrient broth and potato dextrose broth, respectively. The concentrations of the cultures were adjusted turbidometrically at a wavelength of 600 nm which gave 10^5–10^6 colony forming units (CFU) per mL. The compounds to be tested were dissolved in dimethyl sulphoxide (DMSO) at concentration of 1 mg/mL. About 10 µL of each sample solution was loaded on Whatman No. 1 filter paper disc (0.6 mm). The disc was placed on the surface of the agar plate (nutrient agar or potato dextrose agar) previously inoculated with bacteria. The agar plates were then inverted and incubated for 24 hours at 37 ℃. The antimicrobial activity was recorded by measuring the zone of inhibition (IZ) in mm around each disc, against the test organisms. The experiments were repeated in triplicate and the results were expressed as average values. The antibiotic streptomycin sulfate (10 µg/disc) was used as positive control and DMSO as negative control in the assay (Omar et al., 2013).

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3.6.3.3 Antioxidant Inhibitory Activity

In this activity, the standard 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was used with slight modification. In this technique, 96 micro-well flat bottom plates were used instead of cuvettes to consume minimal amounts of sample, vehicle and DPPH solutions. Each compound was prepared at 1 mg/mL in DMSO. Each well was filled in with 100 µL of diluent (DMSO). The first row (row A) of the 96 wells was filled in with 100 µL of diluted sample at 1 mg/mL. Concentration in the first row was 500 µg/mL of sample in each well and was serially diluted (two fold dilution) in 96 micro-well plates to varying concentrations, topping from 500 µg/mL down to the lowest 7.8 µg/mL. Then, 5 µL of the DPPH solution (2.5 mg/mL in DMSO) was added to each well. The DPPH solution should be kept in the dark at 4 ℃. This procedure should not be done under direct light. The plate was shaken to ensure thorough mixing before placed in the dark. After 30 minutes, the optical density of each well was read using ELISA Reader (EL340 Biokinetic Reader, Bio-Tek Instrumentation) at wavelength 517 nm. Percentage inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = 1 - \text{OD(DPPH + Sample)/OD(DPPH)} \times 100$$

The IC_{50} value was determined as the concentration of each sample required or able to scavenge 50 % of the DPPH. All tests and analyses were run in triplicate and averaged. Antioxidant assay by using DPPH method was used to assess the capacity of the compounds to scavenge stable free radical DPPH at different concentration. The

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radical scavenging effect was examined and compared with natural antioxidants α-tocopherol (Sigma, USA), ascorbic acid (Sigma USA), and quercetin (Sigma, USA) used as positive controls (Table 3.1) (Hashim et al., 2012).

Table 3.1

The IC_{50} Values of the Standard Against DPPH (Free Radical).

Standard	IC_{50}(μg/mL)
Ascorbic acid	20 ±1.2
α-tocopherol	60 ±2.9
Quercetin	40 ±5.8

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CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter is focusing on analyzing the data that has been obtained from the instruments that has been tested. A few chemical compounds have been isolated from the bark of *Alstonia spathulata* and root of *Kopsia singapurensis*. The isolation of pure compounds was accomplished through various chromatography methods such as column chromatography (CC), thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC). The structure of the isolated compounds were then elucidated by nuclear magnetic resonance (NMR), UV, IR and MS (GC-MS). Structures of the compounds were also determined by comparison data with the previous work. Discussion on the structural elucidation of all isolated compounds is briefly presented in the following section of this chapter.

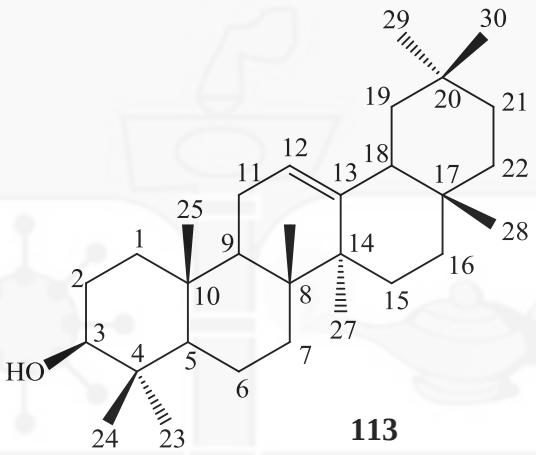
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4.2 Chemical constituents from the bark of *Alstonia spathulata*

The phytochemical investigation of the hexane crude extract and CH_2C1_2 residue extract from the bark of *Alstonia spathulata* has resulted in the isolation of five triterpenes: β-amyrin **113**, β-amyrin acetate **114**, β-sitosterol **115**, lupeol acetate **116**, stigmasterol **117** and a mixture of lupeol **118**, α-amyrin **119** and β-amyrin **113**.

4.2.1 Compound A: β-amyrin 113



113

Compound **A** was obtained as white amorphous. The IR spectrum showed abosrption peaks at 3360 cm^{-1} (O-H), 1709 cm^{-1} (olefin) and 1294 cm^{-1} (C-O). The mass spectrum of **113** showed a molecular ion peak at *m/z* 449 $[M+Na]^+$ corresponding to molecular formula $C_{30}H_{50}O$. From ^1H NMR spectrum (Table 4.1 and Figure 4.1), methyl signals were observed at δ 0.98, δ 0.91, δ 0.77, δ 0.95, δ 1.12, δ 0.81, δ 0.85 and δ 0.85 were corresponding to proton attached to C- 23, C-24, C 25, C-26, C-27, C-28, C-29 and C-30, respectively. A methine proton, signal at δ 3.20 (H-3, *dd, J*=11.0, 5.0 Hz) attached to a hydroxyl group and a signal δ 5.l6 (*t, J* = 3.6 Hz) was attributed to an olefinic proton at C-12.

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Analysis of ^{13}C NMR spectrum (Table 4.1 and Figure 4.2) revealed the presence of 30 carbons. There are seven quaternary carbons δ 38.9 (C-4), δ 39.9 (C-8), δ 37.0 (C-10), δ 145.3 (C-13), δ 41.8 (C-14), δ 32.8 (C-17), δ 31.2 (C-20); five methine carbons δ 79.1 (C-3), δ 55.3 (C-5), δ 47.7 (C-9), δ 121.8 (C-12), δ 47.2 (C-18); ten methylene carbons δ 38.7 (C-1), δ 27.3 (C-2), δ 18.5 (C-6), δ 32.7 (C-7), δ 23.6 (C-11), δ 26.2 (C-15), δ 27.0 (C-16), δ 46.9 (C-19), δ 34.8 (C-21), δ 37.2 (C-22); eight methyl carbons δ 28.2 (C-23), δ 15.6 (C-24), δ 15.7 (C-25), δ 16.9 (C-26), δ 26.1 (C-27), δ 28.5 (C-28), δ 33.5 (C-29), δ 23.8 (C-30). A pair of downfield carbon signal at δ 121.8 (C-12) and δ 145.3 (C-13) represented the alkene carbon and hydroxyl carbon signal at δ 79.1 (C-3) were also observed.

The positions of carbon resonance were further confirmed by ^{1}H-^{1}H COSY (Figure 4.3), HSQC (Figure 4.4) and HMBC (Figure 4.5). ^{1}H-^{1}H COSY suggested the following cross peaks: C-1 – C-3, C-5 – C-7, C-9 – C-11 – C-12, C-15 – C-16, C-18 – C-19 and C-21 – C-22. A series of ^{1}H – ^{13}C long range correlation signals were observed in HMBC spectral. Its HMBC correlations include: H-3 to C-4 and C-24; H-11 to C-13; H-12 to C-11 and C-18; H-23 to C-3 and C-5; H-24 to C-5; H-27 to C-13; H-29 to C-20, C-21 and C-30. Other correlations of COSY and HMBC were summarized in Figure 4.6. ^{1}H and ^{13}C NMR spectral data as well as mass spectrum were in agreement with those reported for β-amyrin (Paz Lima et al., 2004; Thanakijcharoenpath & Theanphong, 2007). Thus; compound **113** was identified as β-amyrin.

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Table 4.1

^{1}H NMR [400 MHz, δ_H (J, Hz)] and ^{13}C NMR [100 MHz, δ_C] of 113 in $CDCl_3$

	β-amyrin		β-amyrin [125 MHz, $CDCl_3$] [Thanakijcharoenpath & Theanphong, 2007]	
Position	δ_H (*J* Hz), ppm	δ_C, ppm	δ_H (*J* Hz), ppm	δ_C, ppm
1	1.61 (*m*)	38.7		38.6
	0.98 (*s*)			
2	1.58 (*m*)	27.3		27.2
	0.77 (*m*)			
3	3.20 (*dd*, 11.0, 5.0 Hz)	79.1	3.20 (*dd*, 10.7, 5.0 Hz)	79.0
4		38.9		38.8
5	0.74 (*m*)	55.3		55.2
6	1.53 (*m*)	18.5		18.4
	1.37 (*m*)			
7	1.50 (*m*)	32.7		32.5
	1.31 (*m*)			
8		39.9		39.8
9	1.54 (*m*)	47.7		47.6
10		37.0		36.9
11	1.85 (*m*)	23.6		23.6
12	5.16 (*t*, 3.6 Hz)	121.8	5.16 (*t*, 3.0 Hz)	121.7
13		145.3		145.2
14		41.8		41.7
15	1.74 (*m*)	26.2		26.1
	0.95 (*m*)			
16	1.97 (*m*)	27.0		26.9
17		32.6		32.6
18	1.94 (*m*)	47.2		47.2
19	1.64 (*m*)	46.9		46.8
	1.00 (*m*)			
20		31.2		31.1
21	1.31 (*m*)	34.8		34.7
	1.10 (*m*)			
22	1.38 (*m*)	37.2		37.1
	1.21 (*m*)			
23	0.98 (*s*)	28.2	0.98 (s)	28.1
24	0.91 (*s*)	15.6	0.92 (s)	15.5
25	0.77 (*s*)	15.7	0.77 (s)	15.6
26	0.95 (*s*)	16.9	0.95 (s)	16.8
27	1.12 (*s*)	26.1	1.12 (s)	26.0
28	0.81 (*s*)	28.5	0.81 (s)	28.4
29	0.85 (*s*)	33.5	0.85 (s)	33.3
30	0.85 (*s*)	23.8	0.85 (s)	23.7

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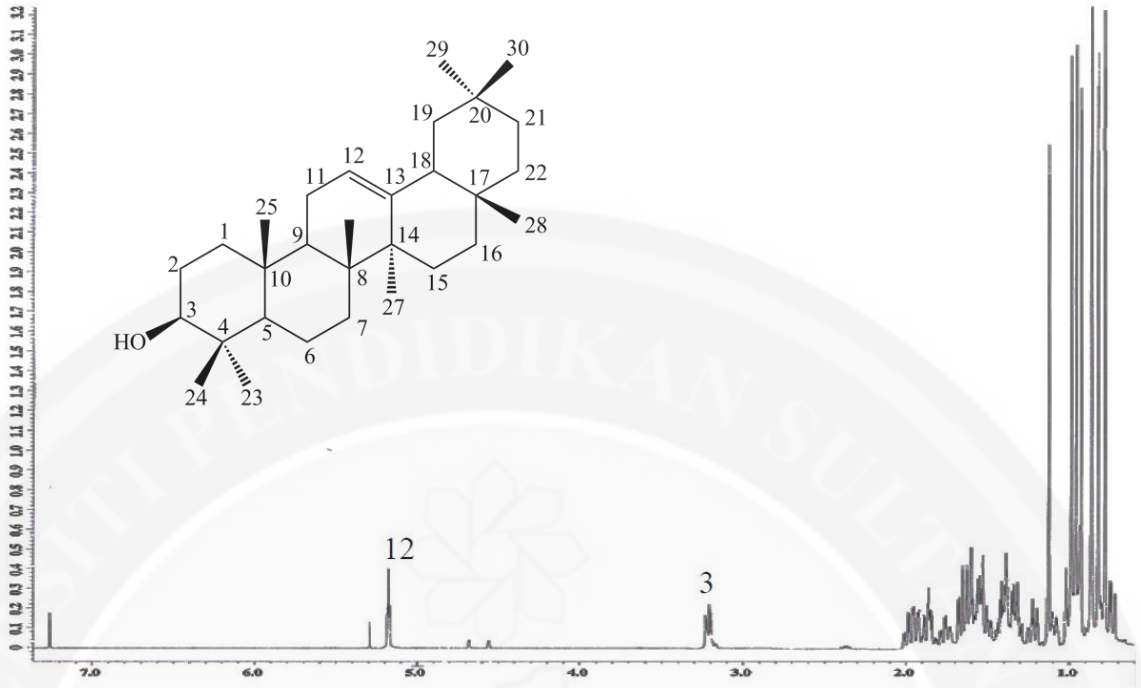


Figure 4.1. ^{1}H NMR spectrum of **113**

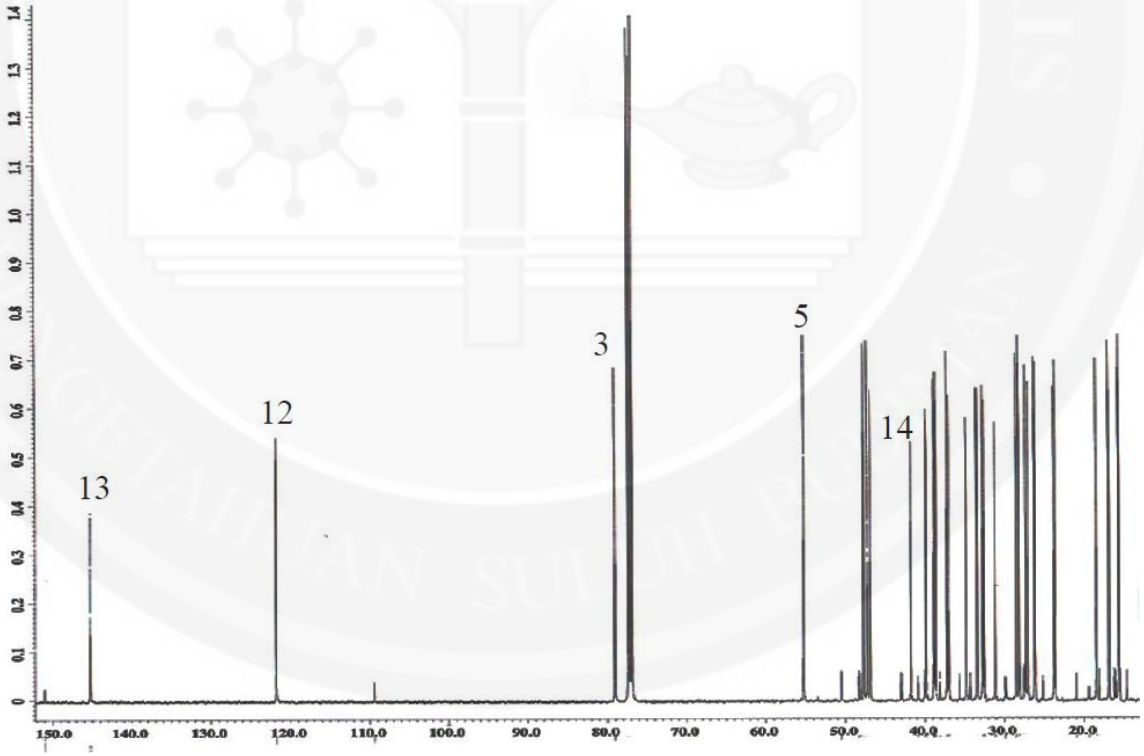


Figure 4.2. ^{13}C NMR spectrum of **113**

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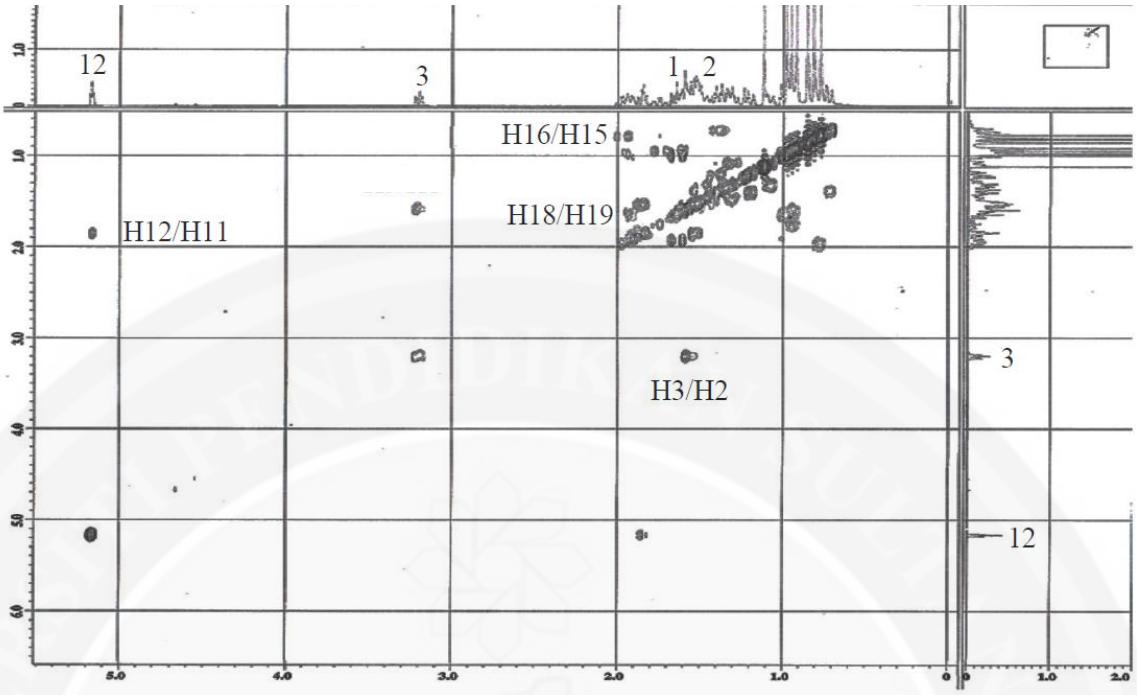


Figure 4.3. COSY spectrum of **113**

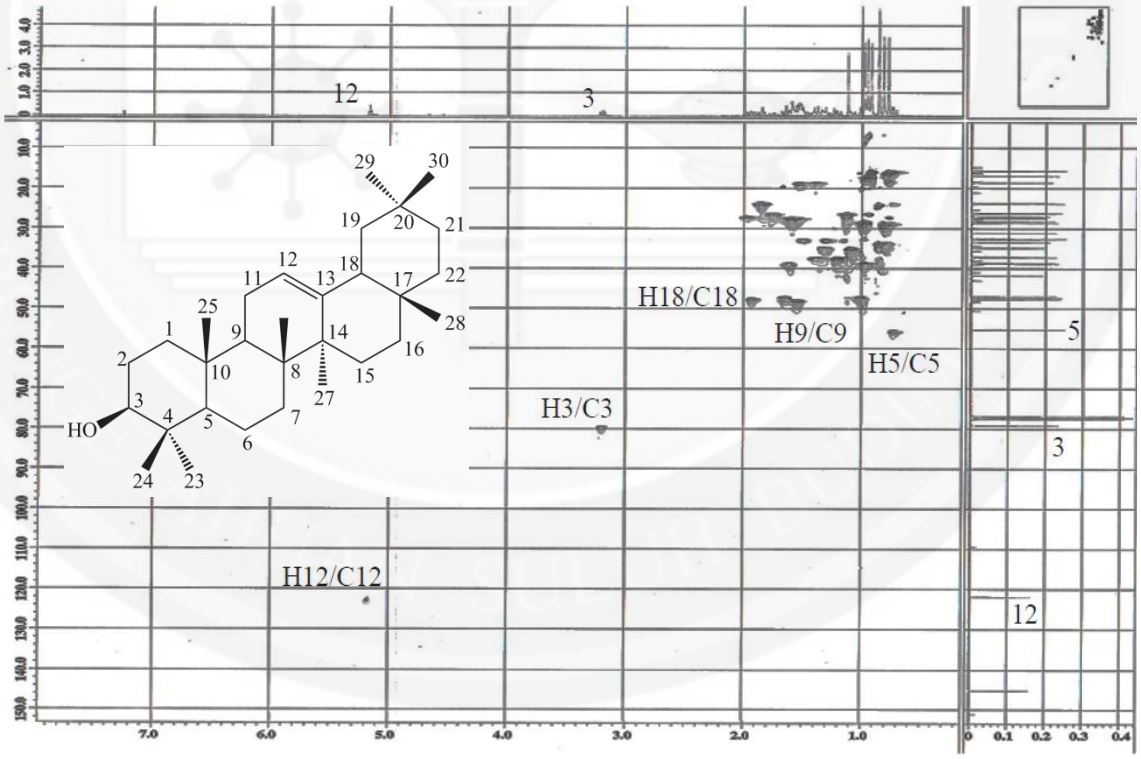


Figure 4.4. HSQC spectrum of **113**

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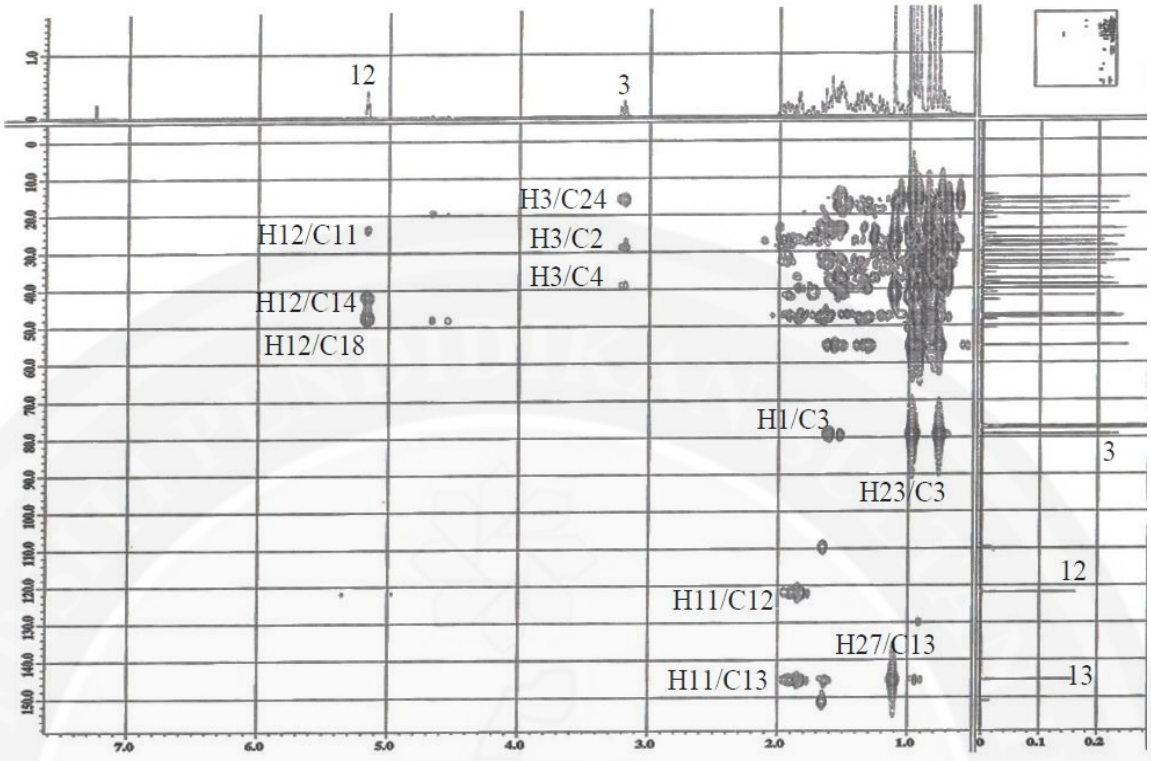


Figure 4.5. HMBC spectrum of **113**

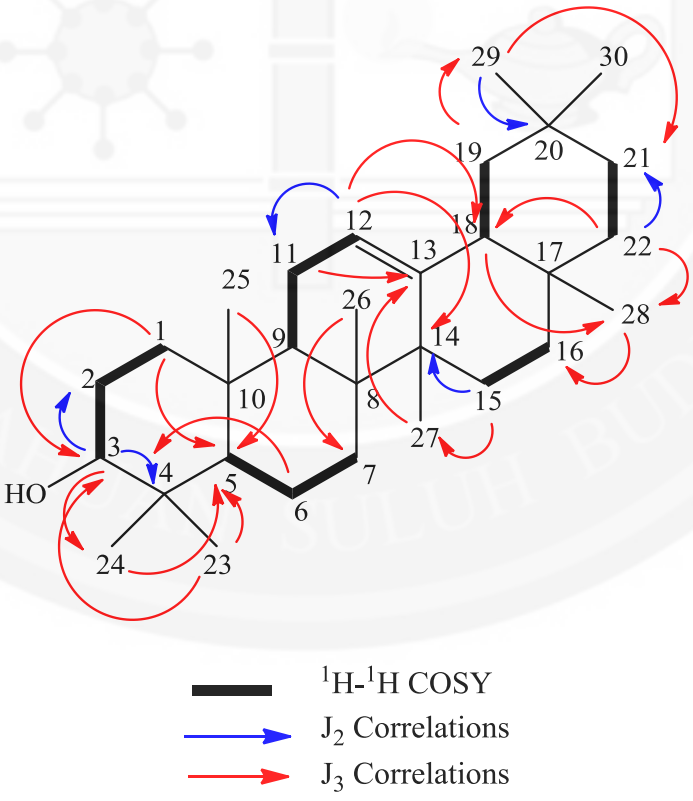
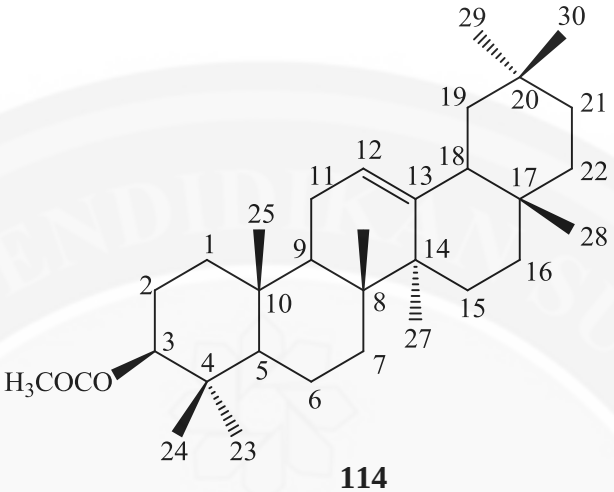


Figure 4.6. Selected COSY and HMBC of **113**

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4.2.2 Compound B: β-amyrin acetate 114



114

Compound **B** was isolated as colourless needles (m.p.: 239.0-240.0 ^{o}C). The IR spectrum displayed stretching band for ester carbonyl (1712 cm^{-1}) and C-O (1264 cm^{-1}). The mass spectrum of **114** showed a molecular ion peak at *m/z* 468 $[M]^{+}$ corresponding to molecular formula $C_{32}H_{52}O_2$. The ^{1}H NMR spectrum (Table 4.2 and Figure 4.7) of **114** exhibited for eight singlet methyl groups at δ 0.88, δ 0.86, δ 0.96, δ 0.97, δ 1.13, δ 0.83, δ 0.87, δ 0.87 were corresponding to proton attached to C- 23, C-24, C 25, C-26, C-27, C-28, C-29 and C-30 respectively. It was identified as triterpene acetate due to the presence of an acetate methyl signal at δ 2.05 as a singlet. A methine proton, exhibited downfield shift signal at δ 4.50 (H-3, *t*, *J* = 8.0 Hz) which is attached to an acetoxyl group, compared with the corresponding proton in **113**. A signal δ 5.l8 (*t*, *J* = 3.5 Hz) was attributed to an olefinic proton at C-12.

^{13}C NMR spectrum (Table 4.2 and Figure 4.8) of **114** showed the presence of 32 carbon atoms indicating a mono acetyl derivative. This attribution was corroborated by the presence of signals at δ 171.1 (C-31) which is characteristic of an

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ester carbon group. In addition, a pair of characteristic signals at δ 121.6 (C-12) and δ 145.2 (C-13) indicated it is an oleanan type triterpene, as well as presence of oxygenated carbons at δ 81.0 (C-3). There are seven quaternary carbons δ 37.7 (C-4), δ 39.8 (C-8), δ 36.8 (C-10), δ 145.2 (C-13), δ 41.7 (C-14), δ 32.5 (C-17), δ 31.0 (C-20); five methine carbons δ 81.0 (C-3), δ 55.2 (C-5), δ 47.6 (C-9), δ 121.6 (C-12), δ 47.2 (C-18); ten methylene carbons δ 38.3 (C-1), δ 23.5 (C-2), δ 18.3 (C-6), δ 32.7 (C-7), δ 23.6 (C-11), δ 26.1 (C-15), δ 26.9 (C-16), δ 46.8 (C-19), δ 34.7 (C-21), δ 37.1 (C-22); nine methyl carbons δ 28.0 (C-23), δ 16.7 (C-24), δ 15.6 (C-25), δ 16.8 (C-26), δ 26.0 (C-27), δ 28.4 (C-28), δ 33.4 (C-29), δ 23.7 (C-30), δ 21.4 (C-32) and the present of a carbonyl group at δ 171.1 (C-31).

The structure of the compound was further supported by ^{1}H-^{1}H COSY (Figure 4.9), HSQC (Figure 4.10) and HMBC (Figure 4.11). ^{1}H-^{1}H COSY revealed the cross peak include: C-1 – C-3, C-5 – C-7, C-9 – C-11 – C-12, C-15 – C-16, C-18 – C-19 and C-21 – C-22. A series of ^{1}H – ^{13}C long range correlation signals were observed in HMBC spectral. Its HMBC correlations include: H-3 to C-2, C-23 and C-31; H-12 to C-9 and C-11; H-18 to C-12 and C-13; H-22 to C-18, C-20 and C-21; H-23 to C-5 and C-24; H-27 to C-13; H-32 to C-32. Figure 4.12 showed the summarized of other correlations. All spectral data of **114** were found to be in full agreement with those reported for β-amyrin acetate (Muhammad et al., 2007; Ragasa, Tsai, & Shen, 2009; Ahmed et al., 2011; Segovia et al., 2011). Therefore; compound **114** was identified as β-amyrin acetate.

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Table 4.2

^{1}H NMR [600 MHz, δ_H (J, Hz)] and ^{13}C NMR [150 MHz, δ_C] of 114 in $CDCl_3$

	β-amyrin acetate		β-amyrin acetate	
Position	δ_H (*J* Hz), ppm	δ_C, ppm	δ_H (*J* Hz), ppm [125 MHz, $CDCl_3$] [Muhammad et al., 2007]	δ_C, ppm [75 MHz, $CDCl_3$] [Segovia et al., 2011]
1	1.63 (*m*) 1.06 (*m*)	38.3		38.2
2	1.88 (*m*) 1.60 (*m*)	23.5		26.6
3	4.50 (*t*, 8.0 Hz)	81.0	4.52 (*t*, 8.0 Hz)	80.9
4		37.7		37.7
5	0.84 (*m*)	55.2		55.2
6	1.53 (*m*) 1.40 (*m*)	18.3		18.2
7	1.52 (*m*) 1.33 (*m*)	32.6		32.6
8		39.8		38.4
9	1.57 (*m*)	47.6		47.5
10		36.8		36.8
11	1.80 (*m*) 1.63 (*m*)	23.6		23.6
12	5.18 (*t*, 3.5 Hz)	121.6	5.20 (*t*, 3.5 Hz)	121.6
13		145.2		145.2
14		41.7		41.7
15	1.76 (*m*) 0.85 (*m*)	26.1		26.1
16	1.99 (*m*) 0.79 (*m*)	26.9		26.9
17		32.5		32.9
18	1.93 (*d*, 4.2 Hz)	47.2		47.1
19	1.66 (*m*) 1.02 (*m*)	46.8		46.8
20		31.1		31.0
21	1.08 (*m*) 1.33 (*m*)	34.7		34.7
22	1.42 (*m*) 1.20 (*m*)	37.1		37.1
23	0.88 (*s*)	28.0	0.88 (s)	28.0
24	0.86 (*s*)	16.7	0.88 (s)	15.5
25	0.96 (*s*)	15.6	0.98 (s)	15.7
26	0.97 (*s*)	16.8	0.99 (s)	16.8
27	1.13 (*s*)	26.0	1.15 (s)	25.9
28	0.83 (*s*)	28.4	0.85 (s)	28.3
29	0.87 (*s*)	33.4	0.89 (s)	33.3
30	0.87 (*s*)	23.7	0.89 (s)	23.6
31		171.1		170.9
32	2.05 (*s*)	21.4	2.06 (s)	21.3 (Ragasa et al., 2009)

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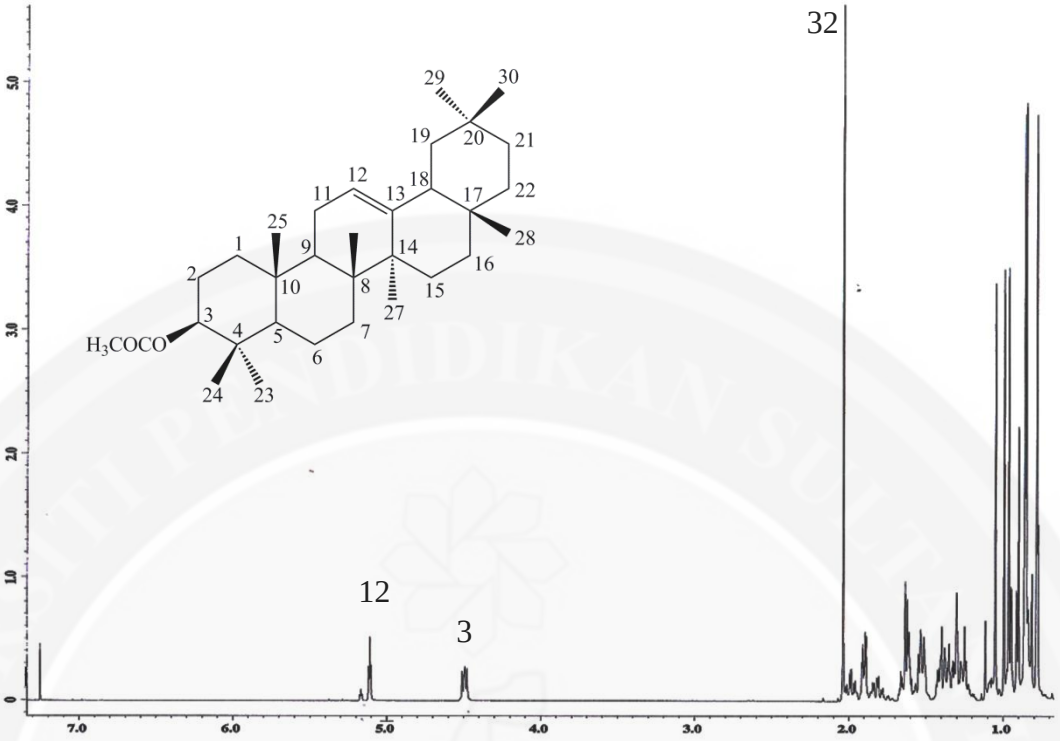


Figure 4.7. ^{1}H NMR spectrum of **114**

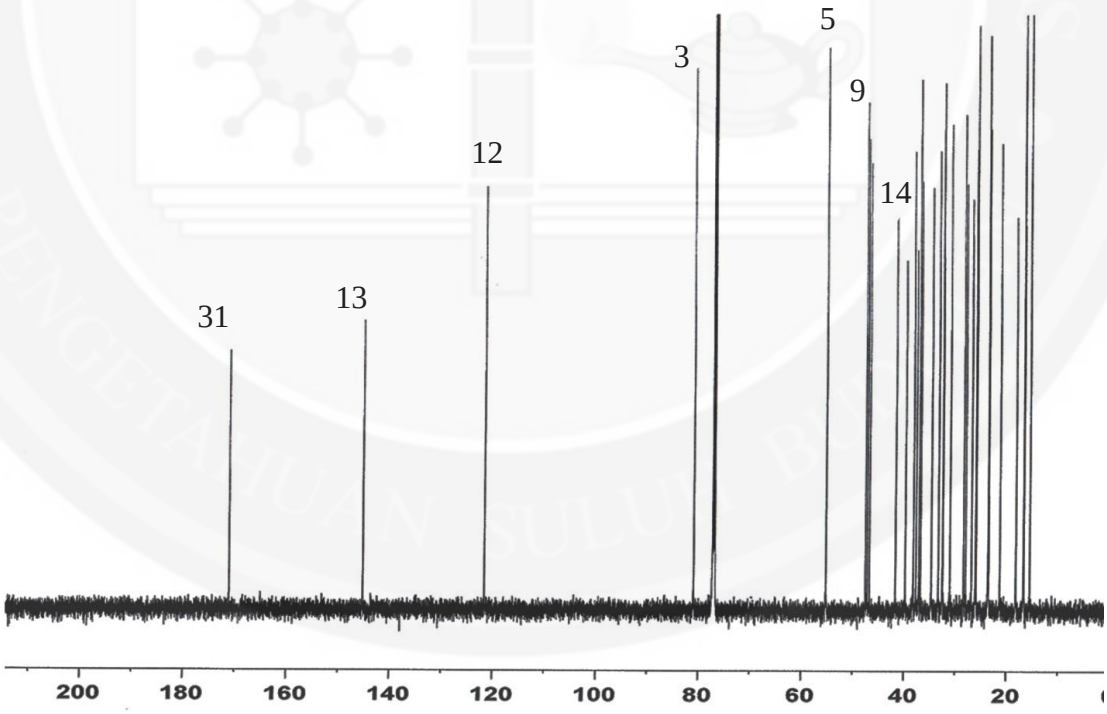


Figure 4.8. ^{13}C NMR spectrum of **114**

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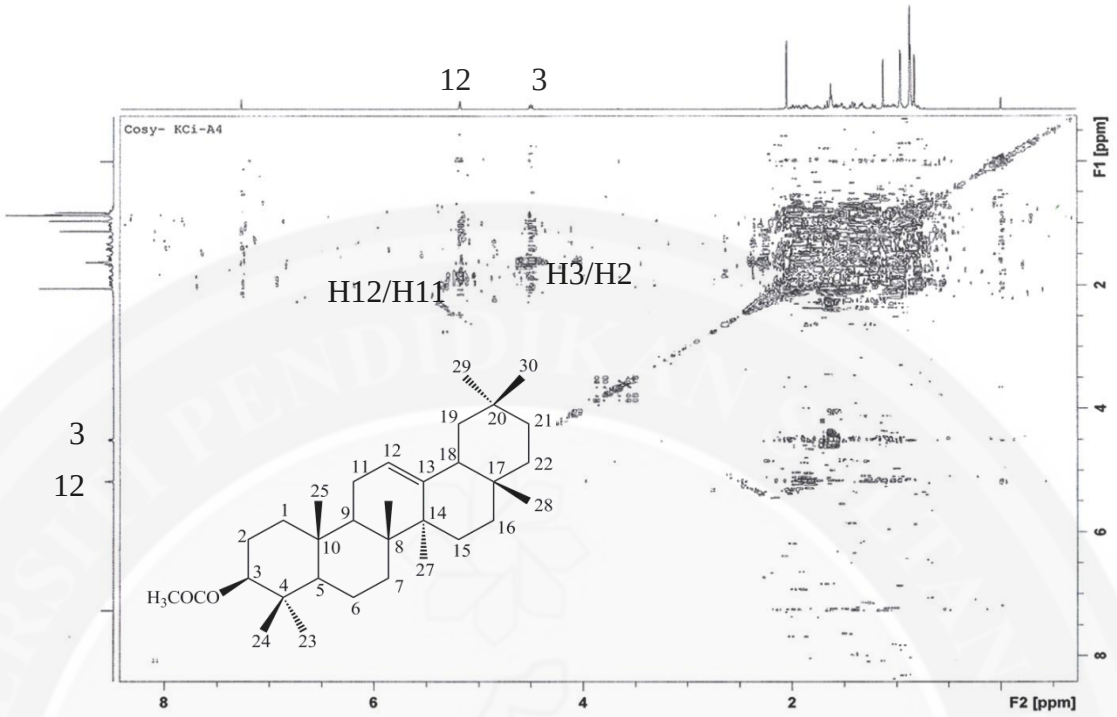


Figure 4.9. COSY spectrum of **114**

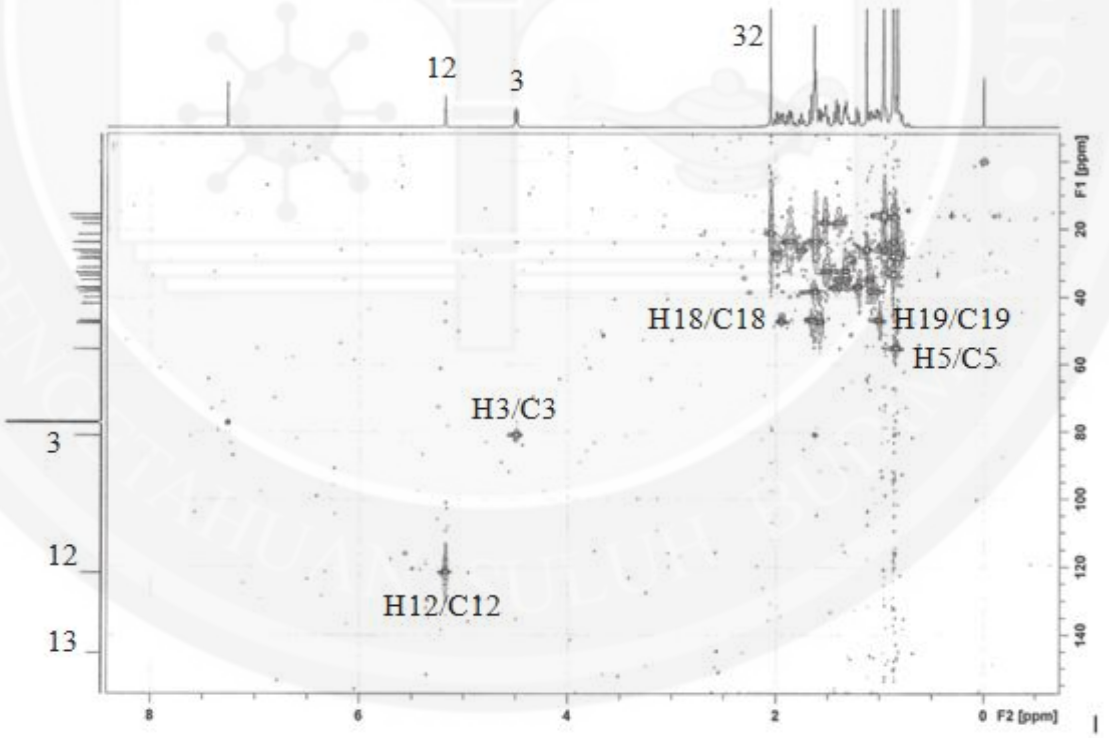


Figure 4.10. HSQC spectrum of **114**

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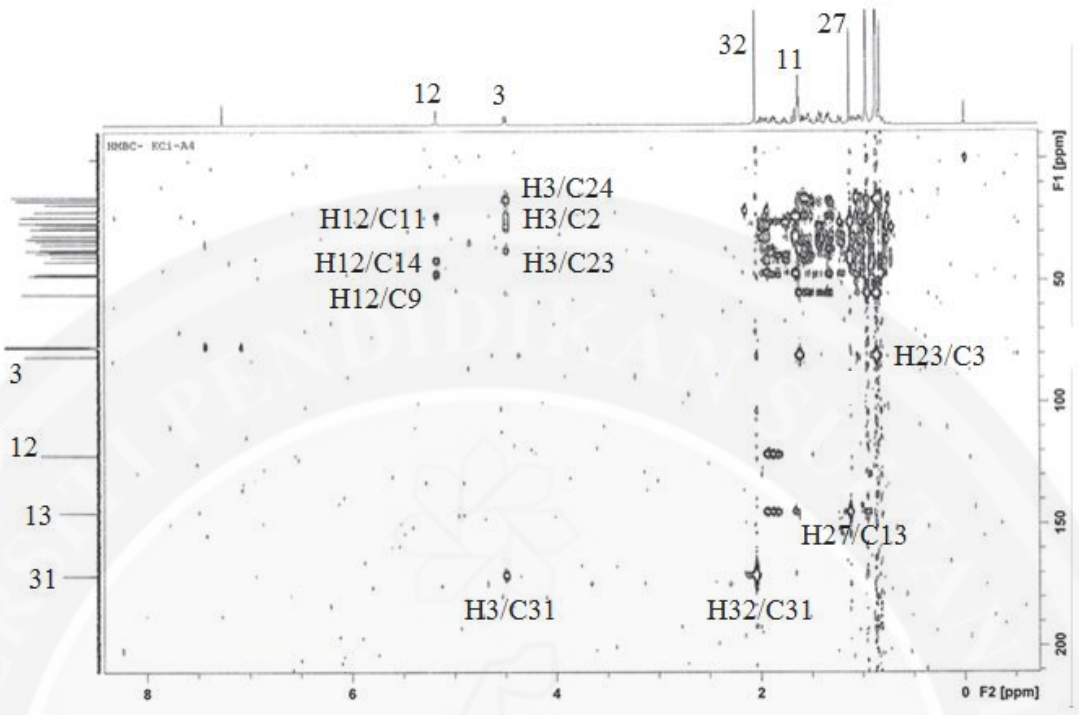


Figure 4.11. HMBC spectrum of **114**

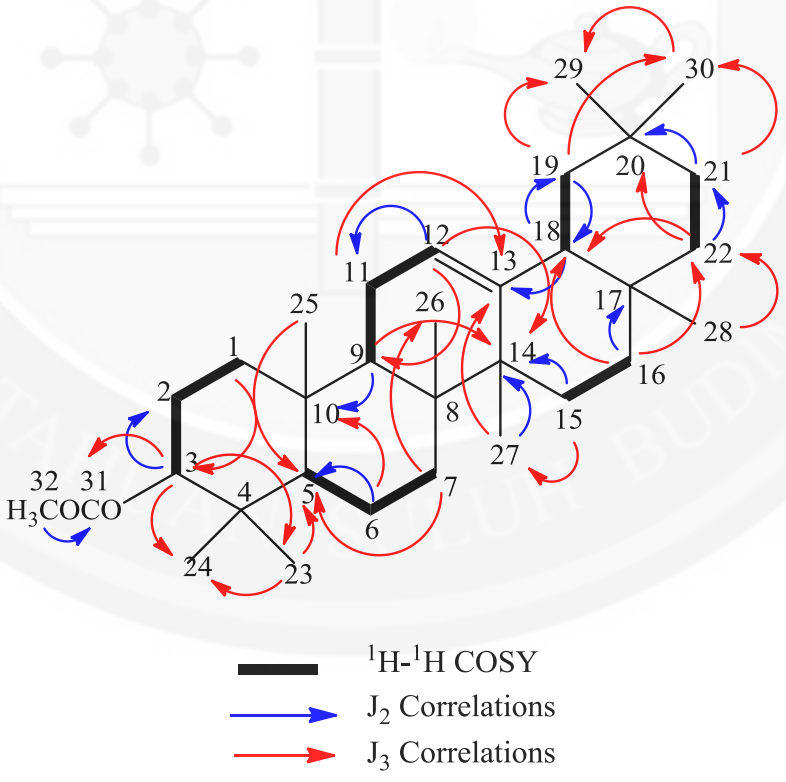
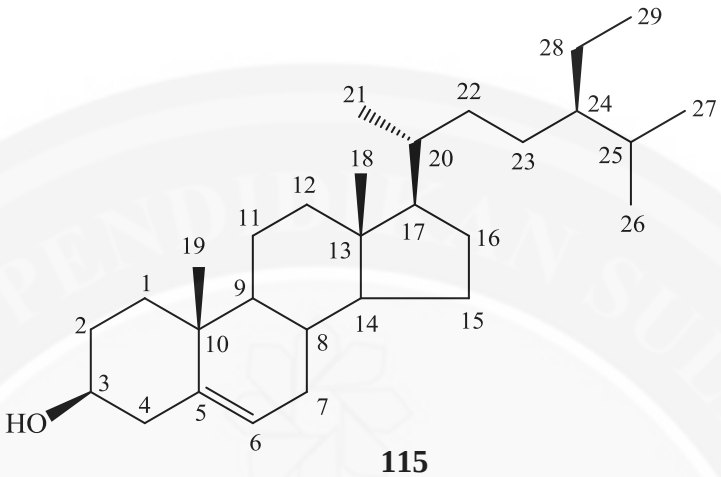


Figure 4.12. Selected COSY and HMBC of **114**

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4.2.3 Compound C: β-sitosterol 115



115

Compound **C** was obtained as white amorphous. On subjection to IR spectroscopic analysis, the observed absorption bands are 3347.1 cm^{-1} that is characteristic of O-H strecthing. Absorption at 1642.9 cm^{-1} is due to olefinic strecthing (C=C) and absorption at 1032.3 cm^{-1} attributed to C-O strecthing. The mass spectrum data showed the molecular ion peak at *m/z* 414 $[M]^+$ that correspond to the molecular formula $C_{29}H_{50}O$.

The ^1H NMR (Table 4.3 and Figure 4.13) has revealed the existence of signals for two angular methyl proton at δ 0.68 (H-18) and δ 1.01 (H-19); three methyl doublets at δ 0.92 (*d, J* = 7 Hz, H-21), δ 0.84 (*d, J* = 6.90 Hz, H-26), δ 0.81 (*d, J* = 6.85 Hz, H-27) and a methyl triplet at δ 0.85 (*t, J* = 7.45 Hz, H-29). A hydroxyl methine proton appear at δ 3.55 as a multiplet corresponding to proton H-3. The typical signal for the olefinic proton H-6 of the steroidal skeleton was evident as a triplet at δ 5.35 (*t, J* = 2.20 Hz) intergrating for one proton.

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Total of 29 carbon atoms was revealed from the ^{13}C NMR spectrum (Table 4.3 and Figure 4.14). This compound is having six methyl carbons: δ 12.0 (C-18), δ 19.5 (C-19), δ 18.9 (C-21), δ 19.9 (C-26), δ 19.0 (C-27) and δ 12.1 (C-29); eleven methylene carbons: δ 37.3 (C-1), 31.7 (C-2), δ 42.4 (C-4), δ 32.0 (C-7), δ 21.2 (C-11), δ 39.9 (C-12), δ 24.4 (C-15), δ 28.3 (C-16), δ 34.0 (C-22), δ 26.1 (C-23) and 23.1 (C-28); nine methine carbons: δ 71.9 (C-3), δ 121.8 (C-6), δ 32.0 (C-8), δ 50.2 (C-9), δ 56.8 (C-14), δ 56.1 (C-17), δ 36.2 (C-20), δ 45.9 (C-24) and δ 29.2 (C-25) and three quaternary carbons: δ 140.8 (C-5), δ 36.6 (C-10), and δ 42.4 (C-13). The ^{13}C NMR has shown recognizable signals δ 140.8 and δ 121.8ppm, which are assigned C-5 and C-6 respectively. The δ value at 71.9ppm is due to presence of hydroxy group at C-3. Signals at δ 12.0 and δ 19.5 corresponds to angular carbon atom C-18 and C-19.

The unambiguous assignment was further verify by the ^{1}H-^{1}H COSY (Figure 4.15), HMQC (Figure 4.16) and HMBC (Figure 4.17). ^{1}H-^{1}H COSY revealed the following cross peaks: C-1 – C-4, C-6 – C-7, C-14 – C-15 – C-16 – C-17, C-25 – C-27 and C-28 – C-29. In HMBC experiments, correlations of protons and carbons were observed. This correlations comfirmed the attribution of all ^{13}C signals of **115**. Its HMBC correlations include: H-1 to C-5 and C-10, H-11 to C-9 and C-12; H-18 to C-12, C-13, C-14 and C-17; H-23 to C-20 and C-22; H-26 to C-24 and C-25. All the correlations were summarized in Figure 4.18. The above spectral data and comparison of the ^{13}C NMR signal with those described in the literatures (Agrawal, Jain, Gupta & Thakur, 1985; Pretsch, Buhlmann & Badertscher, 2009; Mouffok, Haba, Lavaud, Long & Benkhaled, 2012) showed the **115** to be the β-sitosterol.

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Table 4.3

^{1}H NMR [500 MHz, δ_H (J, Hz)] and ^{13}C NMR [125 MHz, δ_C] of 115 in $CDCl_3$

	β-sitosterol		β-sitosterol [100 MHz, $CDCl_3$] [Mouffok et al., 2012]	
Position	δ_H (*J* Hz), ppm	δ_C, ppm	δ_H (*J* Hz), ppm	δ_C, ppm
1	1.08 (*m*) 1.86 (*m*)	37.3		37.2
2	1.53 (*m*) 1.86 (*m*)	31.7		31.6
3	3.55 (*m*)	71.9	3.58 (*tt*, 11.3, 5.3 Hz)	71.8
4	2.29 (*m*) 2.23 (*m*)	42.4		42.3
5		140.8		140.7
6	5.35 (*t*, 2.20 Hz)	121.8	5.40 (*dd*, 5.2, 2.3 Hz)	121.7
7	1.49 (*m*) 1.95 (*m*)	32.0		31.9
8	2.01 (*m*)	32.0		31.8
9	0.91 (*m*)	50.2		50.1
10		36.6		36.5
11	1.44 (*m*) 1.50 (*m*)	21.2		21.1
12	1.15 (*m*) 2.02 (*m*)	39.9		39.7
13		42.4		42.3
14	1.00 (*m*)	56.8		56.7
15	1.59 (*m*)	24.4		24.3
16	1.26 (*m*) 1.83 (*m*)	28.3		28.2
17	1.08 (*m*)	56.1		56.0
18	0.68 (*s*)	12.0	0.74 (*s*)	11.8
19	1.01 (*s*)	19.5	1.06 (*s*)	19.4
20	1.35 (*m*)	36.2		36.1
21	0.92 (*d*, 7.0 Hz)	18.9	0.93 (*d*, 6.50 Hz)	18.8
22	1.03 (*m*) 1.33 (*m*)	34.0		33.9
23	1.16 (*m*)	26.1		26.0
24	0.91 (*m*)	45.9		45.8
25	1.67 (*m*)	29.2		29.1
26	0.84 (*d*, 6.90 Hz)	19.9	0.88 (*d*, 6.90 Hz)	19.8
27	0.81 (*d*, 6.85 Hz)	19.0	0.87 (*d*, 6.90 Hz)	19.0
28	1.25 (*m*)	23.1		23.0
29	0.85 (*t*,7.45 Hz)	12.1	0.89 (*t*,7.40 Hz)	12.0

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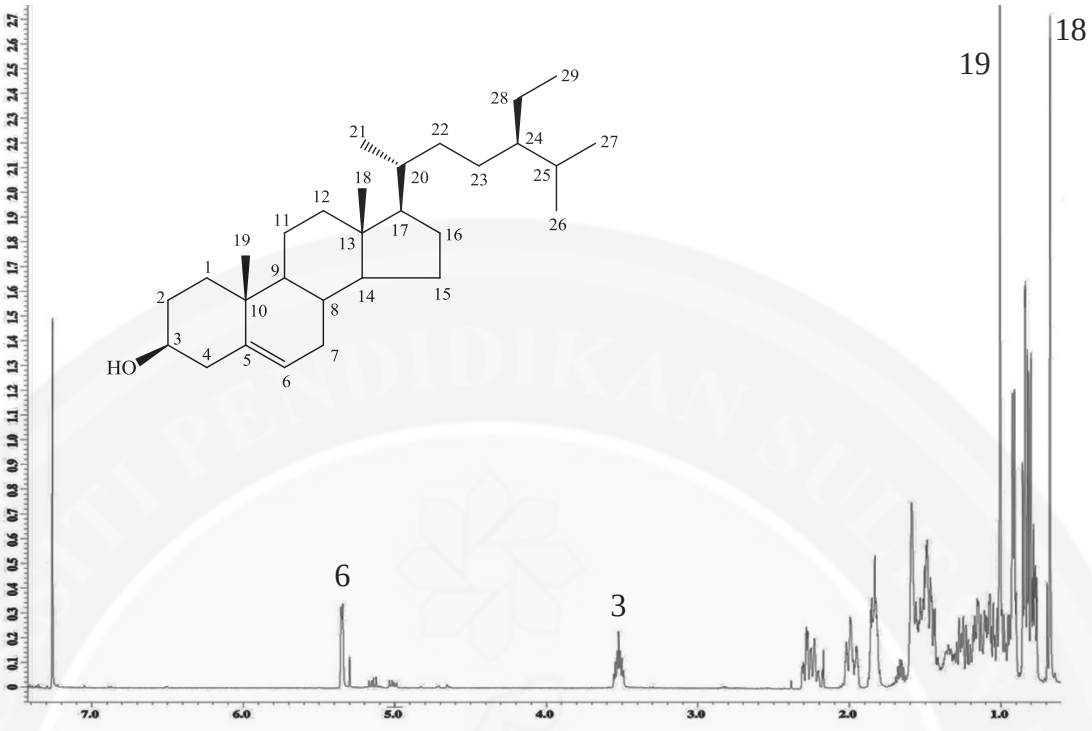


Figure 4.13. ^{1}H NMR spectrum of **115**

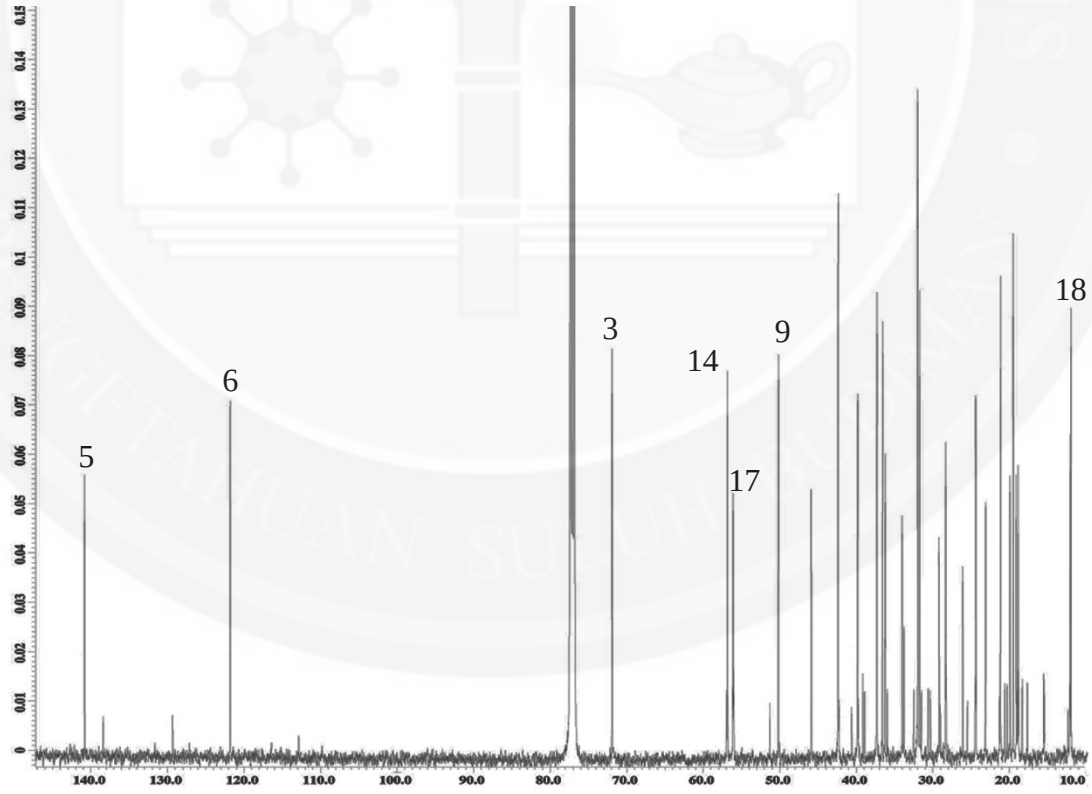


Figure 4.14. ^{13}C NMR spectrum of **115**

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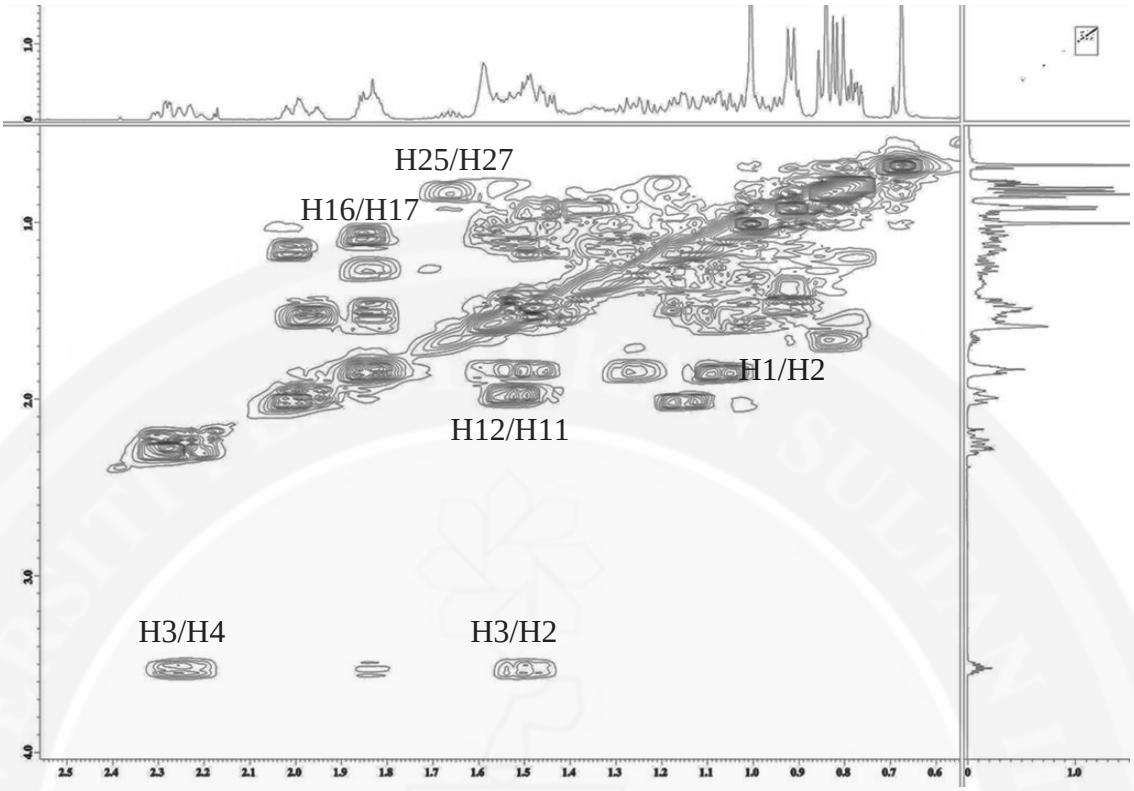


Figure 4.15. COSY spectrum of **115**

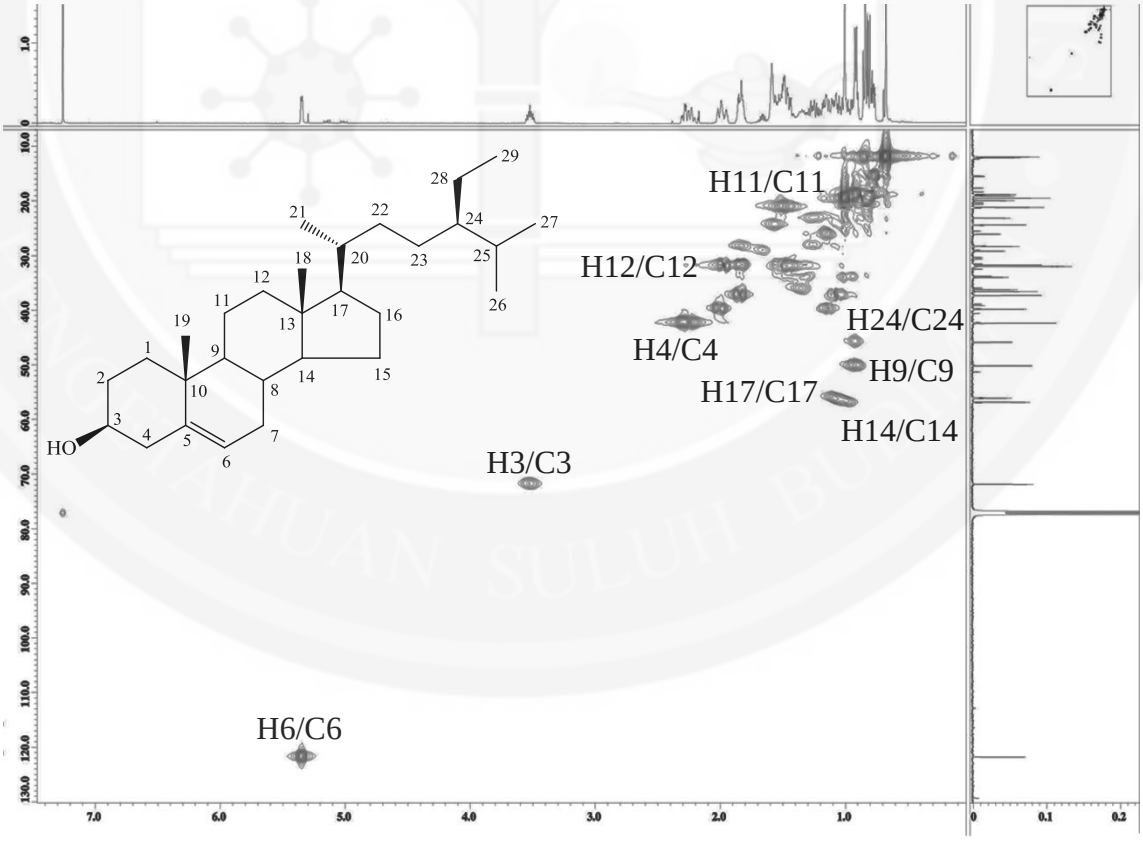


Figure 4.16. HMQC spectrum of **115**

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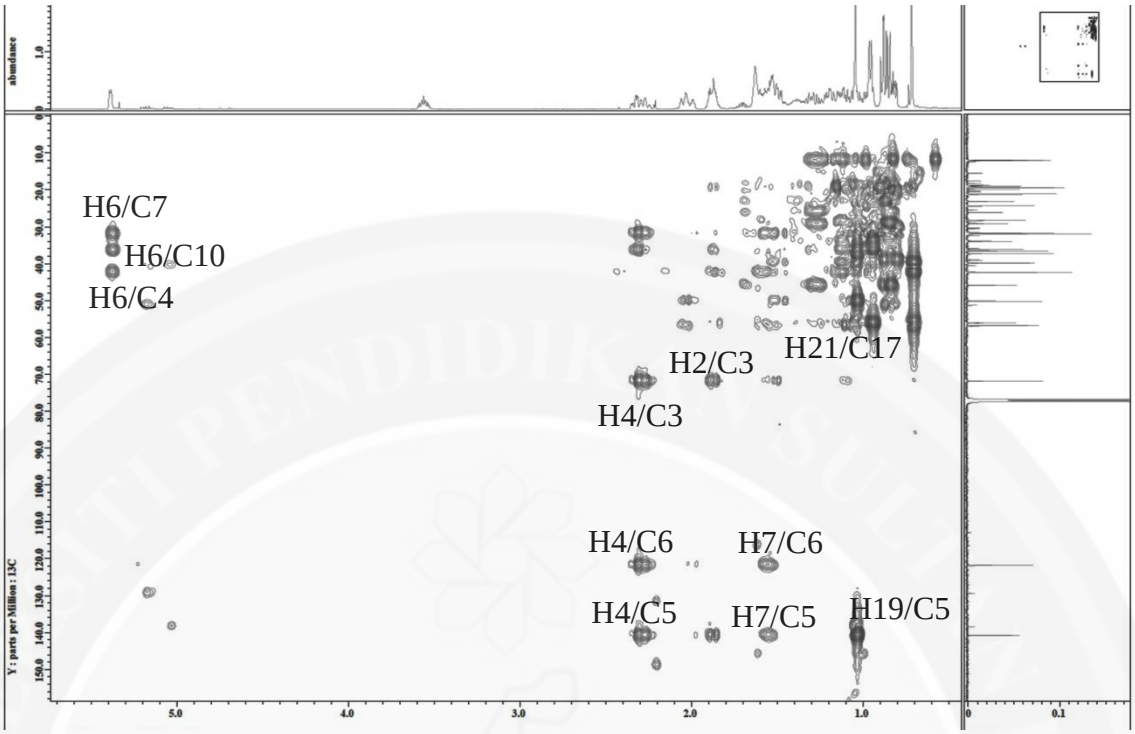


Figure 4.17. HMBC spectrum of **115**

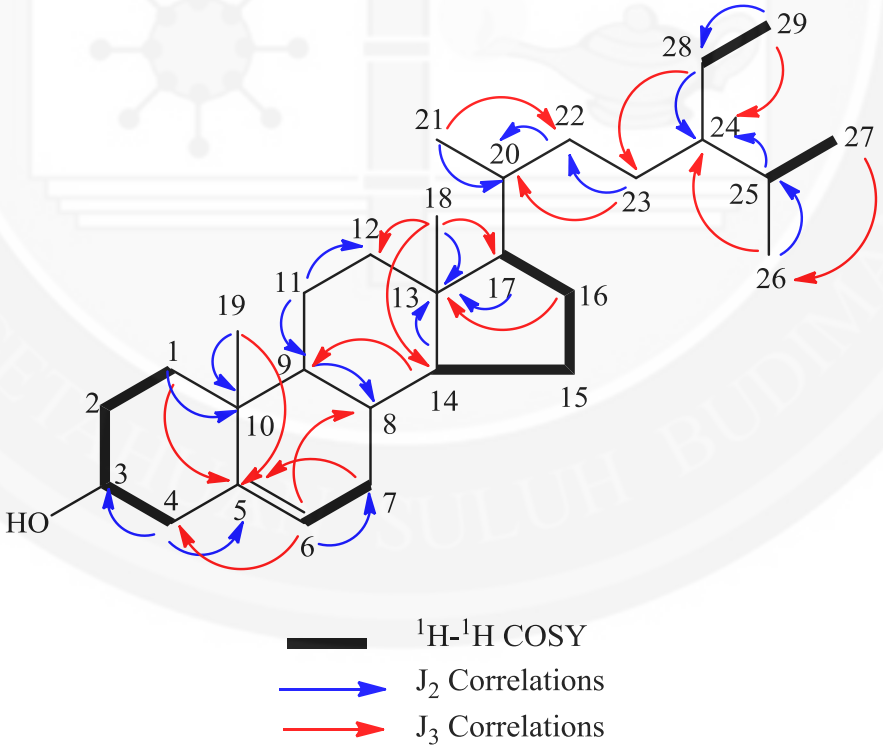
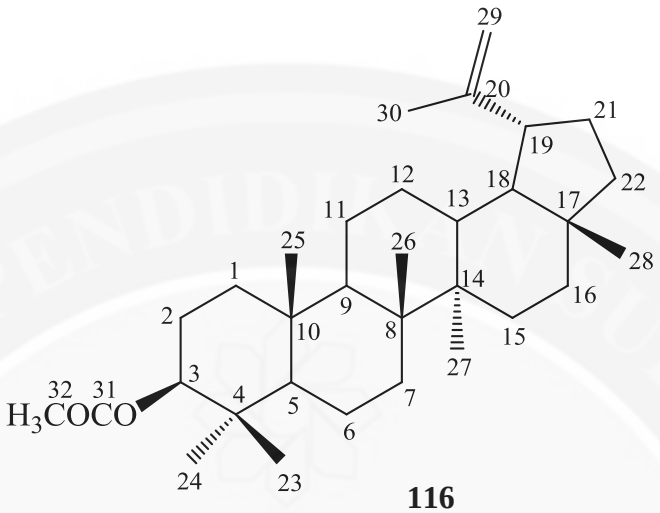


Figure 4.18. Selected COSY and HMBC of **115**

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4.2.4 Compound D: Lupeol acetate 116



116

Compound **D** was obtained as colourless amorphous. The IR spectrum showed stretching band for ester carbonyl (1712 cm^{-1}) and C-O (1263 cm^{-1}). The mass spectrum of **116** showed a molecular ion peak at *m/z* 468 $[M]^+$ corresponding to molecular formula $C_{32}H_{52}O_2$. The ^1H NMR spectrum (Table 4.4 and Figure 4.19) of **116** exhibited six methyl singlets at δ 0.84, 0.83, 0.85, 1.03, 0.94, 0.78 and a vinylic methyl at 1.68 ppm which corresponding to proton attached to C- 23, C-24, C 25, C-26, C-27, C- 28 and C-30 respectively. It was identified as triterpene acetate due to the presence of an acetate methyl signal at δ 2.05 as a singlet. H-3 showed proton signal at δ 4.47 (*dd*, *J* = 10.7 Hz, 5.5 Hz). The presence of two exo-methylene at δ 4.57 and δ 4.69 (H-29) and one methyl signal at δ 1.68 (H-30) reminiscent lupane skeleton.

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Analysis of ^{13}C NMR spectrum (Table 4.4 and Figure 4.20) showed the presence of thirty two carbon atoms indicating a mono acetyl derivative. There are six quaternary carbons δ 37.8 (C-4), δ 40.9 (C-8), δ 37.1 (C-10), δ 42.8 (C-14), δ 43.0 (C-17), δ 151.0 (C-20); six methine carbons δ 81.0 (C-3), δ 55.4 (C-5), δ 50.3 (C-9), δ 38.0 (C-13), δ 48.3 (C-18), δ 48.0 (C-19); eleven methylene carbons δ 38.4 (C-1), δ 23.7 (C-2), δ 18.0 (C-6), δ 34.2 (C-7), δ 20.9 (C-11), δ 25.1 (C-12), δ 27.4 (C-15), δ 35.6 (C-16), δ 29.8 (C-21), δ 40.0 (C-22), δ 109.4 (C-29); eight methyl carbons δ 28.0 (C-23), δ 16.5 (C-24), δ 16.2 (C-25), δ 16.0 (C-26), δ 14.5 (C-27), δ 18.2 (C-28), δ 19.3 (C-30), δ 21.3 (C-32) and the present of a carbonyl group at δ 171.1 (C-31).

The assignment was further substantiated by ^{1}H-^{1}H COSY (Figure 4.21), HSQC (Figure 4.22) and HMBC (Figure 4.23). ^{1}H-^{1}H COSY suggested the following fragments: C-1 – C-3, C-5 – C-7, C-9 – C-11 – C-12, C-15 – C-16, C-18 – C-19 and C-21 – C-22. A series of ^{1}H – ^{13}C long range correlation signals were observed in HMBC spectral. The HMBC correlations showed unambiguous assignment of the protons and carbons. It's showed cross signal from: H-1 to C-3 and C-5; H-3 to C-2, C-23, C-24 and C-31; H-11 to C-9, C-12 and C-13; H-19 to C-20, C-21 and C-30. Correlations from H-32 and H-3 verify that an acetoxyl group was connected to C-3. Figure 4.24 showed all the correlations of **116**. On the basic of the above spectral features, **116** was identified as lupeol acetate. The identified of **116** as lupeol acetate was confirmed comparison of these data with publish values of (Jamal & Ammar, 2009; Prachayasittikul et al., 2010; Ajithabai et al., 2011).

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Table 4.4

^{1}H NMR [600 MHz, δ_H (J, Hz)] and ^{13}C NMR [150 MHz, δ_C] of 116 in $CDCl_3$

	Lupeol acetate		Lupeol acetate [75 MHz, $CDCl_3$] [Prachayasittikul et al., 2010]	
Position	δ_H (*J* Hz), ppm	δ_C, ppm	δ_H (*J* Hz), ppm	δ_C, ppm
1	0.99 (*m*)	38.4		38.3
2	1.62 (*m*)	23.7		23.7
3	4.47 (*dd*, 10.7, 5.5 Hz)	81.0	4.44 (*dd*, 10.8, 5.8 Hz)	80.9
4		37.8		37.7
5	0.79 (*m*)	55.4	0.76 (*dd*, 10.8, 5.8 Hz)	55.3
6	1.50 (*m*)	18.2		18.1
	1.38 (*m*)			
7	1.49 (*m*)	34.2		34.2
	1.38 (*m*)			
8		40.9		40.8
9	1.28 (*m*)	50.3		50.3
10		37.1		37.0
11	1.39 (*m*)	20.9		20.9
	1.21 (*m*)			
12	1.66 (*m*)	25.1		25.0
	1.05 (*m*)			
13	1.65 (*m*)	38.0		38.0
14		42.8		42.8
15	1.67 (*m*)	27.4		27.4
	1.00 (*m*)			
16	1.47 (*m*)	35.6		35.5
	1.36 (*m*)			
17		43.0		42.9
18	1.35 (*m*)	48.3		48.0
19	2.40 (*m*)	48.0	2.33 (*dt*, 11.1, 5.6 Hz)	48.2
20		151.0		150.9
21	1.91 (*m*)	29.8		29.8
	1.33 (*m*)			
22	1.38 (*m*)	40.0		39.9
	1.18 (*m*)			
23	0.84 (*s*)	28.0	0.82 (*s*)	28.2
24	0.83 (*s*)	16.5	0.82 (*s*)	15.9
25	0.85 (*s*)	16.2	0.82 (*s*)	16.1
26	1.03 (*s*)	16.0	1.00 (*s*)	16.4
27	0.94 (*s*)	14.5	0.91 (*s*)	14.5
28	0.78 (*s*)	18.0	0.81 (*s*)	17.9
29	4.69 (*br, s*)	109.4	4.66 (*br, s*)	109.3
	4.57 (*br, s*)		4.54 (*br, s*)	
30	1.68 (*s*)	19.3	1.66 (*s*)	19.0
31		171.1		171.0
32	2.05 (*s*)	21.3	2.01 (*s*)	21.3

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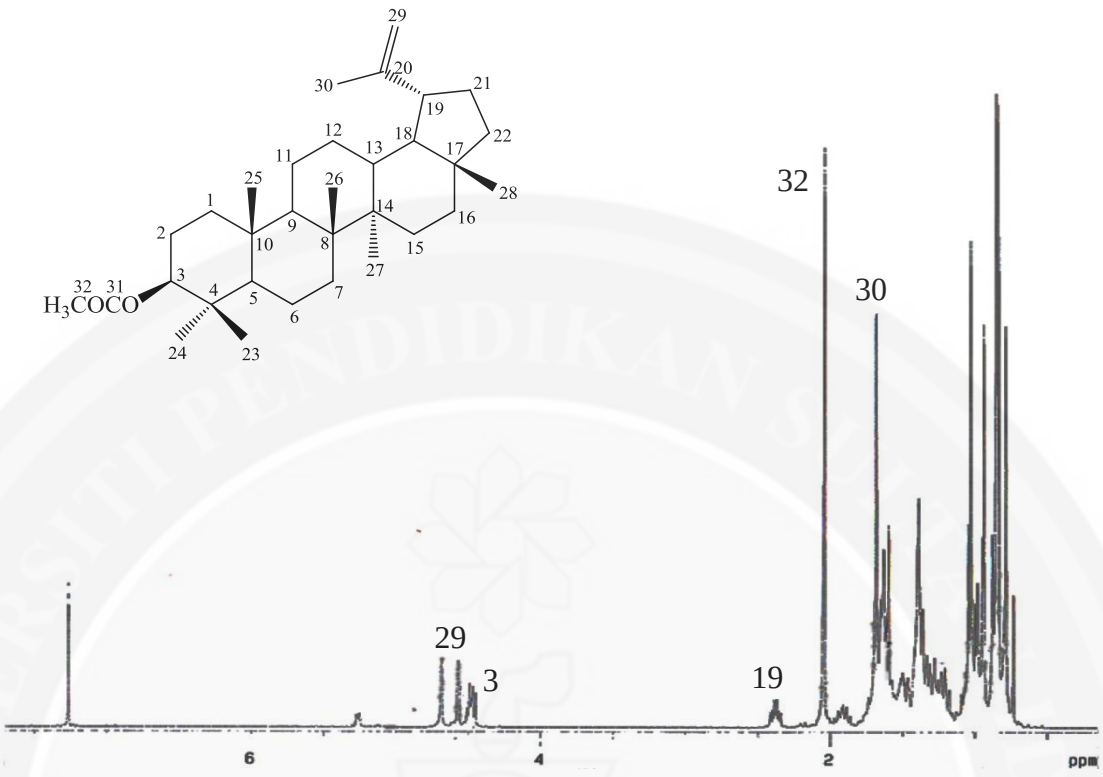


Figure 4.19. ^{1}H NMR spectrum of **116**

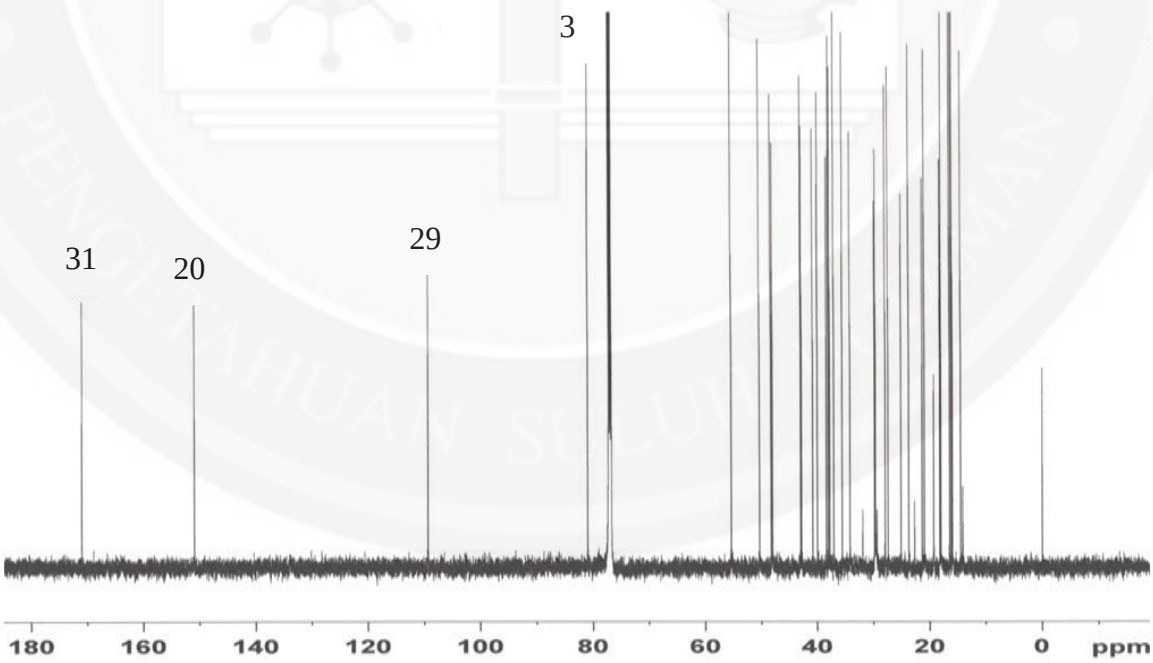


Figure 4.20. ^{13}C NMR spectrum of **116**

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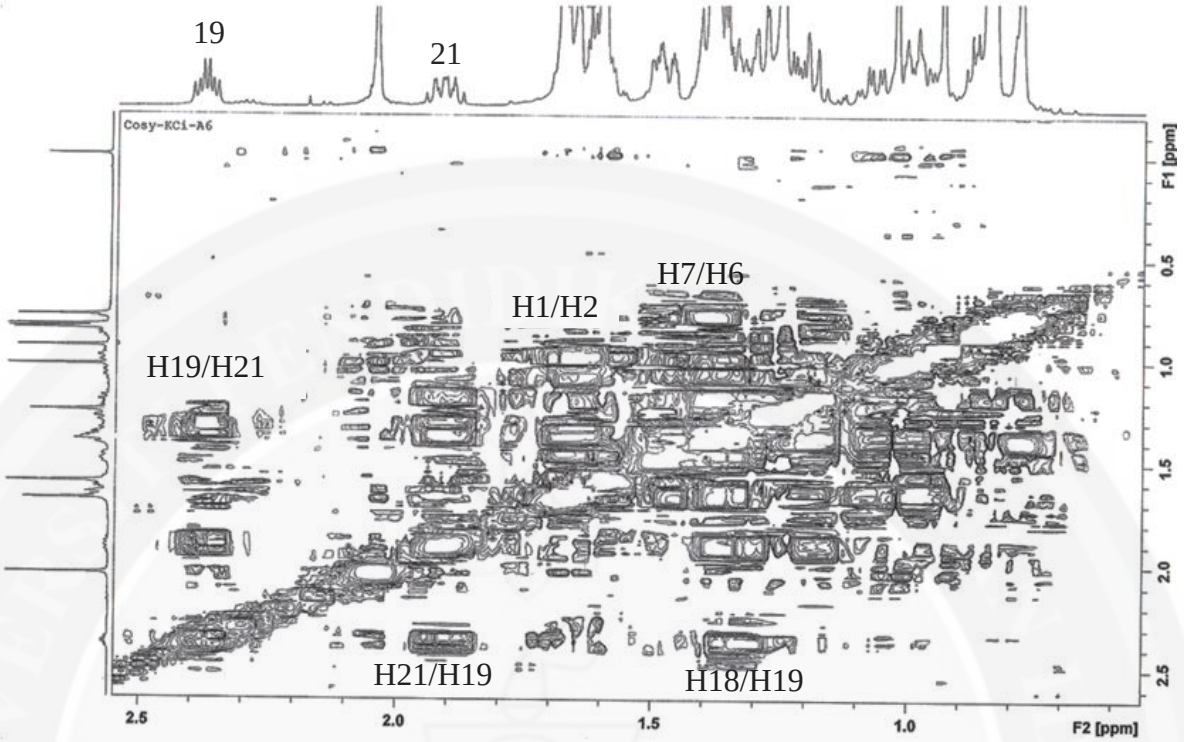


Figure 4.21. COSY spectrum of **116**

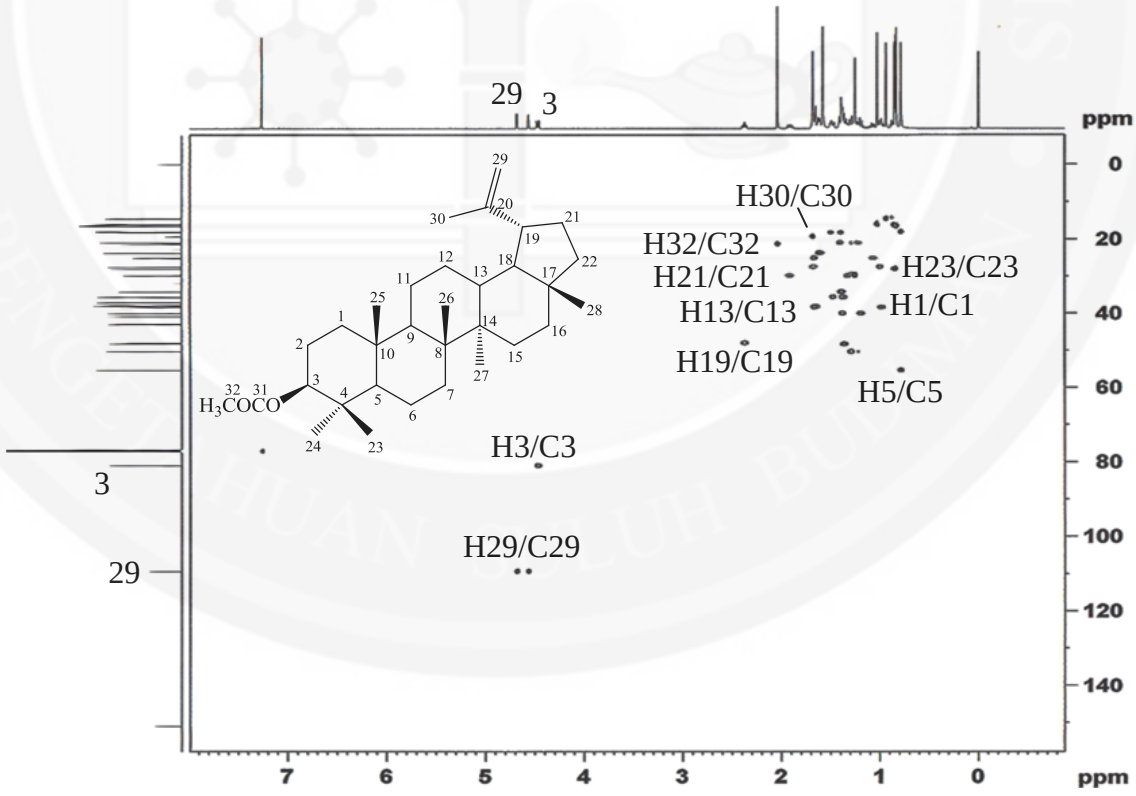


Figure 4.22. HSQC spectrum of **116**

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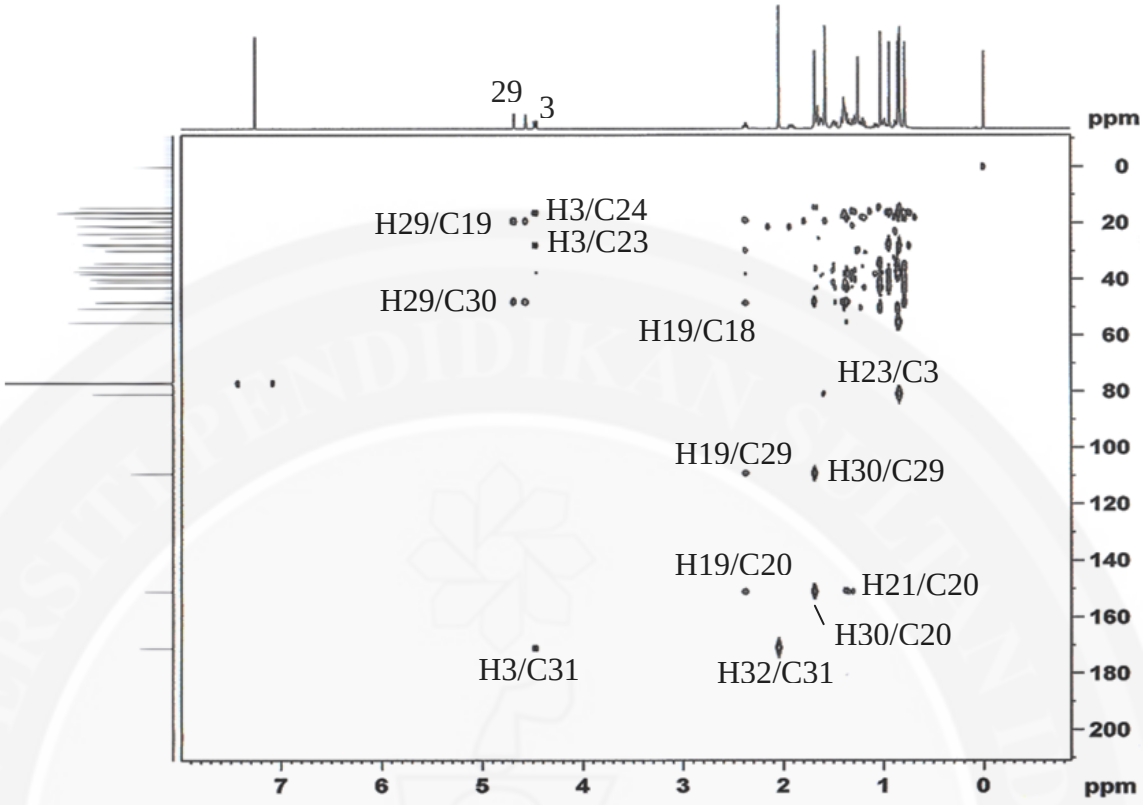


Figure 4.23. HMBC spectrum of **116**

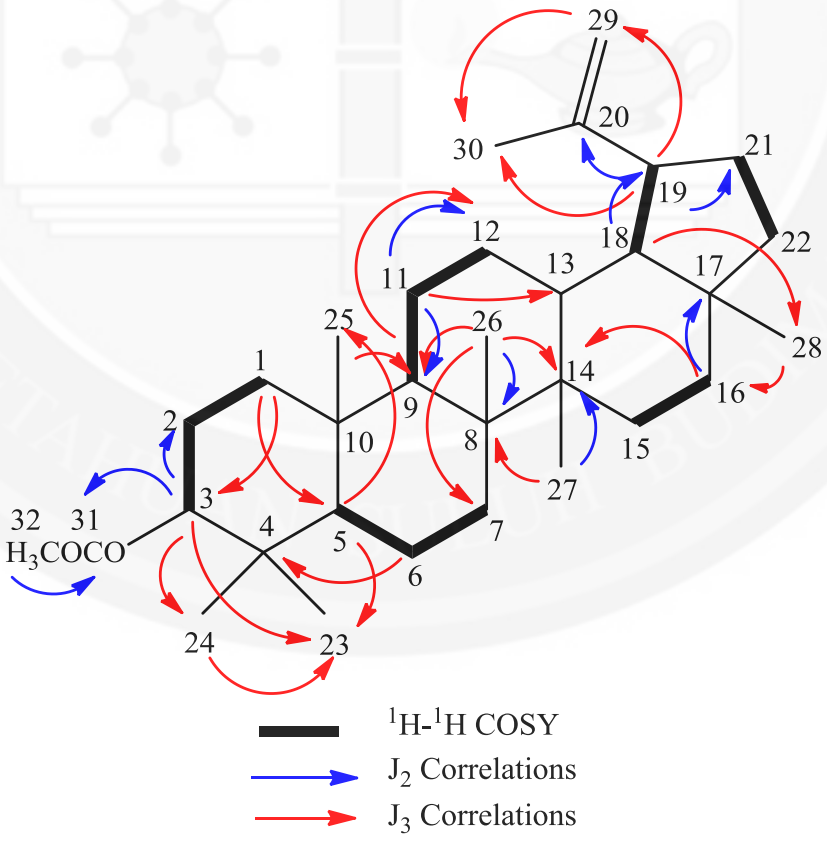
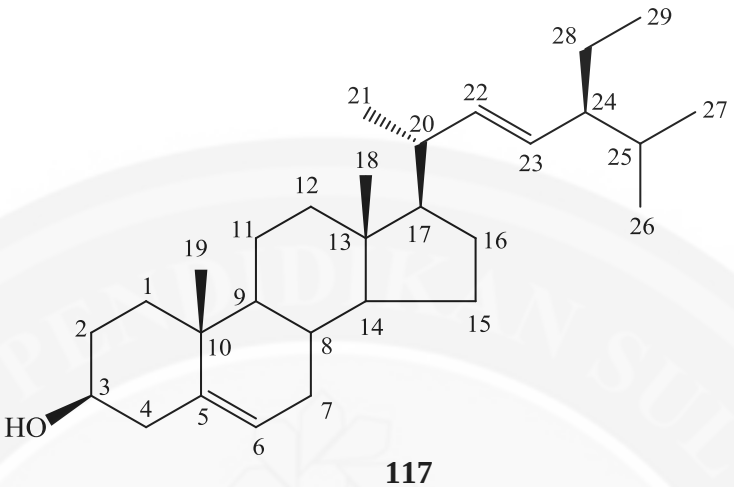


Figure 4.24. Selected COSY and HMBC of **116**

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4.2.5 Compound E: Stigmasterol 117



117

Compound **E** was obtained as colourless needles (m.p: 151.0 - 152.0˚C). The mass spectral data of **117** gave a molecular formula $C_{29}H_{48}O$, *m/z* 413 $[M+H]^+$. The IR spectrum displayed stretching band for O-H (3298 cm^{-1}), olefin (1640 cm^{-1}) and C-O (1019 cm^{-1}). ^1H NMR spectrum (Table 4.5 and Figure 4.25) consist of two methyl groups at δ 0.68 (H-18) and δ 1.00 (H-19) as a singlet, methyl doublets at δ 0.92 (*d, J* = 6.3 Hz, H-21), δ 0.85 (*d, J* = 6.9 Hz, H-26) and δ 0.79 (*d, J* = 6.8 Hz, H-27) and a methyl tripet at δ 0.80 (H-29). A hydroxyl methine proton resonated at δ 3.52 as a multiplet corresponding to proton (H-3). The position and multiplicity of which was indicative of H-3 of the steroidal nucleus. Three olefinic protons in which the last two are of *trans* configuration were observed at δ 5.35, δ 5.14 (*dd, J* = 15.5 Hz and 8.6 Hz) and δ 5.00 (*dd, J* = 15.5 Hz and 9.2 Hz) attributable to H-6, H-22 and H-23 respectively.

^{13}C NMR spectrum (Table 4.5 and Figure 4.26) of compound **117** exhibited twenty nine signals. Signal for six methyl carbons C-18, C-19, C-21, C-26, C-27 and C-29 could be seen in the most upfield region of the NMR spectrum at δ 12.0, δ 19.5,

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δ 21.2, δ 21.2 δ 19.1 and δ 12.1. The position of three quaternary carbons appreared at δ 140.8 (C-5), δ 36.6 (C-10), δ 42.3 (C-13); nine methylene carbons: δ 37.3 (C-1), δ 31.7 (C-2), δ 42.4 (C-4), δ 31.9 (C-7), δ 21.2 (C-11), δ 39.8 (C-12), δ 24.4 (C-15), δ 29.0 (C-16), δ 29.8 (C-28); and eleven methine carbons: δ 71.9 (C-3), δ 121.8 (C-6), δ 31.9 (C-8), δ 50.2 (C-9), δ 56.8 (C-14), δ 56.0 (C-17), δ 40.6 (C-20), δ 138.4 (C-22), δ 129.3 (C-23), δ 51.3 (C-24), δ 45.9 (C-25). Signal at δ 140.8, δ 121.8, δ 138.3 and δ 129.3 were observed for four olefinic carbons, corresponding to C-5, C-6, C-22 and C-23 respectively. A deshielded signal of δ 71.9 confirmed the presence of hydroxyl group attached to C-3. This compound was found to be a C-5 unsaturated modified tetracyclic triterpene, which showed only 29 carbons instead of 30 carbons for tetracyclic triterpenes.

The unambiguous assignment was further comfirmed with the help of ^{1}H-^{1}H COSY (Figure 4.27), HMQC (Figure 4.28) and long range coupling in HMBC (Figure 4.29). The ^{1}H-^{1}H COSY revealed the following fragmenst: C-1 – C-4, C-6 – C-8 –C-14 – C-17 – C-20 – C-22 – C-26, C-25 – C-27, C-24 – C-28 – C-29 and C-9 – C-11 – C-12. The HMBC showed the correlations of the protons and carbons. It showed the cross peak from H-3 to C-1 and C-2; H-4 to C-3, C-5, C-6 and C-10; H-6 to C-4 and C-7; H-18 to C-13 and C-17; H-22 to C-21, C-23 and C-24; H-23 to C-20 and C-24. Other correlations were showed in Figure 4.30. Comparison of these ^{1}H and ^{13}C NMR data with literature (Jamal, Yaacob, & Laily, 2009; Pateh et al., 2009) showed that **117** is stigmasterol. Stigmasterol **117** have same skeleton with β-sitosterol **115**, both of the compound are steroid. The only difference between the two compound is the presence of C_{22}=C_{23} double bond in **117** and C_{22}-C_{23} single bond in **115**.

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Table 4.5

^{1}H NMR [500 MHz, δ_H (J, Hz)] and ^{13}C NMR [125 MHz, δ_C] of 117 in $CDCl_3$

	Stigmasterol		Stigmasterol [100 MHz,$CDCl_3$] (Pateh et al., 2009)	
Position	δ_H (*J* Hz), ppm	δ_C, ppm	δ_H (*J* Hz), ppm	δ_C, ppm
1	1.07 (*m*)	37.3		37.3
	1.83 (*m*)			
2	1.52 (*m*)	31.7		31.6
	1.83 (*m*)			
3	3.52 (*m*)	71.9	3.52 (*m*)	71.8
4	2.23 (*m*)	42.4		42.3
	2.28 (*dd*, 4.6, 1.7 Hz)			
5		140.8		140.8
6	5.35 (*br*, *s*)	121.8	5.36 (*br*, *s*)	121.7
7	1.46 (*m*)	31.9		31.9
	1.99 (*m*)			
8	1.52 (*m*)	31.9		31.9
9	0.92 (*m*)	50.2		51.2
10		36.6		36.5
11	1.52 (*m*)	21.2		21.1
12	1.15 (*m*)	39.8		39.7
	2.02 (*m*)			
13		42.3		42.3
14	0.99 (*m*)	56.8		56.9
15	1.02 (*m*)	24.4		24.4
	1.58 (*m*)			
16	1.25 (*m*) 1.68 (*m*)	29.0		28.4
17	1.07 (*m*)	56.0		56.1
18	0.68 (*s*)	12.0	0.70 (*s*)	11.0
19	1.00 (*s*)	19.5	1.01 (*s*)	21.2
20	2.02 (*m*)	40.6		40.5
21	0.92 (*d*, 6.3 Hz)	21.2	1.02 (*d*, 7.5 Hz)	21.2
22	5.14 (*dd*, 15.5, 8.6 Hz)	138.3		138.3
23	5.00 (*dd*, 15.5, 9.2 Hz)	129.3		129.3
24	1.52 (*m*)	51.3		51.2
25	1.50 (*m*)	32.0		31.9
26	0.85 (*d*, 6.9 Hz)	21.2	0.80 (*d*, 6.5 Hz)	21.2
27	0.79 (*d*, 6.8 Hz)	19.1	0.85 (*d*, 6.5 Hz)	19.0
28	1.00 (*m*)	25.5		25.4
29	0.80 (*t*, 6.9 Hz)	12.1	0.80 (*t*, 7.5 Hz)	12.1

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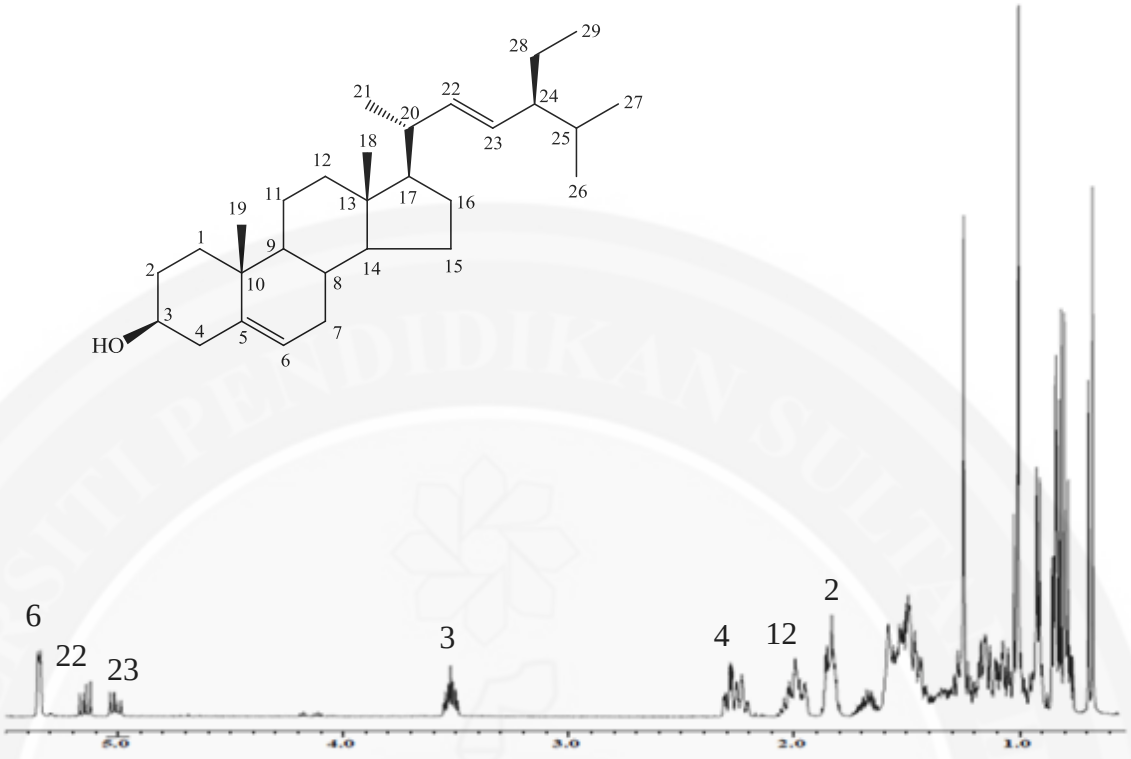


Figure 4.25. ^{1}H NMR spectrum of **117**

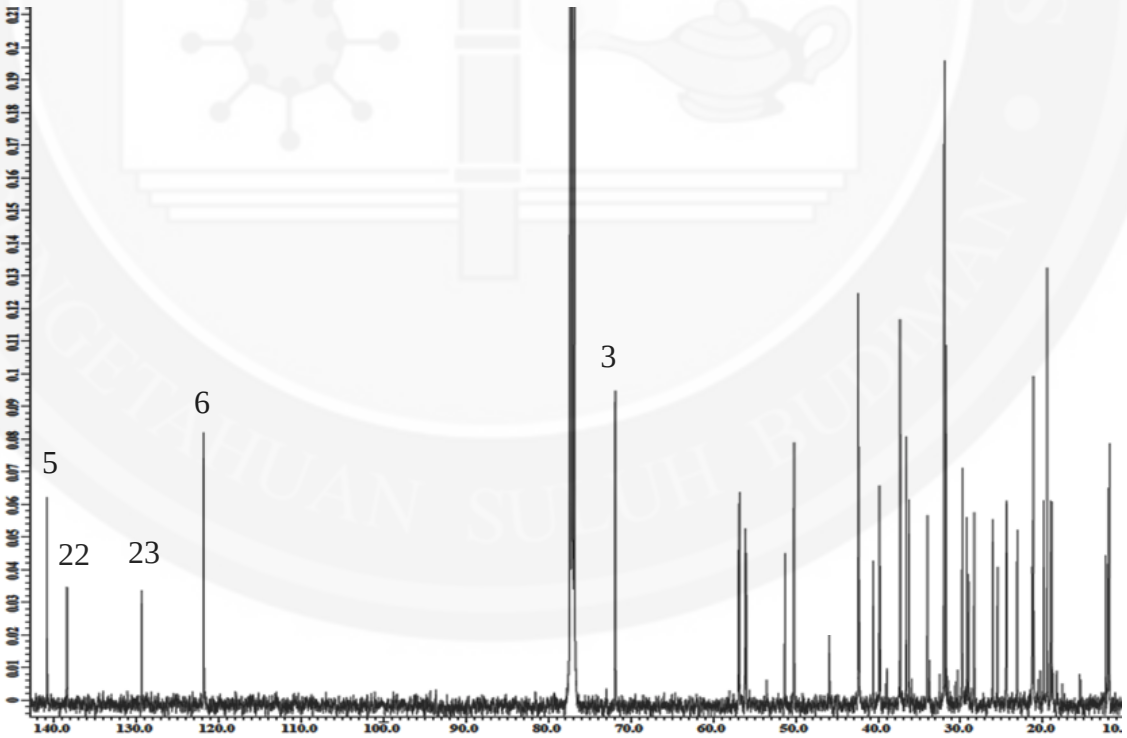


Figure 4.26. ^{13}C NMR spectrum of **117**

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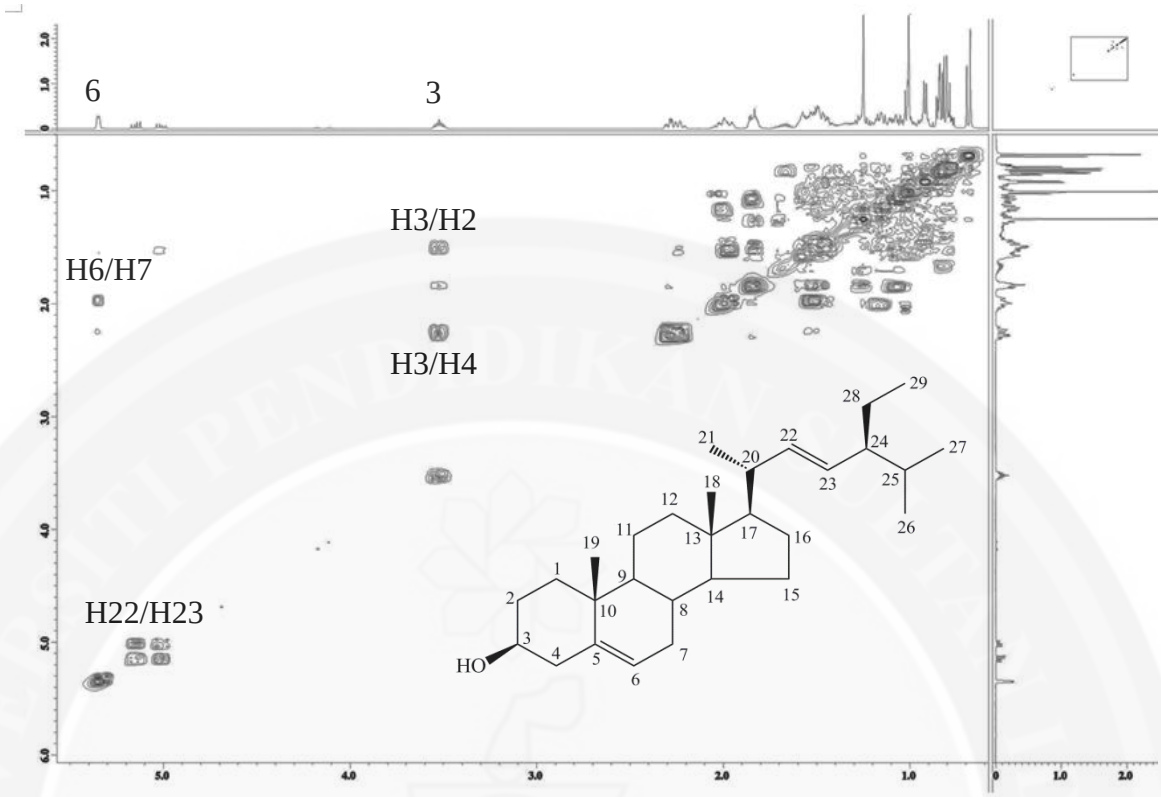


Figure 4.27. COSY spectrum of **117**

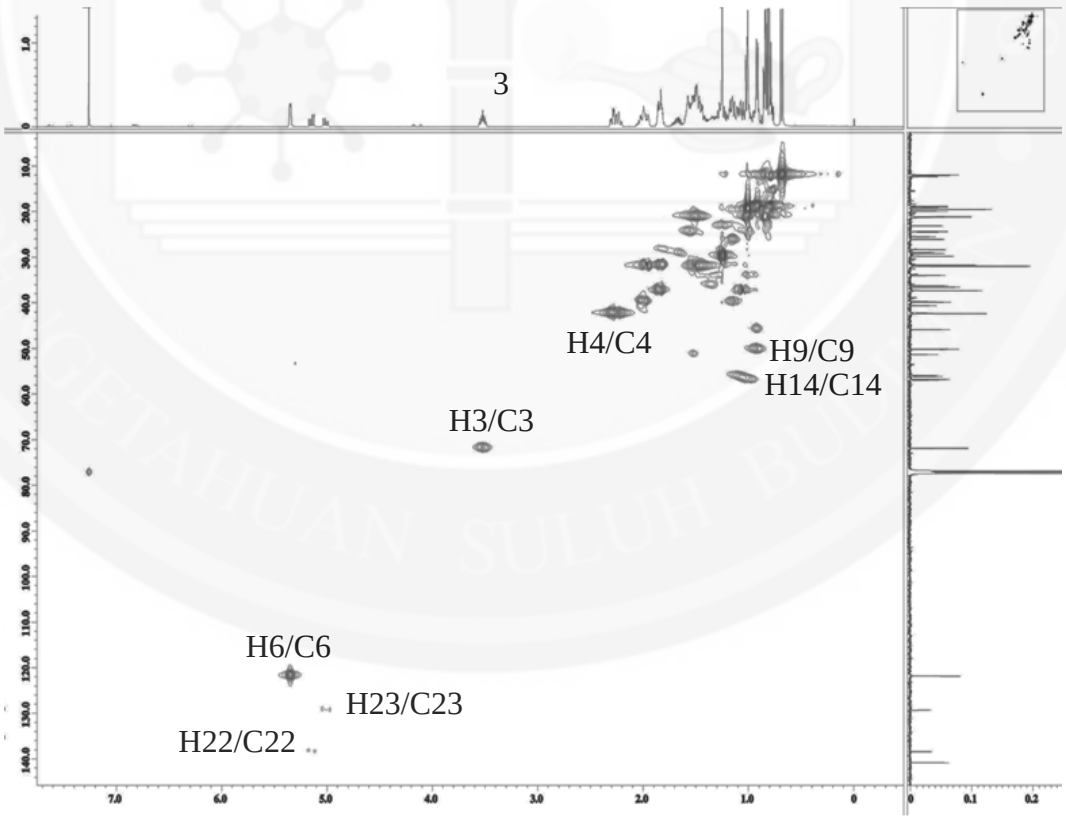


Figure 4.28. HMQC spectrum of **117**

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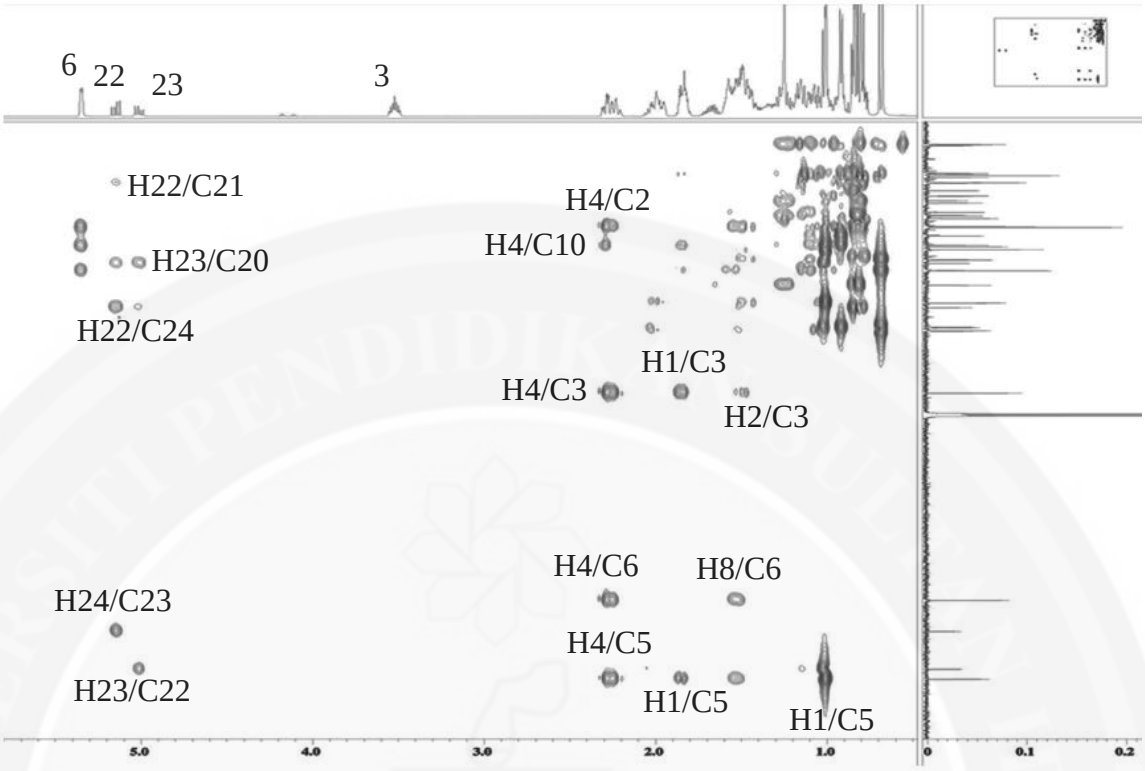


Figure 4.29. HMBC spectrum of **117**

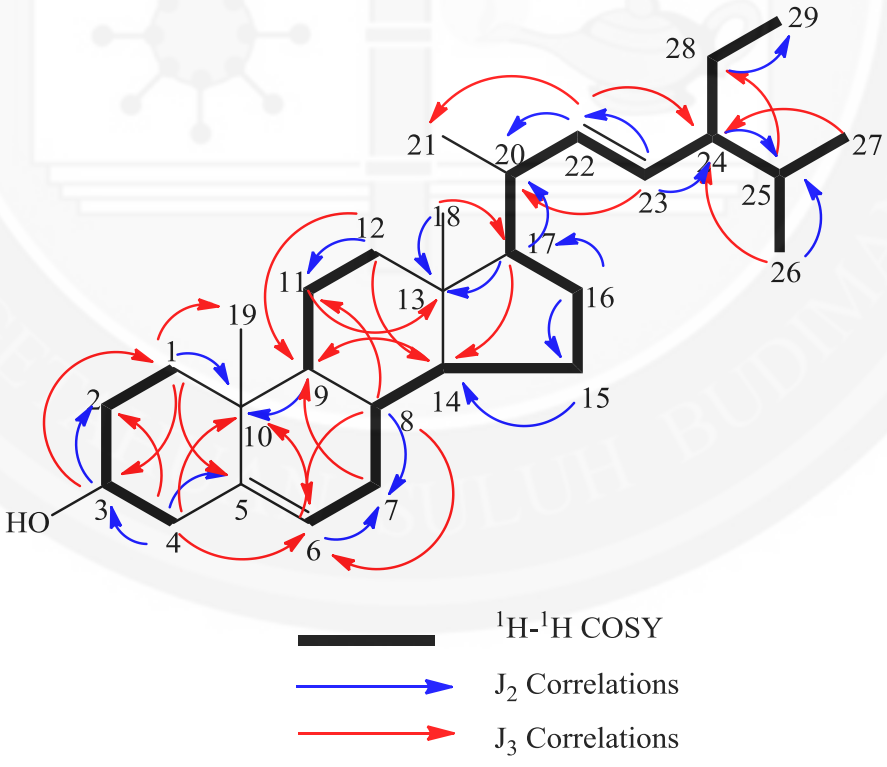
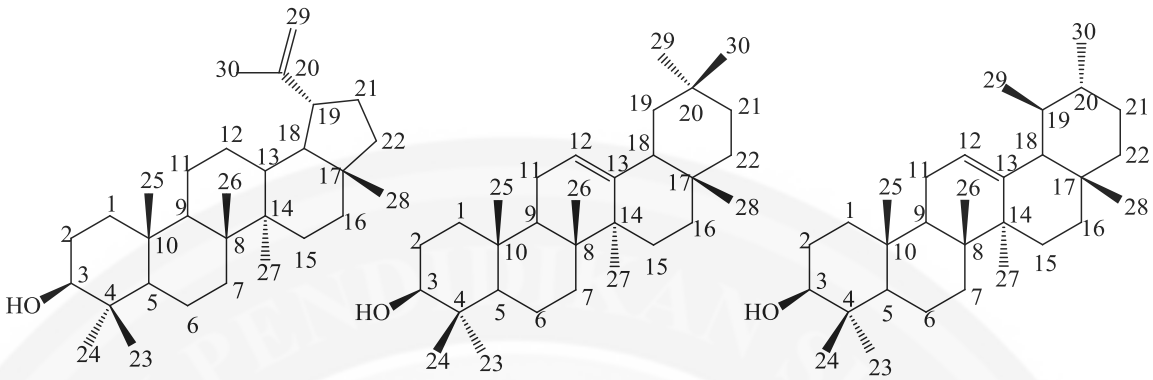


Figure 4.30. Selected COSY and HMBC of **117**

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4.2.6 Compound F: Mixture of Lupeol 118, β-Amyrin 113 and α-Amyrin 119



118

113

119

Compound **F** was isolated as a white amorphous mixture. The mixture was shown as a single spot on the TLC plate with R_f value 0.58. From the ^{13}C NMR spectral data, three layers of carbon were clearly showed. Structures of the compound of these triterpene mixtures were revealed by spectral data, mainly ^{13}C NMR through chemical shift and multiplicity of the signals of carbons atoms and deduced by comparison with those corresponding compounds with the previous work (Sobrinho et al., 1991).

The IR spectra data of the mixture showed absorption at 3278 cm^{-1}(O-H), 1709 (olefin) cm^{-1} and 1096 cm^{-1} (C-O). The mass spectrum of **F** showed a molecular ion peak at *m/z* 426 $[M]^+$ corresponding to molecular formula $C_{30}H_{50}O$.

118 was found to be lupane type triterpene due to ^1H NMR spectra (Figure 4.31) of **118** displayed a pair of broad singlets due to exo-methylene protons (H-29) at δ 4.57 and δ 4.69. A signal at δ 3.20 represented to the H-3 for all three triterpenes. As results of three compounds were mix together, signal at δ 3.20 could not observe as double of doublet. In addition, signal at δ 151.2 (C-20) and δ 109.5 (C-29) are the

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characteristic of triterpenes with lupane skeleton. The ^{13}C NMR (Table 4.6 and Figure 4.32) of the compound showed 30 signals for the terpenoid of lupane skeleton which includes a carbon bonded to the hydroxyl group at C-3 position appeared at δ 79.1, while the olefinic carbons of the exo-cyclic double bond appeared at δ 151.2 (C-20) and δ 109.5 (C-29). Among the 30 carbons, the ^{13}C NMR data indicated the presence of six quaternary carbons δ 38.9 (C-4), δ 40.9 (C-8), δ 37.3 (C-10), δ 42.9 (C-14), δ 43.1 (C-17), δ 151.1 (C-20); six methine carbons δ 79.1 (C-3), δ 55.3 (C-5), δ 50.5 (C-9), δ 38.2 (C-13), δ 48.4 (C-18), δ 48.1 (C-19); eleven methylene carbons δ 38.8 (C-1), δ 27.5 (C-2), δ 18.4 (C-6), δ 34.4 (C-7), δ 21.0 (C-11), δ 25.3 (C-12), δ 27.5 (C-15), δ 35.7 (C-16), δ 29.9 (C-21), δ 40.1 (C-22), δ 109.9 (C-29); seven methyl carbons δ 28.1 (C-23), δ 15.5 (C-24), δ 16.2 (C-25), δ 16.1 (C-26), δ 14.6 (C-27), δ 18.1 (C-28), δ 19.4 (C-30). Thus, the structure of **118** was assigned as lupeol that was consistent to the reported literature values.

Two proton signals were very characteristic of the two different triterpene skeletons. A downfield signal at δ 5.l8 (*t*, *J* = 6.9 Hz) and signal at δ 5.l2 (*t*, *J* = 7.5 Hz) was attributed to an olefinic proton at H-12 which was assigned to β-amyrin **113** and α-amyrin **119** respectively. Signal at δ 3.20 was also assignable to the carbinylic proton (H-3) for both compounds.

As the chemical shift of a sp^2 carbon atom is very characteristic for each triterpenoid skeleton, ^{13}C NMR spectroscopy has been very frequently employed for the structural analysis of triterpene mixture (Mahato, Nandy & Roy, 1992). A pair of downfield carbon signal at δ 121.8 and δ 145.3 represented the C-12 and C-13

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unsaturation of the β-amyrin **113**. The ^{13}C NMR spectrum (Table 4.7) confirmed **119** to be α-amyrin. The presence of signals at δ 124.5 (C-12) and δ 139.7 (C-13) support this proposal. The both β-amyrin **113** and α-amyrin **119** were further confirmed by DEPT spectrum (Figure 4.33). Position at C-20 of β-amyrin **113** showed that it is quaternary carbon. However, DEPT spectrum revealed that both C-19 and C-20 were tertiary carbon for α-amyrin **119**.

Analysis of α-amyrin **119** ^{13}C NMR spectrum (Table 4.7) also revealed the presence of 30 carbon atoms. There are six quaternary carbons: δ 38.9 (C-4), δ 40.1 (C-8), δ 37.0 (C-10), δ 139.7 (C-13), δ 42.2 (C-14) and δ 33.8 (C-17); seven methine carbons: δ 79.1 (C-3), δ 55.2 (C-5), δ 47.8 (C-9), δ 124.5 (C-12), δ 59.1 (C-18), δ 39.8 (C-19) and δ 39.7 (C-20); nine methylene carbons: δ 38.8 (C-1), δ 27.5 (C-2), δ 18.4 (C-6), δ 33.0 (C-7), δ 23.5 (C-11), δ 28.1 (C-15), δ 26.7 (C-16), δ 31.3 (C-21) and δ 41.6 (C-22); eight methyl carbons: δ 28.8 (C-23), δ 15.7 (C-24), δ 15.8 (C-25), δ 16.9 (C-26), δ 23.4 (C-27), δ 28.2 (C-28), δ 17.6 (C-29) and δ 21.5 (C-30). Olefin carbon showed at δ 124.5 (C-12) and δ 139.7 (C-13) as well as hydroxyl carbon signal at δ 79.1 (C-3) were also observed.

The ^{13}C NMR spectrum of β-amyrin **113** (Table 4.8) showed a total of 30 carbon atoms. There are seven quaternary carbons: δ 39.8 (C-4), δ 40.1 (C-8), δ 37.0 (C-10), δ 145.3 (C-13), δ 41.8 (C-14), δ 32.6 (C-17), δ 31.2 (C-20); five methine carbons δ 79.1 (C-3), δ 55.3 (C-5), δ 47.7 (C-9), δ 121.8 (C-12), δ 47.3 (C-18); ten methylene carbons δ 38.7 (C-1), δ 27.4 (C-2), δ 19.4 (C-6), δ 32.7 (C-7), δ 23.8 (C-11), δ 28.2 (C-15), δ 27.0 (C-16), δ 46.9 (C-19), δ 34.8 (C-21), δ 36.7 (C-22); eight

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methyl carbons δ 29.7 (C-23), δ 15.6 (C-24), δ 15.7 (C-25), δ 16.9 (C-26), δ 26.2 (C-27), δ 28.5 (C-28), δ 33.4 (C-29), δ 23.6 (C-30). The alkene carbon appeared at δ 121.8 (C-12) and δ 145.3 (C-13) and hydroxyl carbon signal at δ 79.1 (C-3) were also observed.

Based on the above spectral data, compound F was assigned as mixture of three compounds: lupeol (50%), β-amyrin (20%) and α-amyrin (30%) further supported by the physical and spectral data reported from the literature (Menezes, Borsatto, Pereira, Matos & Kaplan, 1998; Thanakijcharoenpath & Theanphong, 2007; Mouffok et al., 2012).

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Table 4.6

^{1}H NMR [500 MHz, δ_H (J, Hz)] and ^{13}C NMR [125 MHz, δ_C] of 118 in $CDCl_3$

Position	δ_C, ppm	DEPT	Lupeol [100 MHz, $CDCl_3$] [Mouffok et al., 2012]
1	38.8	CH_2	38.7
2	27.5	CH_2	27.5
3	79.1	CH	79.3
4	38.9	Q	39.8
5	55.3	CH	55.5
6	18.4	CH_2	19.0
7	34.4	CH_2	34.2
8	40.9	Q	41.1
9	50.5	CH	50.9
10	37.3	Q	37.2
11	21.0	CH_2	21.2
12	25.3	CH_2	25.3
13	38.2	CH	38.5
14	42.9	Q	42.8
15	27.5	CH_2	27.2
16	35.7	CH_2	35.9
17	43.1	Q	43.2
18	48.4	CH	48.5
19	48.1	CH	47.8
20	151.1	Q	151.2
21	29.9	CH_2	30.1
22	40.1	CH_2	40.3
23	28.1	CH_3	28.4
24	15.5	CH_3	15.6
25	16.2	CH_3	16.2
26	16.1	CH_3	16.1
27	14.6	CH_3	14.8
28	18.1	CH_3	18.1
29	109.9	CH_2	109.5
30	19.4	CH_3	19.8

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Table 4.7

^{1}H NMR [500 MHz, δ_H (J, Hz)] and ^{13}C NMR [125 MHz, δ_C] of 119 in $CDCl_3$

Position	δ_C, ppm	DEPT	α-Amyrin [50.2 MHz, $CDCl_3$] [Menezes, 1998]
1	38.8	CH_2	38.7
2	27.5	CH_2	27.2
3	79.1	CH	78.3
4	38.9	Q	38.7
5	55.2	CH	55.2
6	18.4	CH_2	18.3
7	33.0	CH_2	32.9
8	40.1	Q	40.0
9	47.8	CH	47.7
10	37.0	Q	36.9
11	23.5	CH_2	23.3
12	124.5	CH	124.3
13	139.7	Q	139.3
14	42.2	Q	42.0
15	28.1	CH_2	28.7
16	26.7	CH_2	26.6
17	33.8	Q	33.7
18	59.1	CH	58.9
19	39.8	CH	39.6
20	39.7	CH	39.6
21	31.3	CH_2	31.2
22	41.6	CH_2	41.5
23	28.8	CH_3	28.1
24	15.7	CH_3	15.6
25	15.8	CH_3	15.6
26	16.9	CH_3	16.8
27	23.4	CH_3	23.3
28	28.2	CH_3	28.1
29	17.6	CH_3	17.4
30	21.5	CH_3	21.3

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Table 4.8

^{1}H NMR [500 MHz, δ_H (J, Hz)] and ^{13}C NMR [125 MHz, δ_C] of 113 in $CDCl_3$

Position	δ_C, ppm	DEPT	β -amyrin [125 MHz, $CDCl_3$] [Thanakijcharoenpath & Theanphong, 2007]
1	38.7	CH_2	38.6
2	27.4	CH_2	27.2
3	79.1	CH	79.0
4	39.8	Q	38.8
5	55.3	CH	55.2
6	19.4	CH_2	18.4
7	32.7	CH_2	32.5
8	40.1	Q	39.8
9	47.7	CH	47.6
10	37.0	Q	36.9
11	23.8	CH_2	23.6
12	121.8	CH	121.7
13	145.3	Q	145.2
14	41.8	Q	41.7
15	28.2	CH_2	26.1
16	27.0	CH_2	26.9
17	32.6	Q	32.6
18	47.3	CH	47.2
19	46.9	CH_2	46.8
20	31.2	Q	31.1
21	34.8	CH_2	34.7
22	36.7	CH_2	37.1
23	29.7	CH_3	28.1
24	15.6	CH_3	15.5
25	15.7	CH_3	15.6
26	16.9	CH_3	16.8
27	26.2	CH_3	26.0
28	28.5	CH_3	28.4
29	33.4	CH_3	33.3
30	23.6	CH_3	23.7

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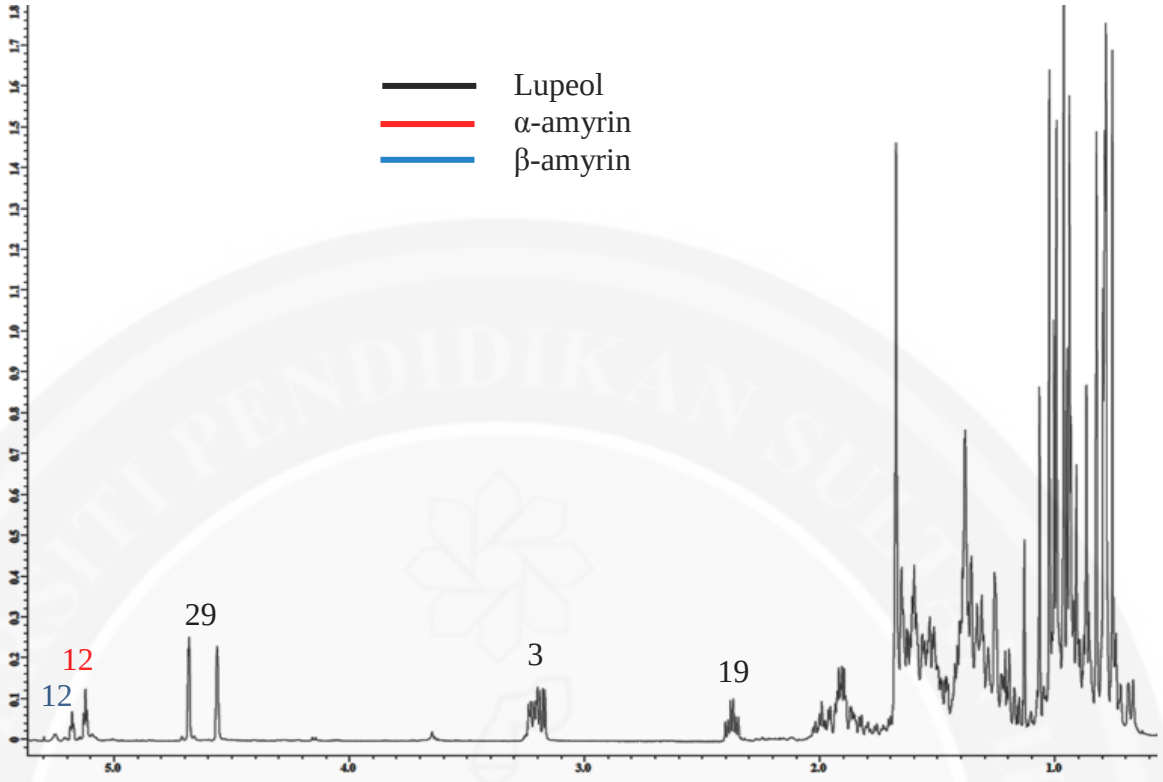


Figure 4.31. ^{1}H NMR spectrum of compound **F**

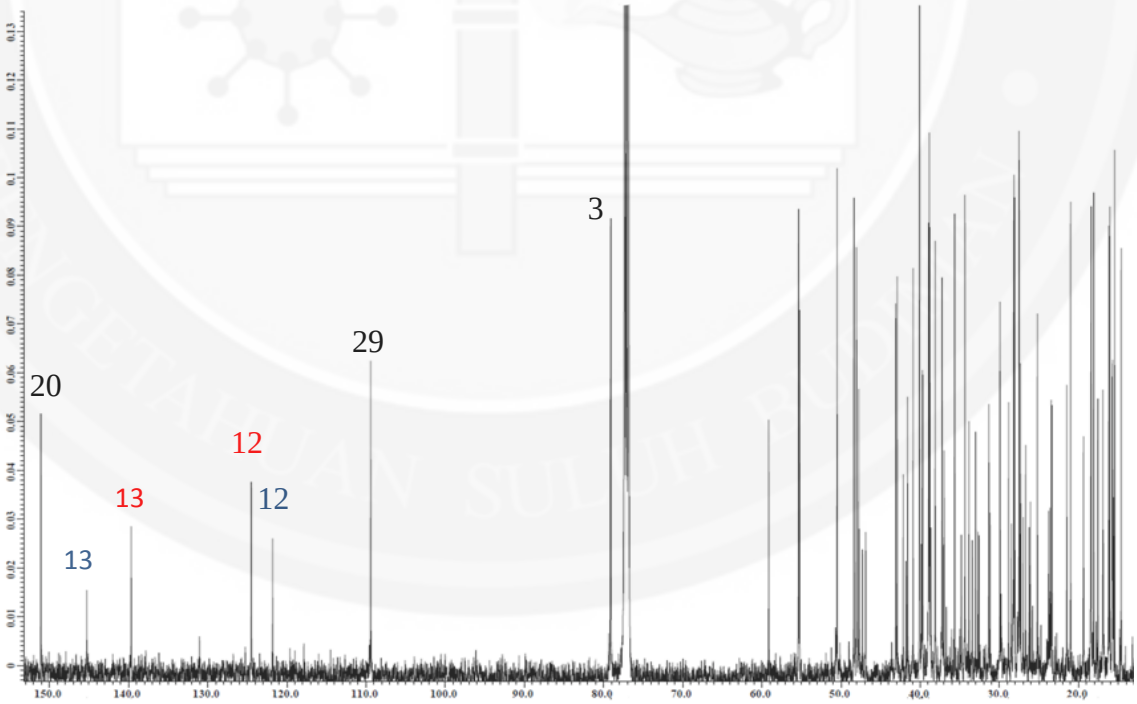


Figure 4.32. ^{12}C NMR spectrum of compound **F**

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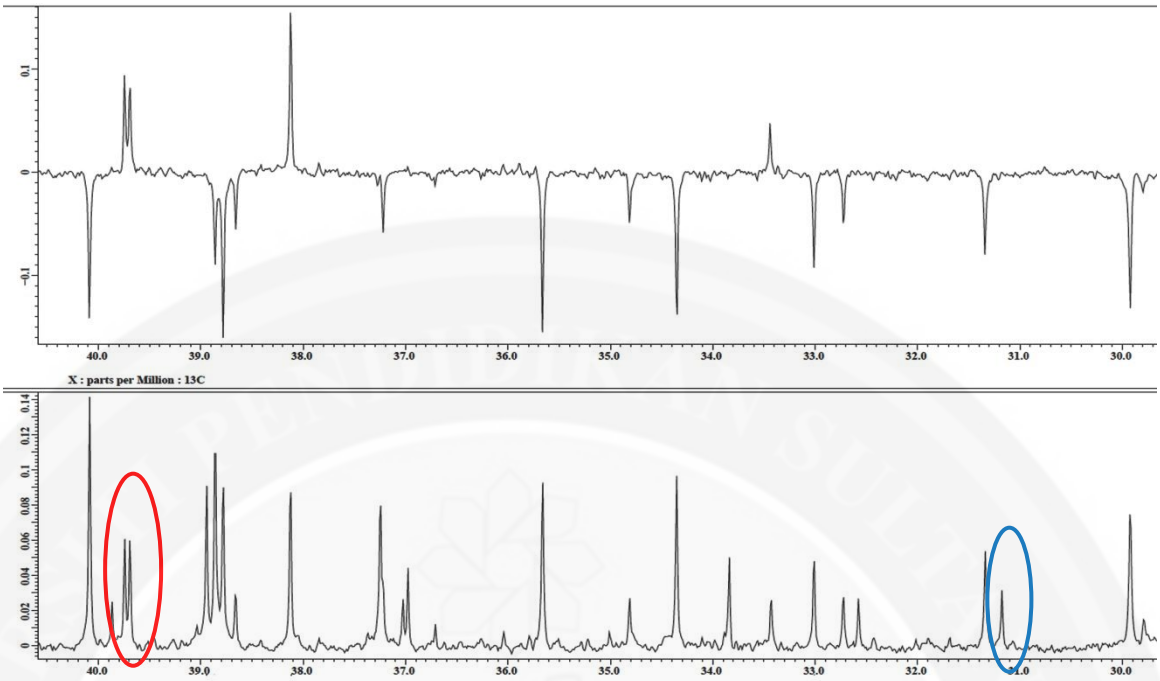


Figure 4.33. DEPT spectrum of compound **F**

4.3 Chemical constituents from the roots of *Kopsia singapurensis*

The phytochemical investigation on the hexane crude extract from the root of *Kopsia singapurensis* has resulted in the isolation of the four known triterpenes: β-amyrin **113**, β-amyrin acetate **114**, lupeol acetate **116** and stigmasterol **117**.

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4.3.1 Compound G: β-amyrin 113

Triterpene **G** was isolated as a white amorphous. The presence of the hydroxyl group was confirmed by its absorption frequency around 3300 cm^{-1} in the IR spectrum. Absorption band at 1709 cm^{-1} and 1294 cm^{-1} indicated the strecthing of olefin and C-O respectively. The MS, ^{1}H and ^{13}C NMR data were assembled to β-amyrin isolated from *A. spathulata* and has been discussed in Section 4.2.1.

4.3.2 Compound H: β-amyrin acetate 114

Triterpene **H** was isolated as colourless needles (m.p.: 239.0-240.0 ^{o}C). IR spectrum exhibited the presence of the highly conjugated hydroxyl group at 3360cm^{-1}. Moreover, MS showed a molecular ion peak at *m/z* 468 $[M]^{+}$. The MS, ^{1}H and ^{13}C NMR data were assemble to β-amyrin acetate isolated from *A. spathulata* and has been discussed in Section 4.2.2.

4.3.3 Compound I: Lupeol acetate 116

Compound **I** was obtained as white amorphous. The IR spectrum showed stretching band for ester carbonyl (1712cm^{-1}) and C-O (1263 cm^{-1}). The MS, ^{1}H and ^{13}C NMR data were assemble to lupeol acetate isolated from *A. spathulata* and has been discussed in Section 4.2.4.

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4.3.4 Compound J: Stigmasterol 117

Compound **J** was obtained as colourless needles (m.p: 151.0 - 152.0˚C). MS data showed a molecular ion peak at *m/z* 413 $[M+H]^+$ corresponding to molecular formula $C_{29}H_{48}O$. Absorption band at 3300 cm^{-1} and 1711 cm^{-1} indicated the strecthing of hydroxyl group and olefin respectively. All the spectral data showed that **117** was stigmasterol and assemble to stigmasterol isolated from *A. spathulata.* ^{1}H and ^{13}C NMR data were previously discussed at Section 4.2.5.

4.4 Results and Discussion of Bioactivity

Compounds **113**, **114**, **116** and **117** had been evaluated for cytotoxicity against MCF-7 cell line, antibacterial and antioxidant activity. Compound **115** were not evaluated due to the small amount of compounds available, not enough for assayed. β-amyrin **113** and β-amyrin acetate **114**, lupeol acetate **116** and stigmasterol **117** exhibited cytotoxic effects in the MTT assay with the IC_{50} values of 15.5, 22.5, 26.0, 14.5 µg/mL respectively.

Antibacterial activity against *Bacillus cereus* of triterpenes from *A. spathulata* was evaluated using the disc diffusion method. Antibacterial activity was indicated by a clear zone of growth inhibition. Results (Table 4.9) showed that all compounds were inactive against the gram-positive bacteria, *Bacillus cereus*. From Table 4.9, triterpenes showed weak antioxidant activity with IC_{50} >500 µg/mL,

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compare with the standard natural antioxidants α-tocopherol, ascorbic acid and quercetin.

Table 4.9

Cytotoxic Effects Against MCF-7 Cell Line, Antibacterial and Antioxidant Activity on Isolated Compounds.

	Cytotoxic	Antibacterial activity	Antioxidant activity
	IC_{50} values (µg/mL)	Diameter of inhibition zone (mm)	IC_{50} values (µg/mL)
Compounds	MCF-7	*B.cereus*	DPPH
β-amyrin **113**	15.5	NI	>500
β-amyrin acetate **114**	22.5	NI	>500
Lupeol acetate **116**	26.0	NI	>500
Stigmasterol **117**	14.5	NI	>500

Note. NI = No inhibition.

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CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Introduction

This chapter involves conclusion and recommendations for future research in order to improve the research that was done. It is hoped that further research can produce more findings which are beneficial and relevant with this research.

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5.2 Conclusion

This study was aimed on investigation of the chemical constituents of two different species from Apocynaceae family: *Alstonia spathulata* and *Kopsia singapurensis*. Phytochemical study, which employed various chromatographic techniques, resulted in the isolation of a series of triterpenoids. These compounds from both species were successfully isolated and characterized using modern spectroscopic techniques.

The study on bark of *A. spathulata* has resulted in the isolation of five known triterpenes which are β-amyrin, β-amyrin acetate, β-sitosterol, lupeol acetate and stigmasterol. A mixture of lupeol, α-amyrin and β-amyrin was also isolated from this plant.

Chemical compounds isolated from roots of *K. singapurensis* were same as *A. spathulata*. Four known triterpenes were isolated: β-amyrin, β-amyrin acetate, lupeol acetate and stigmasterol. Structural elucidation was carried by 1D, 2D NMR and comparison data with literature values. This is the first time the isolation processes focusing on the triterpene compounds have been conducted from both species.

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5.3 Recommendations

This study can be further improved for future research. It can be explored in order for future study to be more beneficial and valuable. There are a few recommendations are listed as below:

i. With the abundance of terpenoids isolated from both plants species, it may be expected to have biological activity. Therefore, future research should be also involving the more biological activity on isolated compound.

ii. Due to the similarities in chemical structure, triterpene mixtures are very difficult to separate and purified. Pentacyclic triterpenes, for example, can differ only in double bond positions or methyl groups (Mahato & Kundu, 1994). It is possible to purified triterpenes with classic chromatographic columns. However, high resolution chromatography procedures, such as HPLC, are more recommended to separate these compounds.

iii. It is hope that the future researcher can study the chemical constituents other than triterpenes for *A. spathulata*. This is because there are not many publications on this species yet.

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REFERENCES

Agrawal, P.K., Jain, D.C., Gupta, R.K. & Thakur, R.S. (1985). Carbon-13 NMR spectroscopy of steroidal sapgenins and steroids saponins. *Phytochemistry Reviews*, *24*(11), 2476-2496.

Ahmad, K., Hirasawa, Y., Nugroho, A.E., Hadi, A.H.A. & Morita, H. (2012). New aspidofractine, aspidosprematan and akuamiline indole alkaloids from the roots of *Kopsia Singapurensis* Ridl. *Heterocycles 86*(2), 1611-1619.

Ahmed, E., Sharif, A., Hussain, S., Malik, A., Mukhtar-ul-Hassan, Munawar, M. A., Nagra, S. A., Anwar, M., Ashraf, M., Afza, N., Athar, A. (2011). Phytochemicaland Antimicrobial Studies of *Grewia tenax*. *J. Chem. Soc. Pak., 33*(5), 676-681.

Ajithabai, M.D., Sreedevi, S., Jayakumar, G., Mangalam, S.N., Nair Deepa, P.N. & Sunitha Rani, S.P. (2011). Phytochemical Analysis and Radical Scavenging Activity of the Extracts of *Costus picatus* Linn and *Coccinia indica* W&A, two Ethnic MedicinalPlants used in the Treatment of *Diabetes mellitus. Free Radicals and Antioxidants, 1*(1), 77-83.

Ambasta, S.P., Ramchandran, K., Kashyapa, K. & Chand, R. (1992). *The Useful Plants of India*. New Delhi: Council of Science and Industrial Research (CSIR).

Ashour, M., Wink, M. & Gershenzon, J. (2010). *Annual plant Reviews*. United Kingdom: Blackwell Publishing Ltd.

Awang, K., Ahmad, K., Thomas, N.F., Hirasawa, Y., Takeya, K. Mukhtar, M.R., Mohamad, K., Morita, H. (2008). Singaporentine A: A New Indole Alkaloid from *Kopsia singapurensis* Ridl. *Heterocycles*, *75*(12), 3051-3056.

Awang, K., Pais, M., Sevenet, T., Schaller, H., Nasir, A.M., Hadi, A.H. (1991). Eburnamiol and larutensine, alkaloids from *Kopsia larutensis*. *Phytochemistry, 30*(9), 3164-3167.

Awang, K., Thoison, O., Hadi, A.H.A., Pais, M., Sevenet, T. (1993). Alkaloids from *Kopsia singapurensis*. *Natural Products Letters, 3*(4), 283-289.

UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKA
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Balandrin, M.F., Klocke, J.A., Wurtele, E.S., & Bollinger, W.H. (1985). Natural plant chemicals: Sources of industrial and medicinal materials. *Science, 228*, 1154-60.

Barbosa, L.F., Mathias, L., Braz-Filho, R. & Vieira, I.J.C. (2010). Chemical Constituents from *Aspidosperma illustre* (Apocynaceae). *Journal of the Brazilian Chemical Society, 21*(8), 1434-1438.

Bhattacharyya, B. & Johri, B.M. (1998). *Flowering plants taxonomy and phylogeny*. India: Narosa Publishing House.

Buckingham (2007). Biochemistry of plant secondary metabolism. In Wink, M. (Ed.), *Dictionary of Natural Product Volume 2*. London: Chapman and Hall/CRC.

Chua, L.S.L. (1998). *Kopsia singaporensis*. IUCN Red List of Threatened Species. Version 2013.1. Retrieved from http://www.iucnredlist.org/details/31758/0

Corner, E.J.H (1952). *Wayside trees of Malaysia, Volume 1* (pp. 137-146). Singapore: Government printing office.

Cota, B.B., De Oliveira, A.B., De Souza-Filho, J.D., Braga, F.C. (2003). Antimicrobial activity and constituents of *Coccoloba acrostichoides*. *Fitoterapia*, *74*, 729-31.

Davison, G.W.H, Ng, P.K.L & Chew, H.H. (2008). *The Singapore Red Data Book: Threatened Plants and Animals of Singpore*. Singapore: Nature Society.

Dudareva, N., Andersson, S., Orlova, I., Gatto, N., Reichelt, M., Rhodes, D., Boland, W., Gershenzon, J. (2005). The nonmevalonate pathway supports both monoterpene and sesquiterpene formation in snapdragon flowers. *Proceedings of the National Academy of Science USA 102*, 933 – 938.

Ebizuka, Y., Katsube, Y., Tsutsumi, T., Kushiro, T. & Shibuya, M. (2003). Functional genomics approach to the study of triterpene biosynthesis. *Pure and Applied Chemistry, 75*(2-3), 369-374.

Endress, M.E. & Bruyns, P.V. (2000). A revised classification of the Apocynaceae s.l.: *Botonical Review*. *66*, 1–56.

UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKA
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Endress, M.E. (2004). Apocynaceae: Brown and Now. *Telopea*, *10*(2), 525-541.

Extrasythese, (2007). *β-amyrin acetate*. Retrieved from http://www.extrasynthese.com/products-catalog/triterpenoids/beta-amyrin acetate-p2932326-c2303.html

Gao, S. & Singh, J. (1998). In vitro percutaneous absorption enhancement of lipophilic drug tamoxifen by terpenes. *Journal of Control Release*, *51*(2-3), 193-199.

Groombridge, B. & Jenkins, M.D. (2002). *World Atlas of Biodiversity: Earth's Living Resources in the 21st Century*. United Kingdom: University of California Press.

Hamilton, A.C. (editor) (2008). *Medicinal plants in conservation and development: Case studies and lessons learnt*. United Kingdom: Plantlife International.

Hashim, N.M., Rahmani, M., Ee, G.C.L., Sukari, M.A., Yahayu, M., Amin, M.A.M., Ali, A.M., & Go, R. (2012). Antioxidant, antimicrobial and tyrosinase inhibitory activities of xanthones isolated from *Artocarpus obtusus* FM Jarrett. *Molecules*, *17*, 6071–6082.

Hodgkiss, R.J. (2011). *Apocynaceae*. Retrieved from http://www.succulent-plant.com/families/apocynaceae.html

Holdsworth, D. (1986). Medicinal plants of Papua New Guinea. In Steiner, R.P. (Ed.), *Folk Medicine: The art and the science*. United state: Maple press company.

Hudlicky, T. (2008). *Triterpene.* Retrieved from http://accessscience.com/content/Triterpene/710800

Jaganath, I.B & Ng, L.T. (2000). *Herbs: The Green Pharmacy of Malaysia*. Kuala Lumpur: Vinpress Sdn. Bhd.

Jamal, A.K., Yaacob, W.A. & Laily, B.D. (2009). Triterpenes from the roots bark of *Phyllantus Columnaris. Australian Journal of Basic and Applied Sciences*, *3*(2), 1428-1431.

Jamal, A.N. & Ammar, A. (2009). Chemical study on stem bark of *Etlingera hornstedia*. *International Journal of Agriculture and crop Sciences*, *1*(1), 21-23.

UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKA
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James, J. T., & Dubery, I. A. (2009). Pentacyclic triterpenoids from the medicinal herb, Centella asiatica (L.) Urban. *Molecules*, *14*(10), 3922-3941.

Joselin, J., Brintha, T.S.S., Florence,A.R., & Jeeva, S. (2012). *Screening of select ornamental flowers of the family Apocynacea for phytochemical constituents*. Retrieved from http://scholar.googleusercontent.com/scholar?q=cache:mtJYMh02zroJ:scholar.google.com/+phyto+study+of+family+apocynaceae&hl=en&as_sdt=0,5&as_vis=1

Jussieu, A. L. D.E. (1789). *Genera plantarum*. Zurich: Viduam Herissant.

Kam, T.S., Tan, P.S., Hoong, P.Y. & Chuah, C.H. (1993). Methyl chanofruticosinates from leaves of *Kopsia arborea*. *Phytochem*istry, *32*(2), 489- 491.

Kam, T.S., Yoganathan, K. & Chuah, C.H. (1994). Kopsinitarines A, B and C, novel cage alkaloids from a Malaysian Kopsia. *Tetrahedron Letter*, *35*(25), 4457-4460.

Kam, T.S., Yoganathan, K., Li, H.Y. & Harada, N. (1996). Tenuisines A – C and tenuiphylline, novel bisindoles from *Kopsia tenuis*. *Tetrahedron*, *53*(37), 12661-12670.

Kam, T.S., Subramaniam, G. & Chen, W. (1999a). Alkaloids from *Kopsia dasyrachis*. *Phytochemistry*, *51*(1), 159-169.

Kam, T.S., Choo, Y.M., Chen, W. & Yao, J.X. (1999b). Indole and monoterpene alkaloids from the leaves of *Kopsia dasyrachis*. *Phytochemistry*, *52*(5), 959-963.

Kam, T.S. & Choo, Y.M. (2003). Kopsifolines A, B and C, indole alkaloids with a novel hexacyclic carbon skeleton from Kopsia. *Tetrahedron Letter*, *44*(6), 1317-1319.

Kam, T.S. & Choo, Y.M. (2004b). Venalstonine and Dioxokopsan Derivatives from *kopsia*. *Phytochemistry*, *65*, 2119-2122.

UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKA
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Koay, Y.C., Wong, K.C., Osman, H., Eldeen, I., & Asmawi, M.Z. Chemical Constituents and Biological Activities of *Strobilanthes crispus* L. (2013). *Rec. Nat. Prod*., *7*(1), 59-64.

Laule, O., Fürholz, A., Chang, H.S., Zhu, T., Wang, X., Heifetz, P.B., Gruissem, W., Lange, M. (2003). Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in Arabidopsis thaliana. *Proceedings Natianal Academy Science USA, 100*(11), 6866 – 6871. doi: 10.1073/pnas.1031755100

Lim, W.H., Kho, B.L., Tay, T.H & Low, W.L., (1998). *Plant of Putrajaya wetlands*. Putrajaya: Agriquest Sdn. Bhd.

Low, Y.Y., Subramanium, G, Lim, K.H., Wong, R.C.S., Robinson, W.T., Kam, T.S. (2009). Mersiphyllines A and B from *Kopsia*: determination of Relative Configuration at a Quaternary Center via Formation of an Alkaloid-borane Complex. *Tetrahedron Letters*, *65*(34), 6873-6876.

Mahato, S. B. & Kundu A. P. (1994). ^{13}C NMR Spectra of Pentacyclic Triterpenoids-A Compilation and some Salient Feature. *Phytochemistry*, *37*(6), 1517-1575.

Mahato, S.B., Nandy, A.K & Roy,G. (1992). Triterpenoids. *Phytochemistry*, *31*, 2199.

Mander, L. & Liu, H.W. (2010). *Comprehensive Natural Products II: Chemistry and Biology (Vol. 1)*. United Kingdom: Elsevier Ltd.

Menezes, F.S., Borsatto, A.S., Pereira, N.A, Matos, F.J.A & Kaplan, M.A.C. (1998). Chamaedrydiol, an ursane triterpene from *Marsypianthes chamaedrys*. *Phytochemistry*, *48*(2), 323-325.

Middleton, D.J. (2002). The apocynaceae of the Crocker Range National Park Sabah. *ASEAN Review of Biodiversity Environental Conservation (ARBEC)*, 1-15. Retrieved from http://www.arbec.com.my/pdf/art5julysep02.pdf

Middleton, D. J. (2004). A Revision of *Kopsia* (Apocynaceae: Rauvolfioideae). *Harvard Paper in Botany*, *9*(1), 89-142.

Middleton, D.J. (2009). An update on the Apocynaceae in Thailand. *Thai Forest Bullentin (Botany)*. (Special issues), 143-155.

UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKA
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Monachino, J. (1949). A Revision of the Genus Alstonia (Apocynaceae). *Pacific Science*, *3*, 134-182.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, *65*, 55-63.

Mouffok, S., Haba, H., Lavaud, C., Long, C. & Benkhaled, M. (2012). Chemical constituents of *Centaurea omphalotricha* Coss. & Duriue ex Batt. & Trab. *Records of Natural Products*, *6*(3), 292-295.

Muhammad, I.A., Ejaz, A., Abdul, J., Abdul, M., Muhammad, A., Muhammad, M., & Muhammad, A.R. (2007). Antimicrobial Constituents from *Fagonia cretica*. *Jour. Chem. Soc. Pak.*, *29*(6), 634-639.

Omar, H., Hashim, N.M., Zajmi, A., Nordin, N., Abdelwahab, S.I., Azizan, A.H.S., Hadi, A.H.A. & Ali, H.M. (2013). Aporphine alkaloids from the leaves of *Phoebe grandis* (Nee) Mer. (Lauraceace) and their cytotoxic and antibacterial activities. *Molecules*, *18*, 8994-9009.

Orient Longman (1994). *Indian medicinal plants Volume I*. India: Orient Longman Private Limited.

Paduch, R., Kandefer-Szerszeń, M., Trytek, M., & Fiedurek, J. (2007). Terpenes: substances useful in human healthcare. *Archivum Immunologiae et Therapiae Experimentalis*, *55*(5), 315-327.

Pateh, U. U., Haruna, A. K., Garba, M., Iliya, I., Sule, I. M., Abubakar, M. S., Ambi, A. A. (2009). Isolation of Stigmasterol, β-Sitosterol and 2 HydroxyhexadecanoicAcid Methyl Ester from the Rhizomes of *Stylochiton lancifolius* Pyer and Kotchy (Areceae). *Nigerian Journal of Pharmaceutical Sciences*, *8*(1), 19-25.

Paz Lima,M. et al. (2004). Phytochemistry of *Trattinnickia burserifolia*, *T. rhoifolia*, and *Dacryodes hopkinsii*: Chemosystematic Implications. *Journal of Brazilian Chemical Society*, *15*(3), 385-394.

Perry, L.M. and Metzger, J., (1980). *Medicinal plants of East and Southeast Asia: Attributed Properties and Uses*. Cambridge: MIT press.

UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKA
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Piel. J., Donath. J., Bandemer, K., Boland, W. (1998). Mevalonate-independent biosynthesis of terpenoid volatiles in plants: Induced and constitutive emission of volatiles. *Angew Chem Int Ed., 37*(18), 2478 – 2481.

Pongbunrod, S. (1979). *Mai-Tet-Murng-Thai: Medicinal characteristic of foreign and Thai traditional medicines, 1st Ed.* (pp. 376-377). Bangkok: Khrungthon Press.

Prachayasittikul, S., Saraban, P., Cherdtrakulkiat, R., Ruchirawat, S. & Virapong Prachayasittikul, V. (2010). New bioactive triterpenoids and antimalarial activity of diospyros rubra lec. *Experimental and Clinical Sciences Journal*, *9*, 1-10.

Pretsch, E., Buhlmann, P. & Badertscher, M. (2009). *Structure Determination of Organic Compounds: Tables of Spectral Data*. Verlag Berlin Heidelberg: Springer.

Ragasa, C.Y., Tsai, P.W., & Shen, C.C. (2009). Terpenoids and Sterols from the Endemic and Endangered Philippine Trees, *Ficus pseudopalma* and *Ficus ulmifolia*. *Philippine Journal of Science*, *138*(2): 205-209.

Razborsek, M.I., Voncina, D.B., Dolecek, V., & Voncinab, E. (2007). Determination of major phenolic acids, phenolic diterpenes and triterpenes in Roasmary (*Rosmarinus officinalis* L.) by gas chromatography and mass spectrometry. *Acta Chim. Slov., 54*, 60-67.

Rios, J.L. (2010). Effects of triterpenes on the immune system. *Journal of Ethnopharmacology*, *128*(1), 1-14.

Ruzicka, L., Eschenmoser, A. & Heusser, H. (1953). The isoprene rule and the biogenesis of terpenic compounds. *Journal Cellular and Molecular Life Sciences*, *9*(10), 357-367.

Ruzicka, L. (1959). History of the isoprene rule: Faraday lecture. *Proceeding chemical society* (pp. 341-360). London, United Kingdom. doi: 10.1039/PS9590000341

UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKA
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Saleem, M., Kweon, M.H., Yun, J.M., Adhami, V.M., Khan, N., Syed, D.N., Mukhtar, H. (2005). A novel dietary triterpene Lupeol induces fas-mediated apoptotic death of androgen-sensitive prostate cancer cells and inhibits tumor growth in a xenograft model. *Cancer Research*, *65*(23), 11203-11213.

Sennblad, B. & Bremer, B. (2002). Classification of Apocynaceae s.l. According to a New Approach Combining Linnaean and Phylogenetic Taxonomy. *Systematic Biology*, *51*(3), 389-409.

Schuhr, C.A., Radykewicz, T., Sagner, S., Latzel, C., Zenk, M.H., Arigoni, D., Bacher, A., Rohdich, F., & Eisenreich, W. (2003). Quantitative assessment of crosstalk between the two isoprenoid biosynthesis pathways in plants by NMR spectroscopy. *Phytochemistry review*, *2*, 3-16.

Segovia, J.F.O., Oliveira, V.L., Goncalves, M.C.A.l., Resck I.S., Silva, C.A.M., Silveira, D., Gavrilov, A.V., Gavrilova, L.A., Kanzakl, L.I.B. (2011). Botanical characterisation, geographical distribution and phytochemistry analysis of manilkara huberi (ducke) stanhl autochtonous in amapa state, Brazil. *Proceedings of the National Academy of Belarus Biological Series: Vol 2*, 34-39.

Sevenet, T., Allorge, L., David, B., Awang, K., Hadi, A.H., Kan-Fan, C., Quirion, J.C., Remy, F., Schaller, H. & Teo, L.E. (1994). A preliminary chemotaxonomic review of Kopsia (Apocynaceae). *Journal of Ethnopharmacology*, *41*(3), 147-183.

Sidiyasa,K. & Baas, P. (1998). Ecological and systematic wood anatomy of Alstonia (Apocynaceae). *International Association of Wood Anatomists*, *19*(2), 207-229.

Smith, N., Mori, S.A., Henderson, A., Stevenson, D.W. & Heald, S.V. (2004). *Flowering plants of the Neotropics*. United Kingdom: Princeton University Press.

Sobrinho, D., Hauptli, M.B., Appolinario, E.V., Kollenz, C.L.M., Carvalho, M.G. & Braz-filho, R. (1991). Triterpenoids isolated from *Parahancornia amapa*. *Journal of Brazilian Chemical Society*, *2*(1), 15-20.

UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKA
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Springob, K. & Kutchan, T.M. (2009). Introduction to the different classes of natural product. In Osbourn, A.E. & Virginia, L. (Eds). *Plant-derived Natural Products: Synthesis, Function, and Application* (pp. 3-44). United state: Springer.

Sutha, S., Devi, V. . K., Tresina, S. P., Maruthupandian, A., & Mohan, V. R. (2011). Pharmacognostical studies on Alstonia venenata R.Br. an ethnomedicinal plant. *International Journal of PharmTech Research*, *3*(3), 1697-1704.

Subramanium, G., Choo, Y.M., Hiruku, O., Komiyama, K. & Kam, T.S. (2008). The alkaloids of the Mersinine Group: A New Subclass of the Monoterpenoid Indole Alkaloids from *Kopsia*. *Tetrahedron Letters*, *64*(7), 1397-1408.

Subramanium, G., Hiruku, O., Hayashi, M., Koyano, T., Komiyama, K. & Kam, T.S. (2007). Biologically Active Aspidofractinine, Rhazanilam, Akuammiline, and Vincorine Alkaloids from *Kopsia*. *Journal of Natural Products*, *70*(11), 1783 1789.

Subramanium, G., Hiruku, O., Hayashi, M., Koyano, T., Komiyama, K. & Kam, T.S. (2008). Biologically Active Aspidofractinine Alkaloids from *Kopsia singapurensis*. *Journal of Natural Products*, *71*(1), 53-57.

Subramanium, G. & Kam, T.S. (2008). New Indole Alkaloids from Kopsia. Alkaloid Variation in Kopsia singapurensis. *Helvetica Chimica Acta*, *91*(5), 930-937.

Tan, S.J. et al. (2010). Strychnan and Secoangustilobine A Type Alkaloids from *Alstonia spatulata.* Revision of the C-20 Configuration of Scholaricine. *Journal of Natural Product*, *73*(11), 1891–1897.

Tao, L.P., Leeuwenberg, A.J.M. & Middleton, D.J. (1995). Apocynaceae. *Flora of China*, *16*, 143-188.

Tijjani,A. Ndukwe, IG. & Ayo, RG. (2012). Isolation and Characterization of Lup-20(29)-ene-3, 28-diol (Betulin) from the Stem-Bark of *Adenium obesum* (Apocynaceae). *Journal of Pharmaceutical*, *11*(2), 259-262.

Thanakijcharoenpath, W. and Theanphong, O. (2007). Triterpenoids from the stem of *Diospyros glandulosa*. *The Thai Journal of Pharmaceutical Sciences*, *31*, 1-8. The Plant List (2010). *Alstonia*, *Version 1*. Retrieved from http://www.theplantlist.org/tpl/search?q=alstonia

UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKA
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The Plant List (2010). *Kopsia, Version 1*. Retrieved from http://www.theplantlist.org/browse/A/Apocynaceae/Kopsia/

U.S Department of Health and Human Services (2003). *Toxicological and profile for pyrethrins and pyrethroids*. Retrieved from http://www.atsdr.cdc.gov/toxprofiles/tp155.pdf

Uzir, S., Mohamaed, M.A., Hadi, A.H., Awang, K., Wiart, C., Gallard, J.F. & Pais, M. (1997). Terengganensines A and B, dihydroeburnane alkaloids from *Kopsia terengganensis*. *Tetrahedron Letter*, *38*(9), 1571-1574.

Ramachandran, K. (1992). *The Useful Plants of India*. New Delhi: Publication and Information Directorate, CSIR.

Wallach, O. (1887). Zur Kentni ß der Terpene und der ätherischen Oele, *Liebig's Ann. Chem.*, *239*, 1–54.

Webster's Online Dictionary (1913). *Definition: Alstonia*. Retrieved from http://www.websters online-dictionary.org/definitions/alstonia

Whitmore, T.C. (1973). *Tree flora of Malaysia: A manuals for forests, Vol. II*. Kuala Lumpur: Longman.

Wiart, C. (2006). *Medicinal plants of Asia and the Pacific*. United States*:* Taylor and Francis Group, LLC.

Wuthammawech, W. (1997). *Thai pharmacy: Traditional medicine collection, 2nd Ed.* (p.342). Bangkok: O.S. Printing House.

Zwenger, S., & Basu, C. (2008). Plant terpenoids : Applications and future potentials. *Biotechnology and Molecular Biology Reviews*, *3*(1), 1-7.

UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKA
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APPENDICES

Participation in Seminar, Proceeding and Conferences

1. Kolokium Kebangsaan Pasca Siswazah Sains dan Matematik 2012, 6 Oktober 2012. Universiti Pendidikan Sultan Idris, Tanjong Malim, Perak (Oral Presenter).

2. 17th Malaysian Chemical Congress (17MCC) 2012, 15-17 October 2012. Putra World Trade Centre, Kuala Lumpur, Malaysia (Poster Presenter).

3. Short Course Biosynthesis of Natural Product, 9- 11 October 2012. Faculty of Applied Sciences University Teknologi MARA, 40450 Shah Alam, Selangor. (Participate).

4. International Conference of Natural Product (ICNP) 2013, 4-6 March 2013. Shah Alam Convention Centre, Shah Alam, Malaysia (Oral Presenter).

5. International Post Graduate Conference on Science and Mathematics (IPCSM) 2013. Convention Hall, E-Learning Building, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia. (Poster Presenter).

Publication

1. Ahamd, K., Tee C.T, Awang, K., Tan, S.P. Lee, Y.S. & Nafiah, A. (2013). Triterpenoids from the Bark of *Alstonia Spathulata* Bl. (Apocynaceae). *Malaysian Journal of Chemistry*, *15*(1), 041 – 046.

2. Lee Yean Shan, Tee Chuan Thing, Tan Siow Ping, Khalijah Awang, Najihah Mohd Hashim, Mohd Azlan Nafiah & Kartini Ahmad (2014). Cytotoxic, antibacterial and antioxidant activity of triterpenoids from *Kopsia singapurensis* Ridl. Journal of Chemical and Pharmaceutical Research, 2014, 6(5):815-822.

UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKA
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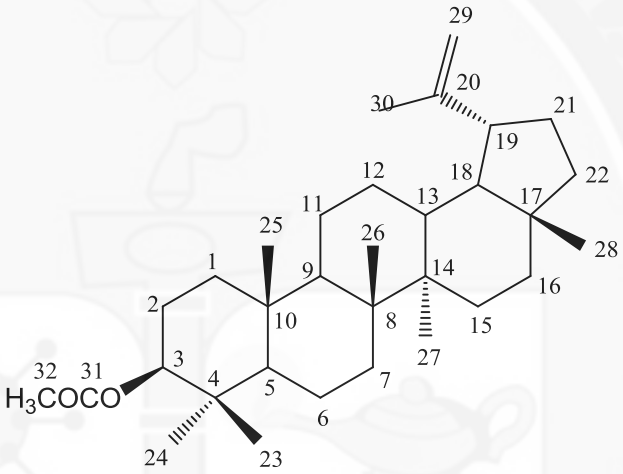
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Chemical constituent from *Alstonia spathulata* Bl. (Apocynaceae)

Kartini Ahmad[1], Tee Chuan Thing, Khalijah Awang[2], Mohd Azlan Nafiah[1]
[1]Department of Chemistry, Faculty of Science and Mathematics, University Pendidikan Sultan Idris, 35900 Tanjung Malim, Perak.
[2]Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur.
*kartini@fsmt.upsi.edu (Corresponding author)

Abtsract

In Malaysia, *Alstonia spathulata* Bl. is locally known as *pulai basong* which distribute in peat forest. The separations of the chemical components from hexane crude extract (bark) of *Alstonia spathulata* were carried out using different chromatographic techniques (column chromatography and thin layer chromatography). One compound were isolated and identified as lupeol acetate **(1)**. This compound have never been reported and isolated from this species. The structures of this compound were elucidated based on the basis of detailed 1D-NMR (1H, 13C and DEPT) and 2D-NMR (COSY, HSQC, HMBC) spectroscopic analysis. The data analysis was also compared with the literature.



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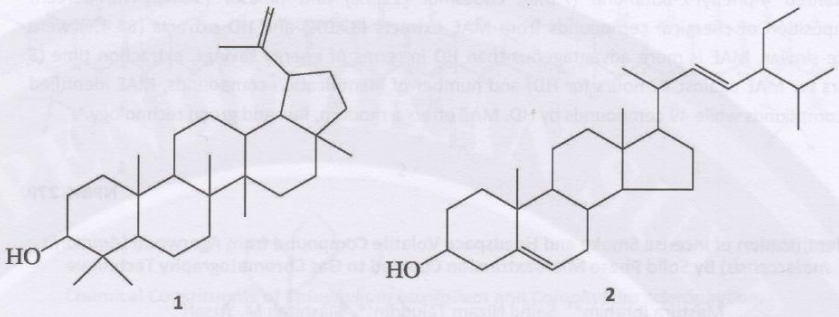
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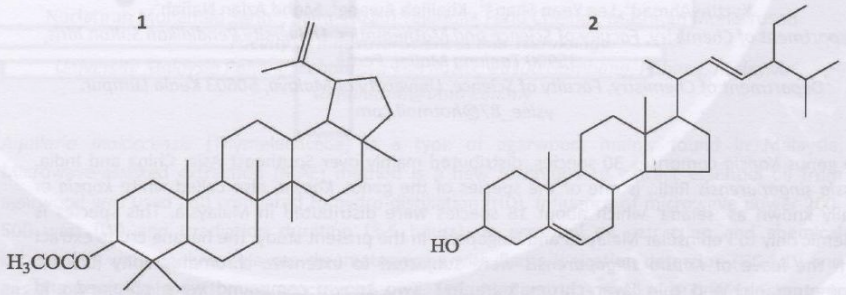
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Triterpenoids from the bark of *Alstonia spathulata* Bl. (Apocynaceae)

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In Malaysia, *Alstonia spathulata* Bl. is locally known as *pulai basong* which distribute in peat forest. The separations of the chemical components from hexane crude extract (bark) of *Alstonia spathulata* were carried out using different chromatographic techniques (column chromatography and thin layer chromatography). Two compounds were isolated and identified as lupeol acetate **(1)** and stigmasterol **(2)**. These compounds have never been reported and isolated from this species. The structures of these compounds were elucidated based on the basis of detailed 1D-NMR (1H, 13C and DEPT) and 2D-NMR (COSY, HSQC, HMBC) spectroscopic analysis, involving also comparison with data from the literature.

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Triterpenoids from the bark of *Alstonia spathulata* Bl. (Apocynaceae)

*Kartini Ahmad[1], Tee Chuan Thing[1], Tan Siow Ping[1], Khalijah Awang[2], Mohd Azlan Nafiah[1]

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Abstract

In Malaysia, *Alstonia spathulata* Bl. is locally known as *pulai basong* which distribute in peat forest. The separations of the chemical components from hexane crude extract (bark) of *Alstonia spathulata* were carried out using different chromatographic techniques (column chromatography and thin layer chromatography). Two compounds were isolated and identified as lupeol acetate **(1)** and stigmasterol **(2)**. These compounds have never been reported and isolated from this species. The structures of these compounds were elucidated based on the basis of detailed 1D-NMR (1H, 13C and DEPT) and 2D-NMR (COSY, HSQC, HMBC) spectroscopic analysis, involving also comparison with data from the literature.

Introduction

Alstonia is genus that under tribe of Alstonieae in Rauvolfioideae subfamily of Apocynaceae and widely distributed at subtropical Africa, Central America, Southeast Asia, Polynesia and Australia, with most species in the Malesian region (Webster Online Dictionary). *Alstonia* is the genus of laticiferous shrubs or trees. The plants contain a milky sap which rich in poisonous alkaloids. *A. spathulata* are swamp dweller. The tree is pagoda-like crown. The bark is smooth, grayish and shows abundant of milky latex ((Lim, kho, Tay & Low, 1998; Wiart, 2006). In Malaysia, *Alstonia spathulata* Bl. is locally known as *pulai basong*. In Cambodia, Loas, Vietnam, and, Malaysia, the latex from *Alstonia spathulata* Bl. is applied externally to sores and diseased skin. The bark is used to lower fever and to expel worms from the intestine (Wiart, 2006). The aim of this study is to isolate and identify the chemical compounds from the bark of *Alstonia spathulata*. In this paper, we report the isolation and characterization of lupeol acetate **1** and stigmasterol **2** which is the first time isolated from this species.

Objectives

- To extract, isolate and purify chemical compounds.
- To elucidate the structure of the isolated compounds using modern spectroscopic methods.

Methodology



Alstonia spathulata (Bark)

Hexane Crude Extract

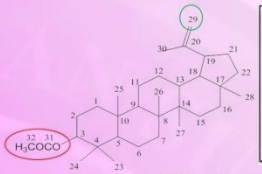
Column Chromatograph
(Hexane: CH_2Cl_2:Methanol)

Compound 1: Lupeol acetate

Compound 2: Stigmasterol

Results & discussion

Compound **1**



^{1}H NMR Spectrum of **1**

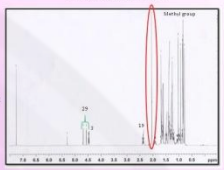
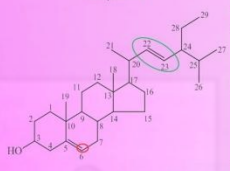


Table 1: ^{13}C NMR Data of compound **1**

^{13}C	1	Lupeol acetate	^{13}C	1	Lupeol acetate	^{13}C	1	Lupeol acetate
1	38.4	38.6	12	25.1	24.0	23	28.0	27.6
2	23.7	21.7	13	38.0	36.2	24	16.5	16.7
3	81.0	81.2	14	42.8	43.0	25	16.2	16.4
4	37.8	38.0	15	27.4	25.3	26	16.0	16.2
5	55.4	55.6	16	35.6	35.8	27	14.5	14.7
6	18.2	18.4	17	43.0	43.2	28	18.0	18.2
7	34.2	34.4	18	48.3	48.5	29	109.4	109.6
8	40.9	41.0	19	48.0	48.2	30	19.3	19.5
9	50.3	50.5	20	151.0	151.2	31	171.1	171.3
10	37.1	37.3	21	29.8	30.0	32	21.3	28.2
11	20.9	21.1	22	40.0	40.2			

Compound **2**



^{1}H NMR Spectrum of **2**

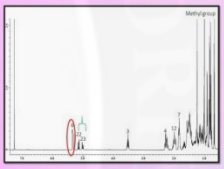


Table 1: ^{13}C NMR Data of compound **2**

^{13}C	1	stigmasterol	^{13}C	1	stigmasterol	^{13}C	1	stigmasterol
1	37.3	37.3	11	21.2	21.1	21	21.2	21.2
2	31.7	31.6	12	39.8	39.7	22	138.3	138.3
3	71.9	71.8	13	42.3	42.3	23	129.3	129.3
4	42.4	42.3	14	56.8	56.9	24	51.3	51.2
5	140.8	140.8	15	24.4	24.4	25	45.9	31.9
6	121.8	121.7	16	29.0	28.4	26	21.2	21.2
7	31.9	31.9	17	56.0	56.1	27	19.1	19.0
8	31.9	31.9	18	12.0	11.0	28	29.8	25.4
9	50.2	51.2	19	19.5	21.2	29	12.1	12.1
10	36.6	36.5	20	40.6	40.5			

Conclusion

The research work through a systematic chemical investigation has determined and identified for the first time the presence of lupeol acetate (**1**) and stigmasterol (**2**) from the bark of *Alstonia spathulata*.

References

- Ajithabai, M.D., Sreedevi, S., Jayakumar, G., Mangalam, S.N., Deepa, P.N.N., Suritha, R.S.P. (2011). Phytochemical Analysis and Radical Scavenging Activity of the Extracts of *Costus picatus* Linn and *Coccinia indica* W&A, two Ethnic Medicinal Plants used in the Treatment of *Diabetes mellitus*. *Free Radicals and Antioxidants*, 1(1), 77-83.
- Jamal, A.N. and Ammar, A. (2009). Chemical study on stem bark of *Ettingera hornstedia*. *International Journal of Agriculture and crop Sciences*, 1(1), 21-23.
- Jamal, A.E., Yaacob, W.A. & Lally, B.D. (2009). Triterpenes from the roots bark of *Phyllantus Columnaris*. *Australian Journal of Basic and Applied Sciences*, 3(2), 1428-1431.
- Lim, W.H., Kho, B.L., Tay, T.H & Low, W.L. (1998). Plant of Putrajaya wetlands. Putrajaya: Agriquest Sdn. Bhd.
- Patel, U.H., Harsha, A.K., Garba, M., Biya, I., Sule, I.M., Abubakar, M.S., Ando, A.A. (2009). Isolation of Stigmasterol, β-Sitosterol and 2-Hydroxybenzadecanoic Acid Methyl Ester from the Rhizomes of *Stylochiton lancifolius* Pyer and Kotchy (Araceae). *Nigerian Journal of Pharmaceutical Sciences*, 8(1), 19-25.
- Webster's Online Dictionary, Definition: Alstonia. Retrieved on April 29, 2012 from http://www.websters-online-dictionary.org/definition/alstonia
- Wiart, C. (2006). Medicinal plants of Asia and the Pacific. *United States*: Taylor and Francis Group, LLC.

Acknowledgement

We would like to thank the University Pendidikan Sultan Idris for the provision of laboratory facilities and technical assistance as well as the excellent technical assistance of University Malaya.

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antimicrobial activity results showed that the essential oils of *G. cylindrostigma* exhibited strongest inhibitory activity against *S. epidermidis* and *Candida albicans*. Based on the results of the assay on standard samples, compounds such as geranyl acetate, geraniol, linalool and terpinen-4-ol in the oils might be responsible for the high antimicrobial activity.

Keywords: *Goniothalamus cylindrostigma*; Essential oil; Antimicrobial

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OR-32

Oleanane Type Triterpenoids from the Bark of *Alstonia spathulata* Bl. (Apocynaceae)

Ahmad K[1], Tee CT[1], Awang K[2], Tan SP[1], Nafiah MA[1]

[1]Department of Chemistry, Faculty of Science and Mathematics, University Pendidikan Sultan Idris, 35900 Tanjung Malim, Perak; [2]Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur.
Email: kartini@fsmt.upsi.edu

In Malaysia, *Alstonia spathulata* Bl. is locally known as 'pulai basong' which distribute in peat forest. The separation of the chemical components from hexane crude extract (bark) of *Alstonia spathulata* was carried out using different chromatographic techniques (column chromatography and thin layer chromatography). Two compounds were isolated and identified as beta-amyrin 1a and beta-amyrin acetate 1b. The compounds are reported for the first time from this plant. The structures of these compounds were elucidated based on the basis of detailed 1D-NMR (^{1}H, ^{13}C and DEPT) and 2D-NMR (COSY, HSQC, HMBC) spectroscopic analysis, involving also comparison with data from the literature.

Keywords: Apocynaceae, *Alstonia spathulata*, beta-amyrin, beta-amyrin acetate

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OR-33

Chemical Constituents from the Nonpolar Fraction of *Goniothalamus macrophyllus*

Norkamilah Abdullah[1], Choo Chee Yan[1]*, Hitotsuyanagi Y[2] , Takeya K[2]

[1]MedChem Herbal Research Group, Faculty of Pharmacy,Universiti Teknology MARA, 42300 Puncak Alam, Selangor, Malaysia; [2]Tokyo University of Pharmacy and Life Science,1432-1 Horinouchi, Hachioji,Tokyo 192-0392,Japan.
Email: choo715@puncakalam.uitm.edu.my

Goniothalamus is one of the species from the Annonaceae family consisting of 115 species distributed throughout the tropics and subtropics. *Goniothalamus macrophyllus* (Blume)Hook. f. & Thoms. is a medicinal plant used widely in peninsular Malaysia and known by the local as "pokok gajah beranak". Phytochemical studies on goniothalamus species have led to the isolation and characterization of styryllactones, acetogenins, alkaloids and flavanoids. The objective of the study is to identify compounds from the nonpolar fraction from the roots of *G. macrophyllus*. Dried roots of *G. macrophyllus* were ground into fine powder using a cutter mill. The ground roots were soaked in 80 % aqueous methanol at room temperature, filtered and the solvent were removed under reduced pressure at 40 ^{0}C to afford crude methanolic extract. The crude extract was further suspended in aqueous methanol and sequentially partitioned with *n*-hexane, chloroform and *n*-butanol. New compounds were isolated and purified from the nonpolar fraction through a combination of preparative HPLC and TLC preparative. The absolute stereo structures were discussed and confirmed by using one (1D) and two-dimensional (2D) NMR, MS, IR, UV-Vis spectroscopy. In conclusion, the nonpolar fraction afforded new compounds.

Keywords : *Goniothalamus macrophyllus*,Annonaceae, Pokok gajah beranak

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OR-34

Depigmenting Efficacy of Commercially Available Skin-Lightening Creams: Comparative Analysis and *In-Vivo* Evaluation

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[1] Faculty of Pharmacy, University of Sargodha, 40100, Sargodha, Pakistan

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P-007

PHYTOCHEMICAL STUDY OF BARK FROM *Alstonia spathulata* Bl. (Apocynaceae)

Tee Chuan Thing[1], Lee Yean Shan[1], Tan Siow Ping[1], Khalijah Awang[2], Mohd Azlan Hafizh[1] Kartini Ahmad[1]

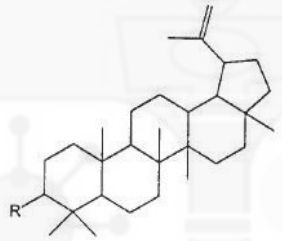
[1]Department of Chemistry, Faculty of Science and Mathematics, University Pendidikan Sultan Idris, 35900 Tanjung Malim, Perak.

[2]Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur

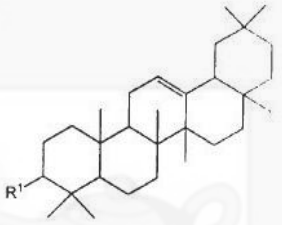
* Corresponding author

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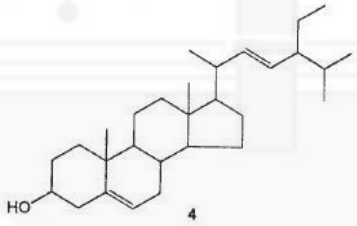
In Malaysia, *Alstonia spathulata* Bl. is locally known as 'pulai basong' which distributed in peat forest. The separation of the chemical components from hexane and DCM crude extract of *Alstonia spathulata* was carried out using different chromatographic techniques (column chromatography and thin layer chromatography). A total of five known compound were isolated from the bark extracts and identified as: lupeol acetate **1**, β-amyrin **2**, β-amyrin acetate **3**, stigmasterol **4** and β-sitosterol **5**. The structures of these compounds were established using 1D-NMR (1H, 13C and DEPT) and 2D-NMR (COSY, HSQC, HMBC) spectroscopic analysis, involving also comparison with data from the literature.



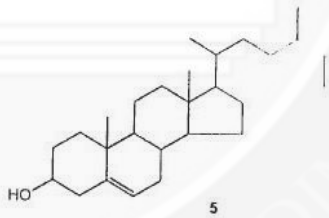
1 R = $OCOCH_3$



2 R = OH
3 R = $OCOCH_3$



4



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Triterpenoids from the bark of *Alstonia spathulata* Bl. (Apocynaceae)

Tee Chuan Thing[1], Mohd Azlan Nafiah[2], Tan Siow Ping[1], Khalijah Awang[2], Kartini Ahmad[1]*

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Abstract

In Malaysia, *Alstonia spathulata* Bl. is locally known as 'pulai basong' which distribute in peat forest. The separation of the chemical components from hexane and DCM crude extract of *Alstonia spathulata* was carried out using different chromatographic techniques (column chromatography and thin layer chromatography). A total of five known compound were isolated from the bark extracts and identified as: β-amyrin **1**, β-amyrin acetate **2**, lupeol acetate **3**, stigmasterol **4** and β-sitosterol **5**. The structures of these compounds were established using 1D-NMR (1H, 13C and DEPT) and 2D-NMR (COSY, HSQC, HMBC) spectroscopic analysis, involving also comparison with data from the literature.

Introduction

Alstonia is genus that under tribe of Alstonieae in Rauvolfioideae subfamily of Apocynaceae and widely distributed at subtropical Africa, Central America, Southeast Asia, Polynesia and Australia, with most species in the Malesian region (Webster's Online Dictionary, 2012). *A. spathulata* is swamp dweller. The tree is pagoda-like crown. The bark is smooth, grayish and shows abundant of milky latex (Lim, kho, Tay & Low, 1998; Wiart, 2006). In Malaysia, *Alstonia spathulata* Bl. is locally known as 'pulai basong'. In Cambodia, Laos, Vietnam, and, Malaysia, the latex from *Alstonia spathulata* Bl. is applied externally to sores and diseased skin. The bark is used to lower fever and to expel worms from the intestine (Wiart, 2006). The aim of this study is to isolate and identify the chemical compounds from the bark of *Alstonia spathulata*. In this paper, we report the isolation and characterization of β-amyrin **1**, β-amyrin acetate **2**, lupeol acetate **3**, stigmasterol **4** and β-sitosterol **5** which are the first time isolated from this species.

Objectives

- To extract, isolate and purify chemical compounds.
- To elucidate the structure of the isolated compounds using modern spectroscopic methods.

Conclusion

The research work through a systematic chemical investigation has determined and identified for the first time the presence of β-amyrin **1**, β-amyrin acetate **2**, lupeol acetate **3**, stigmasterol **4** and β-sitosterol **5**, from the bark of *Alstonia spathulata*. The work was carried out by means of various physical (solvent extraction and column chromatography) and spectral techniques. The assignment will be further supported by 2D-NMR (COSY, HSQC, and HMBC) spectroscopic analysis. The structures of the compounds were also elucidated by comparison with literature.

References

Agrawal, P.K., Jain, D.C., Gupta, R.K. & Thakur, R.S. (1985). Carbon-13 NMR spectroscopy of steroidal sapgenins and steroidal saponins. *Phytochemistry Reviews*, 24(11), 2476-2496.
2. Ajaiyeoba, et al. (2011). Phytochemical Analysis and Radical Scavenging Activity of the Extracts of *Costus picatus* Linn and *Cocelnia indica* W&A, two Ethnic MedicinalPlants used in the Treatment of *Diabetes mellitus*. *Free Radicals and Antioxidants*, 1(1), 77-83.
3. Jamal, A.K. and Ammar, A. (2009). Chemical study on stem bark of *Etlingera hornstedia*. *International Journal of Agriculture and Crop Sciences*. 1(1), 23-23.
4. Jamal, A.K., Yaqoob, W.A. & Laily, B.D. (2009). Triterpenes from the roots bark of *Phyllantus Columnaris*. *Australian Journal of Basic and Applied Sciences*, 3(2), 1428-1431.
5. Lim, W.H., Kho, B.L., Tay, T.H & Low, W.L., (1998). Plant of Putrajaya wetlands. Putrajaya: Agriquest Sdn. Bhd.
6. Mahato, S.B., Nandy, A.K. and Roy,G. (1992). Triterpenoids. *Phytochemistry*, 31, 2199.
7. Mouffok, S., Haba, H., Lavaud, C., Long, C. & Benkhaled, M. (2012). Chemical constituents of *Centaurea amphalotricha* Coss. & Durieu ex Batt. & Trab. *Records of Natural Products*, 6(3), 292-295.
8. Menezes, F.S., Borsatto, A.S., Pereira, N.A, Matos, F.J.A & Kaplan, M.A.C. (1998). Chamaedrydiol, an ursane triterpene from *Marsypianthes chamaedrys*.*Phytochemistry*, 48(2), 323-325.
9. Paz Lima, M. et al. (2004). Phytochemistry of *Trattinnickia burserifolia*, *T. rhoifolia*, and *Dacryodes hopkinsii*: Chemosystematic Implications. *Journal of the Brazilian Chemical Society*. 15(3), 385-394.
10. Fateh, U. U., Haruna, A. K., Garba, M., Iliya, I., Sule, I. M., Abubakar, M. S., Ambi, A. A. (2009). Isolation of Stigmasterol, β -Sitosterol and 2 Hydroxyhexadecanoic Acid Methyl Ester from the Rhizomes of *Stylochiton lancifolius* Pyer and Kotchy (Areceae). *Nigerian Journal of Pharmaceutical Sciences*, 8(1),19-25.
11. Pretsch, E., Buhlmann, P. & Badertscher, M. (2009). Structure Determination of Organic Compounds: Tables of Spectral Data. Verlag Berlin Heidelberg: Springer.
12. Thanakijcharoenpath, W. and Theanphong, O. (2007). Triterpenoids from the stem of *Diospyros blandulosa*. *The Thai Journal of Pharmaceutical Sciences*. 31, 1-8.
13. Segovia, J.F.O. et al. (2011). Botanical characterisation, geographical distribution and phytochemistry analysis of manilkara huberi (ducke) stanhl autochtonous in amapa state, Brazil. *Proceedings of the National Academy of Belarus Biological Series*: Vol 2, 34-39.
14. Sobrinho, D., Hauptli, M.B., Appolinario, L.V., Kollenz, C.L.M., Carvalho, M.G. & Braz-Filho, R. (1991). Triterpenoids isolated from Parahancornia amapa. *Journal of Brazilian Chemical Society*, 2(1),1-10.
15. Wiart, C. (2006). Medicinal plants of Asia and the Pacific. *United States*: Taylor and Francis Group, LLC.

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- We would like to thank the University Pendidikan Sultan Idris for the provision of laboratory facilities and technical assistance as well as the excellent technical assistance of University Malaya.

Methodology



Results and Discussion

Table 1: ^{1}H NMR [600 MHz, δ_H (*J*, Hz)] of compound 1, 2, 3 4 and [500 MHz, δ_H (*J*, Hz)] of 5 in $CDCl_3$

Position	1	2	3	4	5
1	1.63 (m) 0.98 (t)	1.63 (m) 1.06 (m)	0.99 (m)	1.07 (m) 1.03 (m)	1.08 (m) 1.66 (m)
2	1.55 (m) 0.77 (m)	1.58 (m) 1.60 (m)	1.42 (m)	1.52 (m) 1.23 (m)	1.53 (m) 1.86 (m)
3	3.20 (dd, *J*=11.0, 5.0 Hz)	4.58 (t, 8.8 Hz)	4.47 (dd, 10.7 Hz and 5.5 Hz)	3.02 (m)	3.55 (m)
4				2.23 (m) 2.28 (dd 4 Hz and 1.7 Hz)	2.29 (m) 2.23 (m)
5	0.74 (m)	0.84 (m)	0.79 (m)		
6	1.53 (m)	1.53 (m)	1.50 (m)	5.35 (br s)	5.35 (m)
7	1.37 (m) 1.50 (m)	1.40 (m) 1.52 (m)	1.38 (m) 1.49 (m)	1.46 (m)	1.43 (m)
8	1.31 (m)	1.33 (m)	1.33 (m)	1.99 (m) 1.52 (m)	1.95 (m) 2.01 (m)
9	1.54 (m)	1.57 (m)	1.28 (m)	0.92 (m)	0.91 (m)
10					
11	1.35 (m) 1.45 (m)	1.00 (m) 1.43 (m)	1.39 (m) 1.21 (m)	1.52 (m)	1.44 (m) 1.59 (m)
12	5.18 (t, 3.5 Hz)	5.18 (t, 3.5 Hz)	1.66 (m) 1.85 (m)	1.15 (m) 2.02 (m)	1.15 (m) 2.02 (m)
13			1.65 (m)		
14				0.99 (m)	1.08 (m)
15	1.74 (m) 0.95 (m)	1.76 (m) 0.93 (m)	1.67 (m) 1.00 (m)	1.02 (m) 1.53 (m)	1.59 (m)
16	1.97 (m)	1.99 (m) 0.76 (m)	1.47 (m) 1.26 (m)	1.25(m) 1.68 (m) 1.07 (m)	1.24 (m) 1.83 (m) 1.08 (m)
17					0.88 (s)
18	1.94 (m)	1.93 (d, 4.2 Hz)	1.35 (m)	0.68 (s)	
19	1.64 (m) 1.00 (m)	1.46 (m) 1.02 (m)	2.40 (m)	1.00 (s)	1.01 (s)
20				2.02 (m)	1.35 (m)
21	1.31 (m) 1.16 (m)	1.08 (m) 1.33 (m)	1.91 (m) 1.33 (m)	1.18 (d, 5.7Hz)	0.92 (d,7Hz)
22	1.38 (m)	1.42 (m)	1.38 (m)	5.14 (dd, 15.5 Hz and 8.6 Hz)	1.03 (m)
23	1.21 (m) 0.98 (s)	1.20 (m) 0.88 (s)	1.18 (m) 0.94 (s)	5.10 (dd, 15.5 Hz and 9.2 Hz)	1.33 (m) 1.16 (m)
24	0.77 (s)	0.96 (s)	0.83 (s)	1.52 (m)	0.91 (m)
25	0.91 (s)	0.86 (s)	0.85 (s)	0.92 (m)	1.67 (m)
26	0.95 (s)	0.97 (s)	1.03 (s)	0.85 (d, 4.1 Hz)	0.84 (d, 6.9Hz)
27	1.12 (s)	1.13 (s)	0.94 (s)	0.79 (d, 6.9Hz)	0.83 (d, 6.9Hz)
28	0.81 (s)	0.83 (s)	0.78 (s)	1.25 (m)	1.25 (m)
29	0.85 (s)	0.87 (s)	4.69 (br s) 4.57 (br s)	0.80 (t, 6.9Hz)	0.85 (t,7.45Hz)
30	0.85 (s)	0.87 (s)	1.68 (s)		
31	-	-	-	-	-
32	-	2.05 (s)	2.05 (s)	-	-

Table 2: ^{13}C NMR [600 MHz, δ_H (*J*, Hz)] of compound 1, 2, 3, 4 and [500 MHz, δ_C (*J*, Hz)] of 5 in $CDCl_3$

Position	1	2	3	4	5
1	38.7	38.3	38.4	37.3	37.2
2	27.3	23.7	23.7	31.7	31.6
3	79.1	81.0	81.0	71.9	71.8
4	38.9	39.8	37.8	42.4	42.3
5	55.3	55.2	55.4	140.8	140.7
6	18.5	18.3	18.2	121.8	121.7
7	32.7	32.6	34.2	31.9	31.9
8	38.9	37.7	40.9	31.9	31.8
9	47.7	47.6	50.3	50.2	50.1
10	37.0	36.8	37.1	36.6	36.5
11	23.6	23.6	20.9	21.2	21.1
12	121.8	121.6	25.1	39.8	39.7
13	145.3	145.2	38.0	42.3	42.3
14	41.8	41.7	42.8	56.8	56.7
15	26.2	26.1	27.4	24.4	24.3
16	27.0	26.9	35.6	29.0	28.2
17	32.6	32.5	43.0	56.0	56.0
18	47.2	47.2	48.3	12.0	11.8
19	46.9	46.8	48.0	19.5	19.4
20	31.2	31.1	151.0	40.6	36.1
21	34.8	34.7	29.8	21.2	18.8
22	37.2	37.1	40.0	138.3	33.9
23	28.2	28.0	28.0	129.3	26.0
24	15.7	15.6	16.5	51.3	45.8
25	15.6	16.7	16.2	45.9	29.1
26	16.9	16.8	16.8	21.2	19.8
27	26.1	26.0	14.5	19.1	19.0
28	28.5	28.4	18.0	25.3	23.0
29	33.5	33.4	109.4	12.1	12.0
30	23.8	23.7	19.3	-	-
31	-	171.1	171.1	-	-
32	-	21.4	21.3	-	-

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Triterpenoids from the Bark of *Alstonia Spathulata* Bl. (Apocynaceae)

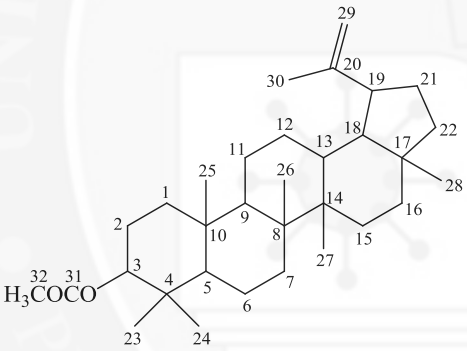
***Kartini Ahmad[1], Tee Chuan Thing, Khalijah Awang[2], Tan Siow Ping[1], Lee Yean Shan[1], Mohd Azlan Nafiah[1]**

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[2]Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur.
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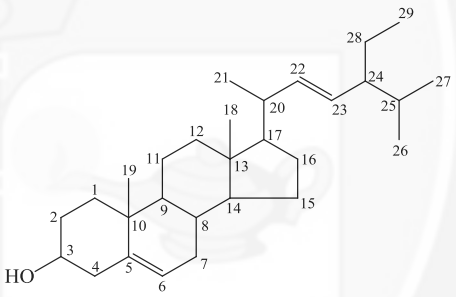
Abstract : In Malaysia, *Alstonia spathulata* Bl. is locally known as 'pulai basong' which distribute in peat forest. The separation of the chemical components from hexane crude extract (bark) of *Alstonia spathulata* was carried out using different chromatographic techniques (column chromatography and thin layer chromatography). Two compounds were isolated and identified as lupeol acetate **1** and stigmasterol **2**. These compounds have never been reported and isolated from this species. The structures of these compounds were elucidated based on the basis of detailed 1D-NMR (^{1}H, ^{13}C and DEPT) and 2D-NMR (COSY, HSQC, HMBC) spectroscopic analysis, involving also comparison with data from the literature.

Keywords: Apocynaceae, *Alstonia spathulata*, lupeol acetate, stigmasterol

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1



2

Introduction

Alstonia is genus that under tribe of Alstonieae in Rauvolfioideae subfamily of Apocynaceae and widely distributed at subtropical Africa, Central America, Southeast Asia, Polynesia and Australia, with most species in the Malesian region [1]. *Alstonia* is the genus of laticiferous shrubs or trees. The plant contain a milky sap which rich in poisonous alkaloids. *A. spathulata* is swamp dweller. The tree is pagoda-like crown. The bark is smooth, grayish and shows abundant of milky latex [2, 3]. In Malaysia, *Alstonia spathulata* Bl. is locally known as 'pulai basong'. In Cambodia, Laos, Vietnam, and, Malaysia, the latex from *Alstonia spathulata* Bl. is applied externally to sores and diseased skin. The bark is used to lower fever and to expel worms from the intestine [3]. The aim of this study is to isolate and identify the chemical compounds from the bark of *Alstonia spathulata.* In this paper, we report the isolation and characterization of lupeol acetate **1** and stigmasterol **2** which are the first time isolated from this species.

Materials and methods

General Methods

Nuclear Magnetic Resonance spectra (NMR) were recorded in deuterated chloroform (CDCl3) on a JEOL 600MHz and 500MHz. Chemical shifts (δ) were reported in ppm and coupling constants (*J*) in Hz. Mass spectra (MS) were determined by using the GC-mass spectroscopy (GC-MS Agilent 5975 Series). The infrared spectra (IR) were recorded on a Nicolet 6700 FTIR spectrophotometer, with CH_2Cl_2 as dilution solvent of the sample. Column chromatography were prepared by using Silica Gel 60F, 70-230 mesh ASTM and, 230-400 mesh ASTM and Silica Gel 60 containing Gypsum F_{254} as stationary phase. Analytical thin layer

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chromatography (TLC) was performed on commercially precoated aluminium supported silica gel $60F_{254}$ TLC sheets.

Plant Material

The species selected for the current study is *Alstonia spathulata* Bl. which was collected from Mersing, Johor and identified by the phytochemical group, Chemistry Department, Faculty Science, University of Malaya. The Voucher specimens were deposited at the Chemistry Department, Faculty Science, University of Malaya, Kuala Lumpur, Malaysia and the Herbarium of the Forest Research Institute, Kepong, Malaysia.

Extraction and Isolation

Dried grounded bark of the *Alstonia spathulata* was extracted with hexane for 17 hours at room temperature. The extract was dried by using rotary evaporator. For preliminary fractionation, the hexane crude extract was subjected to column chromatography over silica gel and eluted with different combinations solvent systems of hexane, dichloromethane and methanol mixture by increasing its polarity. Eluents were collected in fractions and were concentrated using rotary-evaporator. Each concentrated fractions was analysed by using aluminium supported TLC plate with a suitable solvent system and test with 5% sulphuric acid. The fractions having spots with same retention factor (R_f) value were grouped together. Each grouped of fractions were treated separately by extensive column chromatography or preparative TLC for further purification process until single spot show on the TLC.

Results and Discussion

Compound **1** was obtained as colourless amorphous. The IR spectrum showed stretching band for ester carbonyl (1712cm^{-1}) and C-O (1263 cm^{-1}) (Figure 1). The mass spectrum of **1** showed a molecular ion peak at m/z 468 $[M]^+$ corresponding to molecular formula $C_{32}H_{52}O_2$. The ^{1}H NMR spectrum (Table 1 and Figure 2) of **1** exhibited six methyl singlets at δ 0.84, 0.83, 0.85, 1.03, 0.94, 0.78 and a vinylic methyl at 1.68 ppm which corresponding to proton attached to C- 23, C-24, C 25, C-26, C-27, C- 28 and C-30 respectively. It was identified as triterpene acetate due to the presence of an acetate methyl signal at δ 2.05 as a singlet. H-3 showed proton signal at δ 4.47 (*dd*, *J* = 10.7 Hz, 5.5 Hz). The presence of two exo-methylene at δ 4.57 and δ 4.69 (H-29) and one methyl signal at δ1.68 (H-30) reminiscent lupane skeleton.

Analysis of ^{13}C NMR spectrum (Table 1 and Figure 3) showed the presence of thirty two carbon atoms indicating a mono acetyl derivative. There are six quaternary carbons δ 37.8 (C-4), δ 40.9 (C-8), δ 37.1 (C-10), δ 42.8 (C-14), δ 43.0 (C-17), δ 151.0 (C-20); six methine carbons δ 81.0 (C-3), δ 55.4 (C-5), δ 50.3 (C-9), δ 38.0 (C-13), δ 48.3 (C-18), δ 48.0 (C-19); eleven methylene carbons δ 38.4 (C-1), δ 23.7 (C-2), δ 18.0 (C-6), δ 34.2 (C-7), δ 20.9 (C-11), δ 25.1 (C-12), δ 27.4 (C-15), δ 35.6 (C-16), δ 29.8 (C-21), δ 40.0 (C-22), δ 109.4 (C-29); eight methyl carbons δ 28.0 (C-23), δ 16.5 (C-24), δ 16.2 (C-25), δ 16.0 (C-26), δ 14.5 (C-27), δ 18.2 (C-28), δ 19.3 (C-30), δ 21.3 (C-32) and the present of a carbonyl group at δ 171.1 (C-31). The alkene carbon appeared at δ 151.0 (C-20) and δ 109.4 (C-29). ^{1}H and ^{13}C NMR spectral data and mass spectrum were in agreement with those reported for lupeol acetate [4, 5]. Thus, compound **1** was identified as lupeol acetate.

Compound **2** was obtained as colourless needles (m.p: 151.0 - 152.0˚C). The mass spectral data of **2** gave a molecular formula $C_{29}H_{42}O$, m/z 413$[M+H]^+$. The IR spectrum displayed stretching band for O-H (3298 cm^{-1}), olefin (1640 cm^{-1}) and C-O (1019 cm^{-1}) (Figure 4). ^{1}H NMR spectrum (Table 2 and Figure 5) consisted of two methyl singlets at δ 0.68 (H-18) and δ 1.00 (H-19), methyl doublets at δ 1.18 (H-21), δ 0.85 (*d*, *J* = 4.1 Hz, H-26) and δ 0.79 (*d*, *J* = 6.8Hz, H-27) and a methyl tripet at δ 0.80 (H-29). A hydroxyl methine proton resonated at δ 3.52 as a multiplet corresponding to proton (H-3). Three olefinic protons in which the last two are of trans configuration were observed at δ 5.35, δ 5.14 (*dd*, *J* = 15.5 Hz and 8.6 Hz) and δ 5.00 (*dd*, *J* = 15.5 Hz and 9.2 Hz) attributable to H-6, H-22 and H-23 respectively.

^{13}C NMR spectrum (Table 2 and Figure 6) of compound **2** exhibited twenty nine signals. Signal for six methyl carbons C-18, C-19, C-21, C-26, C-27 and C-29 could be seen in the most upfield region of the NMR spectrum at δ 12.0, δ 19.5, δ 21.2, δ 21.2 δ 19.1 and δ 12.1. The position of three quaternary carbons appreared at δ 140.8 (C-5), δ 36.6 (C-10), δ 42.3 (C-13); nine methylene carbons: δ 37.3 (C-1), δ 31.7 (C-2), δ 42.4 (C-4), δ 31.9 (C-7), δ 21.2 (C-11), δ 39.8 (C-12), δ 24.4 (C-15), δ 29.0 (C-16), δ 29.8 (C-28); and eleven methine carbons: δ 71.9 (C-3), δ 121.8 (C-6), δ 31.9 (C-8), δ 50.2 (C-9), δ 56.8 (C-14), δ 56.0 (C-17), δ 40.6 (C-20), δ 138.4 (C-22), δ 129.3 (C-23), δ 51.3 (C-24), δ 45.9 (C-25). Signal at δ 140.8, δ 121.8, δ 138.3 and δ 129.3 were observed for four olefinic carbons, corresponding to C-5, C-6, C-22 and C-23 respectively. A deshielded signal of δ 71.9 confirmed the presence of hydroxyl group attached to C-3. This compound was found to be a C-5 unsaturated modified tetracyclic triterpene, which showed only 29 carbons instead of 30 carbons for tetracyclic triterpenes. Comparison of these ^{1}H and ^{13}C NMR data with literature [6, 7] showed that compund **2** is stigmasterol.

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Table 1 : ^{1}H NMR [600 MHz, δ_H (*J*, Hz)] and ^{13}C NMR [100 MHz, δ_C] of 1 in $CDCl_3$

Position	δ_H (*J* Hz), ppm	δ_C, ppm	DEPT	Lupeol acetate [75, 125 MHz, CDCl3] [5]
1	0.99 (*m*)	38.4	CH_2	38.6
2	1.62 (*m*)	23.7	CH_2	21.7
3	4.47 (*dd*, 10.7 and 5.5 Hz)	81.0	CH	81.2
4		37.8	Q	38.0
5	0.79 (*m*)	55.4	CH	55.6
6	1.50 (*m*)	18.2	CH_2	18.4
	1.38 (*m*)			
7	1.49 (*m*)	34.2	CH_2	34.4
	1.38 (*m*)			
8		40.9	Q	41.0
9	1.28 (*m*)	50.3	CH	50.5
10		37.1	Q	37.3
11	1.39 (*m*)	20.9	CH_2	21.1
	1.21 (*m*)			
12	1.66 (*m*)	25.1	CH_2	24.0
	1.05 (*m*)			
13	1.65 (*m*)	38.0	CH	36.2
14		42.8	Q	43.0
15	1.67 (*m*)	27.4	CH_2	25.3
	1.00 (*m*)			
16	1.47 (*m*)	35.6	CH_2	35.8
	1.36 (*m*)			
17		43.0	Q	43.2
18	1.35 (*m*)	48.3	CH	48.5
19	2.40 (*m*)	48.0	CH	48.2
20		151.0	Q	151.2
21	1.91 (*m*)	29.8	CH_2	30.0
	1.33 (*m*)			
22	1.38 (*m*)	40.0	CH_2	40.2
	1.18 (*m*)			
23	0.84 (*s*)	28.0	CH_3	27.6
24	0.83 (*s*)	16.5	CH_3	16.7
25	0.85 (*s*)	16.2	CH_3	16.4
26	1.03 (*s*)	16.0	CH_3	16.2
27	0.94 (*s*)	14.5	CH_3	14.7
28	0.78 (*s*)	18.0	CH_3	18.2
29	4.69 (*br, s*)	109.4	CH_2	109.6
	4.57 (*br, s*)			
30	1.68 (*s*)	19.3	CH_3	19.5
31		171.1	C=O	171.3
32	2.05 (*s*)	21.3	CH_3	28.2

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Table 2 : ^{1}H NMR [500 MHz, δ_H (*J*, Hz)] and ^{13}C NMR [100 MHz, δ_C] of 2 in $CDCl_3$

Position	δ_H (*J* Hz), ppm	δ_C, ppm	DEPT	Stigmasterol, [100MHz, $CDCl_3$] [2]
1	1.07 (*m*) 1.83 (*m*)	37.3	CH_2	37.3
2	1.52 (*m*) 1.83 (*m*)	31.7	CH_2	31.6
3	3.52 (*m*)	71.9	CH	71.8
4	2.23 (*m*) 2.28 (*dd* 4.6 and 1.7 Hz)	42.4	CH_2	42.3
5		140.8	Q	140.8
6	5.35 (*br, s*)	121.8	CH	121.7
7	1.46 (*m*) 1.99 (*m*)	31.9	CH_2	31.9
8	1.52 (*m*)	31.9	CH	31.9
9	0.92 (*m*)	50.2	CH	51.2
10		36.6	Q	36.5
11	1.52 (*m*)	21.2	CH_2	21.1
12	1.15 (*m*) 2.02 (*m*)	39.8	CH_2	39.7
13		42.3	Q	42.3
14	0.99 (*m*)	56.8	CH	56.9
15	1.02 (*m*) 1.58 (*m*)	24.4	CH_2	24.4
16	1.25(*m*) 1.68 (*m*)	29.0	CH_2	28.4
17	1.07 (*m*)	56.0	CH	56.1
18	0.68 (*s*)	12.0	CH_3	11.0
19	1.00 (*s*)	19.5	CH_3	21.2
20	2.02 (*m*)	40.6	CH	40.5
21	1.18 (*d*, 5.7Hz)	21.2	CH_3	21.2
22	5.14 (*dd*, 15.5 and 8.6 Hz)	138.3	CH	138.3
23	5.00 (*dd*, 15.5 and 9.2 Hz)	129.3	CH	129.3
24	1.52 (*m*)	51.3	CH	51.2
25	0.92 (*m*)	45.9	CH	31.9
26	0.85 (*d*, 4.1 Hz)	21.2	CH_3	21.2
27	0.79 (*d*, 6.8Hz)	19.1	CH_3	19.0
28	1.25 (*m*)	29.8	CH_2	25.4
29	0.80 (*t*, 6.9Hz)	12.1	CH_3	12.1

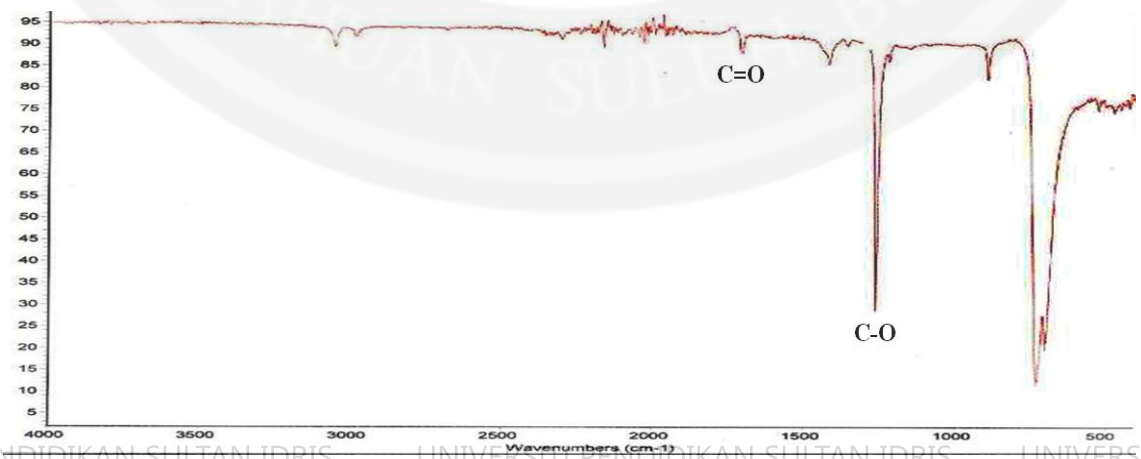


Figure 1 : IR spectrum of compound 1

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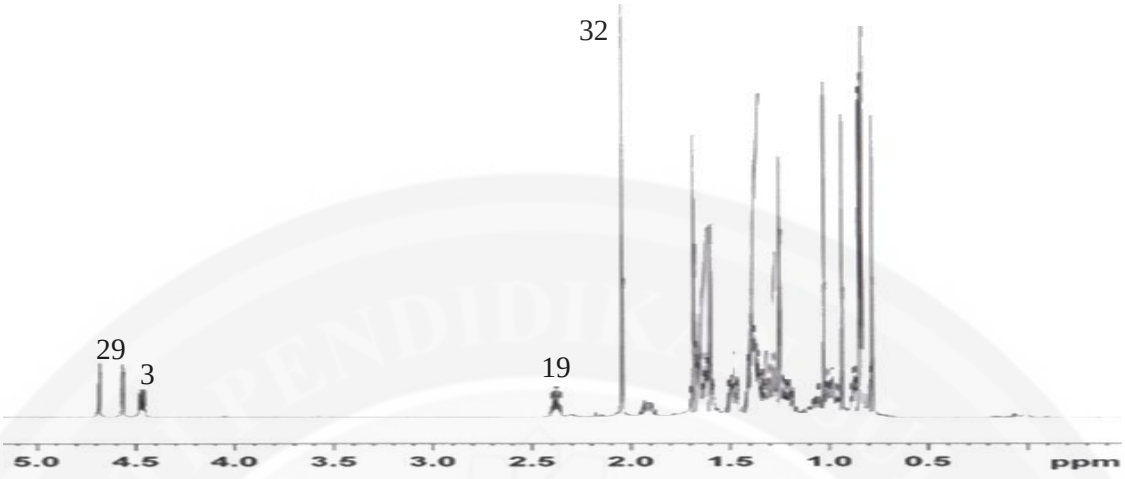


Figure 2 : ^{1}H NMR spectrum of compound 1

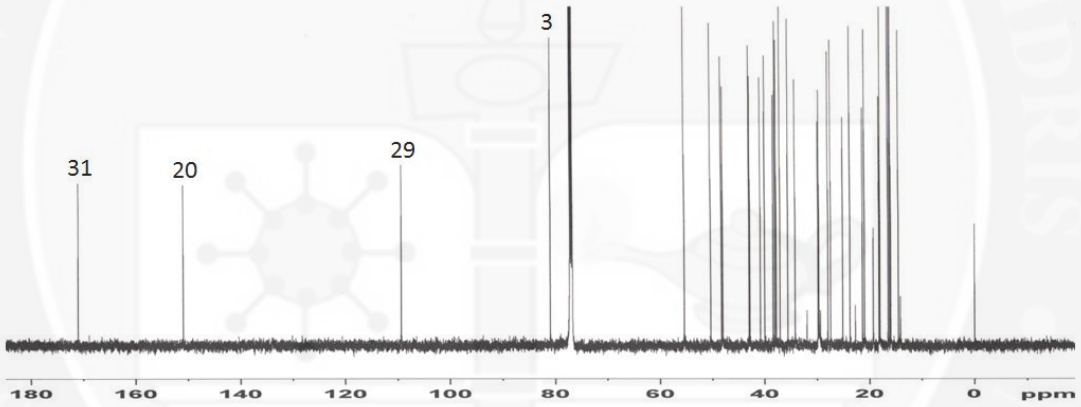


Figure 3 : ^{13}C NMR spectrum of compound 1

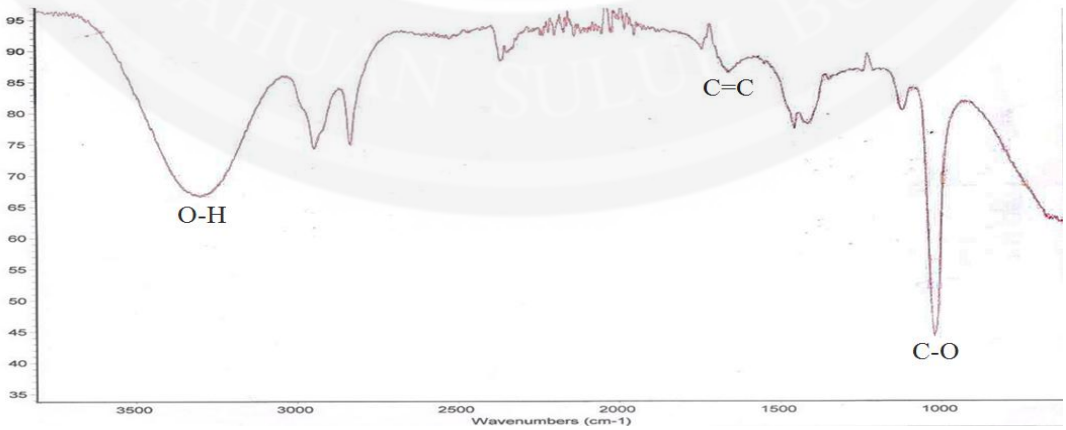


Figure 4 : IR spectrum of compound 2

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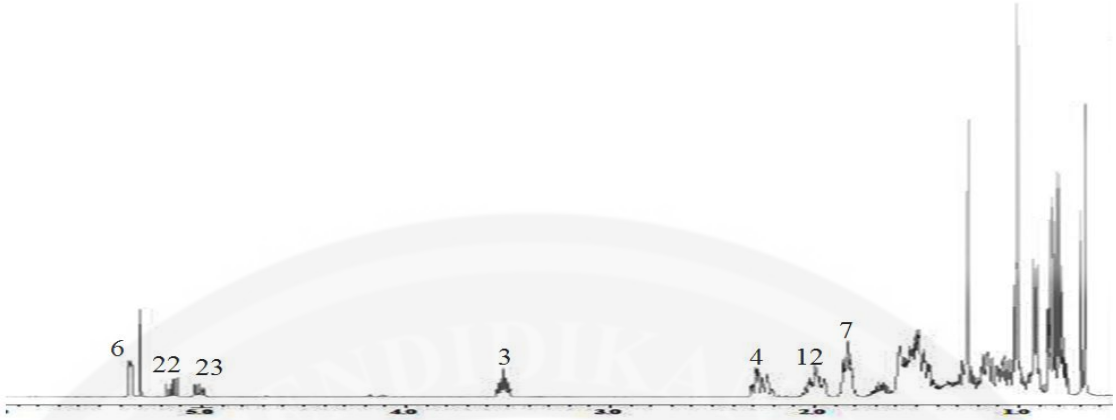


Figure 5 : ^{1}H NMR spectrum of compund 2

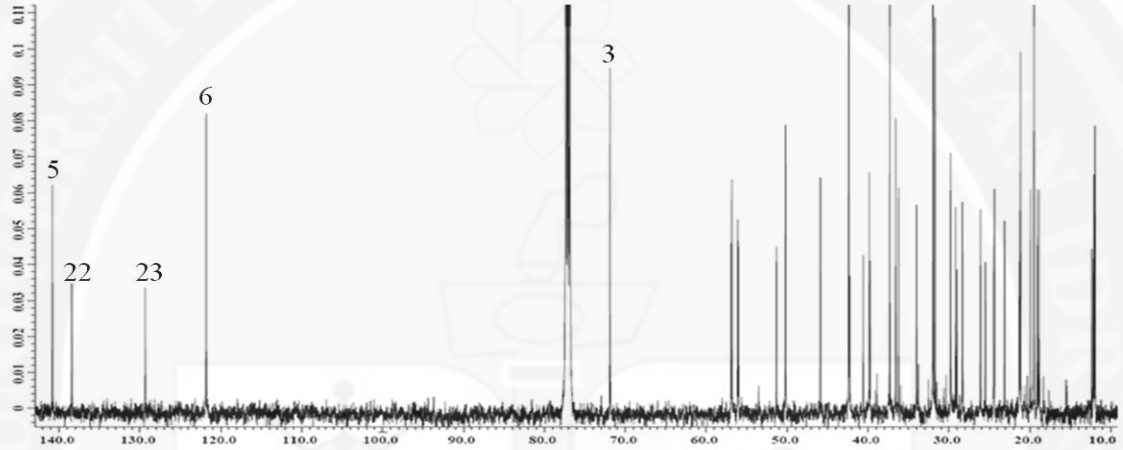


Figure 6 : ^{13}C NMR spectrum of compound 2

Conclusions

The research work through a systematic chemical investigation has determined and identified for the first time the presence of lupeol acetate **1** and stigmasterol **2** from the bark of *Alstonia spathulata.* The work was carried out by means of various physical (solvent extraction and column chromatography) and spectral techniques. The assignment will be further supported by 2D-NMR (COSY, HSQC, and HMBC) spectroscopic analysis. The structures of the compounds were also elucidated by comparison with literature.

Acknowledgement

We would like to thank the University Pendidikan Sultan Idris for the provision of laboratory facilities and technical assistance as well as the excellent technical assistance of University Malaya.

References

1. Webster's Online Dictionary. Definition: Alstonia. Retrieved on April 29, 2012 from http://www.websters-online-dictionary.org/definitions/alstonia
2. Pateh, U. U., Haruna, A. K., Garba, M., Iliya, I., Sule, I. M., Abubakar, M. S., Ambi, A. A. (2009) Isolation of Stigmasterol, β-Sitosterol and 2 Hydroxyhexadecanoic Acid Methyl Ester from the Rhizomes of *Stylochiton lancifolius* Pyer and Kotchy (Areceae). *Nigerian Journal of Pharmaceutical Sciences,* **8(1)**, 19-25.
3. Wiart, C. (2006) Medicinal plants of Asia and the Pacific. *United States:* Taylor and Francis Group, LLC.
4. Jamal, A. N. and Ammar, A. (2009) Chemical study on stem bark of *Etlingera hornstedia*. *International Journal of Agriculture and crop Sciences*, **1(1)**, 21-23.
5. Ajithabai, M. D., Sreedevi, S., Jayakumar, G., Mangalam, S. N., Deepa, P. N. N., Sunitha, R. S. P. (2011) Phytochemical Analysis and Radical Scavenging Activity of the Extracts of *Costus picatus* Linn and *Coccinia indica* W&A, two Ethnic Medicinal Plants used in the Treatment of *Diabetes mellitus. Free Radicals and Antioxidants*, **1(1)**, 77-83.
6. Jamal, A. K., Yaacob, W. A. & Laily, B. D. (2009) Triterpenes from the roots bark of *Phyllantus Columnaris. Australian Journal of Basic and Applied Sciences*, **3(2)**, 1428-1431.
7. Lim, W. H., Kho, B. L., Tay, T. H. & Low, W. L., (1998) Plant of Putrajaya wetlands. Putrajaya: Agriquest Sdn. Bhd.

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Research Article

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Cytotoxic, antibacterial and antioxidant activity of triterpenoids from *Kopsia singapurensis* Ridl.

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ABSTRACT

*Kopsia singapurensis Ridl. is one of the species of the genus Kopsia, also called white kopsia or locally known as 'selada' which about 18 species were distributed in Malaysia. This species is endemic only to Peninsular Malaysia and Singapore. This plant is known to produce a large number of biologically active compounds. In the present study, six known compounds were obtained from the hexane crude extract from the leave and bark of Kopsia singapurensis and identified as: lupeol **1**, lupeol acetate **2**, β-amyrin **3** β-amyrin acetate **4,** β-amyrone **5** and stigmasterol **6**. The structures of these compounds were elucidated by combination of various spectroscopic methods such as MS, UV, IR, 1D and 2D NMR, involving also comparison with data from the literature. All the isolated compounds exhibited cytotoxic effects against MCF-7 cell line, compound **6** was the most active compared to the rest of the compounds in the cytotoxic activity with IC_{50}= 14.5µg/mL, while all tested compounds showed no significant on antibacterial (gram-positive bacteria, Bacillus cereus) and antioxidant activity using the DPPH method.*

Key words: *Kopsia singapurensis* Ridl, cytotoxicity assay, antibacterial activity, antioxidant activity

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INTRODUCTION

Plants of the genus *Kopsia* is classified as member of the tribe Vinceae in subfamily Rauvolfioideae. The genus *Kopsia* (belonging to Apocynaceae family) comprises 30 species, distributed mainly over Southeast Asia, China and India [1]. *Kopsia singapurensis* Ridl.; one of the species of the genus *Kopsia*, also called white *kopsia* or locally known as 'selada' which about 18 species were distributed in Malaysia. In Singapore, this tree can be found and protected in the Nee Soon Swamp Forest of the Central Catchment's Nature Reserve [2]. This species is endemic only to Peninsular Malaysia and Singapore and rich in indole alkaloids [1-3]. Previous studies [4-6] have been reported that the alkaloids obtained from the plants in the family of Apocynaceae have antioxidant and anticancer properties. Various medicinal uses of this genus have been reported; the roots of *K. larutensis* King & Gamble, *K. macrophylla* Hook f., *K. singapurensis* Ridl. and *K. pauciflora* Hook f. were used to treat poulticing ulcerated noses in tertiary syphilis [7]. However, the isolation of terpenoids was only reported from the species *K. fruticosa* [8] and *K. longiflora* [9]. There is no report on the isolation of terpenoids from this species in the literature to date.

The purpose of this study is to isolate and identify the chemical compounds from the leaves and bark of *K. singapurensis* and to determine the bioactivities of the isolated compounds. In this paper, we report the isolation and characterization of known compounds from *K. singapurensis* namely lupeol **1**, lupeol acetate **2**, β-amyrin, **3** β-amyrin acetate **4,** β-amyrone **5** and stigmasterol **6**. The isolated compounds were then tested for cytotoxic activity by

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using MCF-7 cell line, antibacterial activity, *Bacillus cereus* was selected for this activity and antioxidant inhibitory activity (standard DPPH assay).

EXPERIMENTAL SECTION

General Methods

Nuclear Magnetic Resonance spectra (NMR) were recorded in deuterated chloroform ($CDCl_3$) on a BRUKER 400MHz, BRUKER 600MHz and JEOL 500MHz. Chemical shifts (δ) were expressed in ppm and the coupling constants (J) are given in Hz. Mass spectra (MS) were measured by using the GC-mass spectroscopy (GC-MS Agilent 5975 Series). The infrared spectra (IR) were recorded on a Nicolet 6700 FTIR spectrophotometer, with CH_2Cl_2 as dilution solvent of the sample. The ultra violet spectral was obtained in dichloromethane (CH_2Cl_2) on a Perkin Elmer UV- visible spectrophotometer and the wavelength of the spectrum was recorded in the range of 200 to 400nm. Solvent used for diluted the sample was CH_2Cl_2.

Column chromatography were prepared by using Silica Gel 60F, 70-230 mesh ASTM and, 230-400 mesh ASTM and Silica Gel 60 containing Gypsum F_{254} as stationary phase. Analytical thin layer chromatography (TLC) was performed on commercially precoated aluminium supported silica gel $60F_{254}$ TLC sheets.

Reagents

10.0% solution of sulphuric acid was used as a spraying reagent to detect the presence of terpenoids spotting on the TLC plates. A positive result was indicated by the formation of purple spot on the TLC plates once the plate was heated to 110ºC for 5 minutes.

Plant Material

The species selected for the current study is *Kopsia singapurensis* Ridl. (KL5334) collected from Kluang, Johor and identified by the phytochemical group, Chemistry Department, Faculty Science, University of Malaya. The Voucher specimens were deposited at the Chemistry Department, Faculty Science, University of Malaya, Kuala Lumpur, Malaysia and the Herbarium of the Forest Research Institute, Kepong, Malaysia.

Extraction and Isolation

The dried ground leaves (2.5kg) and barks (3.3kg) of *Kopsia singapurensis* were defatted separately with hexane by Soxhlet extractor for 17 hours. Then, the extracts were dried on the rotary evaporator. For preliminary fractionation, the both hexane crude extracts were subjected to column chromatography over silica gel and eluted with different combinations solvent systems of hexane, dichloromethane and methanol mixture by increasing its polarity.

Eluents were collected in fractions and were concentrated using rotary-evaporator. Then, each concentrated fraction was analyzed by using aluminium supported TLC plate with a suitable solvent system. After the development of the TLC, 10% sulphuric acid as spraying agents, the fractions having spots with same retention factor (R_f) value were grouped together. Each grouped fraction was treated separately by extensive column chromatography or preparative TLC for further purification process.

Preparation of the Bioactivity

Cell Culture and MTT Cytotoxicity Assay

A preliminary screening against cancer cells was performed using the Microculture Tetrazolium (MTT) method [10] against MCF-7 cell line. The MCF-7 used in this study was obtained from the American Type Cell Collection (ATCC). The cell was cultured using RPMI 1640 culturing media (PAA, Leverkusen, Germany). Besides, cell was trypsinized and counted using hemocytometer and plated in a microtiter plate of 96-wells. After an overnight incubation to allow cell attachment, the medium was changed and 0.2 mL of new supplemented medium was added into each well. Cells were then treated with different drug concentrations and incubated at 37 °C, 5% CO_2 for 24 hours. Each concentration of the samples was assayed in triplicate. The colorimetric assay was performed at an absorbance of 570 nm. Results were expressed as a percentage of control giving a certain percentage of cell viability after 24 hour exposure to the test agent. The potency of cell growth inhibition for test agent was expressed as an IC_{50} value [11].

Antibacterial Activity

A gram positive bacterium, *Bacillus cereus* was selected for the *in vitro* antibacterial activity. The strain was stored in the appropriate medium before use. Antibacterial activity was determined by the disc diffusion method [12] with slight modifications in terms of sample concentration, volume of sample loaded and use of paper discs. The bacteria were cultured at 37 °C for overnight in nutrient broth and potato dextrose broth, respectively. The concentrations of the cultures were adjusted turbidometrically at a wavelength of 600 nm which gave 10^5–10^6 colony forming units

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(CFU) per mL. The compounds to be tested were dissolved in dimethyl sulphoxide (DMSO) at concentration of 1 mg/mL. About 10 µL of each sample solution was loaded on Whatman No. 1 filter paper disc (0.6 mm). The disc was placed on the surface of the agar plate (nutrient agar or potato dextrose agar) previously inoculated with bacteria. The agar plates were then inverted and incubated for 24 hours at 37 °C. The antimicrobial activity was recorded by measuring the zone of inhibition (IZ) in mm around each disc, against the test organisms. The experiments were repeated in triplicate and the results were expressed as average values. The antibiotic streptomycin sulfate (10 µg/disc) was used as positive control and DMSO as negative control in the assay [11].

Antioxidant Inhibitory Activity

In this activity, the standard 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was used with slight modification. In this technique, 96 micro-well flat bottom plates were used instead of cuvettes to consume minimal amounts of sample, vehicle and DPPH solutions. Each compound was prepared at 1 mg/mL in DMSO. Each well was filled in with 100 µL of diluent (DMSO). The first row (row A) of the 96 wells was filled in with 100 µL of diluted sample at 1 mg/mL. Concentration in the first row was 500 µg/mL of sample in each well and was serially diluted (two fold dilution) in 96 micro-well plates to varying concentrations, topping from 500 µg/mL to the lowest 7.8 µg/mL. Then, 5 µL of the DPPH solution (2.5 mg/mL in DMSO) was added to each well. The DPPH solution should be kept in the dark at 4°C. This procedure should not be done under direct light. The plate was shaken to ensure thorough mixing before placed in the dark. After 30 minutes, the optical density of each well was read using ELISA Reader (EL340 Biokinetic Reader, Bio-Tek Instrumentation) at wavelength 517 nm. Percentage inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = 1 - \text{OD(DPPH + Sample)}/\text{OD(DPPH)} \times 100$$

The IC_{50} value was determined as the concentration of each sample required or able to scavenge 50% of the DPPH. All tests and analyses were run in triplicate and averaged. Antioxidant assay by using DPPH method was used to assess the capacity of the compounds to scavenge stable free radical DPPH at different concentration. The radical scavenging effect was examined and compared with natural antioxidants a-tocopherol (Sigma, USA), ascorbic acid (Sigma USA), and quercetin (Sigma, USA) used as positive controls (Table 1) [12].

Table 1: The IC_{50} values of the standard against DPPH (free radical).

Standard	**IC_{50}(µg/mL)**
Ascorbic acid	20 ±1.2
α-tocopherol	60 ±2.9
Quercetin	40 ±5.8

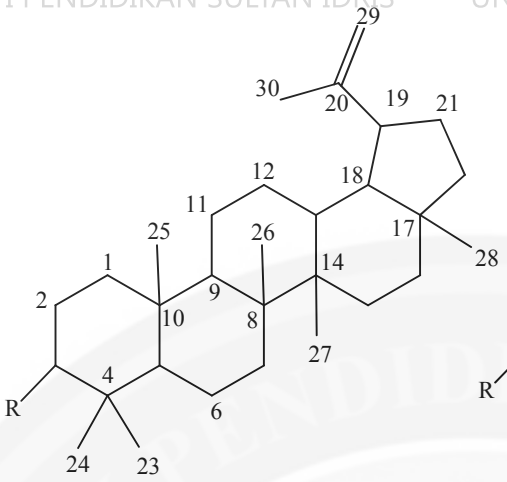
RESULTS AND DISCUSSION

Repeated chromatographic separation and purification of the hexane crude extracts of the leaf and bark of *Kopsia singapurensis* separately provided a total of six compounds (compound **1**, **2**, **3**, **4, 5** and **6**), the structure of the compounds were determined by ^{1}H and ^{13}C NMR spectral analysis as well as by comparison of their spectral data with previously reported values (Table 2 and 3). Compound **1** and **2** were belongs to lupane type triterpenoids while compound **3, 4** and **5** were belongs to oleanane type triterpenoids. Compound **6** was identified as stigmasterol from its physical constants and spectral data.

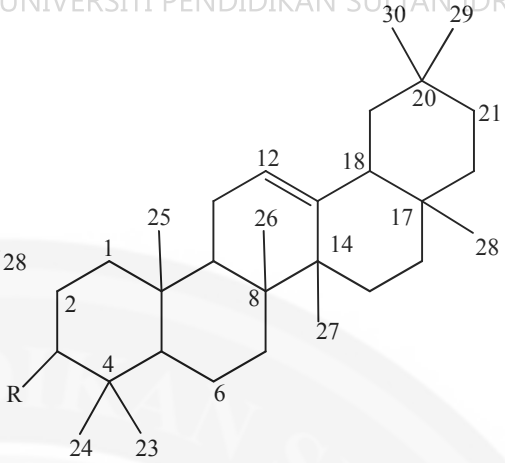
Compound **1** is a pentacyclic triterpenoid. It was obtained as white amorphous. In the GC-MS, the molecular formula $C_{30}H_{50}O$ was established from the molecular ion peak at m/z 426$[M]^{+}$. The IR spectrum of **1** displayed stretching band for O-H (3325cm^{-1}), olefin (1704cm^{-1}) and C-O (1204cm^{-1}) and the UV spectrum revealed the maxima absorption at 229.9 and 206.2nm. The ^{1}H NMR (500 MHz, CDCl3) spectrum showed seven tertiary methyl singlets (H-23, 24, 25, 26, 27, 28 and 30) and a proton of H-3 appeared as double doublet (*dd*) at δ 3.52 corresponding to hydroxyl methine proton. It also showed olefinic protons at δ 4.56 (H-29, *d*, *J* = 3.04Hz) and 4.68 (H-29, *d*, *J* = 2.32Hz). ^{13}C NMR (500 MHz, $CDCl_3$) spectrum of the compound showed 30 carbon signals for the triterpenoid of lupine skeleton which was represented by seven methyl groups. The carbon bonded to the hydroxyl group C-3 appeared at δ 79.1, while the two olefinic carbons appeared at δ 151.0 (C-20) and δ 109.3 (C-29). The spectra were compared with published data of Mouffok et al. works [13].

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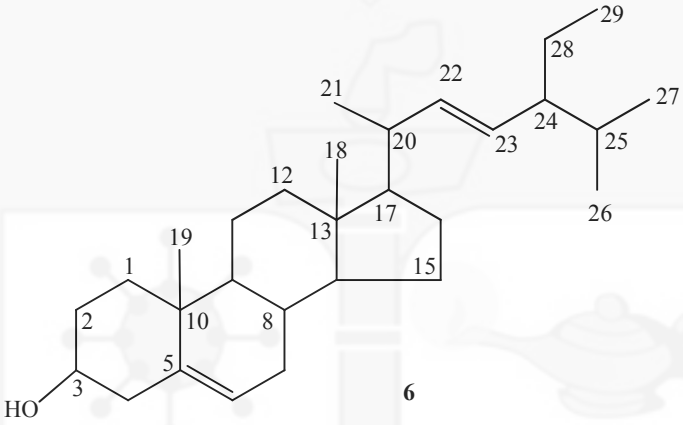
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1 R: OH
2 R: COOH



3 R: OH
4 R: COOH
5 R: $OCOCH_3$



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Compound **2** was isolated as white amorphous. This compound was found to be a lupane type triterpenoid, which is having similar skeleton with compound **1**. The mass spectrum of compound **2** exhibited a molecular ion peak at m/z 468 $[M]^+$ corresponding to its molecular formula $C_{32}H_{52}O_2$. The IR spectrum displayed an ester carbonyl and a C-O stretching band at 1712cm^{-1} and 1264cm^{-1}. The UV spectrum showed maxima absorption at 277.6nm. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) showed the presence of eight tertiary methyl singlets at δ 0.84(H-23), δ 0.83 (H-24), δ 0.85 (H-25), δ 1.03 (H-26), δ 0.94 (H-27), δ 0.78 (H-28), δ 1.68 (H-30) and δ 2.05 (H-32). Two protons appeared at δ 4.57 (*d, 2.0Hz*) and 4.69 (*d, 2.0Hz*) as doublet, representing the exocyclic double bond protons H-29a and H-29b, respectively. The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound 2 has a total of 32 carbon atoms. Compound **2** has an ester carbonyl signal at δ 171.1 (C-31), C-3 at δ 81.2 and the alkene carbons at δ 151.20 and 109.6. The identity of 2 as lupeol acetate was confirmed by comparison published values of Ajithabai, Sreedevi, Jayakumar, Mangalam, Deepa & Sunitha, (2011) [14, 15].

Compound **3** was isolated as white amorphous. The UV spectrum revealed the maximum absorptions at 273.4 and 229.0nm indicating the presence of a triterpenoid with carbon C-12 unsaturated oleanane skeleton [16]. The IR spectrum showed the band at 3360cm^{-1}, 1790cm^{-1} and 1294cm^{-1} which were assigned to functional groups for O-H, C=C and C-O, respectively. The molecular formula of **3** was established as $C_{30}H_{50}O$ from its quasi-molecular ion peak at m/z 449 $[M+Na]^+$ in the GC-MS analysis. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) displayed the eight tertiary methyl singlets at δ 0.98(H-23), δ 0.92 (H-24), δ 0.77 (H-25), δ 1.96 (H-26), δ 1.12 (H-27), δ 0.81 (H-28), δ 0.85 (H-29) and δ 0.85 (H-30). A signal at δ 3.20 (*dd, J = 11.0, 5.0 Hz*) was assigned to H-3 whereas another downfield signal, δ 5.16 (*t, J = 3.6Hz*) was attributed to the olefinic proton (H-12). The ^{13}C NMR spectrum (100 MHz, $CDCl_3$) showed a total of 30 carbon atoms. Compound **3** has a hydroxyl carbon signal at δ 79.1. Besides, the signal at δ 121.8 and δ 145.3 were assignable to C-12 and C-13 (olefinic carbon). The above spectral features are in close agreement to those observed for β-amyrin in literature [17].

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Table 2: ^{1}H NMR spectroscopic data for compound 1-6 [400,500,600 MHz,δH (J, Hz), $CDCl_3$]

Position	1	2	3	4	5	6
1	0.87 *(m)*	0.99 (m)	1.61 *(m)*	1.64 *(m)*	1.85 (m)	1.07 *(m)*
	1.66 (m)		0.98 (s)	1.04 (m)	1.36 (m)	1.83 (m)
2	1.54 (m)	1.62 (m)	1.59 (m);	1.88 (m)	2.33 (m)	1.52 (m)
	1.60 (m)		0.77 (m)	1.60 (m)	2.50 (m)	1.83 (m)
3	3.18 (*dd* , 11.5; 5.1Hz)	4.47 *(dd* , 10.7; 5.5Hz*)*	3.20 *(dd,* 11.0; 5.0Hz*)*	4.50 (*t* , 8.0Hz)		3.52 *(m)*
4						2.23 *(m)*
						2.28 (*dd* 4.6Hz; 1.7Hz)
5	0.68 *(m)*	0.79 *(m)*	0.73 *(m)*	0.84 *(m)*	1.28 (*m*)	
6	1.38 *(m)*	1.50 *(m)*	1.52 *(m)*	1.53 *(m)*	1.46 (*m*)	5.35 (*br, s*)
	1.52 *(m)*	1.38 *(m)*	1.38 *(m)*	1.40 *(m)*		
7	1.38 *(m)*	1.49 *(m)*	1.49 *(m)*	1.52 *(m)*	1.35 (*m*)	1.46 *(m)*
		1.38 *(m)*	1.31 *(m)*	1.33 *(m)*	1.50 (m)	1.99 *(m)*
8						1.52 *(m)*
9	1.29 *(m)*	1.28 *(m)*	1.54 *(m)*	1.58 *(m)*	0.91 (*m*)	0.92 *(m)*
10						
11	1.25 *(m)*	1.39 *(m)*	1.86 *(m)*	1.80 *(m)*	1.87 (*m*)	1.52 *(m)*
	1.38 *(m)*	1.21 *(m)*		1.63 *(m)*		
12	1.07 *(m)*	1.66 *(m)*	5.16 (*t,* 3.6Hz)	5.18 (*t* , 3.5Hz)	5.20 (*t* , 3.6Hz)	1.15 *(m)*
	1.68 *(m)*	1.05 *(m)*				2.02 *(m)*
13	1.68 *(m)*	1.65 *(m)*				
14						0.99 *(m)*
15	1.33 *(m)*	1.67 *(m)*	1.74 *(m)*	1.76 *(m)*	1.08 (*m*)	1.02 *(m)*
	1.68 *(m)*	1.00 *(m)*	0.95 *(m)*	0.85 *(m)*	1.74 (m)	1.58 *(m)*
16	1.47 *(m)*	1.47 *(m)*	1.97 *(m)*	1.98 *(m)*	0.78 (*m*)	1.25 *(m)*
		1.36 *(m)*		0.79 *(m)*		1.68 *(m)*
17						1.07 *(m)*
18	1.38 *(m)*	1.35 *(m)*	1.94 *(m)*	1.93 (*d* , 4.2Hz)	1.92 (*m*)	0.68 (*s*)
19	2.38 *(m)*	2.40 *(m)*	1.64 *(m)*	1.66 *(m)*	1.61 (*m*)	1.00 (*s*)
			1.01 *(m)*	1.00 *(m)*		
20						2.02 *(m)*
21	1.33 *(m)*	1.91 *(m)*	1.31 *(m)*	1.32 *(m)*	1.05 (*m*)	1.18 (*d,*
	1.91 *(m)*	1.33 *(m)*	1.09 *(m)*	1.08 *(m)*	1.31 (m)	5.7Hz)
23	0.96 (*s*)	0.84 *(s)*	0.98 *(s)*	0.88 *(s)*	1.08 (*s*)	5.00 (*dd* , 15.5Hz; 9.2Hz)
24	0.76 (s)	0.83 *(s)*	0.77 *(s)*	0.96 *(s)*	1.04 (*s*)	1.52 *(m)*
25	0.83 (s)	0.85 *(s)*	0.92 *(s)*	0.86 *(s)*	1.05 (*s*)	1.50 *(m)*
26	1.03 (*s*)	1.03 *(s)*	0.95 *(s)*	0,97 *(s)*	1.00 (*s*)	0.85 (*d* ,
27	0.94 (*s*)	0.94 *(s)*	1.12 *(s)*	1.13 *(s)*	1.13 (*s*)	0.79 (*d* ,
28	0.78 (*s*)	0.78 *(s)*	0.81 *(s)*	0.83 *(s)*	0.83 (*s*)	1.00 *(m)*
29	4.56 (*d,* 3.0Hz)	4.69 *(d,* 2.0Hz*)*	0.85 *(s)*	0.88 *(s)*	0.86 (*s*)	0.80 (*t,* 6.9Hz)
	4.68 (*d* , 2.3Hz)	4.57 *(d,* 2.0Hz*)*				
30	1.68 (*s*)	1.68 *(s)*	0.85 *(s)*	0.87 *(s)*	0.86 (*s*)	-
OAc	-	2.05 *(s)*	-	2.05 *(s)*		-

Compound **4** was obtained as colorless needles. This compound was found to be an oleanane type triterpenoid, which is having same skeleton with compound **3**. The IR spectrum displayed stretching band for C=O (1712cm^{-1}), and C-O (1264cm^{-1}). The UV spectrum showed maxima absorption at 277.8 and 229.7nm. The GC-MS of **4** gives an ion peak at m/z 468 $[M]^+$ corresponding to the molecular formula of compound of $C_{32}H_{52}O_2$. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) indicate the eight tertiary methyl singlets which is almost same with compound **3** except for the presence of acetoxyl group at δ 2.05 (H-32). A signal appear at downfield shift, δ 4.50 (t, J = 8.0 Hz),

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compared with the corresponding proton in compound **3**. The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum indicated a total of 32 carbons where the two olefinic carbon signals at δ 121.8 and δ 145.3, an acetoxyl group attached to C-3 (δ 81.0) with two carbon signals at δ 171.1 (C-31) and δ 21.4 (C-32). These spectra features are in close agreement to those observed for β-amyrin acetate by Segovia, et al. (2011) [18]. On this basis, the identity of compound **4** was confirmed as β-amyrin acetate.

Table 3: ^{13}C NMR spectroscopic data for compounds 1-6 [100, 125 MHz, δC, $CDCl_3$]

Position	**1**	**2**	**3**	**4**	**5**	**6**
1	38.8	38.4	38.7	38.3	39.4	37.3
2	27.5	23.7	27.3	23.5	24.3	31.7
3	79.1	81	79.1	81	218	71.9
4	38.9	37.8	39.9	39.8	47.6	42.4
5	55.3	55.4	55.3	55.2	55.4	140.8
6	18.4	18	18.5	18.3	19.7	121.8
7	34.3	34.2	32.7	32.6	32.2	31.9
8	40.8	40.9	38.9	37.7	39.9	31.9
9	50.5	50.3	47.7	47.6	46.9	50.2
10	37.2	37.1	37	36.8	36.8	36.6
11	21	20.9	23.6	23.6	23.7	21.2
12	25.2	25.1	121.8	121.6	121.6	39.8
13	38.1	38	145.3	145.2	145.4	42.3
14	42.9	42.8	41.8	41.7	41.9	56.8
15	27.5	27.4	26.2	26.1	26.2	24.4
16	35.7	35.6	27	26.9	27	29
17	43.1	43	32.6	32.5	32.6	56
18	48.4	48.3	47.2	47.2	47.4	12
19	48.1	48	46.9	46.8	46.8	19.5
20	151	151	31.2	31.1	31.2	40.6
21	29.9	29.8	34.8	34.7	34.8	21.2
22	40.1	40	37.2	37.1	37.2	138.3
23	28.1	28	28.2	28	26.5	129.3
24	15.5	16.5	15.7	15.6	21.6	51.3
25	16.2	16.2	15.6	16.7	15.3	32
26	16.1	16	16.9	16.8	16.8	21.2
27	14.6	14.5	26.1	26	26	19.1
28	18.1	18.2	28.5	28.4	28.5	25.4
29	109.3	109.4	33.5	33.4	33.4	12.1
30	19.4	19.3	23.8	23.7	23.8	-
31	-	171.1	-	171.1		-
32	-	21.3	-	21.4		-

Compound **5** was obtained as white amorphous. This compound was also found to be an oleanane type triterpene, which is having the same skeleton with compound **3** and **4**. The elucidation of compound **5** was then supported by the detail analysis of 1D and 2D NMR, also the GC-MS analysis which exhibited a molecular ion peak at m/z 424 $[M]^+$ in agreement with molecular formula $C_{30}H_{48}O$. The IR spectrum of **5** was almost similar to compound **3** but differ in the presence of carbonyl group at 1709cm^{-1}. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) indicate the eight tertiary methyl singlets which was almost same with compound **3** and **4.** A downfield signal at δ 5.20 (*t*, J = 3.6Hz) was probably positioned for an olefinic proton at C-12. The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum indicated a total of 30 carbons The present of a carbonyl group of C-3 at δ 218.0 was also observed in agreement with the absorption band at 1709cm^{-1} in the IR spectrum and the two olefinic carbon signals at δ 121.6 (C-12) and δ 145.4 (C-13), Detailed analysis of the spectral data obtained led to the conclusion that compound **5** is β-amyrone after comparison with the literature [19].

Compound **6** was isolated as colourless needles. Its molecular formula was assigned as $C_{29}H_{48}O$ from GC-MS at m/z 413$[M+H]^+$. This compound was found to be a C-5 unsaturated modified tetracyclic triterpenoid, which showed

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only 29 carbons instead of 30 carbons for tetracyclic triterpenoids. This was further supported by the UV spectrum which indicated maxima absorption at 276.8nm [16]. The IR spectrum displayed stretching band for O-H (3300 cm^{-1}), olefin (1711 cm^{-1}) and C-O (1263 cm^{-1}). The ^{1}H NMR spectrum of **5** showed characteristic signals for six methyls at δ 0.68 (Me-18, *s*), δ 1.00 (Me-19, *s*), δ 1.18 (Me-21, *d*, *J* = 5.7 Hz), δ 0.85 (Me-26, *d*, *J* = 4.0 Hz), δ 0.79 (Me-27, *d*, *J* = 6.8 Hz) and δ 0.80 (Me-29, *t*, *J* = 6.9 Hz). The proton of H-3 appeared as multiplet at δ 3.52 corresponding to hydroxyl methine proton. The typical signals for olefinic protons at δ 5.35 (H-6, *m*), δ 5.14 (H-22, *dd*, *J* = 15.5 and 8.6 Hz), δ 5.00 (H-23, *dd*, 15.5 Hz and 9.2 Hz). In the ^{13}C NMR spectrum, 29 carbon signals were observed. There are four olefinic carbon signals at δ 140.8 (C-5), δ 121.8 (C-6), δ 138.4 (C-22) and δ 129.3 (C-23). The compound was assigned as stigmasterol based on the physical and spectral data, also comparison with the literature values [15, 20].

All the compounds (**1 - 6**) were evaluated for the cytotoxicity assay agent MCF-7 cell line, antibacterial activity against *Bacillus cereus* and antioxidant activity using DPPH method.

In the cytotoxicity activity, a preliminary screening against cancer cells was performed using the MTT method [10] against MCF-7 cell line. Doxorubicin was used as a positive control in this study. The results showed that the compounds: lupeol **1**, lupeol acetate **2**, β-amyrin **3,** β-amyrin acetate **4,** β-amyrone **5** and stigmasterol **6** exhibited cytotoxic effect against cancer cell line with IC_{50} values ranging from 14.5 – 26.0µg/mL (Table 4). Compound **6**, stigmasterol was the most active compared to the rest of compounds in the cytotoxic activity with the IC_{50} value = 14.5µg/mL.

The preliminary screening results of antibacterial activity against *Bacillus cereus* was summarized in Table 4. Antibacterial activity was indicated by a clear zone of growth inhibition. All the compounds were evaluated for antibacterial activity using disc diffusion method and streptomycin sulfate was used as a positive control in the assay. The results indicated that all the tested isolated compounds were found to be inactive against the gram-positive bacteria, *Bacillus cereus* [21, 22].

Compounds **1 - 6** were tested for the antioxidant assay by using DPPH method. This assay was used to assess the capacity of the compounds to scavenge stable free radical DPPH at different concentration. From Table 4, triterpenoids showed weak antioxidant activity with IC_{50} >500 µg/mL, compare with the standard natural antioxidants a-tocopherol, ascorbic acid and quercetin.

Table 4: Cytotoxic effects against MCF-7 cell line, antibacterial and antioxidant activity on isolated compounds.

Compounds	Cytotoxic	Antibacterial activity	Antioxidant activity
	IC_{50} values (µg/mL)	Diameter of inhibition zone (mm)	IC_{50} values (µg/mL)
	MCF-7	*B.cereus*	DPPH
Lupeol **1**	17	NI	>500
Lupeol acetate **2**	26	NI	>500
β-amyrin **3**	15.5	NI	>500
β-amyrin acetate **4**	22.5	NI	>500
β-amyrone **5**	21.5	NI	>500
Stigmasterol **6**	14.5	NI	>500
*No inhibition (NI)			

CONCLUSION

The phytochemical study was aimed to investigate the chemical constituents of *Kopsia singapurensis* Ridl. (KL 5334) collected from Kluang (Johor) and to determine the bioactivitis of the isolated compounds. Structural elucidation was established by using various modern spectroscopic techniques such as ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR) including 1D-NMR (^{1}H, ^{13}C, and DEPT), 2D-NMR (COSY, HSQC, and HMBC) and MS (GC-MS). The study on the leaves and bark of *K. singapurensis* yielded a total of 5 pentacyclic triterpenoid: lupeol **1**, lupeol acetate **2**, β-amyrin **3**, β-amyrin acetate **4,** β-amyrone **5** and a steroid namely stigmasterol **6** which were common occurrence in plant. The structures of the compounds were also elucidated by comparison with literature values. All the isolated compounds exhibited cytotoxic effects against MCF-7 cell line, compound **6** was the most active compared to the rest of compounds in the cytotoxic activity with IC_{50}= 14.5µg/mL, while all tested compounds showed no significant on antibacterial (gram-positive bacteria, Bacillus cereus) and

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antioxidant activity using the DPPH method. Based on the previous reports on cytotoxic properties of *Kopsia singapurensis* with that of the present results, it clearly indicates that *Kopsia singapurensis* has potential cytotoxic properties and can be used as a source of antitumor agents.

Acknowledgement
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REFERENCES

[1] K Ahmad; Y Hirasawa; AE Nugroho; AHA Hadi; H Morita. *Heterocycles*, **2012**, 86(2), 1611-1619.
[2] DJ Middleton. *Harvard Papers in Botany*. **2004**, 9(1), 89-142.
[3] K Ahmad; Y Hirasawa; AE Nugroho; AHA Hadi; K Takeya; NF Thomas; K Awang; H Morita; SP Tan; MA Nafiah. *The Open Conference Proceedings Journal*, **2013,** 4(Suppl-2, M18), 75-82.
[4] T Kumari; J Shukla; S Joshi. *Journal of Chemical and Pharmaceutical Research*, **2012**, 4(10), 4533-4541.
[5] K Awang; K Ahmad; NF Thomas; Y Hirasawa; K Takeya; MR Mukhtar; K Mohamad; H Morita. *Heterocycles*, **2008**, 75(12), 3051-3056.
[6] SA Gadir. *Journal of Chemical and Pharmaceutical Research*, **2012**, 4(12), 5145-5148.
[7] HI Burkill. A Dictionary of the Economic Products of the Malay Peninsula, Ministry of Agriculture and Cooperatives, Kuala Lumpur, Malaysia, **2008**.
[8] SK Talapatra; B Malabika. *Journal of the Indian Chemical Society*, **1968**, 45(10), 962-963.
[9] S Iseda. *Chemical & Pharmaceutical Bulletin*, **1959**, 7, 129 – 130.
[10] T Mosmann. *J. Immunol. Methods*, **1983**, 65, 55–63. (1983).
[11] H Omar; NM Hashim; A Zajmi; N Nordin; SI Abdelwahab; AHS Azizan; AHA Hadi; HM Ali. *Molecules*, **2013**, 18, 8994-9009.
[12] NM Hashim; M Rahmani; GCL Ee; MA Sukari; M Yahayu; MAM Amin; AM Ali; R Go. *Molecules*, **2012**, 17, 6071–6082.
[13] S Mouffok; H Haba; C Lavaud; C Long; M Benkhaled. *Rec. Nat. Prod.* **2012**, 6(3), 292-295.
[14] MD Ajithabai; S Sreedevi; G Jayakumar; SN Mangalam; PNN Deepa; RSP Sunitha. *Free Radicals and Antioxidants*, **2011**, 1(1), 77-83.
[15] K Ahmad; CT Tee; K Awang; SP Tan; YS Lee; MA Nafiah. *Malaysian Journal of Chemistry*, **2013**, 15(1), 041-046.
[16] M Burnouf-Radosevich; NE Delfel. *Journal of Chromatography*, **1984**, 292, 403-409.
[17] W Thanakijcharoenpath; O Theanphong. *The Thai Journal of Pharmaceutical Sciences*. **2007**, 31, 1-8.
[18] JFO Segovia; et al. *Proceedings of the National Academy of Belarus Biological*, **2011**, *Vol 2*, 34-39.
[19] CS Li; HW Yu; GY Li; GL Zhang. *Chinese Journal of Natural Medicines*, **2010**, 8(4), 270-273.
[20] UU Pateh; AK Haruna; M Garba; I Iliya; IM Sule; MS Abubakar; AA Ambi. *Nigerian Journal of Pharmaceutical Sciences*, **2009**, 8(1), 19-25.
[21] KS Kosmulalage; S Zahid; CC Udenigwe; S Akhtar; A Ata; R Samarasekera. *Z. Naturforsch*, **2007**, 62b, 580-586.
[22] CY Ragasa; J Yumul; J Guanzon; JA Rideout. *KIMIKA*, **2001**, 17(1), 1-4.

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Triterpenoids from the Bark of *Alstonia Spathulata* Bl. (Apocynaceae)

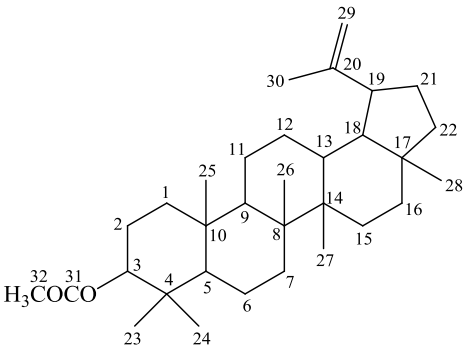
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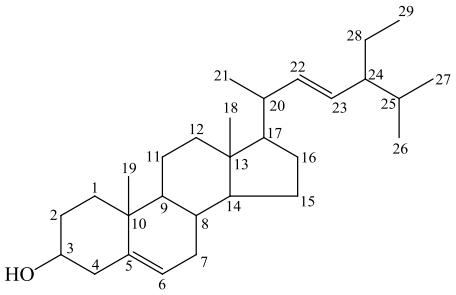
Abstract : In Malaysia, *Alstonia spathulata* Bl. is locally known as 'pulai basong' which distribute in peat forest. The separation of the chemical components from hexane crude extract (bark) of *Alstonia spathulata* was carried out using different chromatographic techniques (column chromatography and thin layer chromatography). Two compounds were isolated and identified as lupeol acetate **1** and stigmasterol **2**. These compounds have never been reported and isolated from this species. The structures of these compounds were elucidated based on the basis of detailed 1D-NMR (^{1}H, ^{13}C and DEPT) and 2D-NMR (COSY, HSQC, HMBC) spectroscopic analysis, involving also comparison with data from the literature.

Keywords: Apocynaceae, *Alstonia spathulata*, lupeol acetate, stigmasterol

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1



2

Introduction

Alstonia is genus that under tribe of Alstonieae in Rauvolfioideae subfamily of Apocynaceae and widely distributed at subtropical Africa, Central America, Southeast Asia, Polynesia and Australia, with most species in the Malesian region [1]. *Alstonia* is the genus of laticiferous shrubs or trees. The plant contain a milky sap which rich in poisonous alkaloids. *A. spathulata* is swamp dweller. The tree is pagoda-like crown. The bark is smooth, grayish and shows abundant of milky latex [2, 3]. In Malaysia, *Alstonia spathulata* Bl. is locally known as 'pulai basong'. In Cambodia, Laos, Vietnam, and, Malaysia, the latex from *Alstonia spathulata* Bl. is applied externally to sores and diseased skin. The bark is used to lower fever and to expel worms from the intestine [3]. The aim of this study is to isolate and identify the chemical compounds from the bark of *Alstonia spathulata.* In this paper, we report the isolation and characterization of lupeol acetate **1** and stigmasterol **2** which are the first time isolated from this species.

Materials and methods

General Methods

Nuclear Magnetic Resonance spectra (NMR) were recorded in deuterated chloroform (CDCl3) on a JEOL 600MHz and 500MHz. Chemical shifts (δ) were reported in ppm and coupling constants (*J*) in Hz. Mass spectra (MS) were determined by using the GC-mass spectroscopy (GC-MS Agilent 5975 Series). The infrared spectra (IR) were recorded on a Nicolet 6700 FTIR spectrophotometer, with CH_2Cl_2 as dilution solvent of the sample. Column chromatography were prepared by using Silica Gel 60F, 70-230 mesh ASTM and, 230-400 mesh ASTM and Silica Gel 60 containing Gypsum F_{254} as stationary phase. Analytical thin layer

chromatography (TLC) was performed on commercially precoated aluminium supported silica gel $60F_{254}$ TLC sheets.

Plant Material

The species selected for the current study is *Alstonia spathulata* Bl. which was collected from Mersing, Johor and identified by the phytochemical group, Chemistry Department, Faculty Science, University of Malaya. The Voucher specimens were deposited at the Chemistry Department, Faculty Science, University of Malaya, Kuala Lumpur, Malaysia and the Herbarium of the Forest Research Institute, Kepong, Malaysia.

Extraction and Isolation

Dried grounded bark of the *Alstonia spathulata* was extracted with hexane for 17 hours at room temperature. The extract was dried by using rotary evaporator. For preliminary fractionation, the hexane crude extract was subjected to column chromatography over silica gel and eluted with different combinations solvent systems of hexane, dichloromethane and methanol mixture by increasing its polarity. Eluents were collected in fractions and were concentrated using rotary-evaporator. Each concentrated fractions was analysed by using aluminium supported TLC plate with a suitable solvent system and test with 5% sulphuric acid. The fractions having spots with same retention factor (R_f) value were grouped together. Each grouped of fractions were treated separately by extensive column chromatography or preparative TLC for further purification process until single spot show on the TLC.

Results and Discussion

Compound **1** was obtained as colourless amorphous. The IR spectrum showed stretching band for ester carbonyl (1712cm^{-1}) and C-O (1263 cm^{-1}) (Figure 1). The mass spectrum of **1** showed a molecular ion peak at m/z 468 $[M]^+$ corresponding to molecular formula $C_{32}H_{52}O_2$. The ^{1}H NMR spectrum (Table 1 and Figure 2) of **1** exhibited six methyl singlets at δ 0.84, 0.83, 0.85, 1.03, 0.94, 0.78 and a vinylic methyl at 1.68 ppm which corresponding to proton attached to C- 23, C-24, C 25, C-26, C-27, C- 28 and C-30 respectively. It was identified as triterpene acetate due to the presence of an acetate methyl signal at δ 2.05 as a singlet. H-3 showed proton signal at δ 4.47 (*dd*, *J* = 10.7 Hz, 5.5 Hz). The presence of two exo-methylene at δ 4.57 and δ 4.69 (H-29) and one methyl signal at δ1.68 (H-30) reminiscent lupane skeleton.

Analysis of ^{13}C NMR spectrum (Table 1 and Figure 3) showed the presence of thirty two carbon atoms indicating a mono acetyl derivative. There are six quaternary carbons δ 37.8 (C-4), δ 40.9 (C-8), δ 37.1 (C-10), δ 42.8 (C-14), δ 43.0 (C-17), δ 151.0 (C-20); six methine carbons δ 81.0 (C-3), δ 55.4 (C-5), δ 50.3 (C-9), δ 38.0 (C-13), δ 48.3 (C-18), δ 48.0 (C-19); eleven methylene carbons δ 38.4 (C-1), δ 23.7 (C-2), δ 18.0 (C-6), δ 34.2 (C-7), δ 20.9 (C-11), δ 25.1 (C-12), δ 27.4 (C-15), δ 35.6 (C-16), δ 29.8 (C-21), δ 40.0 (C-22), δ 109.4 (C-29); eight methyl carbons δ 28.0 (C-23), δ 16.5 (C-24), δ 16.2 (C-25), δ 16.0 (C-26), δ 14.5 (C-27), δ 18.2 (C-28), δ 19.3 (C-30), δ 21.3 (C-32) and the present of a carbonyl group at δ 171.1 (C-31). The alkene carbon appeared at δ 151.0 (C-20) and δ 109.4 (C-29). ^{1}H and ^{13}C NMR spectral data and mass spectrum were in agreement with those reported for lupeol acetate [4, 5]. Thus, compound **1** was identified as lupeol acetate.

Compound **2** was obtained as colourless needles (m.p: 151.0 - 152.0˚C). The mass spectral data of **2** gave a molecular formula $C_{29}H_{42}O$, m/z 413$[M+H]^+$. The IR spectrum displayed stretching band for O-H (3298 cm^{-1}), olefin (1640 cm^{-1}) and C-O (1019 cm^{-1}) (Figure 4). ^{1}H NMR spectrum (Table 2 and Figure 5) consisted of two methyl singlets at δ 0.68 (H-18) and δ 1.00 (H-19), methyl doublets at δ 1.18 (H-21), δ 0.85 (*d*, *J* = 4.1 Hz, H-26) and δ 0.79 (*d*, *J* = 6.8Hz, H-27) and a methyl tripet at δ 0.80 (H-29). A hydroxyl methine proton resonated at δ 3.52 as a multiplet corresponding to proton (H-3). Three olefinic protons in which the last two are of trans configuration were observed at δ 5.35, δ 5.14 (*dd*, *J* = 15.5 Hz and 8.6 Hz) and δ 5.00 (*dd*, *J* = 15.5 Hz and 9.2 Hz) attributable to H-6, H-22 and H-23 respectively.

^{13}C NMR spectrum (Table 2 and Figure 6) of compound **2** exhibited twenty nine signals. Signal for six methyl carbons C-18, C-19, C-21, C-26, C-27 and C-29 could be seen in the most upfield region of the NMR spectrum at δ 12.0, δ 19.5, δ 21.2, δ 21.2 δ 19.1 and δ 12.1. The position of three quaternary carbons appreared at δ 140.8 (C-5), δ 36.6 (C-10), δ 42.3 (C-13); nine methylene carbons: δ 37.3 (C-1), δ 31.7 (C-2), δ 42.4 (C-4), δ 31.9 (C-7), δ 21.2 (C-11), δ 39.8 (C-12), δ 24.4 (C-15), δ 29.0 (C-16), δ 29.8 (C-28); and eleven methine carbons: δ 71.9 (C-3), δ 121.8 (C-6), δ 31.9 (C-8), δ 50.2 (C-9), δ 56.8 (C-14), δ 56.0 (C-17), δ 40.6 (C-20), δ 138.4 (C-22), δ 129.3 (C-23), δ 51.3 (C-24), δ 45.9 (C-25). Signal at δ 140.8, δ 121.8, δ 138.3 and δ 129.3 were observed for four olefinic carbons, corresponding to C-5, C-6, C-22 and C-23 respectively. A deshielded signal of δ 71.9 confirmed the presence of hydroxyl group attached to C-3. This compound was found to be a C-5 unsaturated modified tetracyclic triterpene, which showed only 29 carbons instead of 30 carbons for tetracyclic triterpenes. Comparison of these ^{1}H and ^{13}C NMR data with literature [6, 7] showed that compund **2** is stigmasterol.

Table 1 : ^{1}H NMR [600 MHz, δ_H (*J*, Hz)] and ^{13}C NMR [100 MHz, δ_C] of 1 in $CDCl_3$

Position	δ_H (*J* Hz), ppm	δ_C, ppm	DEPT	Lupeol acetate [75, 125 MHz, CDCl3] [5]
1	0.99 (*m*)	38.4	CH_2	38.6
2	1.62 (*m*)	23.7	CH_2	21.7
3	4.47 (*dd*, 10.7 and 5.5 Hz)	81.0	CH	81.2
4		37.8	Q	38.0
5	0.79 (*m*)	55.4	CH	55.6
6	1.50 (*m*)	18.2	CH_2	18.4
	1.38 (*m*)			
7	1.49 (*m*)	34.2	CH_2	34.4
	1.38 (*m*)			
8		40.9	Q	41.0
9	1.28 (*m*)	50.3	CH	50.5
10		37.1	Q	37.3
11	1.39 (*m*)	20.9	CH_2	21.1
	1.21 (*m*)			
12	1.66 (*m*)	25.1	CH_2	24.0
	1.05 (*m*)			
13	1.65 (*m*)	38.0	CH	36.2
14		42.8	Q	43.0
15	1.67 (*m*)	27.4	CH_2	25.3
	1.00 (*m*)			
16	1.47 (*m*)	35.6	CH_2	35.8
	1.36 (*m*)			
17		43.0	Q	43.2
18	1.35 (*m*)	48.3	CH	48.5
19	2.40 (*m*)	48.0	CH	48.2
20		151.0	Q	151.2
21	1.91 (*m*)	29.8	CH_2	30.0
	1.33 (*m*)			
22	1.38 (*m*)	40.0	CH_2	40.2
	1.18 (*m*)			
23	0.84 (*s*)	28.0	CH_3	27.6
24	0.83 (*s*)	16.5	CH_3	16.7
25	0.85 (*s*)	16.2	CH_3	16.4
26	1.03 (*s*)	16.0	CH_3	16.2
27	0.94 (*s*)	14.5	CH_3	14.7
28	0.78 (*s*)	18.0	CH_3	18.2
29	4.69 (*br, s*)	109.4	CH_2	109.6
	4.57 (*br, s*)			
30	1.68 (*s*)	19.3	CH_3	19.5
31		171.1	C=O	171.3
32	2.05 (*s*)	21.3	CH_3	28.2

Table 2 : ^{1}H NMR [500 MHz, δ_H (*J*, Hz)] and ^{13}C NMR [100 MHz, δ_C] of 2 in $CDCl_3$

Position	δ_H (*J* Hz), ppm	δ_C, ppm	DEPT	Stigmasterol, [100MHz, $CDCl_3$] [2]
1	1.07 (*m*)	37.3	CH_2	37.3
	1.83 (*m*)			
2	1.52 (*m*)	31.7	CH_2	31.6
	1.83 (*m*)			
3	3.52 (*m*)	71.9	CH	71.8
4	2.23 (*m*)	42.4	CH_2	42.3
	2.28 (*dd* 4.6 and 1.7 Hz)			
5		140.8	Q	140.8
6	5.35 (*br, s*)	121.8	CH	121.7
7	1.46 (*m*)	31.9	CH_2	31.9
	1.99 (*m*)			
8	1.52 (*m*)	31.9	CH	31.9
9	0.92 (*m*)	50.2	CH	51.2
10		36.6	Q	36.5
11	1.52 (*m*)	21.2	CH_2	21.1
12	1.15 (*m*)	39.8	CH_2	39.7
	2.02 (*m*)			
13		42.3	Q	42.3
14	0.99 (*m*)	56.8	CH	56.9
15	1.02 (*m*)	24.4	CH_2	24.4
	1.58 (*m*)			
16	1.25(*m*)	29.0	CH_2	28.4
	1.68 (*m*)			
17	1.07 (*m*)	56.0	CH	56.1
18	0.68 (*s*)	12.0	CH_3	11.0
19	1.00 (*s*)	19.5	CH_3	21.2
20	2.02 (*m*)	40.6	CH	40.5
21	1.18 (*d*, 5.7Hz)	21.2	CH_3	21.2
22	5.14 (*dd*, 15.5 and 8.6 Hz)	138.3	CH	138.3
23	5.00 (*dd*, 15.5 and 9.2 Hz)	129.3	CH	129.3
24	1.52 (*m*)	51.3	CH	51.2
25	0.92 (*m*)	45.9	CH	31.9
26	0.85 (*d*, 4.1 Hz)	21.2	CH_3	21.2
27	0.79 (*d*, 6.8Hz)	19.1	CH_3	19.0
28	1.25 (*m*)	29.8	CH_2	25.4
29	0.80 (*t*, 6.9Hz)	12.1	CH_3	12.1

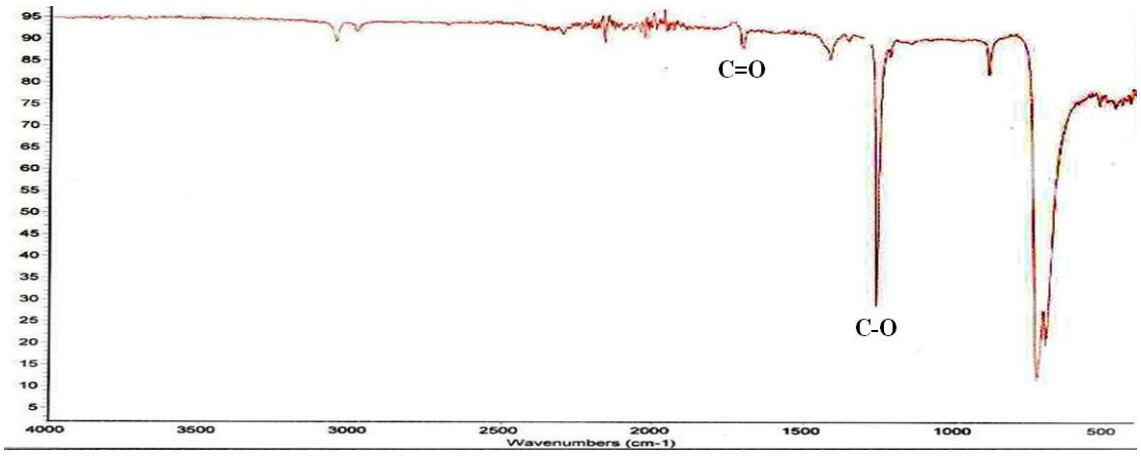


Figure 1 : IR spectrum of compound 1

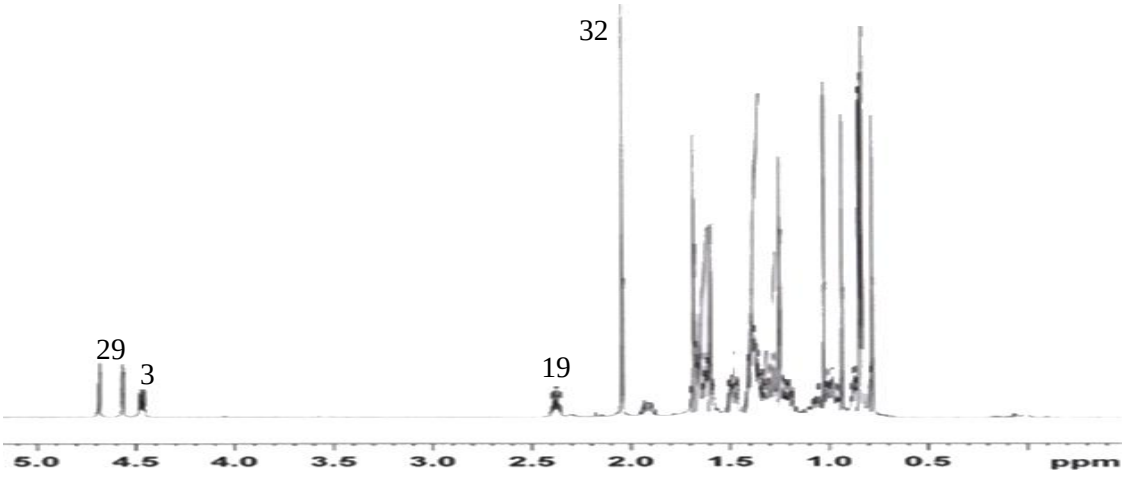


Figure 2 : ^{1}H NMR spectrum of compound 1

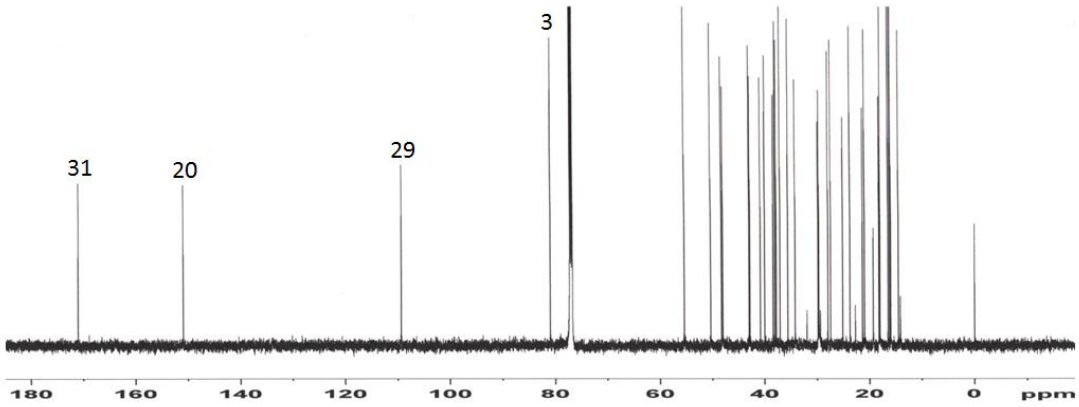


Figure 3 : ^{13}C NMR spectrum of compound 1

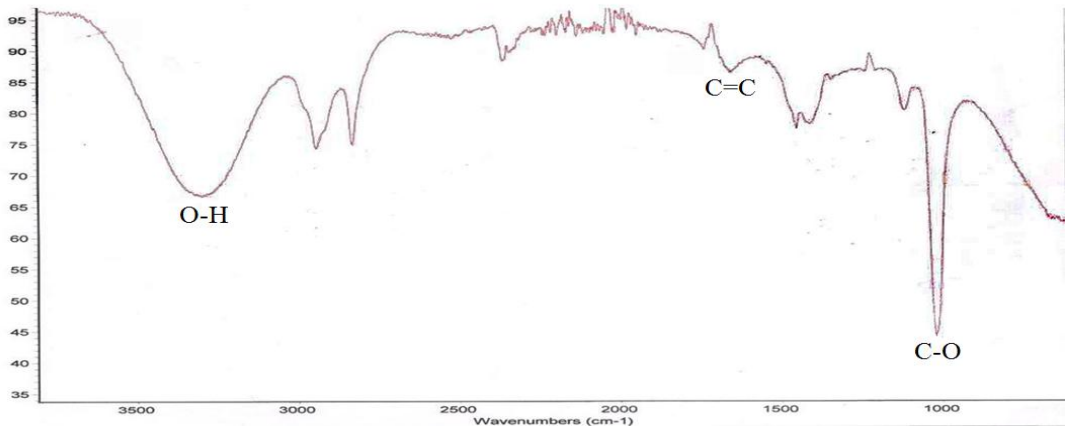


Figure 4 : IR spectrum of compound 2

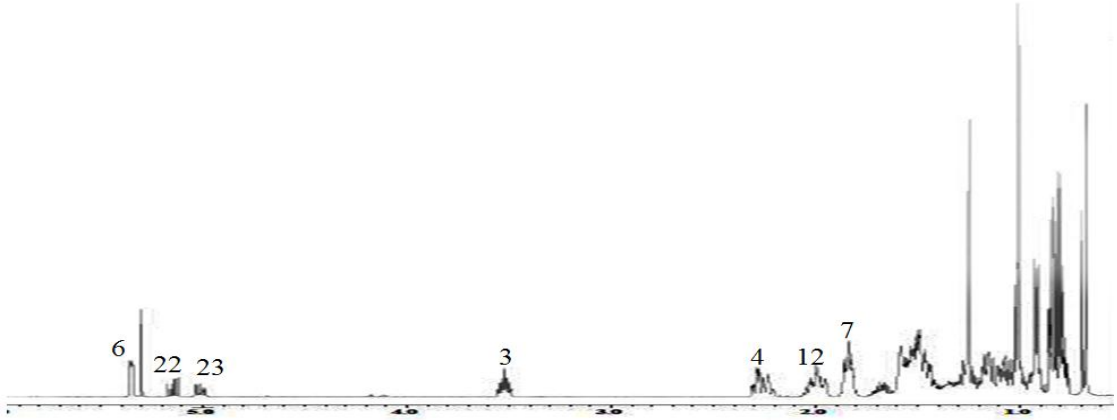


Figure 5 : ^{1}H NMR spectrum of compund 2

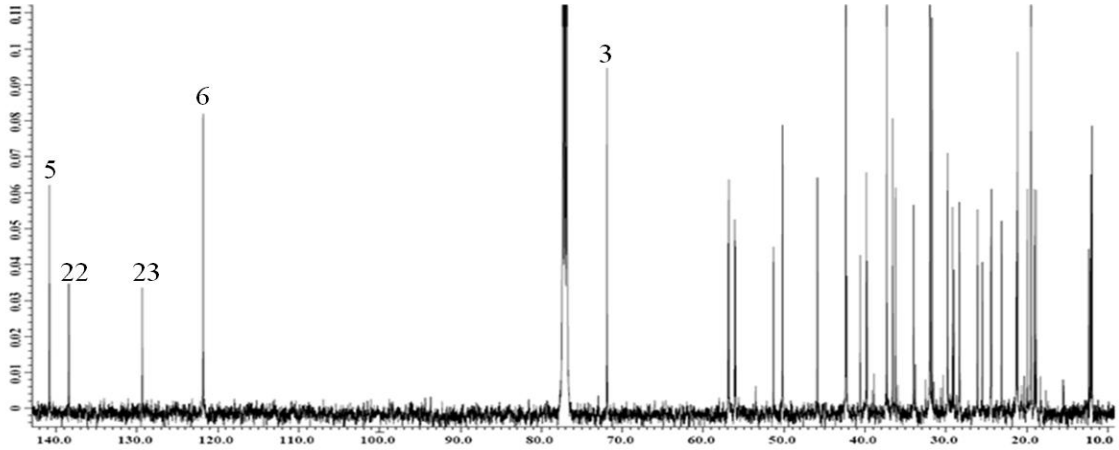


Figure 6 : ^{13}C NMR spectrum of compound 2

Conclusions

The research work through a systematic chemical investigation has determined and identified for the first time the presence of lupeol acetate **1** and stigmasterol **2** from the bark of *Alstonia spathulata.* The work was carried out by means of various physical (solvent extraction and column chromatography) and spectral techniques. The assignment will be further supported by 2D-NMR (COSY, HSQC, and HMBC) spectroscopic analysis. The structures of the compounds were also elucidated by comparison with literature.

Acknowledgement

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References

1. Webster's Online Dictionary. Definition: Alstonia. Retrieved on April 29, 2012 from http://www.websters-online-dictionary.org/definitions/alstonia
2. Pateh, U. U., Haruna, A. K., Garba, M., Iliya, I., Sule, I. M., Abubakar, M. S., Ambi, A. A. (2009) Isolation of Stigmasterol, β-Sitosterol and 2 Hydroxyhexadecanoic Acid Methyl Ester from the Rhizomes of *Stylochiton lancifolius* Pyer and Kotchy (Areceae). *Nigerian Journal of Pharmaceutical Sciences,* **8(1)**, 19-25.
3. Wiart, C. (2006) Medicinal plants of Asia and the Pacific. *United States:* Taylor and Francis Group, LLC.
4. Jamal, A. N. and Ammar, A. (2009) Chemical study on stem bark of *Etlingera hornstedia*. *International Journal of Agriculture and crop Sciences*, **1(1)**, 21-23.
5. Ajithabai, M. D., Sreedevi, S., Jayakumar, G., Mangalam, S. N., Deepa, P. N. N., Sunitha, R. S. P. (2011) Phytochemical Analysis and Radical Scavenging Activity of the Extracts of *Costus picatus* Linn and *Coccinia indica* W&A, two Ethnic Medicinal Plants used in the Treatment of *Diabetes mellitus. Free Radicals and Antioxidants*, **1(1)**, 77-83.
6. Jamal, A. K., Yaacob, W. A. & Laily, B. D. (2009) Triterpenes from the roots bark of *Phyllantus Columnaris. Australian Journal of Basic and Applied Sciences*, **3(2)**, 1428-1431.
7. Lim, W. H., Kho, B. L., Tay, T. H. & Low, W. L., (1998) Plant of Putrajaya wetlands. Putrajaya: Agriquest Sdn. Bhd.

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Research Article

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Cytotoxic, antibacterial and antioxidant activity of triterpenoids from *Kopsia singapurensis* Ridl.

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ABSTRACT

*Kopsia singapurensis Ridl. is one of the species of the genus Kopsia, also called white kopsia or locally known as 'selada' which about 18 species were distributed in Malaysia. This species is endemic only to Peninsular Malaysia and Singapore. This plant is known to produce a large number of biologically active compounds. In the present study, six known compounds were obtained from the hexane crude extract from the leave and bark of Kopsia singapurensis and identified as: lupeol **1**, lupeol acetate **2**, β-amyrin **3** β-amyrin acetate **4,** β-amyrone **5** and stigmasterol **6**. The structures of these compounds were elucidated by combination of various spectroscopic methods such as MS, UV, IR, 1D and 2D NMR, involving also comparison with data from the literature. All the isolated compounds exhibited cytotoxic effects against MCF-7 cell line, compound **6** was the most active compared to the rest of the compounds in the cytotoxic activity with IC_{50}= 14.5µg/mL, while all tested compounds showed no significant on antibacterial (gram-positive bacteria, Bacillus cereus) and antioxidant activity using the DPPH method.*

Key words: *Kopsia singapurensis* Ridl, cytotoxicity assay, antibacterial activity, antioxidant activity

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INTRODUCTION

Plants of the genus *Kopsia* is classified as member of the tribe Vinceae in subfamily Rauvolfioideae. The genus *Kopsia* (belonging to Apocynaceae family) comprises 30 species, distributed mainly over Southeast Asia, China and India [1]. *Kopsia singapurensis* Ridl.; one of the species of the genus *Kopsia*, also called white *kopsia* or locally known as 'selada' which about 18 species were distributed in Malaysia. In Singapore, this tree can be found and protected in the Nee Soon Swamp Forest of the Central Catchment's Nature Reserve [2]. This species is endemic only to Peninsular Malaysia and Singapore and rich in indole alkaloids [1-3]. Previous studies [4-6] have been reported that the alkaloids obtained from the plants in the family of Apocynaceae have antioxidant and anticancer properties. Various medicinal uses of this genus have been reported; the roots of *K. larutensis* King & Gamble, *K. macrophylla* Hook f., *K. singapurensis* Ridl. and *K. pauciflora* Hook f. were used to treat poulticing ulcerated noses in tertiary syphilis [7]. However, the isolation of terpenoids was only reported from the species *K. fruticosa* [8] and *K. longiflora* [9]. There is no report on the isolation of terpenoids from this species in the literature to date.

The purpose of this study is to isolate and identify the chemical compounds from the leaves and bark of *K. singapurensis* and to determine the bioactivities of the isolated compounds. In this paper, we report the isolation and characterization of known compounds from *K. singapurensis* namely lupeol **1**, lupeol acetate **2**, β-amyrin, **3** β-amyrin acetate **4,** β-amyrone **5** and stigmasterol **6**. The isolated compounds were then tested for cytotoxic activity by

using MCF-7 cell line, antibacterial activity, *Bacillus cereus* was selected for this activity and antioxidant inhibitory activity (standard DPPH assay).

EXPERIMENTAL SECTION

General Methods

Nuclear Magnetic Resonance spectra (NMR) were recorded in deuterated chloroform ($CDCl_3$) on a BRUKER 400MHz, BRUKER 600MHz and JEOL 500MHz. Chemical shifts (δ) were expressed in ppm and the coupling constants (J) are given in Hz. Mass spectra (MS) were measured by using the GC-mass spectroscopy (GC-MS Agilent 5975 Series). The infrared spectra (IR) were recorded on a Nicolet 6700 FTIR spectrophotometer, with CH_2Cl_2 as dilution solvent of the sample. The ultra violet spectral was obtained in dichloromethane (CH_2Cl_2) on a Perkin Elmer UV- visible spectrophotometer and the wavelength of the spectrum was recorded in the range of 200 to 400nm. Solvent used for diluted the sample was CH_2Cl_2.

Column chromatography were prepared by using Silica Gel 60F, 70-230 mesh ASTM and, 230-400 mesh ASTM and Silica Gel 60 containing Gypsum F_{254} as stationary phase. Analytical thin layer chromatography (TLC) was performed on commercially precoated aluminium supported silica gel $60F_{254}$ TLC sheets.

Reagents

10.0% solution of sulphuric acid was used as a spraying reagent to detect the presence of terpenoids spotting on the TLC plates. A positive result was indicated by the formation of purple spot on the TLC plates once the plate was heated to 110ºC for 5 minutes.

Plant Material

The species selected for the current study is *Kopsia singapurensis* Ridl. (KL5334) collected from Kluang, Johor and identified by the phytochemical group, Chemistry Department, Faculty Science, University of Malaya. The Voucher specimens were deposited at the Chemistry Department, Faculty Science, University of Malaya, Kuala Lumpur, Malaysia and the Herbarium of the Forest Research Institute, Kepong, Malaysia.

Extraction and Isolation

The dried ground leaves (2.5kg) and barks (3.3kg) of *Kopsia singapurensis* were defatted separately with hexane by Soxhlet extractor for 17 hours. Then, the extracts were dried on the rotary evaporator. For preliminary fractionation, the both hexane crude extracts were subjected to column chromatography over silica gel and eluted with different combinations solvent systems of hexane, dichloromethane and methanol mixture by increasing its polarity.

Eluents were collected in fractions and were concentrated using rotary-evaporator. Then, each concentrated fraction was analyzed by using aluminium supported TLC plate with a suitable solvent system. After the development of the TLC, 10% sulphuric acid as spraying agents, the fractions having spots with same retention factor (R_f) value were grouped together. Each grouped fraction was treated separately by extensive column chromatography or preparative TLC for further purification process.

Preparation of the Bioactivity

Cell Culture and MTT Cytotoxicity Assay

A preliminary screening against cancer cells was performed using the Microculture Tetrazolium (MTT) method [10] against MCF-7 cell line. The MCF-7 used in this study was obtained from the American Type Cell Collection (ATCC). The cell was cultured using RPMI 1640 culturing media (PAA, Leverkusen, Germany). Besides, cell was trypsinized and counted using hemocytometer and plated in a microtiter plate of 96-wells. After an overnight incubation to allow cell attachment, the medium was changed and 0.2 mL of new supplemented medium was added into each well. Cells were then treated with different drug concentrations and incubated at 37 °C, 5% CO_2 for 24 hours. Each concentration of the samples was assayed in triplicate. The colorimetric assay was performed at an absorbance of 570 nm. Results were expressed as a percentage of control giving a certain percentage of cell viability after 24 hour exposure to the test agent. The potency of cell growth inhibition for test agent was expressed as an IC_{50} value [11].

Antibacterial Activity

A gram positive bacterium, *Bacillus cereus* was selected for the *in vitro* antibacterial activity. The strain was stored in the appropriate medium before use. Antibacterial activity was determined by the disc diffusion method [12] with slight modifications in terms of sample concentration, volume of sample loaded and use of paper discs. The bacteria were cultured at 37 °C for overnight in nutrient broth and potato dextrose broth, respectively. The concentrations of the cultures were adjusted turbidometrically at a wavelength of 600 nm which gave 10^5–10^6 colony forming units

(CFU) per mL. The compounds to be tested were dissolved in dimethyl sulphoxide (DMSO) at concentration of 1 mg/mL. About 10 µL of each sample solution was loaded on Whatman No. 1 filter paper disc (0.6 mm). The disc was placed on the surface of the agar plate (nutrient agar or potato dextrose agar) previously inoculated with bacteria. The agar plates were then inverted and incubated for 24 hours at 37 °C. The antimicrobial activity was recorded by measuring the zone of inhibition (IZ) in mm around each disc, against the test organisms. The experiments were repeated in triplicate and the results were expressed as average values. The antibiotic streptomycin sulfate (10 µg/disc) was used as positive control and DMSO as negative control in the assay [11].

Antioxidant Inhibitory Activity

In this activity, the standard 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was used with slight modification. In this technique, 96 micro-well flat bottom plates were used instead of cuvettes to consume minimal amounts of sample, vehicle and DPPH solutions. Each compound was prepared at 1 mg/mL in DMSO. Each well was filled in with 100 µL of diluent (DMSO). The first row (row A) of the 96 wells was filled in with 100 µL of diluted sample at 1 mg/mL. Concentration in the first row was 500 µg/mL of sample in each well and was serially diluted (two fold dilution) in 96 micro-well plates to varying concentrations, topping from 500 µg/mL to the lowest 7.8 µg/mL. Then, 5 µL of the DPPH solution (2.5 mg/mL in DMSO) was added to each well. The DPPH solution should be kept in the dark at 4°C. This procedure should not be done under direct light. The plate was shaken to ensure thorough mixing before placed in the dark. After 30 minutes, the optical density of each well was read using ELISA Reader (EL340 Biokinetic Reader, Bio-Tek Instrumentation) at wavelength 517 nm. Percentage inhibition was calculated using the following formula:

% Inhibition = 1 − OD(DPPH + Sample)/OD(DPPH) × 100

The IC_{50} value was determined as the concentration of each sample required or able to scavenge 50% of the DPPH. All tests and analyses were run in triplicate and averaged. Antioxidant assay by using DPPH method was used to assess the capacity of the compounds to scavenge stable free radical DPPH at different concentration. The radical scavenging effect was examined and compared with natural antioxidants a-tocopherol (Sigma, USA), ascorbic acid (Sigma USA), and quercetin (Sigma, USA) used as positive controls (Table 1) [12].

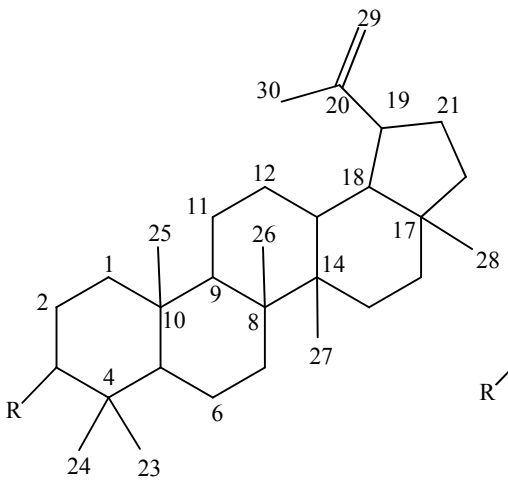
Table 1: The IC_{50} values of the standard against DPPH (free radical).

Standard	**IC_{50}(µg/mL)**
Ascorbic acid	20 ±1.2
α-tocopherol	60 ±2.9
Quercetin	40 ±5.8

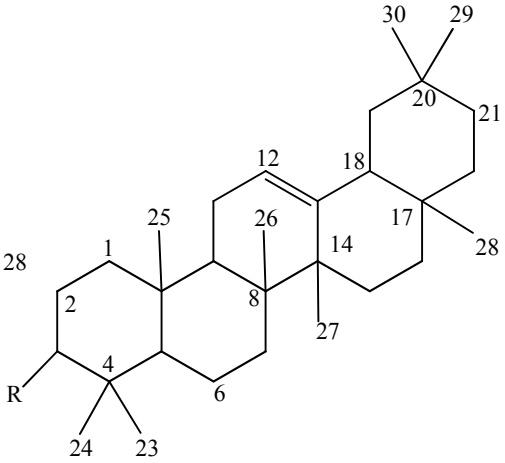
RESULTS AND DISCUSSION

Repeated chromatographic separation and purification of the hexane crude extracts of the leaf and bark of *Kopsia singapurensis* separately provided a total of six compounds (compound **1**, **2**, **3**, **4, 5** and **6**), the structure of the compounds were determined by ^{1}H and ^{13}C NMR spectral analysis as well as by comparison of their spectral data with previously reported values (Table 2 and 3). Compound **1** and **2** were belongs to lupane type triterpenoids while compound **3, 4** and **5** were belongs to oleanane type triterpenoids. Compound **6** was identified as stigmasterol from its physical constants and spectral data.

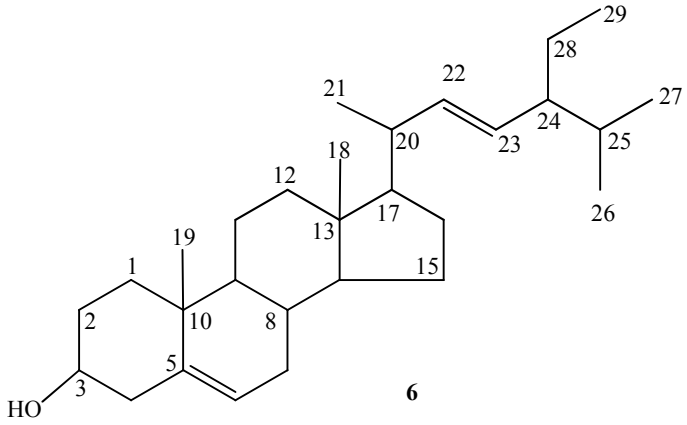
Compound **1** is a pentacyclic triterpenoid. It was obtained as white amorphous. In the GC-MS, the molecular formula $C_{30}H_{50}O$ was established from the molecular ion peak at m/z 426$[M]^{+}$. The IR spectrum of **1** displayed stretching band for O-H (3325cm^{-1}), olefin (1704cm^{-1}) and C-O (1204cm^{-1}) and the UV spectrum revealed the maxima absorption at 229.9 and 206.2nm. The ^{1}H NMR (500 MHz, CDCl3) spectrum showed seven tertiary methyl singlets (H-23, 24, 25, 26, 27, 28 and 30) and a proton of H-3 appeared as double doublet (*dd*) at δ 3.52 corresponding to hydroxyl methine proton. It also showed olefinic protons at δ 4.56 (H-29, *d*, *J* = 3.04Hz) and 4.68 (H-29, *d*, *J* = 2.32Hz). ^{13}C NMR (500 MHz, $CDCl_3$) spectrum of the compound showed 30 carbon signals for the triterpenoid of lupine skeleton which was represented by seven methyl groups. The carbon bonded to the hydroxyl group C-3 appeared at δ 79.1, while the two olefinic carbons appeared at δ 151.0 (C-20) and δ 109.3 (C-29). The spectra were compared with published data of Mouffok et al. works [13].



1 R: OH
2 R: COOH



3 R: OH
4 R: COOH
5 R: $OCOCH_3$



6

Compound **2** was isolated as white amorphous. This compound was found to be a lupane type triterpenoid, which is having similar skeleton with compound **1**. The mass spectrum of compound **2** exhibited a molecular ion peak at m/z 468 $[M]^+$ corresponding to its molecular formula $C_{32}H_{52}O_2$. The IR spectrum displayed an ester carbonyl and a C-O stretching band at 1712cm^{-1} and 1264cm^{-1}. The UV spectrum showed maxima absorption at 277.6nm. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) showed the presence of eight tertiary methyl singlets at δ 0.84(H-23), δ 0.83 (H-24), δ 0.85 (H-25), δ 1.03 (H-26), δ 0.94 (H-27), δ 0.78 (H-28), δ 1.68 (H-30) and δ 2.05 (H-32). Two protons appeared at δ 4.57 (*d, 2.0Hz*) and 4.69 (*d, 2.0Hz*) as doublet, representing the exocyclic double bond protons H-29a and H-29b, respectively. The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound 2 has a total of 32 carbon atoms. Compound **2** has an ester carbonyl signal at δ 171.1 (C-31), C-3 at δ 81.2 and the alkene carbons at δ 151.20 and 109.6. The identity of 2 as lupeol acetate was confirmed by comparison published values of Ajithabai, Sreedevi, Jayakumar, Mangalam, Deepa & Sunitha, (2011) [14, 15].

Compound **3** was isolated as white amorphous. The UV spectrum revealed the maximum absorptions at 273.4 and 229.0nm indicating the presence of a triterpenoid with carbon C-12 unsaturated oleanane skeleton [16]. The IR spectrum showed the band at 3360cm^{-1}, 1790cm^{-1} and 1294cm^{-1} which were assigned to functional groups for O-H, C=C and C-O, respectively. The molecular formula of **3** was established as $C_{30}H_{50}O$ from its quasi-molecular ion peak at m/z 449 $[M+Na]^+$ in the GC-MS analysis. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) displayed the eight tertiary methyl singlets at δ 0.98(H-23), δ 0.92 (H-24), δ 0.77 (H-25), δ 1.96 (H-26), δ 1.12 (H-27), δ 0.81 (H-28), δ 0.85 (H-29) and δ 0.85 (H-30). A signal at δ 3.20 (*dd, J = 11.0, 5.0 Hz*) was assigned to H-3 whereas another downfield signal, δ 5.16 (*t, J = 3.6Hz*) was attributed to the olefinic proton (H-12). The ^{13}C NMR spectrum (100 MHz, $CDCl_3$) showed a total of 30 carbon atoms. Compound **3** has a hydroxyl carbon signal at δ 79.1. Besides, the signal at δ 121.8 and δ 145.3 were assignable to C-12 and C-13 (olefinic carbon). The above spectral features are in close agreement to those observed for β-amyrin in literature [17].

Table 2: ^{1}H NMR spectroscopic data for compound 1-6 [400,500,600 MHz,δH (J, Hz), $CDCl_3$]

Position	**1**	**2**	**3**	**4**	**5**	**6**
1	0.87 *(m)*	0.99 (m)	1.61 *(m)*	1.64 *(m)*	1.85 (m)	1.07 *(m)*
	1.66 (m)		0.98 (s)	1.04 (m)	1.36 (m)	1.83 (m)
2	1.54 (m)	1.62 (m)	1.59 (m);	1.88 (m)	2.33 (m)	1.52 (m)
	1.60 (m)		0.77 (m)	1.60 (m)	2.50 (m)	1.83 (m)
3	3.18 (*dd* , 11.5; 5.1Hz)	4.47 *(dd* , 10.7; 5.5Hz*)*	3.20 *(dd,* 11.0; 5.0Hz*)*	4.50 (*t* , 8.0Hz)		3.52 *(m)*
4						2.23 *(m)*
						2.28 (*dd* 4.6Hz; 1.7Hz)
5	0.68 *(m)*	0.79 *(m)*	0.73 *(m)*	0.84 *(m)*	1.28 (*m*)	
6	1.38 *(m)*	1.50 *(m)*	1.52 *(m)*	1.53 *(m)*	1.46 (*m*)	5.35 (*br, s*)
	1.52 *(m)*	1.38 *(m)*	1.38 *(m)*	1.40 *(m)*		
7	1.38 *(m)*	1.49 *(m)*	1.49 *(m)*	1.52 *(m)*	1.35 (*m*)	1.46 *(m)*
		1.38 *(m)*	1.31 *(m)*	1.33 *(m)*	1.50 (m)	1.99 *(m)*
8						1.52 *(m)*
9	1.29 *(m)*	1.28 *(m)*	1.54 *(m)*	1.58 *(m)*	0.91 (*m*)	0.92 *(m)*
10						
11	1.25 *(m)*	1.39 *(m)*	1.86 *(m)*	1.80 *(m)*	1.87 (*m*)	1.52 *(m)*
	1.38 *(m)*	1.21 *(m)*		1.63 *(m)*		
12	1.07 *(m)*	1.66 *(m)*	5.16 (*t,* 3.6Hz)	5.18 (*t* , 3.5Hz)	5.20 (*t* , 3.6Hz)	1.15 *(m)*
	1.68 *(m)*	1.05 *(m)*				2.02 *(m)*
13	1.68 *(m)*	1.65 *(m)*				
14						0.99 *(m)*
15	1.33 *(m)*	1.67 *(m)*	1.74 *(m)*	1.76 *(m)*	1.08 (*m*)	1.02 *(m)*
	1.68 *(m)*	1.00 *(m)*	0.95 *(m)*	0.85 *(m)*	1.74 (m)	1.58 *(m)*
16	1.47 *(m)*	1.47 *(m)*	1.97 *(m)*	1.98 *(m)*	0.78 (*m*)	1.25 *(m)*
		1.36 *(m)*		0.79 *(m)*		1.68 *(m)*
17						1.07 *(m)*
18	1.38 *(m)*	1.35 *(m)*	1.94 *(m)*	1.93 (*d* , 4.2Hz)	1.92 (*m*)	0.68 (*s*)
19	2.38 *(m)*	2.40 *(m)*	1.64 *(m)*	1.66 *(m)*	1.61 (*m*)	1.00 (*s*)
			1.01 *(m)*	1.00 *(m)*		
20						2.02 *(m)*
21	1.33 *(m)*	1.91 *(m)*	1.31 *(m)*	1.32 *(m)*	1.05 (*m*)	1.18 (*d,*
	1.91 *(m)*	1.33 *(m)*	1.09 *(m)*	1.08 *(m)*	1.31 (m)	5.7Hz)
23	0.96 (*s*)	0.84 *(s)*	0.98 *(s)*	0.88 *(s)*	1.08 (*s*)	5.00 (*dd* , 15.5Hz; 9.2Hz)
24	0.76 (s)	0.83 *(s)*	0.77 *(s)*	0.96 *(s)*	1.04 (*s*)	1.52 *(m)*
25	0.83 (s)	0.85 *(s)*	0.92 *(s)*	0.86 *(s)*	1.05 (*s*)	1.50 *(m)*
26	1.03 (*s*)	1.03 *(s)*	0.95 *(s)*	0,97 *(s)*	1.00 (*s*)	0.85 (*d* ,
27	0.94 (*s*)	0.94 *(s)*	1.12 *(s)*	1.13 *(s)*	1.13 (*s*)	0.79 (*d* ,
28	0.78 (*s*)	0.78 *(s)*	0.81 *(s)*	0.83 *(s)*	0.83 (*s*)	1.00 *(m)*
29	4.56 (*d,* 3.0Hz)	4.69 *(d,* 2.0Hz*)*	0.85 *(s)*	0.88 *(s)*	0.86 (*s*)	0.80 (*t,* 6.9Hz)
	4.68 (*d* , 2.3Hz)	4.57 *(d,* 2.0Hz*)*				
30	1.68 (*s*)	1.68 *(s)*	0.85 *(s)*	0.87 *(s)*	0.86 (*s*)	-
OAc	-	2.05 *(s)*	-	2.05 *(s)*		-

Compound **4** was obtained as colorless needles. This compound was found to be an oleanane type triterpenoid, which is having same skeleton with compound **3**. The IR spectrum displayed stretching band for C=O (1712cm^{-1}), and C-O (1264cm^{-1}). The UV spectrum showed maxima absorption at 277.8 and 229.7nm. The GC-MS of **4** gives an ion peak at m/z 468 $[M]^+$ corresponding to the molecular formula of compound of $C_{32}H_{52}O_2$. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) indicate the eight tertiary methyl singlets which is almost same with compound **3** except for the presence of acetoxyl group at δ 2.05 (H-32). A signal appear at downfield shift, δ 4.50 (t, J = 8.0 Hz),

compared with the corresponding proton in compound **3**. The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum indicated a total of 32 carbons where the two olefinic carbon signals at δ 121.8 and δ 145.3, an acetoxyl group attached to C-3 (δ 81.0) with two carbon signals at δ 171.1 (C-31) and δ 21.4 (C-32). These spectra features are in close agreement to those observed for β-amyrin acetate by Segovia, et al. (2011) [18]. On this basis, the identity of compound **4** was confirmed as β-amyrin acetate.

Table 3: ^{13}C NMR spectroscopic data for compounds 1-6 [100, 125 MHz, δC, $CDCl_3$]

Position	**1**	**2**	**3**	**4**	**5**	**6**
1	38.8	38.4	38.7	38.3	39.4	37.3
2	27.5	23.7	27.3	23.5	24.3	31.7
3	79.1	81	79.1	81	218	71.9
4	38.9	37.8	39.9	39.8	47.6	42.4
5	55.3	55.4	55.3	55.2	55.4	140.8
6	18.4	18	18.5	18.3	19.7	121.8
7	34.3	34.2	32.7	32.6	32.2	31.9
8	40.8	40.9	38.9	37.7	39.9	31.9
9	50.5	50.3	47.7	47.6	46.9	50.2
10	37.2	37.1	37	36.8	36.8	36.6
11	21	20.9	23.6	23.6	23.7	21.2
12	25.2	25.1	121.8	121.6	121.6	39.8
13	38.1	38	145.3	145.2	145.4	42.3
14	42.9	42.8	41.8	41.7	41.9	56.8
15	27.5	27.4	26.2	26.1	26.2	24.4
16	35.7	35.6	27	26.9	27	29
17	43.1	43	32.6	32.5	32.6	56
18	48.4	48.3	47.2	47.2	47.4	12
19	48.1	48	46.9	46.8	46.8	19.5
20	151	151	31.2	31.1	31.2	40.6
21	29.9	29.8	34.8	34.7	34.8	21.2
22	40.1	40	37.2	37.1	37.2	138.3
23	28.1	28	28.2	28	26.5	129.3
24	15.5	16.5	15.7	15.6	21.6	51.3
25	16.2	16.2	15.6	16.7	15.3	32
26	16.1	16	16.9	16.8	16.8	21.2
27	14.6	14.5	26.1	26	26	19.1
28	18.1	18.2	28.5	28.4	28.5	25.4
29	109.3	109.4	33.5	33.4	33.4	12.1
30	19.4	19.3	23.8	23.7	23.8	-
31	-	171.1	-	171.1		-
32	-	21.3	-	21.4		-

Compound **5** was obtained as white amorphous. This compound was also found to be an oleanane type triterpene, which is having the same skeleton with compound **3** and **4**. The elucidation of compound **5** was then supported by the detail analysis of 1D and 2D NMR, also the GC-MS analysis which exhibited a molecular ion peak at m/z 424 $[M]^+$ in agreement with molecular formula $C_{30}H_{48}O$. The IR spectrum of **5** was almost similar to compound **3** but differ in the presence of carbonyl group at 1709cm^{-1}. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) indicate the eight tertiary methyl singlets which was almost same with compound **3** and **4.** A downfield signal at δ 5.20 (*t*, J = 3.6Hz) was probably positioned for an olefinic proton at C-12. The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum indicated a total of 30 carbons The present of a carbonyl group of C-3 at δ 218.0 was also observed in agreement with the absorption band at 1709cm^{-1} in the IR spectrum and the two olefinic carbon signals at δ 121.6 (C-12) and δ 145.4 (C-13), Detailed analysis of the spectral data obtained led to the conclusion that compound **5** is β-amyrone after comparison with the literature [19].

Compound **6** was isolated as colourless needles. Its molecular formula was assigned as $C_{29}H_{48}O$ from GC-MS at m/z 413$[M+H]^+$. This compound was found to be a C-5 unsaturated modified tetracyclic triterpenoid, which showed

only 29 carbons instead of 30 carbons for tetracyclic triterpenoids. This was further supported by the UV spectrum which indicated maxima absorption at 276.8nm [16]. The IR spectrum displayed stretching band for O-H (3300 cm^{-1}), olefin (1711 cm^{-1}) and C-O (1263 cm^{-1}). The ^{1}H NMR spectrum of **5** showed characteristic signals for six methyls at δ 0.68 (Me-18, *s*), δ 1.00 (Me-19, *s*), δ 1.18 (Me-21, *d*, *J* = 5.7 Hz), δ 0.85 (Me-26, *d*, *J* = 4.0 Hz), δ 0.79 (Me-27, *d*, *J* = 6.8 Hz) and δ 0.80 (Me-29, *t*, *J* = 6.9 Hz). The proton of H-3 appeared as multiplet at δ 3.52 corresponding to hydroxyl methine proton. The typical signals for olefinic protons at δ 5.35 (H-6, *m*), δ 5.14 (H-22, *dd*, *J* = 15.5 and 8.6 Hz), δ 5.00 (H-23, *dd*, 15.5 Hz and 9.2 Hz). In the ^{13}C NMR spectrum, 29 carbon signals were observed. There are four olefinic carbon signals at δ 140.8 (C-5), δ 121.8 (C-6), δ 138.4 (C-22) and δ 129.3 (C-23). The compound was assigned as stigmasterol based on the physical and spectral data, also comparison with the literature values [15, 20].

All the compounds (**1 - 6**) were evaluated for the cytotoxicity assay agent MCF-7 cell line, antibacterial activity against *Bacillus cereus* and antioxidant activity using DPPH method.

In the cytotoxicity activity, a preliminary screening against cancer cells was performed using the MTT method [10] against MCF-7 cell line. Doxorubicin was used as a positive control in this study. The results showed that the compounds: lupeol **1**, lupeol acetate **2**, β-amyrin **3,** β-amyrin acetate **4,** β-amyrone **5** and stigmasterol **6** exhibited cytotoxic effect against cancer cell line with IC_{50} values ranging from 14.5 – 26.0µg/mL (Table 4). Compound **6**, stigmasterol was the most active compared to the rest of compounds in the cytotoxic activity with the IC_{50} value = 14.5µg/mL.

The preliminary screening results of antibacterial activity against *Bacillus cereus* was summarized in Table 4. Antibacterial activity was indicated by a clear zone of growth inhibition. All the compounds were evaluated for antibacterial activity using disc diffusion method and streptomycin sulfate was used as a positive control in the assay. The results indicated that all the tested isolated compounds were found to be inactive against the gram-positive bacteria, *Bacillus cereus* [21, 22].

Compounds **1 - 6** were tested for the antioxidant assay by using DPPH method. This assay was used to assess the capacity of the compounds to scavenge stable free radical DPPH at different concentration. From Table 4, triterpenoids showed weak antioxidant activity with IC_{50} >500 µg/mL, compare with the standard natural antioxidants a-tocopherol, ascorbic acid and quercetin.

Table 4: Cytotoxic effects against MCF-7 cell line, antibacterial and antioxidant activity on isolated compounds.

Compounds	Cytotoxic	Antibacterial activity	Antioxidant activity
	IC_{50} values (µg/mL)	Diameter of inhibition zone (mm)	IC_{50} values (µg/mL)
	MCF-7	*B.cereus*	DPPH
Lupeol **1**	17	NI	>500
Lupeol acetate **2**	26	NI	>500
β-amyrin **3**	15.5	NI	>500
β-amyrin acetate **4**	22.5	NI	>500
β-amyrone **5**	21.5	NI	>500
Stigmasterol **6**	14.5	NI	>500
*No inhibition (NI)			

CONCLUSION

The phytochemical study was aimed to investigate the chemical constituents of *Kopsia singapurensis* Ridl. (KL 5334) collected from Kluang (Johor) and to determine the bioactivitis of the isolated compounds. Structural elucidation was established by using various modern spectroscopic techniques such as ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR) including 1D-NMR (^{1}H, ^{13}C, and DEPT), 2D-NMR (COSY, HSQC, and HMBC) and MS (GC-MS). The study on the leaves and bark of *K. singapurensis* yielded a total of 5 pentacyclic triterpenoid: lupeol **1**, lupeol acetate **2**, β-amyrin **3**, β-amyrin acetate **4,** β-amyrone **5** and a steroid namely stigmasterol **6** which were common occurrence in plant. The structures of the compounds were also elucidated by comparison with literature values. All the isolated compounds exhibited cytotoxic effects against MCF-7 cell line, compound **6** was the most active compared to the rest of compounds in the cytotoxic activity with IC_{50}= 14.5µg/mL, while all tested compounds showed no significant on antibacterial (gram-positive bacteria, Bacillus cereus) and

antioxidant activity using the DPPH method. Based on the previous reports on cytotoxic properties of *Kopsia singapurensis* with that of the present results, it clearly indicates that *Kopsia singapurensis* has potential cytotoxic properties and can be used as a source of antitumor agents.

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REFERENCES

[1] K Ahmad; Y Hirasawa; AE Nugroho; AHA Hadi; H Morita. *Heterocycles*, **2012**, 86(2), 1611-1619.
[2] DJ Middleton. *Harvard Papers in Botany*. **2004**, 9(1), 89-142.
[3] K Ahmad; Y Hirasawa; AE Nugroho; AHA Hadi; K Takeya; NF Thomas; K Awang; H Morita; SP Tan; MA Nafiah. *The Open Conference Proceedings Journal*, **2013,** 4(Suppl-2, M18), 75-82.
[4] T Kumari; J Shukla; S Joshi. *Journal of Chemical and Pharmaceutical Research*, **2012**, 4(10), 4533-4541.
[5] K Awang; K Ahmad; NF Thomas; Y Hirasawa; K Takeya; MR Mukhtar; K Mohamad; H Morita. *Heterocycles*, **2008**, 75(12), 3051-3056.
[6] SA Gadir. *Journal of Chemical and Pharmaceutical Research*, **2012**, 4(12), 5145-5148.
[7] HI Burkill. A Dictionary of the Economic Products of the Malay Peninsula, Ministry of Agriculture and Cooperatives, Kuala Lumpur, Malaysia, **2008**.
[8] SK Talapatra; B Malabika. *Journal of the Indian Chemical Society*, **1968**, 45(10), 962-963.
[9] S Iseda. *Chemical & Pharmaceutical Bulletin*, **1959**, 7, 129 – 130.
[10] T Mosmann. *J. Immunol. Methods*, **1983**, 65, 55–63. (1983).
[11] H Omar; NM Hashim; A Zajmi; N Nordin; SI Abdelwahab; AHS Azizan; AHA Hadi; HM Ali. *Molecules*, **2013**, 18, 8994-9009.
[12] NM Hashim; M Rahmani; GCL Ee; MA Sukari; M Yahayu; MAM Amin; AM Ali; R Go. *Molecules*, **2012**, 17, 6071–6082.
[13] S Mouffok; H Haba; C Lavaud; C Long; M Benkhaled. *Rec. Nat. Prod.* **2012**, 6(3), 292-295.
[14] MD Ajithabai; S Sreedevi; G Jayakumar; SN Mangalam; PNN Deepa; RSP Sunitha. *Free Radicals and Antioxidants*, **2011**, 1(1), 77-83.
[15] K Ahmad; CT Tee; K Awang; SP Tan; YS Lee; MA Nafiah. *Malaysian Journal of Chemistry*, **2013**, 15(1), 041-046.
[16] M Burnouf-Radosevich; NE Delfel. *Journal of Chromatography*, **1984**, 292, 403-409.
[17] W Thanakijcharoenpath; O Theanphong. *The Thai Journal of Pharmaceutical Sciences*. **2007**, 31, 1-8.
[18] JFO Segovia; et al. *Proceedings of the National Academy of Belarus Biological*, **2011**, *Vol 2*, 34-39.
[19] CS Li; HW Yu; GY Li; GL Zhang. *Chinese Journal of Natural Medicines*, **2010**, 8(4), 270-273.
[20] UU Pateh; AK Haruna; M Garba; I Iliya; IM Sule; MS Abubakar; AA Ambi. *Nigerian Journal of Pharmaceutical Sciences*, **2009**, 8(1), 19-25.
[21] KS Kosmulalage; S Zahid; CC Udenigwe; S Akhtar; A Ata; R Samarasekera. *Z. Naturforsch*, **2007**, 62b, 580-586.
[22] CY Ragasa; J Yumul; J Guanzon; JA Rideout. *KIMIKA*, **2001**, 17(1), 1-4.