Chemical Constituents of *Albizia myriophylla* Wood and Biological Activities

Sonesay Thammavong

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Thesis Title	Chemical Constituents of *Albizia myriophylla* Wood and Biological Activities
Author	Mr.Sonesay Thammavong
Major Program	Thai Traditional Medicine

Major Advisor :

…….....

(Dr.Nantiya Joycharat)

Co-Advisor :

…….

(Dr.Sukanya Dej-adisai)

Examining Committee :

…...……....Chairperson

(Assoc.Prof.Dr.Sunibhond Pummangura)

……...

(Assoc.Prof.Dr.Sanan Subhadhirasakul)

……....

(Dr.Nantiya Joycharat)

…….....

(Dr.Sukanya Dej-adisai)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Thai Traditional Medicine Degree

……

(Assoc. Prof. Dr. Teerapol Srichana)

Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

…..……..…….Signature

(Dr.Nantiya Joycharat)

Major Advisor

…..……Signature

(Mr.Sonesay Thammavong)

Candidate

I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

….……Signature

(Mr.Sonesay Thammavong)

Candidate

ชื่อวิทยานิพนธ์	องค์ประกอบทางเคมีจากเนื้อไม้ของชะเอมไทยและฤทธิ์ทางชีวภาพ
ผู้เขียน	Mr. Sonesay Thammavong
สาขาวิชา	การแพทย์แผนไทย
ปีการศึกษา	2555

บทคัดย่อ

การตรวจสอบทางพฤกษเคมีของเนื้อไม้ชะเอมไทย (*Albizia myriophylla* Benth.) สามารถแยกสารได้ 6 ชนิด คือ lupinifolin, 8-methoxy-7,3',4'-trihydroxyflavone, 7,8,3',4'-tetrahydroxyflavone, lupeol, สารผสมของ □-sitosterone และ stigmasta-5, 22-dien-3-one และสารผสมของ □-sitosterol และ stigmasterol การพิสูจน์โครงสร้างทางเคมีของสารที่แยกได้อาศัยการวิเคราะห์สเปกตรัมของ UV, IR, MS และ NMR ร่วมกับการเปรียบเทียบข้อมูลของสารที่ทราบโครงสร้างแล้วที่เคยมีรายงานก่อนหน้านี้ สารทั้งหมดยกเว้นสารผสมของ □-sitosterol และ stigmasterol ได้ถูกนำมาศึกษาฤทธิ์ต้านเชื้อ *Streptococcus mutans* และฤทธิ์ความเป็นพิษต่อเซลล์มะเร็งในช่องปาก (KB) จากผลการศึกษาพบว่าสารทั้งหมดที่ทดสอบแสดงฤทธิ์ต้านเชื้อแบคทีเรีย *S. mutans* โดยสาร lupinifolin มีฤทธิ์ดีที่สุดโดยมีค่าความเข้มข้นต่ำสุดที่ยับยั้งการเจริญของเชื้อ (MIC) และความเข้มข้นต่ำสุดของการฆ่าเชื้อแบคทีเรีย (MBC) เท่ากับ 1 และ 2 μg/ml ตามลำดับ นอกจากนี้สาร lupinifolin ยังแสดงฤทธิ์ความเป็นพิษต่อเซลล์มะเร็ง KB โดยมีค่าความเข้มข้นต่ำสุดที่ยับยั้งการเจริญของเซลล์มะเร็งได้ 50% (IC_{50}) เท่ากับ 4.9 μg/ml ในขณะที่สารอื่นไม่แสดงฤทธิ์ความเป็นพิษต่อเซลล์มะเร็งที่ทดสอบที่ความเข้มข้น 50 μg/ml การศึกษานี้เป็นครั้งแรกที่พบสาร ฟลาวาโนน lupinifolin ในพืชสกุล *Albizia* รวมถึงพบฤทธิ์ต้านเชื้อแบคทีเรีย *S. mutans* ของสารดังกล่าว ทั้งนี้สาร lupinifolin อาจมีศักยภาพที่ดีที่จะพัฒนาต่อไปเป็นสารต้านโรคฟันผุได้

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ABSTRACT

Phytochemical investigation of *Albizia myriophylla* wood led to the isolation of six compounds including lupinifolin, 8-methoxy-7,3′,4′-trihydroxyflavone, 7,8,3′,4′-tetrahydroxyflavone, lupeol, a mixture of β-sitosterone and stigmata-5, 22-dien-3-one, and a mixture of β-sitosterol and stigmasterol. The structures of all these isolates were determined by extensive spectroscopic studies, including comparisons of their UV, IR, MS, and NMR data with those previously reported. All of the isolated compounds except for a mixture of β-sitosterol and stigmasterol were evaluated for their antibacterial activity against *Streptococcus mutans* and cytotoxicity against oral cavity cancer (KB) cell line. The results showed that all the tested compounds displayed antibacterial activity against *S. mutans*, of which lupinifolin was found to be the most potent with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 1 and 2 μg/ml, respectively. Furthermore, lupinifolin also exhibited good anticancer activity against KB cell with IC_{50} of 4.9 μg/ml, whereas the other tested compounds did not show activity against cancer cell tested at a concentration of 50 μg/ml. It is the first time that this flavanone, lupinifolin, is described in the genus *Albizia* as well as its anti-*S. mutans* property is established. Lupinifolin may have the great potential to be further developed as a natural anti-cariogenic agent.

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The success of this thesis would not be realized without the generosity and assistance of some persons and various institutions to whom I would like to express my deepest gratitude.

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LIST OF ABBREVIATIONS AND SYMBOLS

$[\alpha]_D$	Specific rotation
δ	Chemical shift in ppm
ω	Molar extinction coefficient
λ_{max}	Maximum wavelength
ν_{max}	Wave number
µg	Microgram
µl	Microliter
µM	Micromolar
br	broad (for NMR signal)
^oC	Degree Celsius
COSY	Correlation Spectroscopy
DEPT	Distortion less Enhancement by Polarization Transfer
DMSO	Dimethylsulfoxide
d	Doublet (for NMR signal)
DMJ	1-Deoxymannojirimycin
DMDP	2,5-Dihydroxymethyl-3-4-dihydroxypyrroldine
EIMS	Electron impact Mass spectroscopy
ESIMS	Electrospray Ionization Mass spectroscopy
g	Gram
HREIMS	High Resolution Electrospray Ionization Mass spectroscopy
HMQC	Heteronuclear MultipleQuantum Coherence
Hz	Hertz
IC_{50}	Inhibitory concentration at 50% of tested subject
IR	Infrared
j	Coupling constant
$[M]^+$	Molecular ion
MS	Mass spectrometry

LIST OF ABBREVIATIONS AND SYMBOLS

min	Minute
ml	Milliliter
m/z	Mas-over-charge ratio
m	Multiplet (for NMR signals)
mg	Milligram
NMR	Nuclear Magnetic Resonance
ppm	Part per million
q	Quartet (for NMR signals)
s	Singlet (for NMR signals)
t	Triplet (for NMR signals)
TLC	Thin Layer Chromatography
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

The genus *Albizia* belongs to Fabaceae/Leguminosae family (Mimosoideae subfamily). Most *Albizia* species are deciduous woody trees and shrubs. They are rarely scandent and with short, recurved hooks. Leaves are bipinnate; stipules usually small, rarely larger and caducous; petiole and rachis with glands; leaflets small in numerous pairs or larger in few pairs. Inflorescence is usually racemose, globose or pedunculate heads. Their small flowers are sessile or pedicellate, bisexual. Calyx is gamosepalous, dentate or shortly lobed. Corolla is gamopetalous, funnel shaped; petals are connate beyond the middle. Stamens are generally indefinite; filaments elongated, white, rose or rarely purple, anthers small, eglandular. Ovary is shortly stipitate or sessile; style filiform; stigma capitate or minute. Pod is broadly linear, thin, compressed, dehiscent or indehiscent. Seeds are ovate or orbicular, compressed, funicle filiform (Shu, 2010).

The genus *Albizia* consists of approximately 150 species. Many members of this genus are endemic to Indian subcontinent (Singh et al., 2004; Wang et al., 2006). Some are widely distributed in Asia, Africa, Australia, and tropical and subtropical America (Zheng et al., 2004*a*; Kim et al., 2007). In Thailand, seven species can be found as follows; *Albizia chinnensis* (Kang Luang), *A. lebbeck* (Preug), *A. lebbekoides* (Kang), *A. lucidior* (Pan Thae), *A. myriophylla* (Cha-em Thai), *A. odoratissima* (Kang khi mot), and *Albizia procera* (Thing Thon) (Smitinand, 2001).

Albizia species are highly valued multipurpose tree legume. They are socially significant for producing high quality timber. Their wood can be used for building and furniture-making. The young leaves are edible (Daniel et al., 2002; Zheng et al., 2004*a*). The seed are a source of oil (Wang et al., 2006) and as a food for livestock and wildlife (Cholticha et al., 2006). Several species of the genus *Albizia* are traditionally used in folk medicine. Some species such as *A. lebbeck* and *A. procera* have shown high potential in soil redevelopment process (Singh et al., 2004). *A. lebbek* is used in folk remedies for abdominal tumors, boils, cough, eye

ailments, flu, and lung ailments. It is also reported to be astringent, pectoral, rejuvenant, and tonic (Hartwell, 1969; Balandrin et al., 1993). The powdered seed is used in scrofulous swellings and its oil for leprosy. In India, the flowers are employed for spermatorrhea. Its leaves are used for the treatment of diarrhea and dysentery (Sudharameshwar and Radhika, 2007). In China, the barks of *A. julibrissin* have been recommended as a sedative and anti-inflammatory drug for treating swelling and pain of the lungs, skin ulcers, wounds bruises, abscesses, boils, hemorrhoids, and fractures (Higuchi et al., 1992; Ikeda et al., 1997; Pharmacopoeia, 2005, Liang et al., 2005). In Asia countries, the bark of *A. julibrissin* is prescribed to treat insomnia, dieresis, and confusion (Chen et al., 2009; Zhu, 1998). *A. julibrissin* grows abundantly in Korea and its stem bark is widely used for the traditional treatment of insomnia, calming the mind, and treatment for injuries (Kim et al., 2004). Its flowers have been commonly used to treat anxiety, depression, and insomnia (Kang et al., 2007). Similarly, the seed of *A. julibrissin, A. lebbeck* and *A. amara* are regarded as astringent and used in the treatment of piles, diarrhea, and gonorrhea (Kang et al., 2000; Kang et al., 2007; Anonymous, 1989). In the Vietnamese systems of traditional medicine, the stems of *A. myriophylla* are used to substitute for licorice due to their sweet taste (Yoshikawa et al., 2002). In Thai traditional medicine, the root of this plant species is used as antitussive and demulcent. The fruit and root are also employed as expectorant. Its wood is recommended as tonic (คณะกรรมการ จัดทำตำราอ้างอิงยาสมุนไพร, 2553). In Ethiopia, *A. gummifera* is documented as anthelmintics in livestock and human (Eguale et al., 2011). In Uganda, the decoction of fresh fruit of *A. anthelmintica* is used for the remedy of helminthiasis, malaria, stomachache, and emetic (Muthee et al., 2011). The stem bark of *A. ver- sicolor* is used for the treatment of venereal diseases, coughs, joint pains, and as a pain reliever (Rukunga and Waterman, 2001). *A. procera* is a tree cultivated in streets and public gardens in Egypt.Its bark is considered useful in pregnancy and stomach (Miyase et al., 2010). The stem bark of *A. coriaria* is used for the treatment of coughs (Namukobe et al., 2011).

Previous phytochemical investigations of plants in the genus *Albizia* have revealed the presence of a variety of compounds with interesting biological activities, including saponins (Haddad et al., 2004; Haddad et al., 2003; Jung et al., 2004*a*; Jung et al., 2004*b*) flavonoids, lignan glycosides, and alkaloids (Ito et al., 1994, Yoshikawa et al., 2002, Asano et al., 2005, Panmei et al., 2007). This genus is known to be a source of bioactive saponins (Krief et al.,

2005; Zheng et al., 2006). Some members of this chemical group were shown to possess high cytotoxicity against many different cancer cell lines including HCT-8 (human colon cancer), Bel-7402 (human hepatoma cancer), BGC-823 (human gastric cancer), A549 (human lung epithelial), and A2780 (human ovarian cancer) (Liu et al., 2010). Apart from saponins, spermine alkaloids found in some plant species of this genus also possessed significant antimalarial activity (Geoffrey et al., 1996) while flavonoids isolated from *Albizia* spp. showed antibacterial, antifungal, and anti-triglyceride activities (Yahagi et al., 2012).

Albizia myriophylla Bent. (Cha-Em Thai; **Figure 1A-D**) of the Leguminosae family, is native to deciduous and semi-deciduous forests in Asia from eastern Pakistan through India, Sri Lanka, Burma, Thailand, Laos, Cambodia, and Vietnam (คณะกรรมการจัดทำตำราอ้างอิงยาสมุนไพร, 2553). It is a small tree that could reach a height of 4 m. The young shoots are dark brown in colour and scarcely villous. The leaves are bipinnate, from 15 to 20 cm long, of bright green colour. The pinnae consist of 10 to 15 pairs. The leaflets are from 30 to 40 pairs, minute, obliquely-linear in shape and smooth. The petioles are common and partial, downy. The panicles are terminal and axillary, villous, composed of globular heads of minute greenish-yellow corollets. The bracts are subulate, villous with calyx and corolla both villous. The filaments are from 10-20, monodelphous. The germ is long-pediculed. The legumes are thin, leafy, smooth, long, broad and obtuse-pointed, from 3 to 6-seeded measuring 15-20 cm long and rather above one broad. The seeds are oval, flat, and smooth in shape and light brown in colour. (William Roxburgh Flora Indica, 1832).

The plant portion of *Albizia myriophylla* including roots, fruits, and wood were used for several therapeutic purposes such as antitussive, expectorant, and tonic (คณะกรรมการจัดทำตำราอ้างอิงยาสมุนไพร, 2553). *A. myriophylla* is among the medicinal plant contained in the Thai herbal formula used against dental caries (ประกอบ, 2547). Previous biological activity study revealed that the ethanolic wood extract of this species exhibited pronounced antibacterial activity against *Streptococcus mutans* with MIC value of 3.9 µg/ml (Joycharat et al., 2012). Previous clinical study has shown that a mouthwash of *A. myriophylla* was significantly active against *mutans Streptococci* in saliva of schoolchildren (Cholticha et al., 2006). Furthermore, biological screenings worldwide showed that many plants of the *Albizia* possess anticancer properties with sometimes surprising efficacy and good selectivity. To our knowledge, few

phytochemical studies have been previously reported on this species. Various chemical classes including phenolic acid (bark), triterpene saponins (stem), lignan glycosides (bark), and alkaloids (wood) (Ito et al., 1994; Yoshikawa et al., 2002; Asano et al., 2005; Panmei et al., 2007) were characterized previously from this plant species. Up to now, only one phytochemical investigation from the wood extract of this species has been reported (Asano et al., 2005). In this case, five iminosugars including DMJ, DMDP, 3-O-β-D-glucopyranosyl-DMDP, and 4-O-β-D-glucopyranosyl-DMJ were characterized from the ethanol extract of this plant part. However, the bioactive constituents of *A. myriophylla* wood have never been recorded. Preliminary evaluation of the antibacterial activity of *A. myriophylla* wood revealed that the crude ethanol extract as well as some of its semi-purified fractions including those of hexane and dichrolomethane exhibited activity against *Streptococcus mutans* ATCC 25175 with MIC values ranging from 256-1024 µg/ml, suggesting the presence of bioactive compounds in this plant species.

As mentioned a bove, *A. myriophylla* was selected for further investigations of its chemical constituents and biological activities. The purposes of this research are as follows:

1. To isolate and purify the compounds from the wood of *Albizia myriophylla*
2. To determine the chemical structures of the isolated compounds
3. To evaluate the cytotoxic and anti-*Streptococcus mutans* activities of the semi-purified fractions and the isolated compounds

A

B

Figure 1 *Albizia myriophylla* Benth.

A) Stem and B) Leaves

C

D

Figure 1 *Albizia myriophylla* Benth.

C) Flowers and D) Fruits

CHAPTER 2

HISTORICAL

1. Chemical constituents of *Albizia* spp.

According to previous phytochemical studies, a number of compounds have been isolated from various plant species of the *Albizia*. They were classified as saponins, alkaloids flavonoids, lingnan glycosides, and phenolic glycosides. The distribution of these compounds in *Albizia* spp. and the chemical structures are summarized in **Table 1**.

Table 1 Chemical constituents of plants in the genus *Albizia*

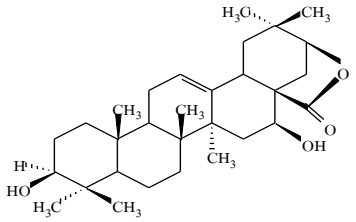
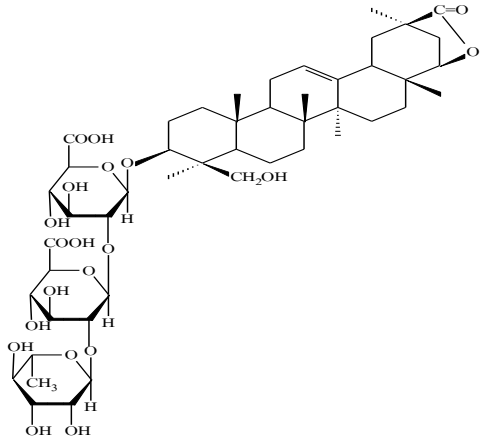
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
Triterpene saponins  1) Acacic acid lactone	-	*A. myriophylla* (Stem) *A. lebbeck* (Bark)	Yoshikawa et al., 2002 Pal et al., 1995
 2) Albiziasaponin A	-	*A. myriophylla* (Stem) *A. lebbeck* (Bark)	Yoshikawa et al., 2002 Pal et al., 1995

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

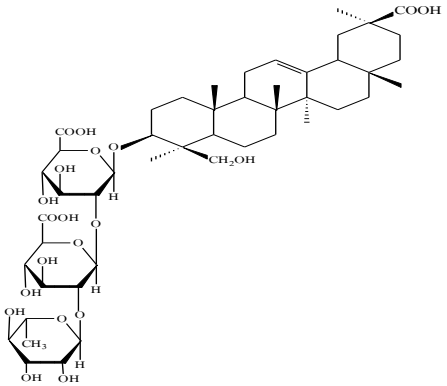
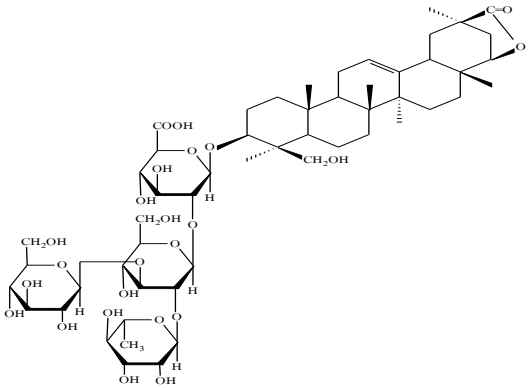
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 3) Albiziasaponin B	-	*A. myriophylla* (Stem) *A. lebbeck* (Bark)	Yoshikawa et al., 2002 Pal et al., 1995
 4) Albiziasaponin C	-	*A. myriophylla* (Stem) *A. lebbeck* (Bark)	Yoshikawa et al., 2002 Pal et al., 1995

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

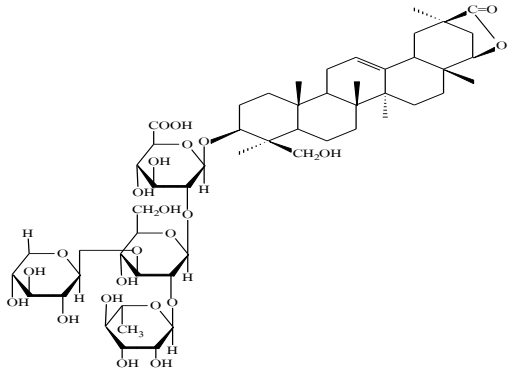
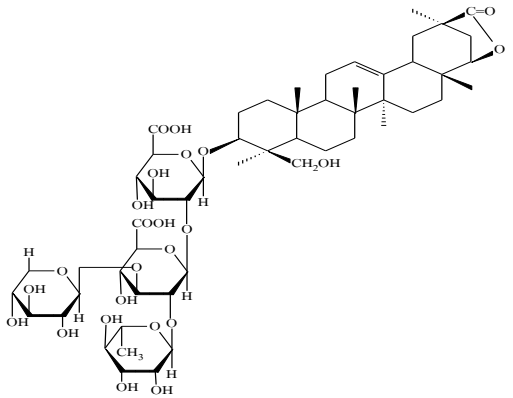
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 5) Albiziasaponin D	-	*A. myriophylla* (Stem)	Yoshikawa et al., 2002
 6) Albiziasaponin E	-	*A. myriophylla* (Stem)	Yoshikawa et al., 2002

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

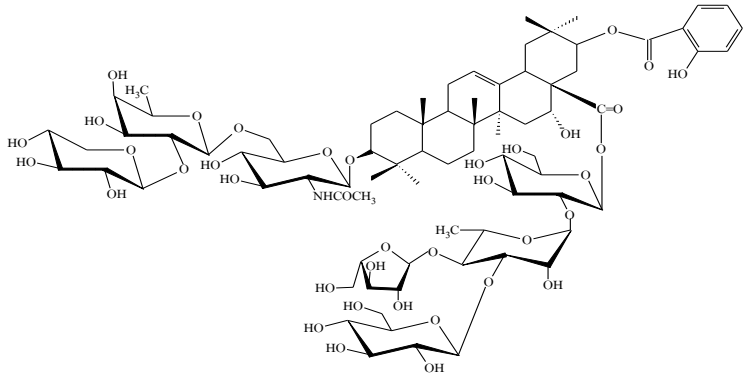
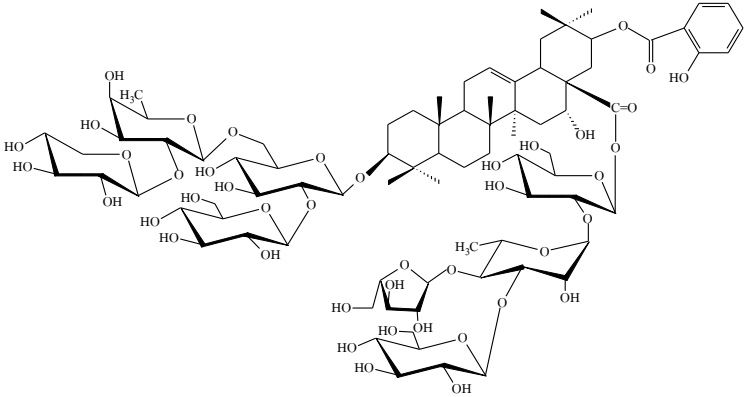
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
	-	*A. adianthifolia* (Root)	Haddad et al., 2004 Haddad et al., 2003
7) Adianthifolioside A			
	-	*A. adianthifolia* (Root)	Haddad et al., 2004 Haddad et al., 2003
8) Adianthifolioside B			

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

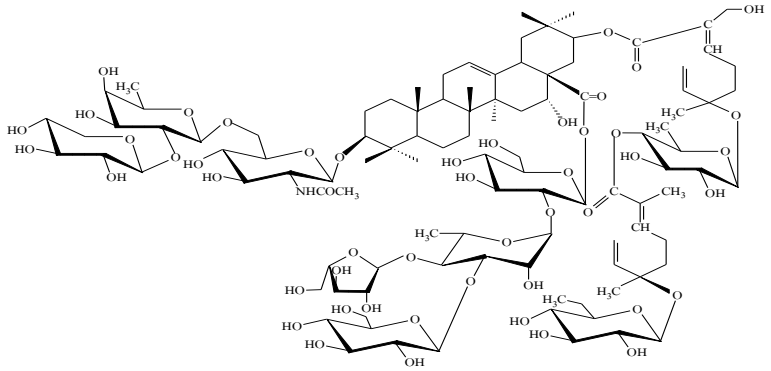
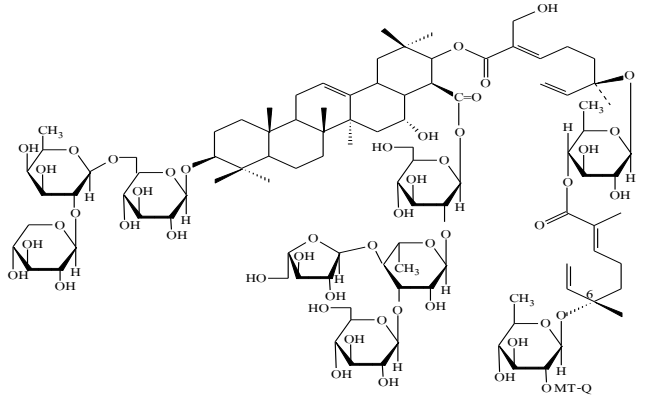
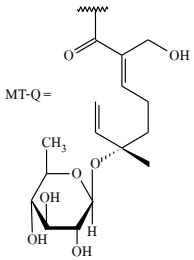
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 9) Adianthifolioside D	-	*A. adianthifolia* (Root)	Haddad et al., 2004 Haddad et al., 2003
 10) Albizioside A		*A. chinnensis* (Stem bark)	Liu et al., 2009

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

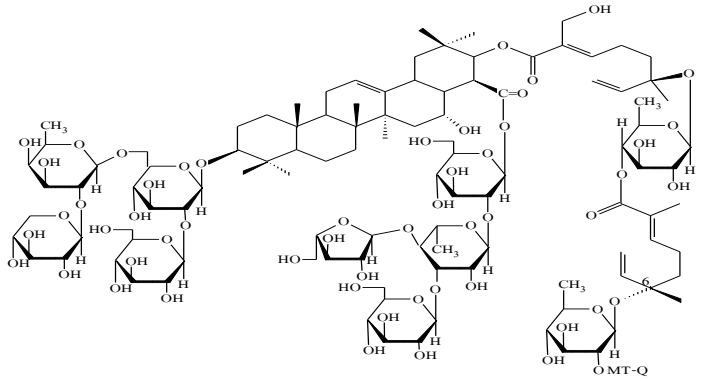
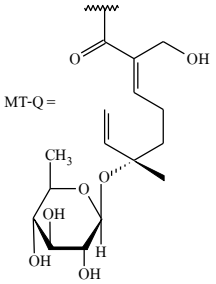
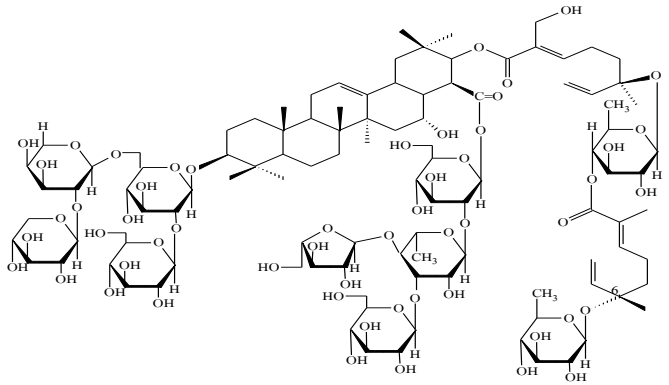
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 11) Albizioside B		*A. chinnensis* (Stem bark)	Liu et al., 2009
 12) Albizioside C	-	*A. chinnensis* (Stem bark)	Liu et al., 2009

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

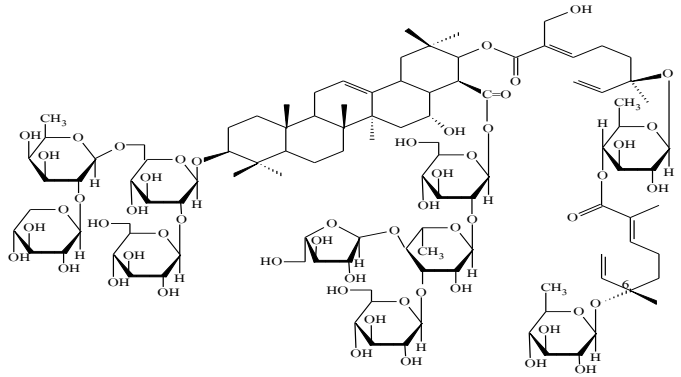
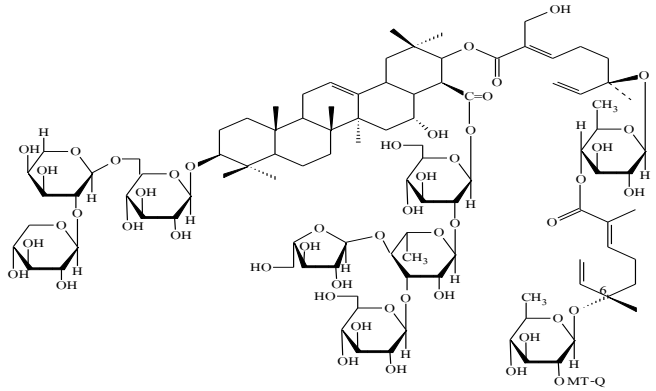
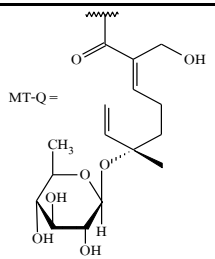
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
	-	*A. chinnensis* (Stem bark)	Liu et al., 2010
13) Albizioside D			
		*A. chinnensis* (Stem bark)	Liu et al., 2010
14) Albizioside E			

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

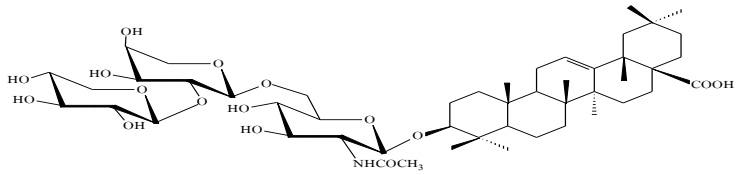
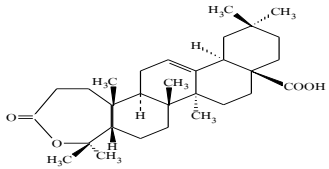
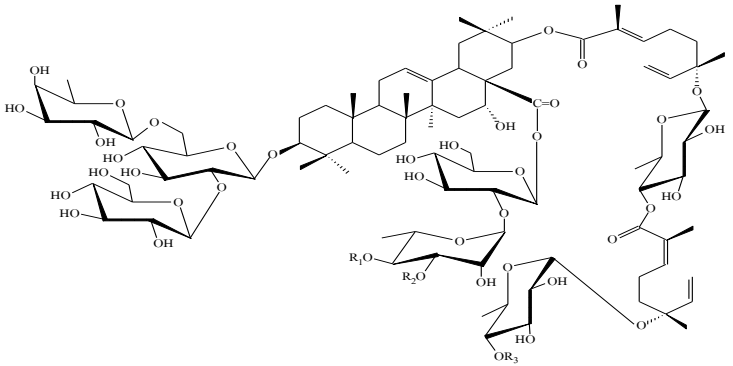
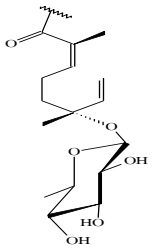
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 15) Albiziatrioside A	-	*A. subdimidiata* (Stem)	Kader et al., 2001
 16) A-Homo-3a-oxa-5b-olean-12-en-3-one-28-oic	-	*A. gummifera* (Stem bark)	Debella et al., 2000
 17) Coriarioside A	R_1=Araf R_2=Glc R_3= 	*A. coriaria* (Root)	Placide et al., 2009

Table 1 Chemical constituents of plants in the genus A*lbizia* (Continued)

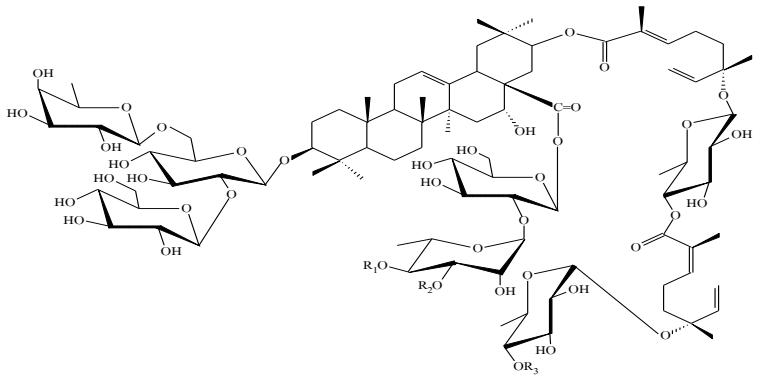
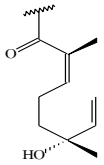
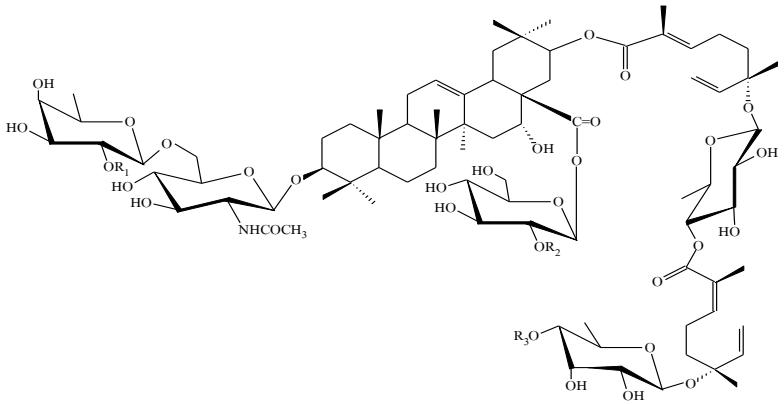
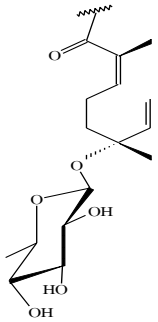
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 18) Coriarioside B	R_1=Xyl R_2=H R_3= 	*A. coriaria* (Root)	Placide et al., 2009
 19) Coriarioside C	R_1=Xyl R_2=Xyl (1→4) Rha R_3= 	*A. coriaria* (Root)	Placide et al., 2010

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

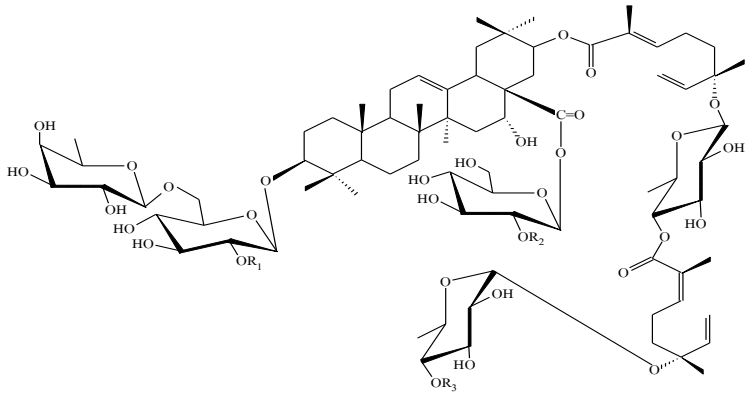
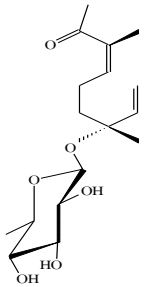
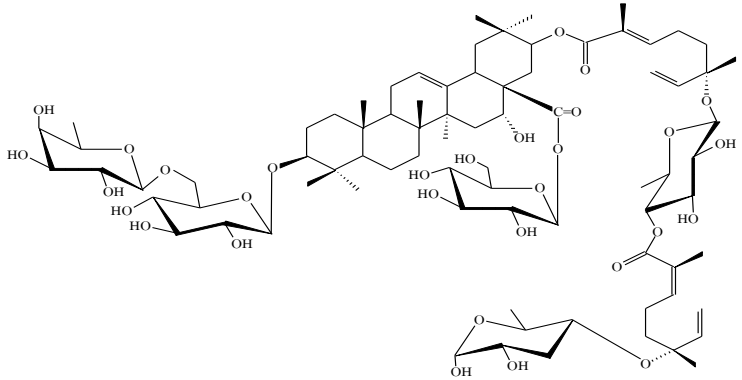
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
	R_1=Glc R_2=Rha R_3= 	*A. coriaria* (Root)	Placide et al., 2010
20) Coriarioside D			
	-	*A. coriaria* (Root)	Placide et al., 2010
21) Coriarioside E			

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

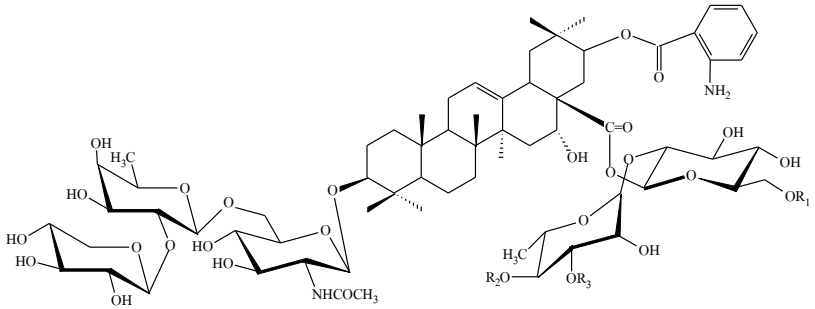
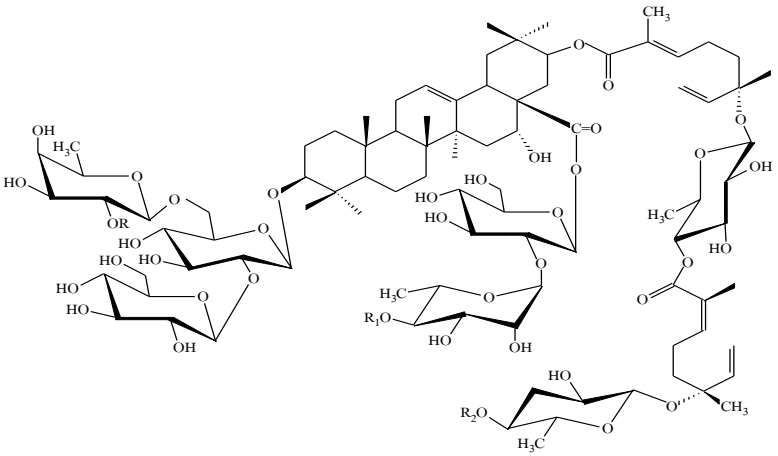
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 22) Grandibracterioside A	R_1=H R_2=Xyl R_3=H	*A. grandibracteata* (Leaf)	Krief et al., 2005
 23) Gummiferaoside A	R=Xyl R_1=Xyl R_2=H	*A. gummifera* (Root)	Cao et al., 2007

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

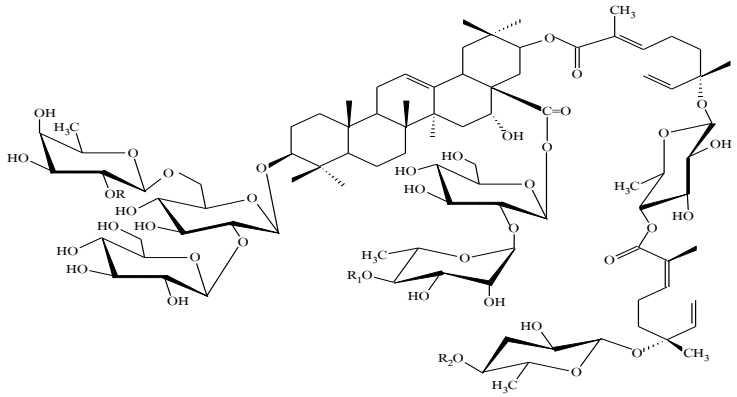
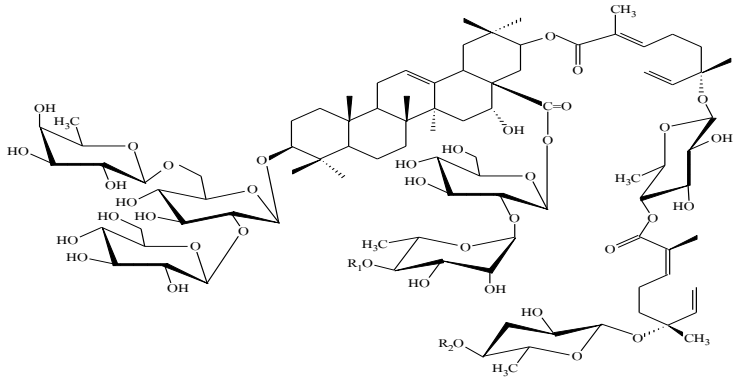
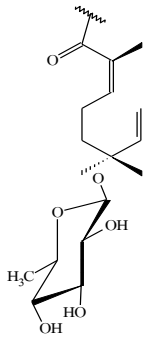
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
	R=Arap R_1=Xyl R_2=H	*A. gummifera* (Root)	Cao et al., 2007
24) Gummiferaoside B			
	R_1=Xyl R_2= 	*A. coriaria* (Root) Cao et al., 2007 *A. gummifera* (Root)	Placide et al., 2009
25) Gummiferaoside C			

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

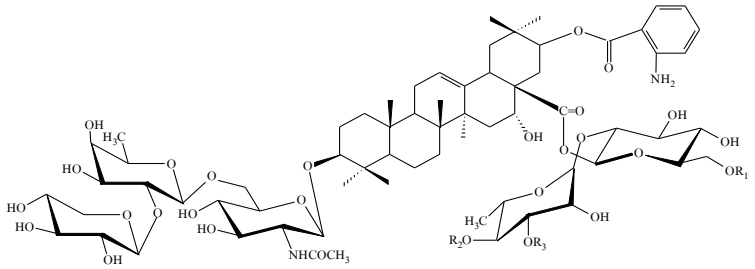
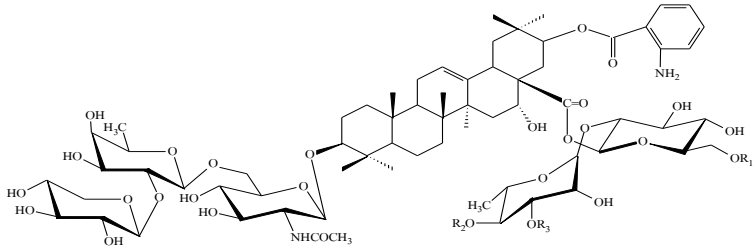
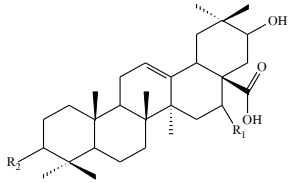
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 26) Grandibracterioside B	R_1=Glc R_2=Xyl R_3=H	*A. grandibracteata* (Leaf)	Krief et al., 2005
 27) Grandibracterioside C	R_1=H R_2=Arap R_3=Glc	*A. grandibracteata* (Leaf)	Krief et al., 2005
 28) Julibroside J_{A2}	R_1=OH R_2=Xyl (1→2) Fuc (1→ 6)-Glc	*A. julibrissin* (Stem bark)	Han et al., 2011

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

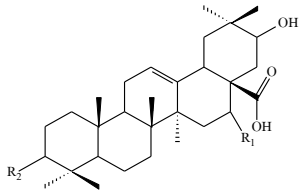
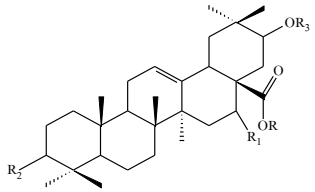
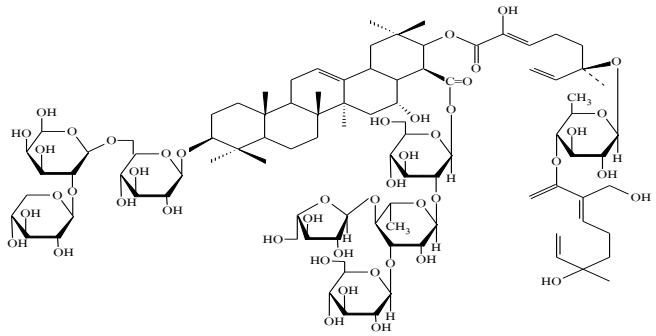
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 29) Julibroside J_{A3}	R_1=OH R_2=Xyl (1→2) Fuc (1→6) Glc (2NHCOCH$_3$)	*A. julibrissin* (Stem bark) *A. adianthifolia* (Root)	Han et al., 2011 Haddad et al., 2002
 30) Julibroside J1	R_1=OH; R_2=Xyl (1→2) Ara (1→6) Glc R_3=Qui (1→6) (6R) MT (1→4) Qui (1→6) MT (C_9-OH) R=Glc (1→3) Ara (1→4) Rha (1→2) Glc	*A. julibrissin* (Stem bark)	Han et al., 2011 Zou et al., 2006 Zou et al., 2000
 31) Julibroside J2		*A. julibrissin* (Stem bark)	Zou et al., 2005

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

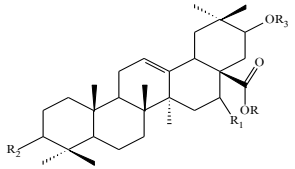
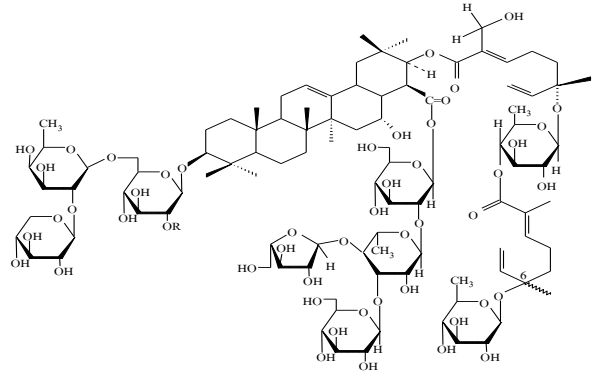
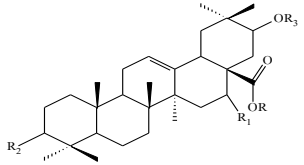
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
	R_1=OH R_2=Xyl (1→2) Fuc (1→6) Glc R_3=Qui (1→6) (6S) MT'(1→4) Qui (1→6) MT R= Glc (1→3) Ara (1→4) Rha (1→2) Glc	*A. julibrissin* (Stem bark)	Han et al., 2011
32) Julibroside J 4			
	R=OH; 6R	*A. julibrissin* (Stem bark)	Zou et al., 2005
33) Julibroside J5			
	R_1=OH R_2=Xyl (1→2) Fuc (1→6) Glc R_3=Qui (6R) MT-Qui-MT (C_9-OH) R=Glc (1→3) Ara (1→4) Rha (1→2) Glc	*A. julibrissin* (Stem bark)	Han et al., 2011
34) Isomer of J5			

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

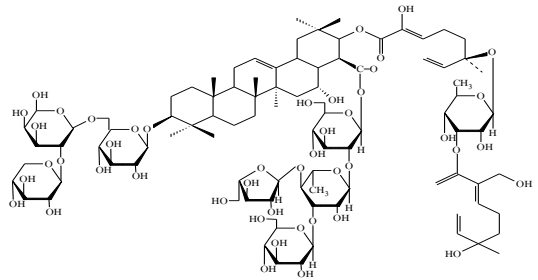
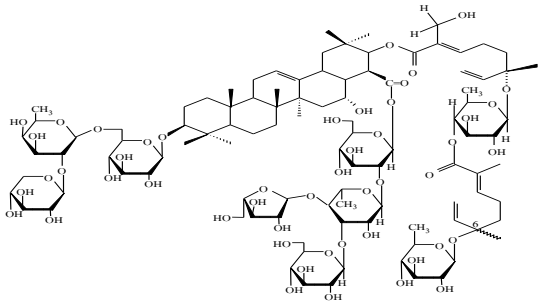
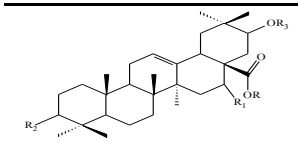
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 35) Julibroside J7	-	*A. julibrissin* (Stem bark)	Zou et al., 2005
 36) Julibroside J8	-	*A. chinnensis* (Stem bark) *A. julibrissin* (Stem bark)	Liu et al., 2010 Zou et al., 2005
 37) Julibroside J9	R_1=OH; R_2=Xyl (1→2) Ara (1→6) Glc R_3=Qui (1→6)(6S) MT'(1→4) Qui (1→6) MT(C_9OH) R=Glc (1→3) Ara (1→4) Rha (1→2) Glc	*A. julibrissin* (Stem bark)	Han et al., 2011 Zou et al., 2000

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

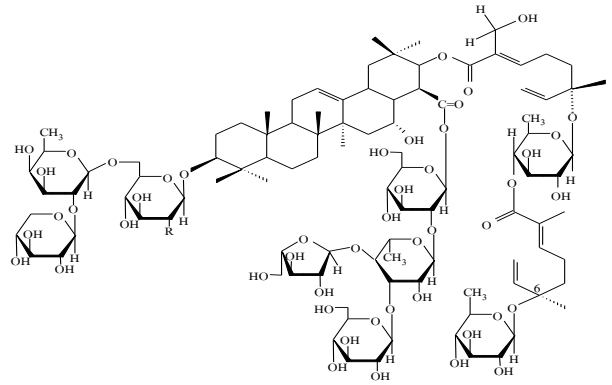
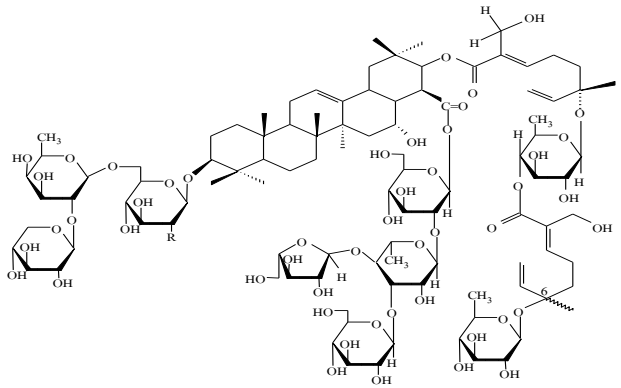
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 38) Julibroside J12	R=$NHCOCH_3$ 6R (MT C-6)	*A. julibrissin* (Stem bark)	Zou et al., 2005
 39) Julibroside J13	R=$NHCOCH_3$ 6S (MT C-6)	*A. julibrissin* (Stem bark)	Zou et al., 2005

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

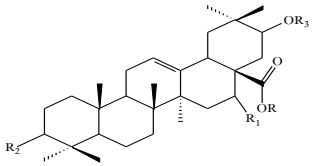
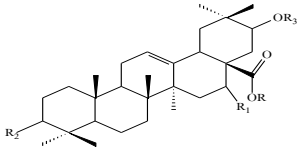
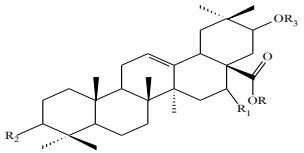
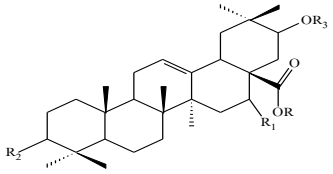
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 40) Julibroside J14	R_1=OH R_2=Xyl (1→2) Ara (1→6) Glc R_3=Qui (1→6)(6R) MT (1→4) Qui (1→6) MT R=Glc (1 →3) Ara (1→4) Rha (1→2) Glc	*A. julibrissin* (Stem bark)	Han et al., 2011 Liang et al., 2005
 41) Isomer of J14	R_1=OH R_2=Xyl (1→2) Ara (1→6) Glc R_3=Qui (6R) MT'-Qui-MT R=Glc (1 →3) Ara (1→4) Rha (1→2) Glc	*A. julibrissin* (Stem bark)	Han et al., 2011
 42) Julibroside J15	R_1=O; R_2=Xyl (1→2) Ara (1→6) Glc R_3=Qui (1→6)(6S) MT (1→4) Qui (1→6) MT R=Glc (1→3) Ara (1→4) Rha (1→2) Glc	*A. julibrissin* (Stem bark)	Han et al., 2011
 43) Isomer of J15	R_1=OH; R_2=Xyl (1→2) Ara (1→6) Glc R_3=Qui (6S) MT'-Qui-MT R=Glc (1 →3) Ara (1→4) Rha (1→2) Glc	*A. julibrissin* (Stem bark)	Han et al., 2011

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

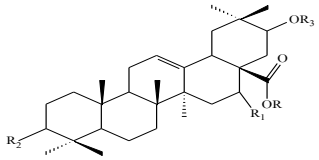
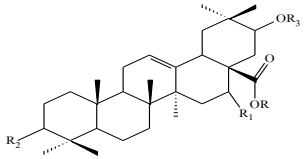
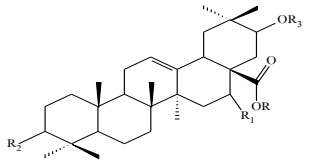
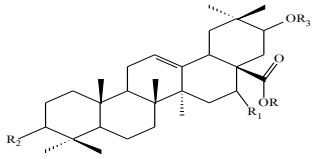
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 44) Julibroside J16	R_1=OH R_2=Xyl (1→2) Fuc (1→6) Glc R_3=Qui (1→6)(6R) MT'(1→4) Qui (1→6) MT R=Glc (1→3) Ara (1→4) Rha (1→2) Glc	*A. julibrissin* (Stem bark)	Han et al., 2011
 45) Isomer of J16	R_1=OH R_2=Xyl (1→ 2) Fuc (1→6) Glc R_3=Qui (6R) MT'-Qui-MT R=Glc (1→3) Ara (1→4) Rha (1→2) Glc	*A. julibrissin* (Stem bark)	Han et al., 2011
 46) Julibroside J20	R_1=OH R_2=Xyl (1→2) Ara (1→6) Glc R_3=Xyl (1→6) MT (C_9-OH) R=Glc (1→3) Ara (1→4) Rha (1→2) Glc	*A. julibrissin* (Stem bark)	Zou et al., 1999
 47) Julibroside J22	R_1=OH; R_2=Xyl (1→2) Ara (1→6) Glc (2NHCOCH$_3$) R_3=Xyl (1→6) MT (C_9-OH) R=Glc (1→3) Ara (1→4) Rha (1→2) Glc	*A. julibrissin* (Stem bark)	Han et al., 2011

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

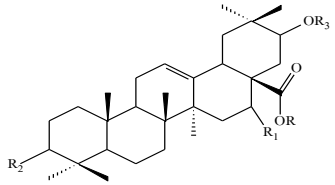
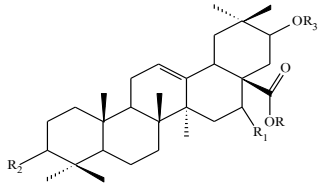
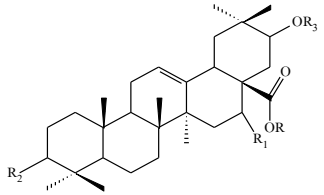
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 48) Julibroside J25	R_1=OH R_2=Xyl (1→2) Ara (1→6) Glc R_3=Qui (1→6) MT (C_9-OH) R=Glc (1→3) Ara (1→4) Rha (1→2) Glc	*A. julibrissin* (Stem bark)	Han et al., 2011
 49) Julibroside J26	R_1=OH R_2=Xyl (1→ 2) Ara (1→6) Glc R_3=Qui (6s) MT-Qui-MT R=Glc (1→3) Ara (1→4) Rha (1→2) Glc	*A. julibrissin* (Stem bark)	Zou et al., 1999
 50) Isomer of J26	R_1=OH R_2=Xyl (1→ 2) Ara (1→6) Glc R_3=Xyl (1→6) MT R=Glc (1→3) Ara (1→4) Rha (1→2) Glc	*A. julibrissin* (Stem bark)	Han et al., 2011

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

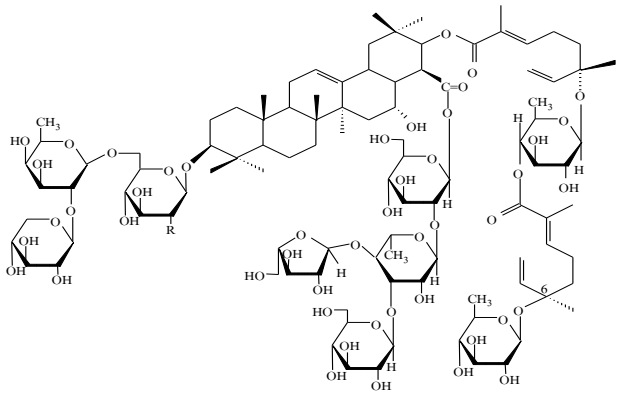
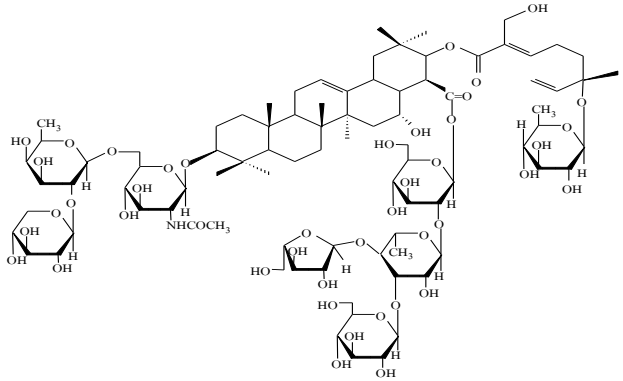
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 51) Julibroside J28	R=$NHCOCH_3$ MT(C-6)=R	*A. julibrissin* (Stem bark)	Liang et al., 2005
 52) Julibroside J29	-	*A. julibrissin* (Stem bark)	Zheng et al., 2006

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

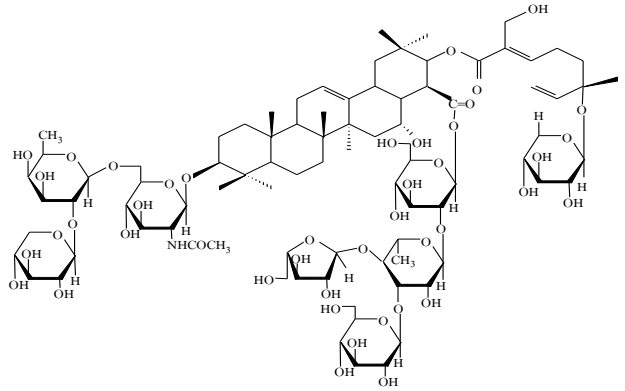
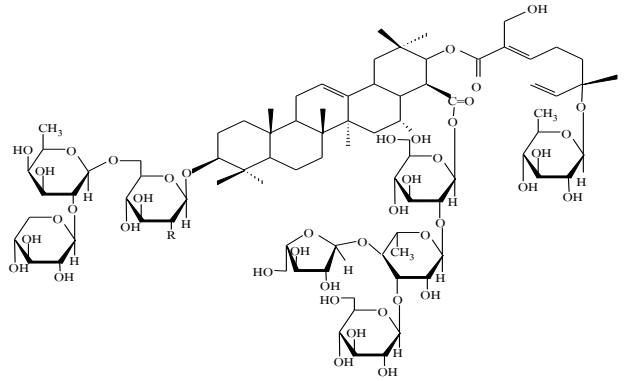
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 53) Julibroside J30	-	*A. julibrissin* (Stem bark)	Zheng et al., 2006
 54) Julibroside J31	R=Oglc	*A. julibrissin* (Stem bark)	Zheng et al., 2006

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

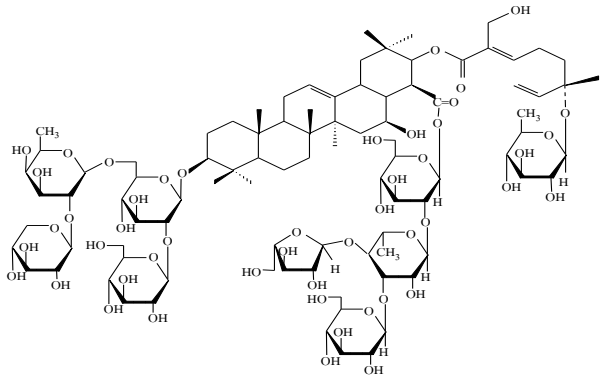
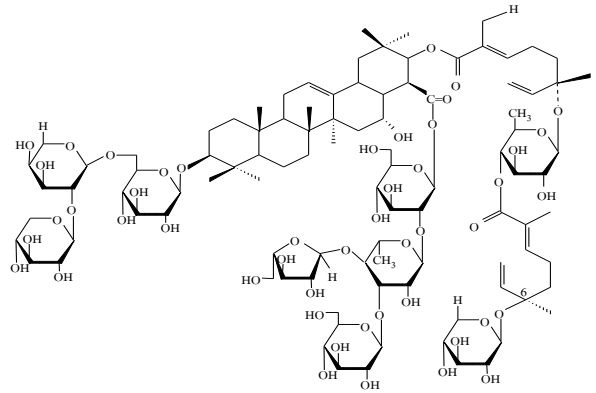
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 55) Julibroside J32	-	*A. julibrissin* (Stem bark)	Zheng et al., 2010
 56) Julibroside J35	-	*A. julibrissin* (Stem bark)	Zheng et al., 2010

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

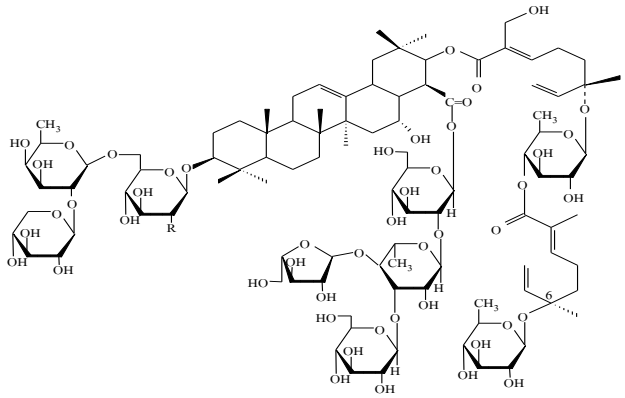
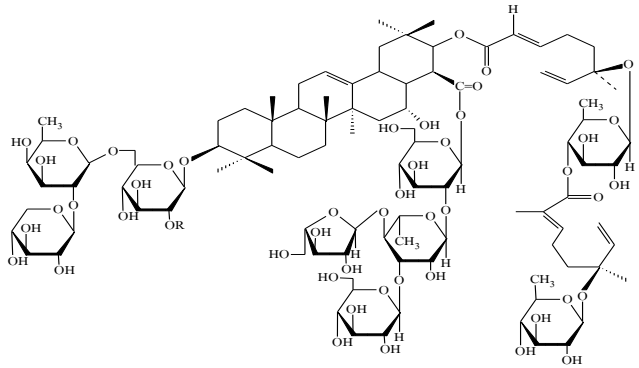
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 57) Julibroside J36	R=O-β-Glc	*A. julibrissin* (Stem bark)	Zhang et al., 2010
 58) Julibroside I	R=Glc	*A. julibrissin* (Stem bark)	Ikeda et al., 1997

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

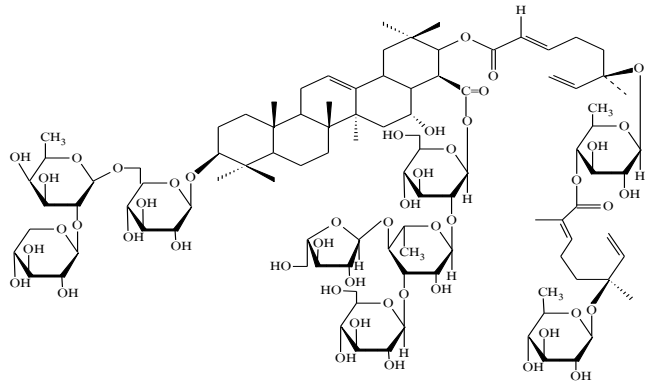
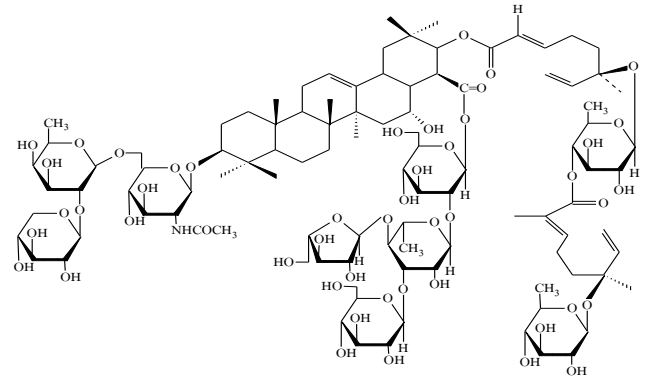
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 59) Julibroside II	-	*A. julibrissin* (Stem bark)	Ikeda et al., 1997
 60) Julibroside III	-	*A. julibrissin* (Stem bark)	Liang et al., 2005 Ikeda et al., 1997

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

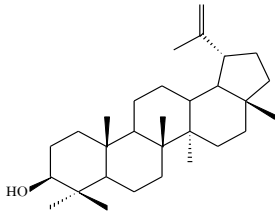
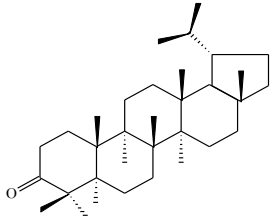
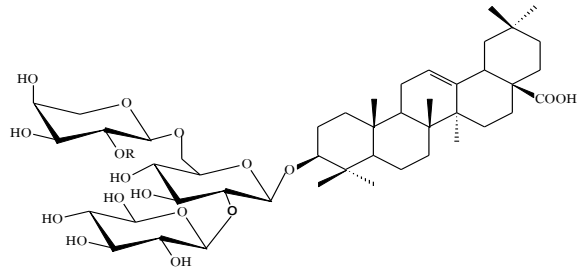
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 61) Lupeol	-	*A. gummifera* (Stem bark)	Rukunga and Waterman, 2001
 62) Lupenone	-	*A. gummifera* (Stem bark)	Rukunga and Waterman, 2001
 63) Pitheduluside G	R=β-D-Xylp	*A. procera* (Bark)	Miyase et al., 2010

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

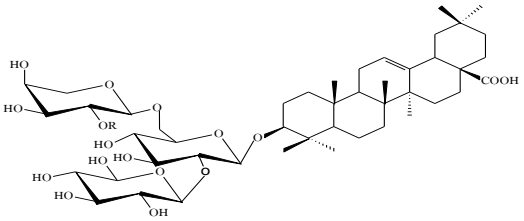
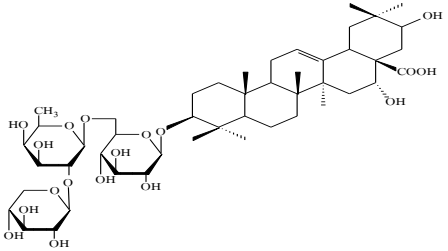
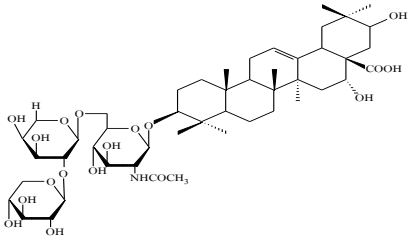
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 64) Pitheduluside F	R=α-D-Arap	*A. procera* (Bark)	Miyase et al., 2010
 65) Prosapogenin-1	-	*A. adianthifolia* (Root) *A. julibrissin* (Stem bark)	Haddad et al., 2002 Ikeda et al., 1997
 66) Prosapogenin-2	-	*A. adianthifolia* (Root) *A. julibrissin* (Stem bark)	Haddad et al., 2002 Ikeda et al., 1997

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

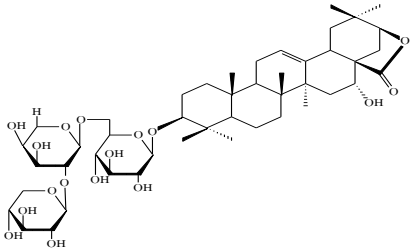
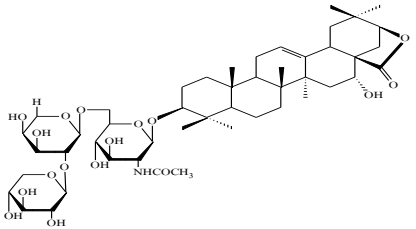
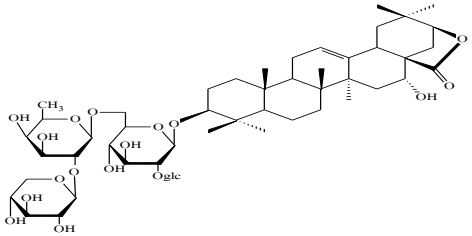
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 67) Prosapogenin-3	-	*A. julibrissin* (Stem bark)	Han et al., 2011 Han et al., 2008 Ikeda et al., 1997
 68) Prosapogenin-4	-	*A. julibrissin* (Stem bark)	Han et al., 2011 Ikeda et al., 1997
 69) Prosapogenin-5	-	*A. julibrissin* (Stem bark)	Ikeda et al., 1997

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

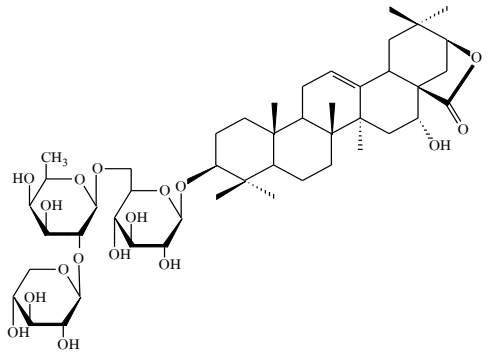
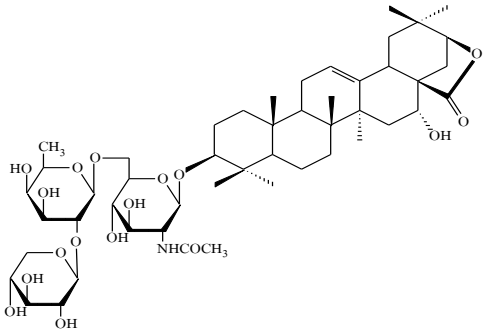
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 70) Prosapogenin-6	-	*A. julibrissin* (Stem bark)	Ikeda et al., 1997
 71) Prosapogenin-7	-	*A. julibrissin* (Stem bark)	Ikeda et al., 1997

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

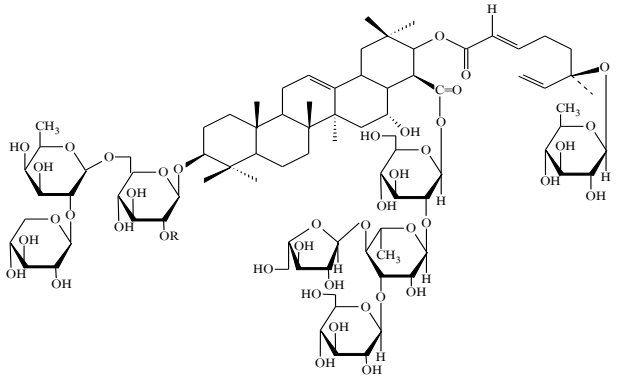
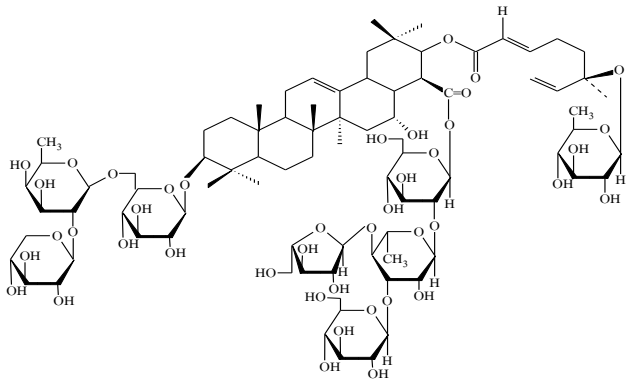
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 72) Prosapogenin-8	R=Glc	*A. julibrissin* (Stem bark)	Ikeda et al., 1997
 73) Prosapogenin-9	-	*A. julibrissin* (Stem bark)	Ikeda et al., 1997

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

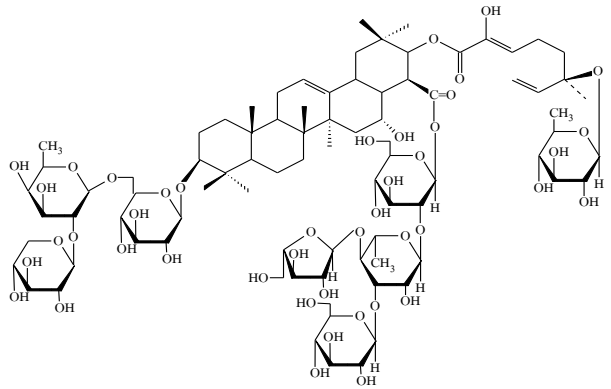
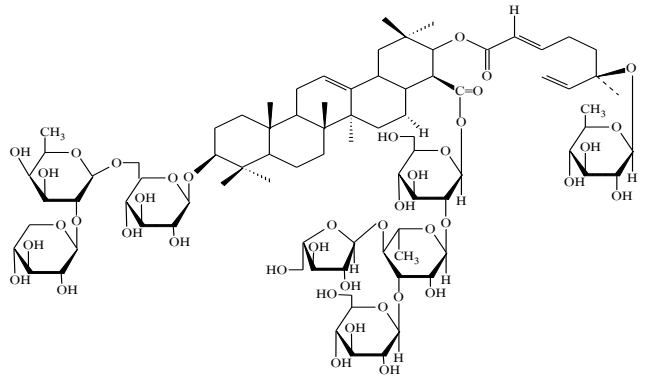
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 74) Prosapogenin-10	-	*A. julibrissin* (Stem bark)	Ikeda et al., 1997
 75) Prosapogenin-11	-	*A. julibrissin* (Stem bark)	Ikeda et al., 1997

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

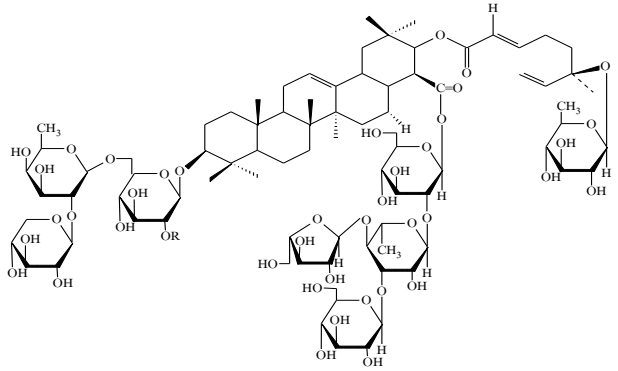
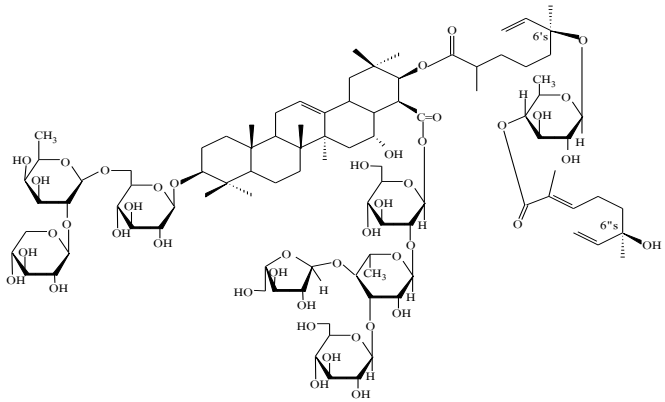
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 76) Prosapogenin-12	R=Glc	*A. julibrissin* (Stem bark)	Ikeda et al., 1997
 77) Proceraoside A	-	*A. procera* (Seed)	Yoshikawa et al., 1998

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

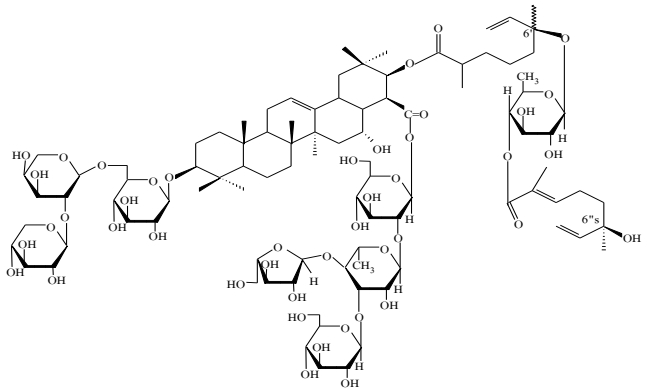
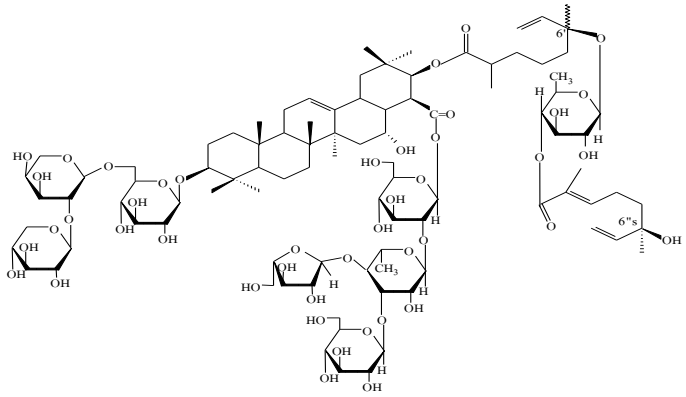
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 78) Proceraoside B	6'S(MT)	*A. procera* (Seed)	Yoshikawa et al., 1998
 79) Proceraoside C	6'R(MT)	*A. procera* (Seed)	Yoshikawa et al., 1998

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

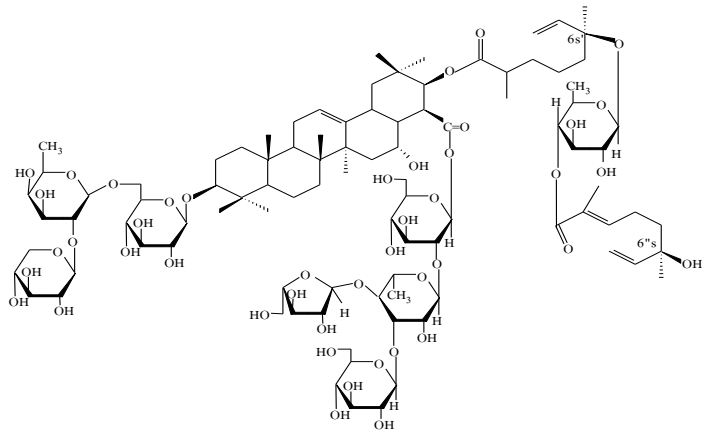
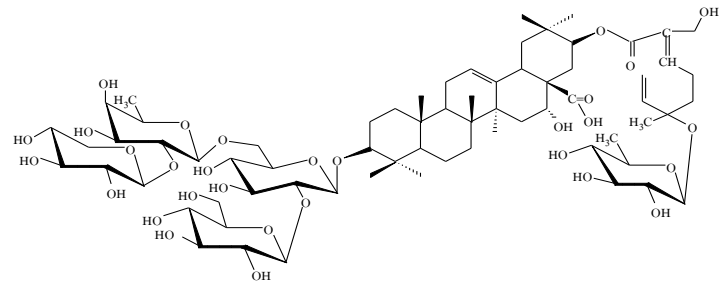
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 80) Proceraoside D	-	*A. procera* (Seed)	Yoshikawa et al., 1998
 81) Prosapogenine prol 1	-	*A. adianthifolia* (Root)	Haddad et al., 2004

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

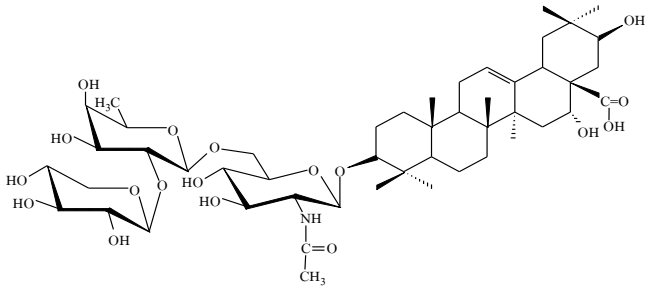
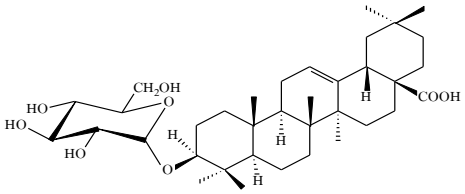
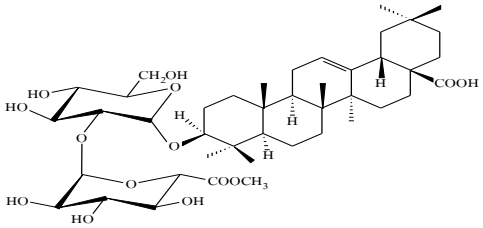
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 82) Prosapogenineprol 2	-	*A. adianthifolia* (Root)	Haddad et al., 2004
 83) Vitalboside A	-	*A. gummifera* (Stem bark)	Rukunga and Waterman, 2001
 84) Vitalboside-A2'-methylglucuronate	-	*A. gummifera* (Stem bark)	Rukunga and Waterman, 2001

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

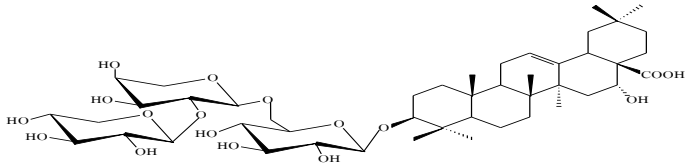
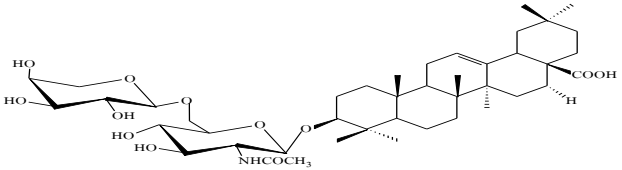
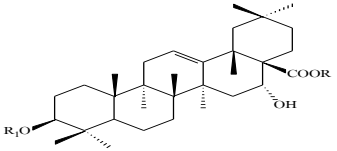
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 85) 3-O-[α-L-arabinopyranosyl (1→2)-α-L-arabinopyranosyl-(1→6)]-β-D-glucopyranosyl oleanolic acid	-	*A. inundata* (Aerial)	Zhang et al., 2011
 86) 3-O-[α-L-arabinopyranosyl (1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl oleanolic acid	-	*A. inundata* (Aerial)	Zhang et al., 2011
 87) 3-O-[β-D-xylopyranosyl (1→2)-α-L-arabinopyranosyl-(1→6)]-[β-D-glucopyranosyl (1→2)]-β-D-glucopyranosyl-echnocystic acid	R_1=Xyl (1→2) Ara (1→6)-Glc (1→2) Glc R=H	*A. lucida* (Bark)	Orsini et al., 1991

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

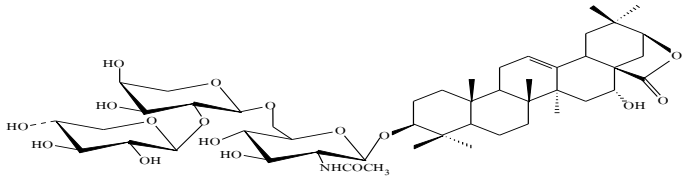
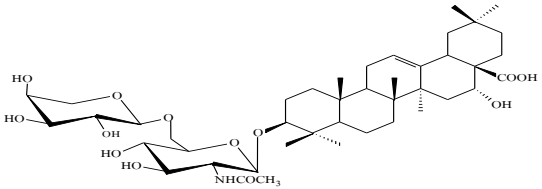
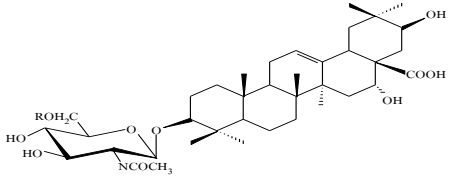
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 88) 3-O-[α-L-arabinopyranosyl (1→2)-α-L-arabinopyranosyl (1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl acacic acid lactone	-	*A. inundata* (Aerial)	Zhang et al., 2011
 89) 3-O-[α-L-arabinopyranosyl (1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl chinocystic acid	-	*A. inundata* (Aerial)	Zhang et al., 2011
 90) 3-O-[α-L-arabinopyranosyl (1→2)-β-D-fucopyranosyl (1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl] echinocystic acid	R=α-L-Arap (1→2)-β-D-Fucp	*A. procera* (Bark)	Melek et al., 2007

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

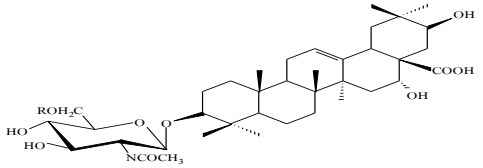
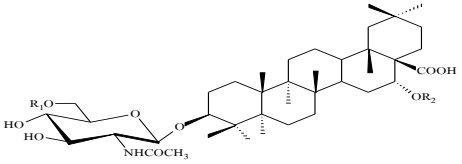
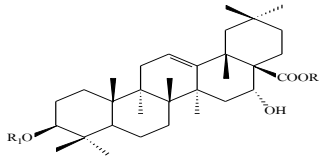
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 91) 3-O-[α-L-arabinopyranosyl (1→2)-α-L-arabinopyranosyl (1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl] echinocystic acid	R=α-L-Arap (1→2)-α-L-Arap	*A. procera* (Bark)	Melek et al., 2007
 92) 3-O-α-L-Arabinopyranosy (1→2)-α-L-arabinopyranosyl (1→6)-2-aceta-2-deoxy-β-D-Glucopyranosylechinocysticacid-16-O-β-D-Glucopyranoside	R_1=α-L-Arap (1→2)-α-L-Arap R_2=β-D-Glcp	*A. procera* (Bark)	Miyase et al., 2010
 93) 3-O-[α-L-arabinopyranosyl (1→6)]-[β-D-glucopyranosyl (1→2)]-β-D-glucopyranosyl echinocystic acid	R_1=Ara (1→6) Glc (1→2) Glc R=H	*A. lucida* (Bark)	Orsini et al., 1991

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

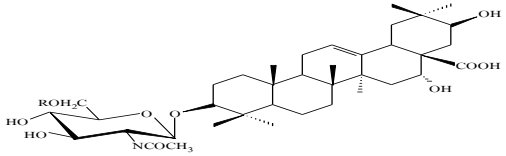
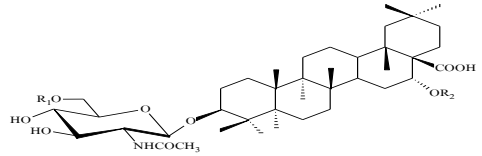
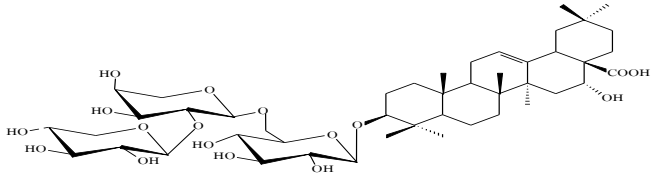
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
	R=β-D-Arap (1→2)-α-D-Fucp	*A. procera* (Bark)	Melek et al., 2007
94) 3-O-[β-D-arabinopyranosyl (1→2)-α-D-fucopyranosyl (1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl] echinocystic acid			
	R_1=β-D-Xylp (1→2)-α-D-Glcp R_2=β-D-Glcp	*A. procera* (Bark)	Miyase et al., 2010
95) 3-O-β-D-Xylopyranosyl (1→2)-α-D-Galactopyranosyl (1→6)-2-acetamido-2-deoxy-β-D-Glucopyranosyl echinocystic acid-16-O-D-Glucopyranoside			
	-	*A. inundata* (Aerial)	Zhang et al., 2011
96) 3-O-[β-D-xylopyranosyl (1→2)-α-L-arabinopyranosyl (l→6)]-β-D-glucopyranosyl oleanolic acid			

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

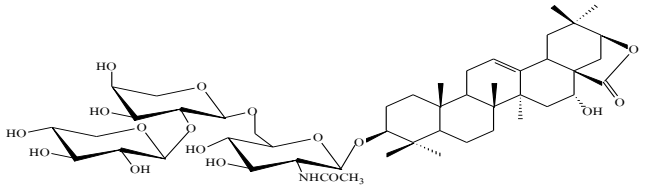
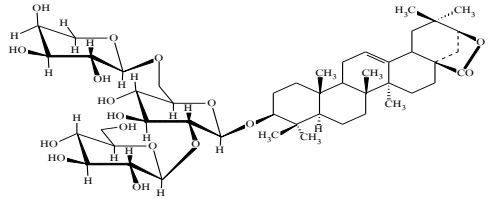
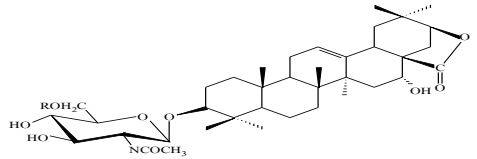
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 97) 3-O-[β-D-xylopyranosyl (1⟶2)-α-L-arabinopyranosyl (1⟶6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl acacic acid lactone	-	*A. inundata* (Aerial)	Zhang et al., 2011
 98) 3-β-[O-D-glucopyranosyl (1⟶2)-[O-α-Larabinopyranosyl (1⟶6)]-β-D-glucopyranosyl oxy]-machaerinic acid lactone	-	*A. gummifera* (Stem bark)	Debella et al., 2000
 99) 3-O-[β-D-xylopyranosyl (1⟶2)-α-L-arabinopyranosyl (1⟶6)-2-acetamido-2-deoxy-β-D-glucopyranosyl] acacic acid lactone	R=β-D-Xylp (1⟶2)-α-L-Arap	*A. procera* (Bark)	Melek et al., 2007

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

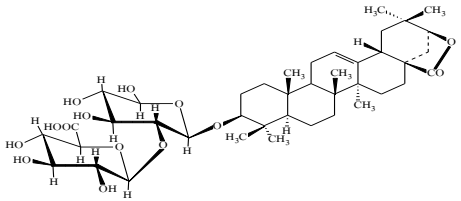
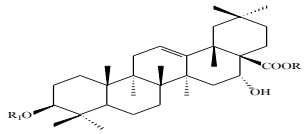
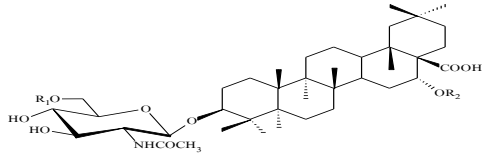
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 100) 3-β-[O-β-D-glucopyranosid uronic acid (1⟶2)-β-D-glucopyranosyl-oxy]-machaerinic acid lactone	-	*A. gummifera* (Stem bark)	Debella et al., 2000
 101) 3-O-[β-D-xylopyranosyl (1⟶2)-β-D-fucopyranosyl (1⟶6)-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid	R_1=Xyl (1⟶2) Fuc (1⟶6)-Glcp-2-$NHCOCH_3$ R=CH_3	*A. lucida* (Bark)	Orsini et al., 1991
 102) 3-O-β-D-xylopyranosyl (1⟶2)-α-L-arabinopyranosyl (1⟶6)-2-aceta-2-deoxy-β-D-glucopyranosyl echinocystic acid-16-O-β-D-glucopyranoside	R_1=β-D-Xylp (1⟶2)-α-L-Arap R_2=β-D-Glcp	*A. procera* (Bark)	Miyase et al., 2010

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

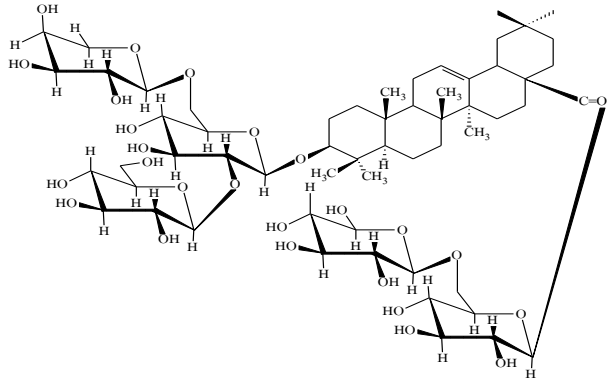
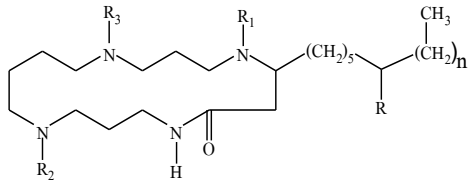
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 103) β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl [3-O-β-D-glucopyranosyl (1→2)-[α-L-arabinopyranosyl (1→6)]-β-D-glucopyranosyl]-oleanolate	-	*A. gummifera* (Stem bark)	Debella et al., 2000
Alkaloids  104) Budmunchiamine A	R=H R_1=R_2=R_3=CH_3 n=2	*A. schimperana* (Stem bark) *A. amara* (Seed)	Rukunga and Waterman, 1996 Pezzuto et al., 1992 Mar et al., 1991

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

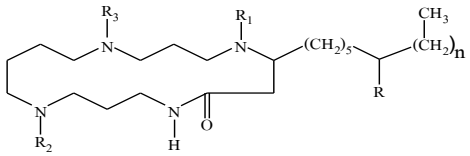
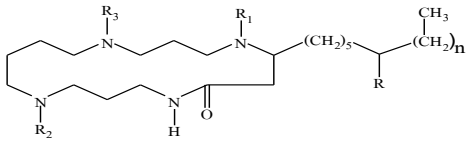
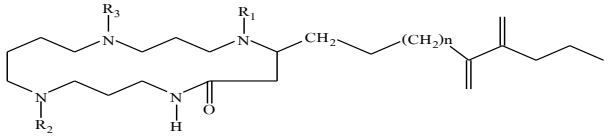
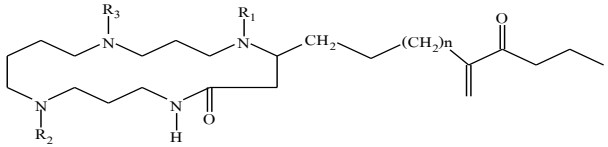
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
	R=H R_1=R_2=R_3=CH_3 n=1	*A. amara* (Seed)	Pezzuto., et al., 1992 Mar et al., 1991
105) Budmunchiamine B			
	R=H R_1=R_2=R_3=CH_3 n=1	*A. amara* (Seed)	Pezzuto., et al., 1992 Mar et al., 1991
106) Budmunchiamine C			
	R_1=H R_2=R_3=CH_3 n=6	*A. amara* (Seed)	Pezzuto et al., 1992
107) Budmunchiamine D			
	R_1=H=R_2=R_3=CH_3 n=6	*A. amara* (Seed)	Pezzuto et al., 1992
108) Budmunchiamine E			

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

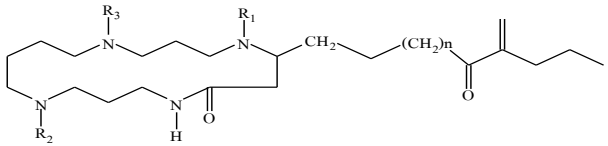
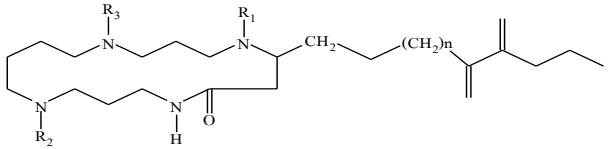
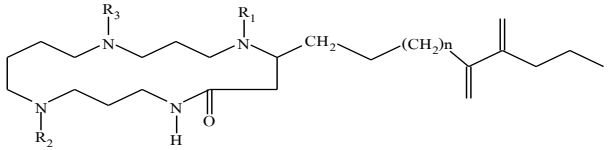
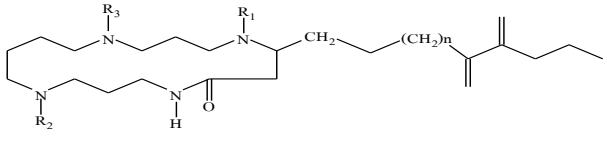
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
	R_1=H R_2=R_3=CH_3 n=6	*A. amara* (Seed)	Pezzuto et al., 1992
109) Budmunchiamine F			
	R_1=R_2=R_3=CH_3 n=4	*A. schimperana* (Stem bark) *A. amara* (Seed)	Rukunga and Waterman,1996 Pezzuto et al., 1992
110) Budmunchiamine G			
	R_1=R_2=R_3=CH_3 n=2	*A. amara* (Seed)	Pezzuto et al., 1992
111) Budmunchiamine H			
	R_1=R_2=R_3=CH_3 n=6	*A. amara* (Seed)	Pezzuto et al., 1992
112) Budmunchiamine I			

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

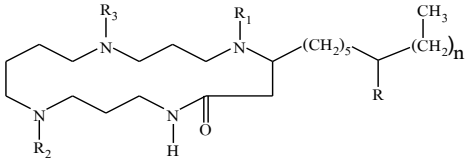
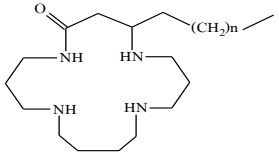
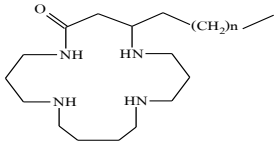
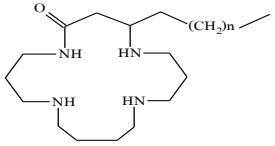
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 113) Budmunchiamine K	R=H $R_1=R_2=R_3=CH_3$ n=8	*A. schimperana* (Stem bark)	Rukunga and Waterman, 1996
 114) Budmunchiamine L1	n=14	*A. adinocephala* (Stem bark, Leaf)	Ovenden et al., 2002
 115) Budmunchiamine L2	n=12	*A. adinocephala* (Stem bark, Leaf)	Ovenden et al., 2002
 116) Budmunchiamine L4	n=11	*A. adinocephala* (Stem bark, Leaf)	Ovenden et al., 2002

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

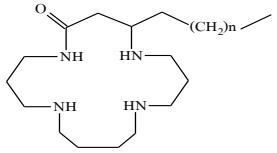
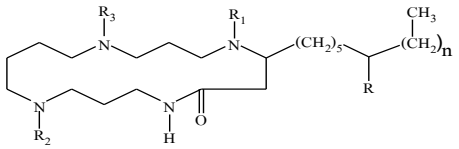
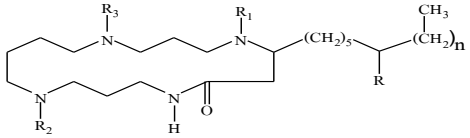
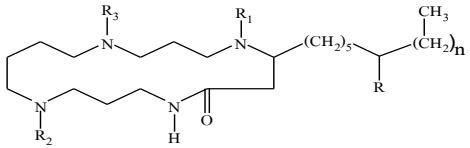
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
	n=13	*A. adinocephala* (Stem bark, Leaf)	Ovenden et al., 2002
117) Budmunchiamine L5			
	R=OH R_1=R_2=R_3=CH_3 n=6	*A. schimperana* (Stem bark)	Rukunga et al., 2007 Rukunga and Waterman, 1996
118) 6-Hydroxybudmunchiamine C			
	R=OH R_1=R_2=R_3=CH_3 n=8	*A. schimperana* (Stem bark)	Rukunga et al., 2007 Rukunga and Waterman, 1996
119) 6-Hydroxybudmunchiamine K			
	R=OH R_1=H R_2=R_3=CH_3 n=8	*A. schimperana* (Stem bark)	Rukunga et al., 2007 Rukunga and Waterman, 1996
120) 6-Hydroxy-5-Hydroxybudmunchiamine K			

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

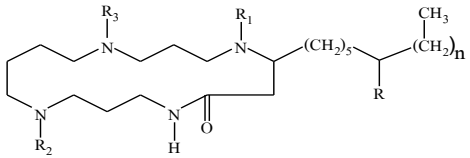
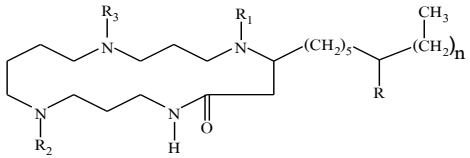
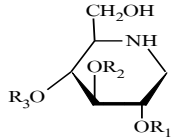
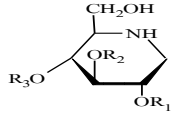
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
	$R=R_3=H$ $R_1=R_2=CH_3$ n=8	*A. gummifera* (Stem bark)	Rukunga et al., 2007 Rukunga and Waterman, 1996
121) 9-Normethylbudmunchiamine K			
	$R_1=R_3=CH_3$ $R=R_2=H$ n=8	*A. gummifera* (Stem bark)	Rukunga and Waterman, 1996
122) 14-Normethylbudmunchiamine K			
	$R_1=R_2=R_3=H$	*A. myriophylla* (Wood)	Asano et al., 2005
123) 1-Deoxymannojirimycin (DMJ)			
	$R_1=R_2=H$ $R_3=\beta$-D-Glc	*A. myriophylla* (Wood)	Asano et al., 2005
124) 4-O-β-D-Glucopyranosyl-DMJ			

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

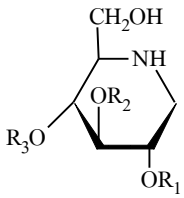
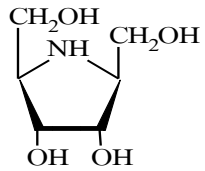
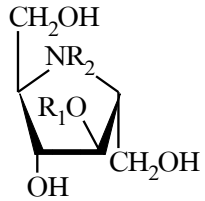
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
	R_1=β-D-Glc R_2=R_3=H	*A. myriophylla* (Wood)	Asano et al., 2005
125) 2-O-β-D-Glucopyranosyl-DMJ			
	-	*A. myriophylla* (Wood)	Asano et al., 2005
126) 2,5-Dideoxy-2,5-amino-D-Glucitol			
	R_1=R_2=H	*A. myriophylla* (Wood)	Asano et al., 2005
127) 2,5-Dihydroxymethyl-3,4-dihydroxypyrrolidine			

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

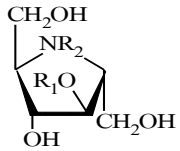
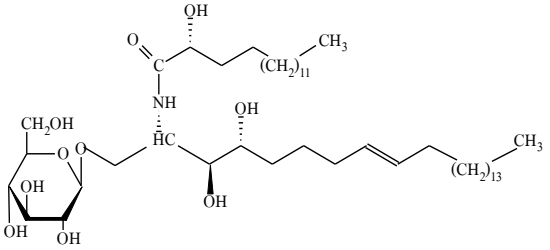
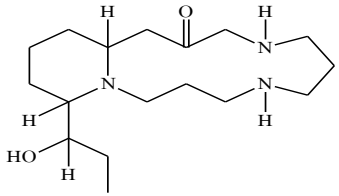
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 128) 2-O-β-D-Glucopyranosyl-DMDP	R_1=β-D-Glc R_2=H	*A. myriophylla* (Wood)	Asano et al., 2005
 129) 1-O-β-D-Glucopyranosyl (2S,3S,4R,8E)-2-[(2′ R)-hydroxyhexadecanoyl amino]-8-tetracosene-1,3,4-triol	-	*A. julibrissin* (Flower)	Kang et al., 2007
 130) Palustrine	-	*A. myriophylla* (Wood)	Phavanantha et al., 1990

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

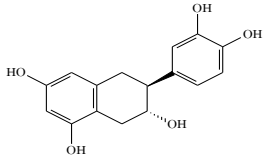
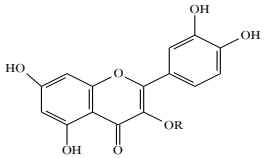
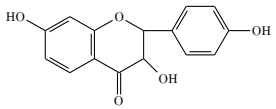
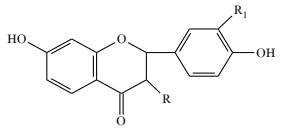
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
Flavonoids  131) Catechin	-	*A. lebbeck* (Stem bark)	Venkatesh et al., 2010
 132) Isoquercitrin	R=β-D-glucopyranose	*A. julibrissin* (Flower)	Kang et al., 2000
 133) Kaempferol	-	*A. chinnensis* (Leaf)	Ghaly et al., 2010
 134) Kaempferol-3-O-α-L-rhamnopyranoside	R=O-α-L-rhamnopyranoside R_1=H	*A. chinnensis* (Leaf)	Ghaly et al., 2010

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

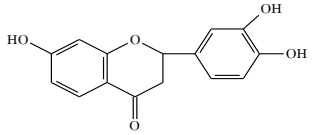
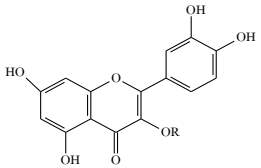
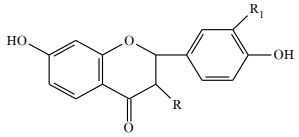
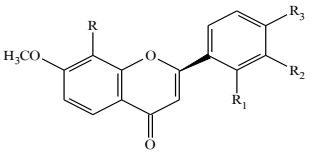
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 135) Luteolin	-	*A. chinnensis* (Leaf)	Ghaly et al., 2010
 136) Quercitrin	R=β-L-rhamnopyranose	*A. julibrissin* (Flower)	Kang et al., 2000
 137) Quercetin-3-O-α-L-Rhamnopyranoside	R=O-α-L-rhamnopyranoside R_1=OH	*A. chinnensis* (Leaf)	Ghaly et al., 2010
 138) 7,8-Dimethoxy-3',4'-methylenedioxyflavone	R=OCH_3 R_1=H R_2-R_3=-O-CH_2-O-	*A. odoratissima* (Root bark)	Rao et al., 2002

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

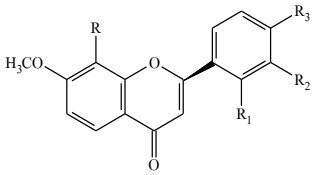
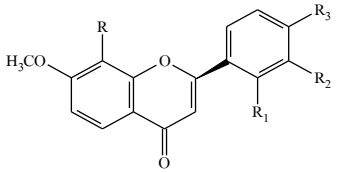
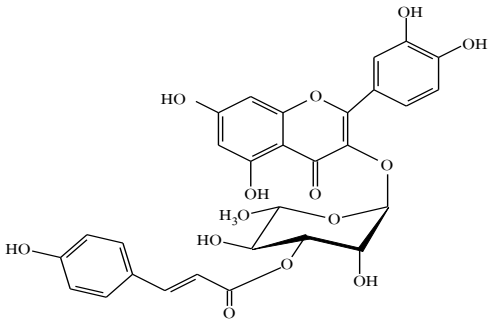
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
	$R=R_2=H$ $R_1=R_3=OCH_3$	*A. odoratissima* (Root bark)	Rao et al., 2002
139) 7,2' 4'-Trimethoxyflavone			
	$R=R_1=H$ $R_2=OH$ $R_3=OCH_3$	*A. odoratissima* (Root bark)	Rao et al., 2002
140) 7,4'-Dimethoxy-3'-hydeoxyflavone			
	-	*A. julibrissin* (Flower)	Yahagi et al., 2012
141) 3''-(E)-P-coumaroylquercitrin			

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

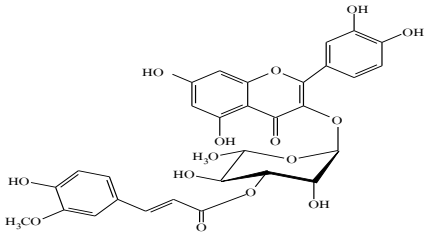
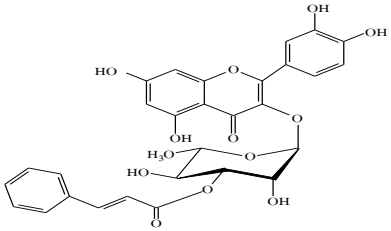
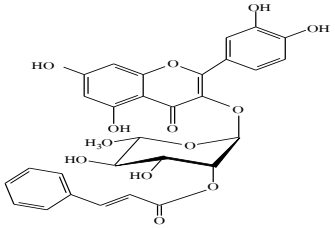
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 142) 3"-(E)-Feruloylquercitrin	-	*A. julibrissin* (Flower)	Yahagi et al., 2012
 143) 3"-(E)-Cinnamoylquercitrin	-	*A. julibrissin* (Flower)	Yahagi et al., 2012
 144) 2"-(E)-Cinnamoylquercitrin	-	*A. julibrissin* (Flower)	Yahagi et al., 2012

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

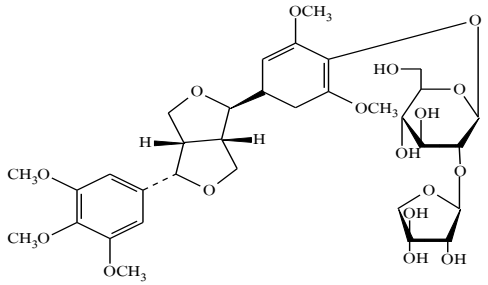
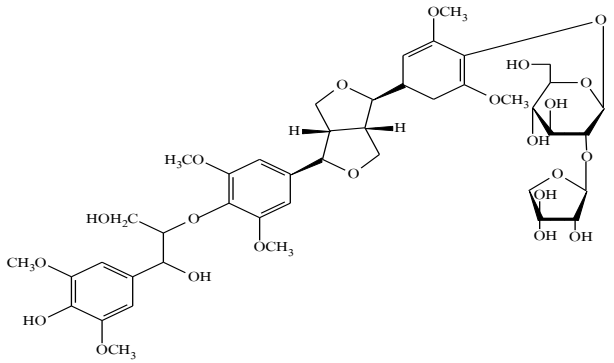
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
Lignan glycosides	-	*A. myriophylla* (Bark)	Ito et al., 1994
 145) Albizizoside A			
 146) Albizizoside B	-	*A. myriophylla* (Bark)	Ito et al., 1994

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

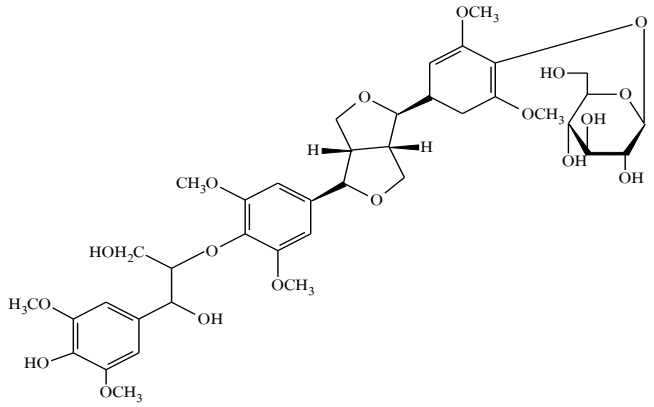
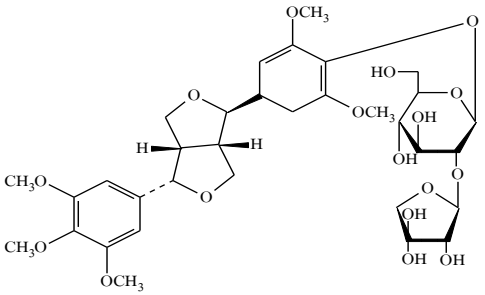
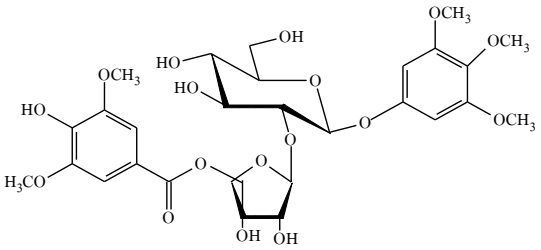
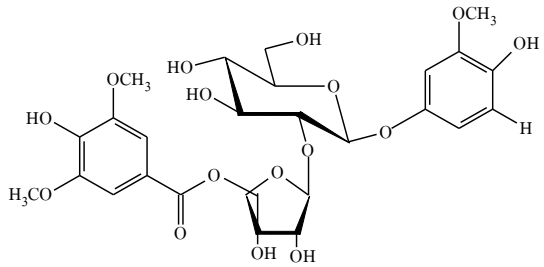
Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
 147) Albizizoside C	-	*A. myriophylla* (Bark)	Ito et al., 1994
 148) Syringaresinol4-O-β-D-apiofuranosyl (1→2)-β-D-gluco-pyranoside	-	*A. myriophylla* (Bark)	Ito et al., 1994

Table 1 Chemical constituents of plants in the genus *Albizia* (Continued)

Chemical group/ Structure	Substituted group (R-group)	Plant (Part)	References
Phenolic compounds	-	*A. julibrissin* (Stem bark)	Jung et al., 2004*a*
 149) Albibrissinoside A			
 150) Albibrissinoside B	-	*A. julibrissin* (Stem bark)	Jung et al., 2004*a*

2. Biological Activities of Plants in the genus *Albizia*

Many experimental evidences have revealed that several species of the genus *Albizia* were shown to possess high cytotoxicity against many different cancer cell lines (Ikeda et al., 1997; Zou et al., 2000 & 2005; Cai et al., 2002; Ueda et al., 2003; Liang et al., 2005; Won et al., 2006; Melek et al., 2007; Roy et al., 2008; Lui et al., 2010; Zhang et al., 2011). Furthermore, biological screenings worldwide showed that many plant species of this genus have demonstrated antimalarial (Desjardins et al., 1979; Ofulla et al., 1995; Freiburghaus et al., 1996; Ovenden et al.,2002; Kohler et al., 2002; Rukunga et al., 2007; Muregi et al., 2008; Elizabeth et al., 2009), immunomodulatory (Barua et al., 2000), antioxidant (Jung et al., 2004(a); D'Souza et al., 2004; Resm et al., 2006; Tamokou et al., 2012), antimicrobial (Collin et al., 1997; Geyid et al., 2005; Duraipandiyan et al., 2006; Runyoro et al., 2006; Babu etal., 2009; Lam et al., 2011), anthelmintic (Galal et al., 1991; EI Garhy et al., 2000; Githiori et al., 2003; Grade et al., 2008; Eguale et al., 2011), antiandrogenic (Gupta et al., 2006), and anti-inflammatory (Tripathi et al., 1979; Besra et al., 2002; Pratibha et al., 2004; Ekenseair et al., 2006; Gnapaty et al., 2006; Qiao et al., 2007; Venkatesh et al., 2010; Yadav et al., 2010) properties. The *Albizia* genus is known to be a rich source of bioactive saponins which were shown previously to possess high cytotoxicity against various cancer cell lines (Krief et al., 2005; Zhang et al., 2006). Apart from saponins, alkaloids found in some plant species of this genus also possessed significant antimalarial activity (Geoffrey et al., 1996). Moreover, some flavonoids isolated previously from the members of *Albizia* have been shown to have antibacterial, antifungal, and antitriglyceride activities (Ghaly et al., 2010; Yahagi et al., 2012). Summary of the bioactive compounds from *Albizia* plants is shown in Table 2.

Table 2 Bioactive compounds from *Albizia* species

Compounds	Plant (part)	Biological activity	References
(1) Adianthifolioside A	*A. adianthifolia* (Root)	Hemolytic activity Cytotoxicity against Jurkat cell	Haddad et al., 2004 Haddad et al., 2003
(2) Adianthifolioside B	*A. adianthifolia* (Root)	Hemolytic activity Cytotoxicity against Jurkat cell	Haddad et al., 2004 Haddad et al., 2003
(3) Adianthifolioside D	*A. adianthifolia* (Root)	Cytotoxicity against Jurkat cell	Haddad et al., 2004
(4) Albibrissinoside B	*A. julibrissin* (Stem bark)	Antioxidant	Jung et al., 2004
(5) Albizoside A	*A. chinnensis* (Stem bark)	Cytotoxicity against HCT-8, Bel-7402, BGC-823, A549, A2780 cancer cells	Liu et al., 2009
(6) Albizoside B	*A. chinnensis* (Stem bark)	Cytotoxicity against HCT-8, Bel-7402, A2780, BGC823, A549 cancer cells	Liu et al., 2009
(7) Albizoside C	*A. chinnensis* (Stem bark)	Cytotoxicity against HCT-8, Bel-7402, BGC-823, A549, A2780 cancer cells	Liu et al., 2009
(8) Albizoside D	*A. chinnensis* (Stem bark)	Cytotoxicity against HCT-8, Bel-7402, BGC-823, A549, A2780 cancer cells	Liu et al., 2010

Table 2 Bioactive compounds from *Albizia* species (Continued)

Compounds	Plant (part)	Biological activity	References
(9) Albizoside E	*A. chinnensis* (Stem bark)	Cytotoxicity against HCT-8, Bel-7402, A2780 cancer cells	Liu et al., 2010
(10) Albiziatrioside A	*A. subdimidiata* (Stem)	Cytotoxicity against A2780 cancer cell	Kader et al., 2001
(11) Budmunchiamine A	*A. amara* (Seed)	Cytotoxicity against KB, MCF7 cancer cells	Mar et al., 1991
(12) Budmunchiamine G	*A. gummifera* (Stem bark)	Antimalarial activity	Rukunga and Waterman, 1996
(13) Budmunchiamine K	*A. gummifera* (Stem bark)	Antimalarial activity	Rukunga et al., 2007
(14) Budmunchiamine L4	*A. adinocephala* (Stem bark)	Antimalarial activity	Ovenden et al., 2002
(15) Budmunchiamine L5	*A. adinocephala* (Stem bark)	Antimalarial activity	Ovenden et al., 2002
(16) Coriarioside A	*A. gummifera* (Roots)	Cytotoxicity against HCT-116 cancer cell	Placide et al., 2009
(17) Grandibracteoside A	*A. gradibracteata* (Leaf)	Cytotoxicity against KB cell and MCF7 cancer cells	Krief et al., 2005
(18) Grandibracteoside B	*A. gradibracteata* (Leaf)	Cytotoxicity against KB cell and MCF7 cancer cells	Krief et al., 2005
(19) Grandibracteoside C	*A. gradibracteata* (Leaf)	Cytotoxicity against KB cell and MCF7 cancer cells	Krief et al., 2005

Table 2 Bioactive compounds from *Albizia* species (Continued)

Compounds	Plant (part)	Biological activity	References
(20) Gummiferaoside A	*A. gummifera* (Roots)	Cytotoxicity against A2780 cancer cell	Cao et al., 2007
(21) Gummiferaoside B	*A. gummifera* (Roots)	Cytotoxicity against A2780 cancer cell	Cao et al., 2007
(22) Gummiferaoside C	*A. gummifera* (Roots)	Cytotoxicity against A2780 cancer cell	Placide et al., 2009 Cao et al., 2007
(23) Julibroside J1	*A. julibrissin* (Stem bark)	Cytotoxicity against KB cancer cell	Zou et al., 2000 Ikeda et al., 1997
(24) Julibroside J2	*A. julibrissin* (Stem bark)	Cytotoxicity against KB cancer cell	Zou et al., 2000 Ikeda et al., 1997
(25) Julibroside J3	*A. julibrissin* (Stem bark)	Cytotoxicity against KB cancer cell	Zou et al., 2000 Ikeda et al., 1997
(26) Julibroside J8	*A. chinnensis* (Stem bark) *A. julibrissin* (Stem bark)	Cytotoxicity against Bel-7402 HeLa, PC-3MIE8, BGC-823, Bel-7402, MDA-1986 cancer cells, Antiangiogenic	Liu et al., 2010 Hua et al., 2009 Zou et al., 2005
(27) Julibroside J9	*A. julibrissin* (Stem bark	Cytotoxicity against KB cancer cell	Zou et al., 2000
(28) Julibroside J12	*A. julibrissin* (Stem bark)	Cytotoxicity against Bel-7402 cancer cell	Zou et al., 2005
(29) Julibroside J13	*A. julibrissin* (Stem bark)	Cytotoxicity against Bel-7402 cancer cell	Zou et al., 2005
(30) Julibroside J21	*A. julibrissin* (Stem bark)	Cytotoxicity against Bel-7402 cancer cell	Zou et al., 2006

Table 2 Bioactive compounds from *Albizia* species (Continued)

Compounds	Plant (part)	Biological activity	References
(31) Julibroside J28	*A. julibrissin* (Stem bark)	Cytotoxicity against HeLa, Bel-7402, PC-3M-1E8 cancer cells	Roy et al., 2008 Liang et al., 2005
(32) Julibroside J29	*A. julibrissin* (Stem bark)	Cytotoxicity against Hela, MDA-1986 cancer cells	Zheng et al., 2006
(33) Julibroside J30	*A. julibrissin* (Stem bark)	Cytotoxicity against Hela, MDA-1986 cancer cells	Zheng et al., 2006
(34) Julibroside J31	*A. julibrissin* (Stem bark)	Cytotoxicity against Hela cancer cell	Zheng et al., 2006
(35) Kaempferol-3-O-α-L-rhamnopyranoside	*A. chinnensis* (Leaf)	Antibacterial activity Antifungal activity	Ghaly et al., 2010
(36) Luteolin	*A. chinnensis* (Leaf)	Antibacterial activity Antifungal activity	Ghaly et al., 2010
(37) 2''-(E)-Cinamoyl-quercitrin	*A. julibrissin* (Flower)	Antitriglyceride activity	Yahagi et al., 2012
(38) 3''-(E)-Pcoumaroyl-quercitrin	*A. julibrissin* (Flower)	Antitriglyceride activity	Yahagi et al., 2012
(39) 3''(E)-Feruloyl-quercitrin	*A. julibrissin* (Flower)	Antitriglyceride activity	Yahagi et al., 2012
(40) 3''-Cinamoylquercitrin	*A. julibrissin* (Flower)	Antitriglyceride activity	Yahagi et al., 2012
(41) 3-O-β-D-Xylopyra-nosyl (1→2)-α-L-arabino-pyranosyl (1→6)-2-aceta-mido-2-deoxy-β-glucopy-ranosyl chinocystic acid	*A. procera* (Bark)	Cytotoxicity against HEPG2 cancer cell	Miyase et al., 2010 Melek et al., 2007

Table 2 Bioactive compounds from *Albizia* species (Continued)

Compounds	Plant (part)	Biological activity	References
(42) 3-O-[β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxyl-β-D-glucopyranosyl] acacic acid lactone	*A. procera* (Bark)	Cytotoxicity against HEPG2 cancer cell	Miyase et al., 2010
(43) 3-O-β-D-Xylopyranosyl-(1→2)-β-D-galactopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic-16-O-β-D-glucopyranoside	*A. procera* (Bark)	Cytotoxicity against HEPG2, MCF7 cancer cells	Miyase et al., 2010 Melek et al., 2007
(44) 3-O-[β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(l→6)]-β-D-glucopyranosyl-oleanolic acid	*A. inundata* (Stem bark)	Cytotoxicity against JMAR, MDA-1986, B16F10 cancer cells	Zhang et al., 2011
(45) 3-O-[α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl acacic acid lactone	*A. inundata* (Bark)	Cytotoxicity against JMAR, MDA-1986, B16F10 cancer cells	Zhang et al., 2011
(46) 3-O-[α-L-arabinopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid	*A. inundata* (Stem bark)	Cytotoxicity against JMAR, MDA-1986 cancer cells	Zhang et al., 2011
(47) 3-O-[α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(l→6)]-β-D-glucopyranosyl olea-nolic acid	*A. inundata* (Stem bark)	Cytotoxicity against JMAR, MDA-1986, B16F10 cancer cells	Zhang et al., 2011

Table 2 Bioactive compounds from *Albizia* species (Continued)

Compounds	Plant (part)	Biological activity	References
(48) 3-O-[α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)]-β-D-glucopyranosyl-oleanolic acid	*A. inundata* (Stem bark)	Cytotoxicity against JMAR, MDA-1986, B16F10 cancer cells	Zhang et al., 2011
(49) 5-Normethylbuchiamine K	*A. gummifera* (Stem bark)	Antimalarial activity	Rukunga et al., 2007
(50) 6-Hydroxybudmunchiamin K	*A. gummifera* (Stem bark)	Antimalarial activity	Rukunga et al., 2007
(51) 6-Hydroxy-5 normethyl-budmunchiamine K	*A. gummifera* (Stem bark)	Antimalarial activity	Rukunga et al., 2007
(52) 9-Normethylbuchiamine K	*A. gummifera* (Stem bark)	Antimalarial activity	Rukunga et al., 2007
(53) 14-Normethylbuchiamine K	*A. gummifera* (Stem bark)	Antimalarial activity	Rukunga and Waterman, 1996
(54) Quercetin-3-O-α-L-rham-nopyranoside	*A. chinnensis* (Leaf)	Antibacterial activity Antifungal activity	Ghaly et al., 2010
(55) Prosagenin 3	*A. julibrissin* (Stem bark)	Cytotoxicity against Jurkat cell	Ikeda et al., 1997
(56) Sulfutine	*A. julibrissin* (Stem bark)	Antioxidant	Jung et al., 2003

HCT-8= human colon cancer, HCT-116= human colorectal cancer cell, BGC-823= human gastric cancer, Bel-7402= human hepatoma cancer, A549= human lung epithelial cancer, A2780= human ovarian cancer, JMAR= human head melanoma cell, MDA 1986= human neck squamous melanoma, B16F10= marine melanoma cell, KB= human oral squamous cell, PC-3M-1E8= human prostate cancer, MCF7= human breast adenocarcinoma, HEPG2= human liver hepatocarcinoma, HeLa= hela cell or human cervical cancer cell

CHAPTER 3

EXPERIMENTAL

1. Source of Plant Material

The wood of *Albizia myriophylla* Benth. was collected from the southern region of Thailand in June 2011. Botanical identification of this plant species was performed by Dr. Oratai Neamsuvan, an ethnobotanist at the Faculty of Traditional Thai Medicine, Prince of Songkla University. Herbarium specimen of this species (NJ0611) has been deposited at the Faculty of Traditional Thai Medicine, Prince of Songkla University, Thailand.

2. General Techniques

2.1 Analytical Thin-Layer Chromatography (TLC)

Technique	:	One dimension, ascending
Adsorbent	:	Silica gel 60 F_{254} (20x20 cm, 0.25 mm. Merck) pre-coated plate
Layer thickness	:	0.2 mm
Distance	:	5.0 cm
Temperature	:	Laboratory temperature (30-35 ^{o}C)
Detection	:	1) Ultraviolet light (254 and 365 nm)
		2) Spraying with anisaldehyde-sulfuric acid solution and heating at 100-110 ^{o}C for 5 min

2.2 Column Chromatography

2.2.1 Liquid Column Chromatography

Column	:	Flat bottom glass column (various diameters)
Adsorbent	:	Silica gel (Merck 60) particle size 70-230 mesh

Solvent	:	Various solvent systems depending on materials
Packing method	:	Dry and wet packing
Sample loading	:	1) Dry packing: The sample was dissolved in a small amount of suitable organic solvent, mixed with small quantity of adsorbent triturated, dried and then placed gently on top of the column. 2) Wet packing: The sample was dissolved in a small amount of eluent and then applied gently on top of the column.
Detection	:	Fractions were examined by TLC technique in the same manner as described in section 2.1. Fractions with similar chromatographic pattern were combined.

2.2.2 Gel Filtration Chromatography

Column size	:	Glass column, 0.5 cm in diameter
Gel Filter	:	Sephadex LH-20 (20-100 μm, Sigma)
Solvent	:	100% MeOH
Packing method	:	Gel filter was suspended in the eluent and left standing to well for 24 hours prior to use, then poured into the column and allowed to set tightly.
Sample loading	:	The sample was dissolved in a small amount of the eluent and Then applied gently on top of the column.
Detection	:	Fractions were examined by TLC technique in the same manner as described in section 2.1.

2.3 Spectroscopy

2.3.1 Ultraviolet (UV) Absorption Spectra

UV absorption spectra were obtained on a Genesys 10 series spectrophotometer (Faculty of Traditional Thai medicine, Prince of Songkhla University).

2.3.2 Infrared (IR) Absorption Spectra

IR absorption spectra (KBr disc and film) were recorded on a EQUINOX 55, Bruker FTIR spectrometer (Scientific Equipment Center, Prince of Songkla University).

2.3.3 Mass Spectra (MS)

Electron impact mass spectra (EIMS) and high-resolution electron impact mass spectra (HREIMS) were obtained with MAT 95 XL mass spectrometer (Scientific Equipment Center, Prince of Songkla University).

2.3.4 Nuclear Magnetic Resonance (NMR) spectra

^{1}H (300 MHz) NMR spectra were obtained on a Bruker Advance DPX-300 FT-NMR spectrometer (Scientific Equipment and Technology, Walailak University).

^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained on a Bruker Advance 400 FT-NMR spectrometer (Department of Chemistry, Faculty of Sciences, Ramkhamhaeng University).

^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra were obtained on a Varian® Unity Inova 500 FT-NMR spectrometer (Scientific Equipment Center, Prince of Songkla University).

The solvents for NMR spectra were deuterated chloroform ($CDCl_3$) and deuterated dimethylsulfoxide (DMSO-d_6). The chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal.

2.4 Physical Properties

2.4.1 Melting points

Melting points were obtained on a BUCHI SMP-20 (Faculty of Pharmaceutical Sciences, Prince of Songkla University).

2.5 Solvents

Organic solvents used in the extraction were commercial grade. For column chromatography, solvents were redistilled prior to use.

3. Extraction and Isolation of Compounds from the Wood of *Albizia myriophylla*

3.1 Extraction and Isolation of Compounds from the Hexane Fraction of *A. myriophylla* Wood

The plant material was rinsed thoroughly and cleaned from foreign matter with tap water, oven-dried at 60 ^{o}C, grounded with an electric grinder, weighed (2.72 kg), and stored in a dry place at room temperature (25-30 ^{o}C). The plant powder was macerated with 95% ethanol (3 x 7 L) for 7 days at room temperature. The filtrates were combined and concentrated by rotary evaporator. The ethanol extract was partition with various solvents ordered by increasing polarity. Each filtrate was pooled and evaporated to dryness under reduced pressure at 40 ^{o}C to yield the hexane fraction (11.79 g, 0.43% yield), dichloromethane fraction (14.13 g, 0.51% yield), ethyl acetate fraction (12.11 g, 0.44% yield), and butanol fraction (16.73 g, 0.61% yield).

The hexane extract (6.29 g) was subjected to column chromatography using silica gel (270 g, 2 x 50 cm) as adsorbent and eluted with acetone-CH_2Cl_2 gradient to give eighty-nine fractions of approximately 60 ml each and then washed down with MeOH. The fractions were then combined on the basis of their TLC profiles, to give eight fractions: fractions A1 (0.45 g), A2 (0.72 g), A3 (0.48 g), A4 (0.13 g), A5 (0.58 g), A6 (0.18 g), A7 (0.49 g), and A8 (0.96 g).

3.1.1 Isolation of Compound HAM1

Fraction A2 (0.72 g) was further chromatographed on a silica gel 60 (45 g, 1.5 x 40 cm) column, eluting with 10% acetone-CH_2Cl_2 gradient to give twenty fractions of approximately 40 ml each and washed down with MeOH. The fractions with similar TLC profiles were then combined to give three fractions: fractions A21 (285 mg), A22 (195 mg), and A23 (300 mg).

Fraction A22 (195 mg), developing with 30% acetone in hexane, displayed one main orange-brown spot on TLC plate under detection with anisaldehyde-sulfuric acid. This fraction was further crystallized in MeOH to yield 54.5 mg of compound HAM1 as colorless needles.

3.1.2 Isolation of Compound HAM2

Fraction A3 (0.48 g) was separated by column chromatography over silica gel 60 (36 g, 1.5 x 50 cm) column, eluting with 10% acetone-CH_2Cl_2 gradient to give sixty-seven fractions of approximately 60 ml each and finally eluting by MeOH. The fractions with similar chromatogram characteristics were combined to afford four fractions: Fractions A31 (137 mg), A32 (56.2 mg), A33 (187.88 mg), and A34 (256 mg).

Fraction A32 (56.2 mg), developing with 20% acetone in CH_2Cl_2, displayed one main brown spot on TLC plate under detection with anisaldehyde-sulfuric acid. This fraction was further purified by gel filtration chromatography using a Sephadex LH-20 column (25 g, 0.5 x 70cm) with MeOH : $CHCl_3$ (1:1) as the eluent to yield 20.1 mg of compound HAM2 as white solid.

3.1.3 Isolation of Compound HAM3

Fraction A33 (187.88 mg) was further chromatographed on a silica gel 60 (24 g, 1 x 50 cm) column, eluting with 25% acetone-CH_2Cl_2 gradient to give forty-five fractions of approximately 40 ml each and then washed down with MeOH. The fractions were then combined according to their TLC profiles to give three fractions: fractions A331 (13 mg), A332 (15.5 mg), and A333 (107 mg).

Crystallization of fraction A332 with MeOH yielded 15.5 mg of compound HAM3 as white needles.

3.1.4 Isolation of Compound HAM4

Fraction A7 (0.49 g) was applied to a silica gel 60 (60 g, 1 x 55 cm) column eluting with 25% acetone-hexane gradient to yield fifty fractions of approximately 60 ml each and then washed down with MeOH. The fractions were then combined on the basis of their TLC profiles to give four fractions: fractions A71 (79 mg), A72 (204 mg), A73 (125 mg), and A74 (64 mg).

Fraction A72 (204 mg), which displayed an anisaldehyde-sulfuric acid positive spot on TLC plate (R_f= 0.53; 40% hexane in acetone), was further purified by silica gel column chromatography (25 g, 1 x 35 cm). Elution was conducted initially with gradient acetone-hexane to give thirty-five fractions of approximately 40 ml each and then washed down with

MeOH. The combined fractions 6-12 from this column yielded 40.3 mg of the compound HAM4 as yellow needles.

3.2 Extraction and Isolation of Compounds from the Dichloromethane Fractoin of *A. myriophylla* Wood

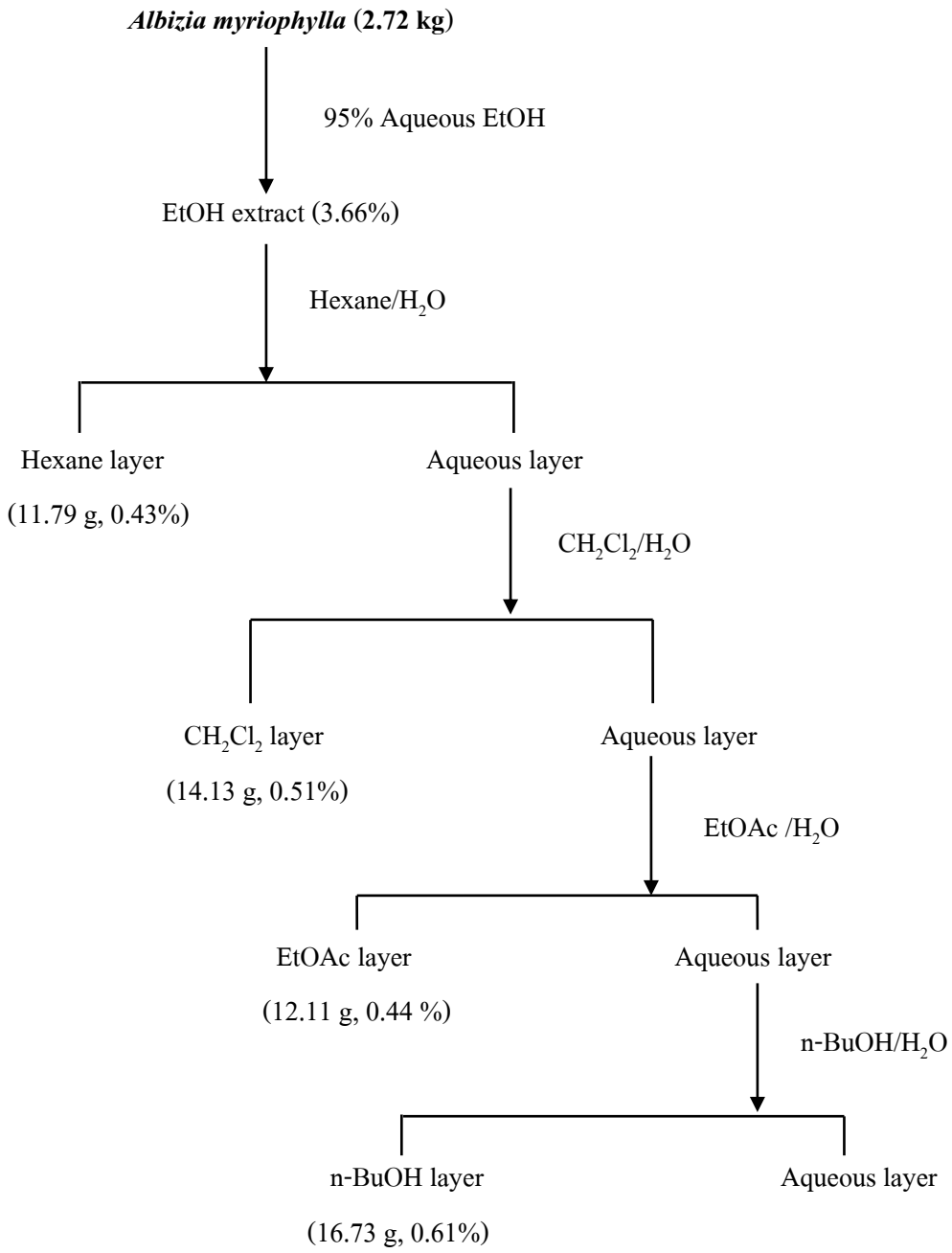
The CH_2Cl_2 extract (12.36 g) was fractionated by column chromatography using silica gel (400 g) as adsorbent and eluted with acetone-CH_2Cl_2 gradient to give one hundred and ten fractions of approximately 60 ml each and then washed down with MeOH. The fractions with similar chromatographic pattern were then combined to give six fractions: Fractions B1 (1.8 g), B2 (1.86 g), B3 (1.5 g), B4 (0.72 g), B5 (1.01g), and B6 (0.84 g).

3.2.1 Isolation of Compounds DAM1 and DAM2

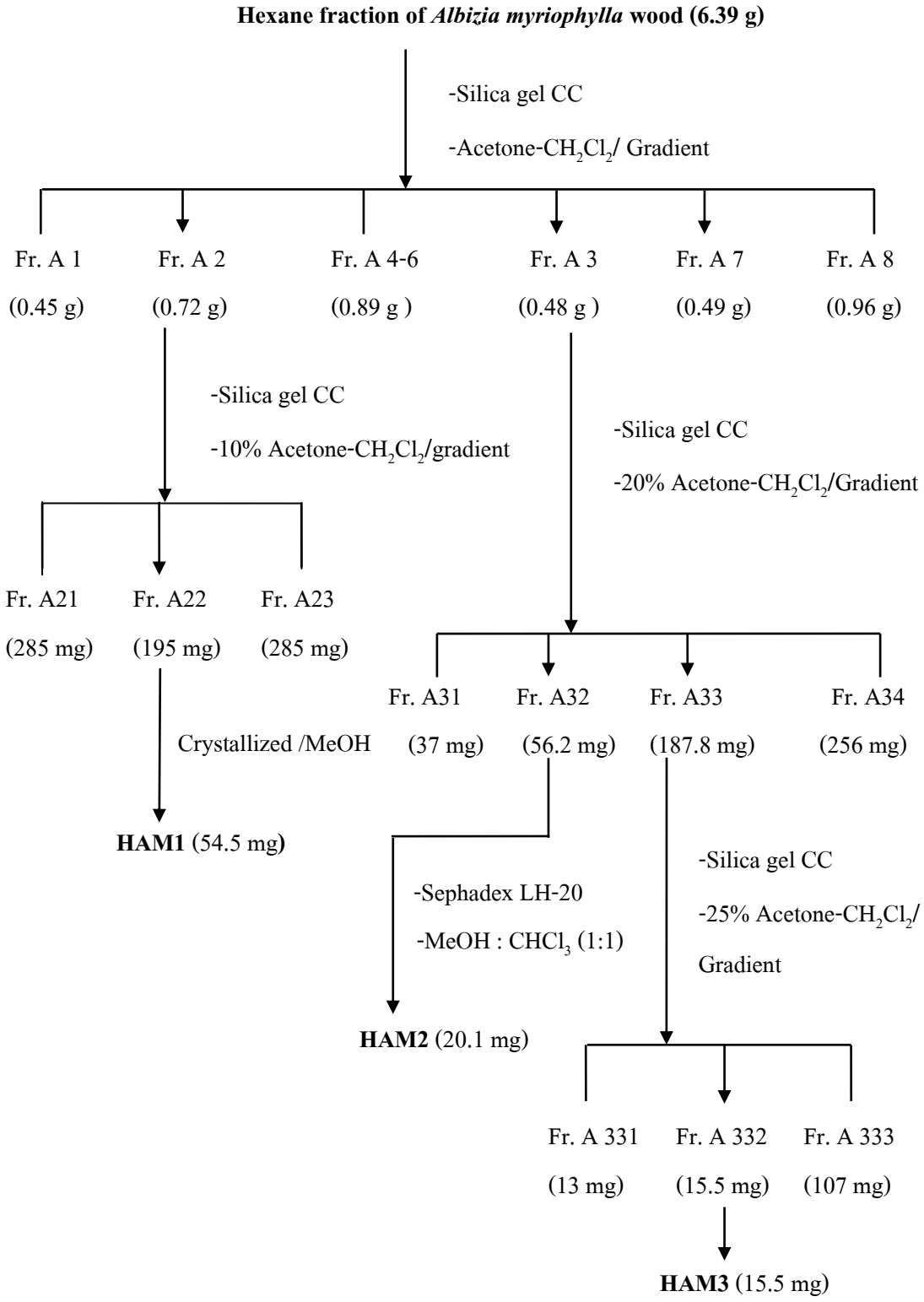
Fraction B3 (1.5 g) was fractionated on a silica gel 60 (170 g, 2 x 70 cm) column (Solvent system: 25% acetone-CH_2Cl_2 gradient) to yield sixty fractions of approximately 50 ml each and then washed down with MeOH. The fractions were then combined according to their TLC profiles to give six fractions: fractions B31 (168.7 mg), B32 (151.8 mg), B33 (117 mg), B34 (127.3 mg), B35 (46.6 mg), and B36 (259 mg).

Fraction B35 (46.6 mg) was further chromatographed on a silica gel 60 (28 g, 1 x 50 cm) column eluted with acetone-CH_2Cl_2 gradient to yield twenty-one fractions of approximately 25 ml each and then washed down with MeOH. Fractions with similar chromatogram characteristics were combined to afford three fractions: Fractions B351 (4 mg), B352 (29 mg), and B353 (9 mg).

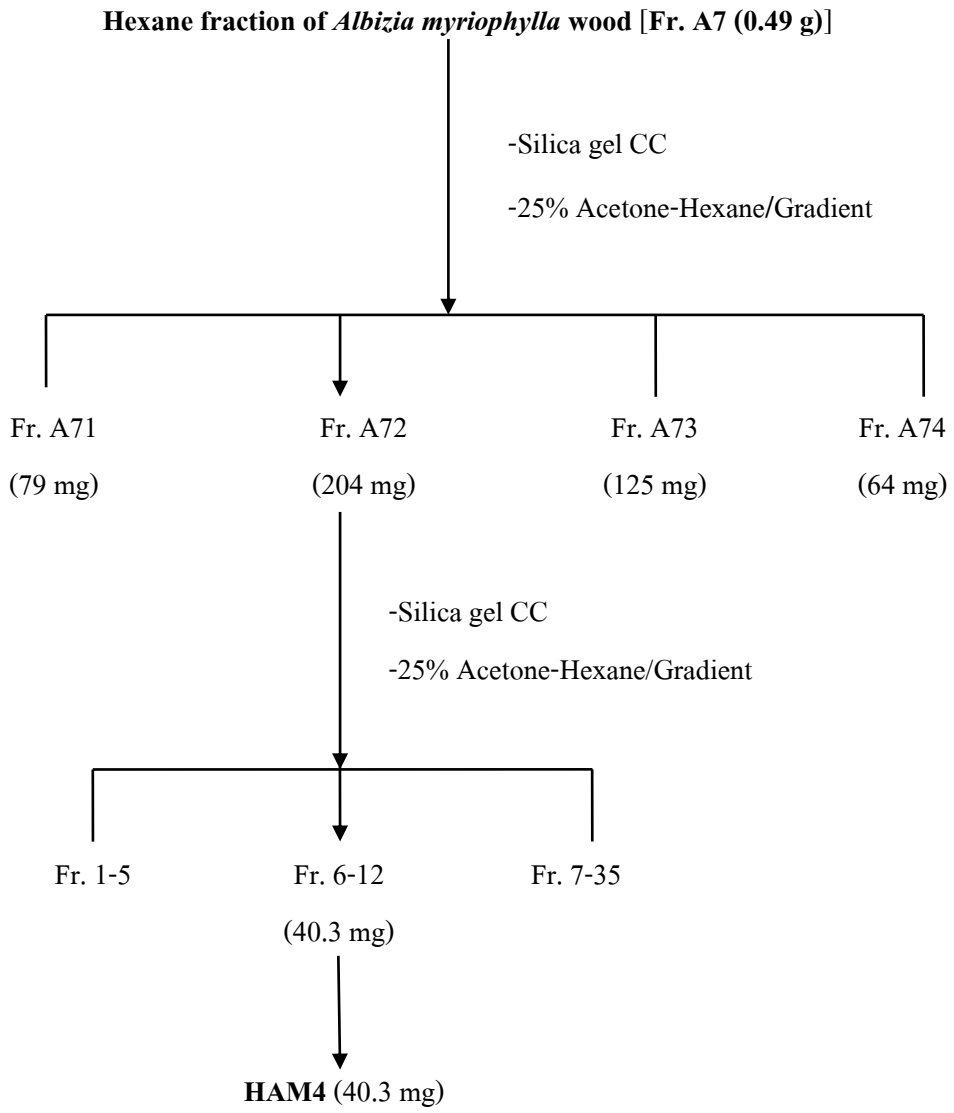
Fraction B352 (29 mg) was further purified by repeated gel filtration chromato-graphy, using three successive Sephadex LH-20 columns (25g, 0.5 x 70 cm) eluted with MeOH to yield compounds DAM1 (9 mg) and DAM2 (5.9 mg).



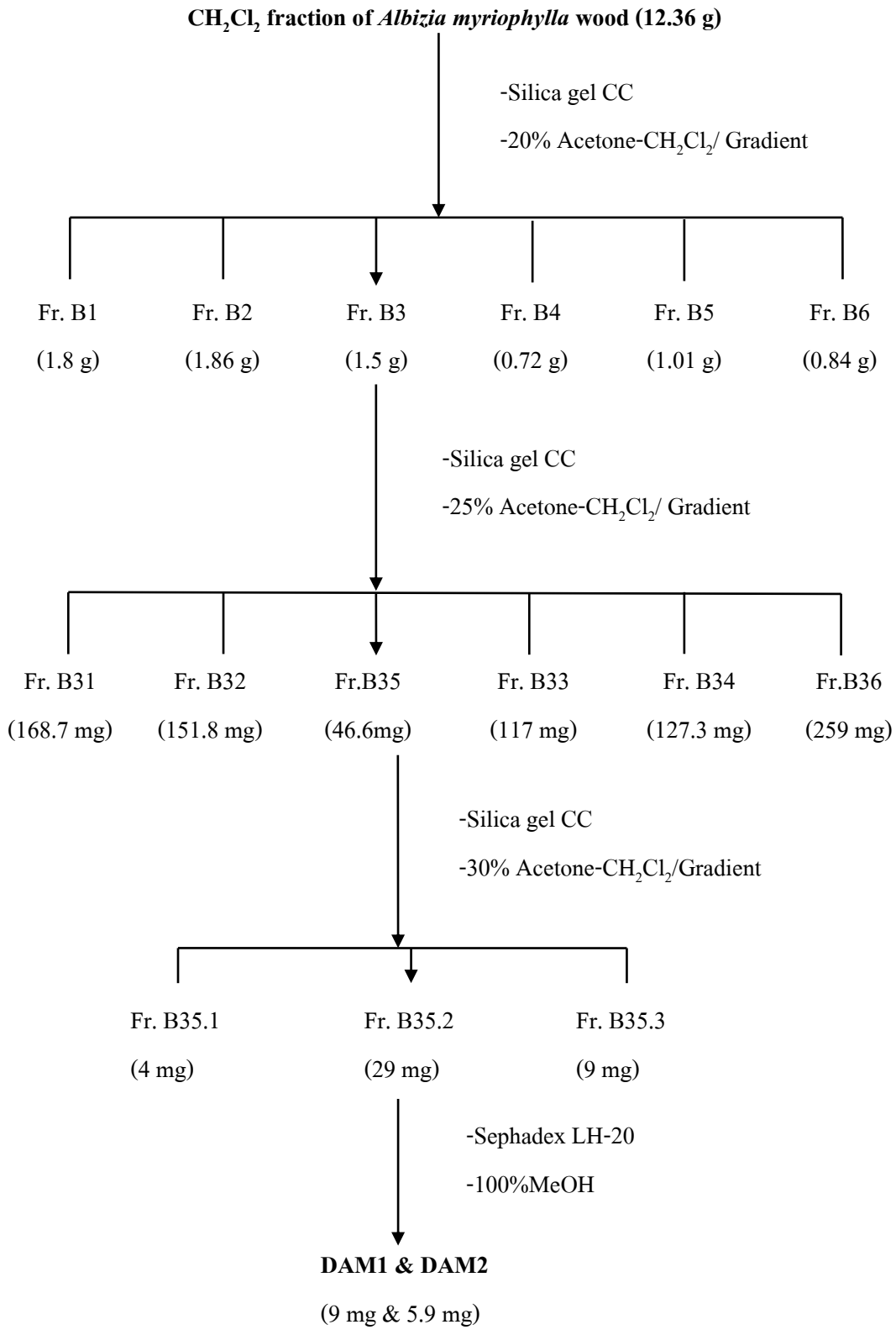
Scheme 1 Extraction of the hexane, CH_2Cl_2, EtOAc, and BuOH fractions of *A. myriophylla* wood



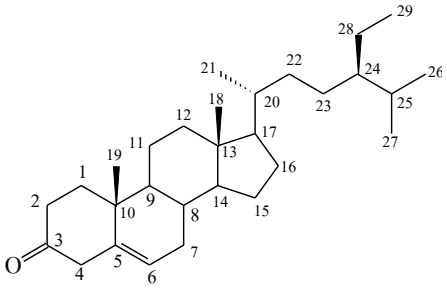
Scheme 2 Separation of hexane fraction of the wood of *A. myriophylla*



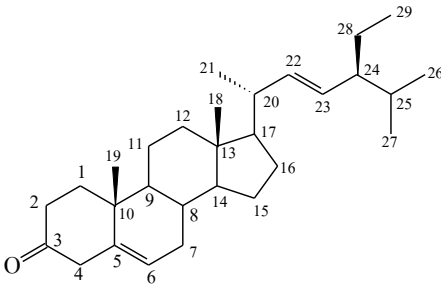
Scheme 2 Separation of hexane fraction of the wood of *A. myriophylla* (Continued)



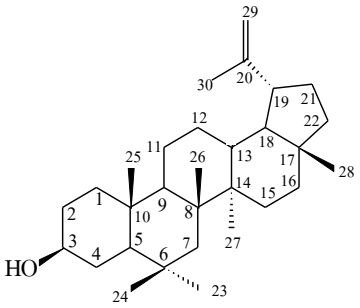
Scheme 3 Separation of CH_2Cl_2 fraction of the wood of *A. myriophylla*



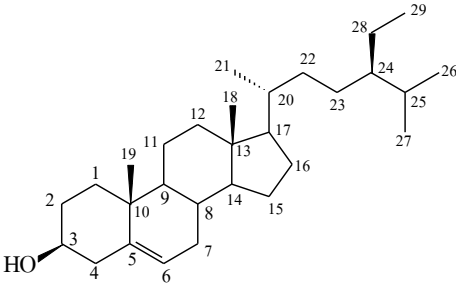
HAM1A



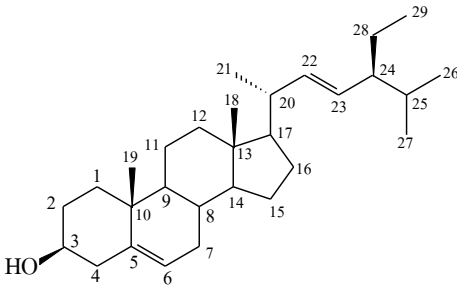
HAM1B



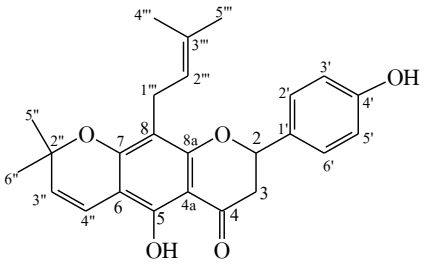
HAM2



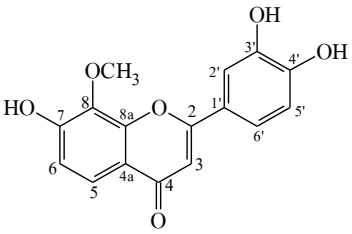
HAM3A



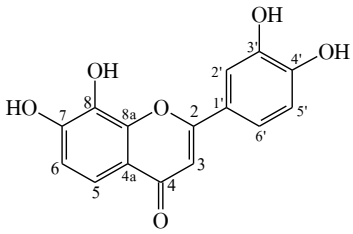
HAM3B



HAM4



DAM1



DAM2

Figure 2 Chemical structures of compounds isolated from *A. myriophylla* wood

4. Physical and Spectral Data of Isolated Compounds

4.1 Compound HAM1

Compound HAM1 was obtained as colorless needles (54.5 mg, 0.05% yield)

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Figure 3; Tables 3 & 4**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Figure 4; Tables 3 & 4**

DEPT 90 : δ ppm, 125 MHz, in $CDCl_3$; **Figure 5**

DEPT 135 : δ ppm, 125 MHz, in $CDCl_3$; **Figure 6**

COSY : δ ppm, 500 MHz, in $CDCl_3$; **Figure 7**

HMQC : δ ppm, 500 MHz, and δ ppm, 125 MHz, in $CDCl_3$; **Figure 8**

HMBC : δ ppm, 500 MHz, and δ ppm, 125 MHz, in $CDCl_3$; **Figure 9**

4.2 Compound HAM2

Compound HAM2 was obtained as white solid (20.1 mg, 0.02% yield)

MP : 193-194 ^{o}C

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Figure 10; Table 5**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Figure 11; Table 5**

DEPT 90 : δ ppm, 125 MHz, in $CDCl_3$; **Figure 12**

DEPT 135 : δ ppm, 125 MHz, in $CDCl_3$; **Figure 13**

4.3 Compound HAM3

Compound HAM3 was obtained as white needles (15.5 mg, 0.01% yield).

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; **Figure 14**

4.4 Compound HAM4

Compound HAM4 was obtained as yellow needles (40.3 mg, 0.04% yield)

UV	:λ_{max} ($CDCl_3$); 275, 298, 313; **Figure 15**
IR	: $V_{max}cm^{-1}$, KB disc; 3422, 2973, 2915, 1643, 1619, 1520, 1452, 1380, 1239, 1196, 1123; **Figure 16**
EIMS	: m/z 406.1775; $[M+H]^+$; **Figure 17**
HREIMS	: m/z 406.1819; **Figure 18**
MP	: 119-121 ^oC
^1H NMR	: δ ppm, 500 MHz, in $CDCl_3$; **Figure 19; Table 6**
^{13} C NMR	: δ ppm, 125 MHz, in $CDCl_3$; **Figure 20; Table 6**
DEPT 90 and 135	: δ ppm, 125 MHz, in $CDCl_3$; **Figure 21**
COSY	: δ ppm, 500 MHz, in $CDCl_3$; **Figure 22**
HMQC	: δ ppm, 500 MHz, and δ ppm, 125 MHz, in $CDCl_3$; **Figure 23**
HMBC	: δ ppm, 500 MHz, and δ ppm, 125 MHz, in $CDCl_3$; **Figures 24 & 25**

4.5 Compound DAM1

Compound DAM1 was obtained as yellow solid (9 mg, 0.009% yield)

UV	:λ_{max} ($CDCl_3$); 221, 250, 349; **Figure 26**
IR	: $V_{max}cm^{-1}$, KB disc; 3458, 3399, 3318, 2561, 2464, 2343, 1599, 1581, 1506, 1440, 1386, 1250, 1207, 1178, 1024; **Figure 27**
EIMS	: m/z 300.0628; $[M+H]^+$; **Figure 28**
HREIMS	: m/z 300.0640; **Figure 29**
MP	: 270-271 ^oC
^1H NMR	: δ ppm, 500 MHz, in $CDCl_3$; **Figure 30; Table 7**
^{13} C NMR	: δ ppm, 125 MHz, in $CDCl_3$; **Figure 31; Table 7**
DEPT 90	: δ ppm, 125 MHz, in $CDCl_3$; **Figure 32**
DEPT 135	: δ ppm, 125 MHz, in $CDCl_3$; **Figure 33**
COSY	: δ ppm, 500 MHz, in $CDCl_3$; **Figure 34 & 35**
HMQC	: δ ppm, 500 MHz, and δ ppm, 125 MHz, in $CDCl_3$; **Figures 36 & 37**
HMBC	: δ ppm, 500 MHz, and δ ppm, 125 MHz, in $CDCl_3$; **Figures 38 & 39**

4.6 Compound DAM2

Compound DAM2 was obtained as yellow solid (5.9 mg, 0.005% yield)

UV : λ_{max} ($CDCl_3$); 209 and 349; **Figure 40**

IR : $V_{max}cm^{-1}$, KB disc; 3435, 2920, 2852, 1626, 1608, 1499, 1270, 1121 and 1020; **Figure 4**1

EIMS : m/z 286; $[M+H]^+$; **Figure 42**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **Figure 43**; **Table 7**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **Figure 44**; **Table 7**

DEPT 135 : δ ppm, 125 MHz, in $CDCl_3$; **Figure 45**

COSY : δ ppm, 500 MHz, in $CDCl_3$; **Figure 46**

HMQC : δ ppm, 500 MHz, and δ ppm, 125 MHz, in $CDCl_3$; **Figures 47 & 48**

HMBC : δ ppm, 500 MHz, and δ ppm, 125 MHz, in $CDCl_3$; **Figures 49-51**

5. Evaluation of Biological Activities

5.1. Determination of Cytotoxic Activity

Cancer cell growth inhibition of the plant extracts and the isolated compounds against epidermis carcinoma (KB), was carried out using resazurin microplate assay (REMA) (Brien et al., 2002). Briefly, cells at a logarithmic growth phase were harvested and diluted to 2.2 x 10^4 cells/ml in fresh medium. Successively, 5 µl of test samples diluted in 5% DMSO and 45 µl of cell suspension were added to 384-well plate, incubated at 37 ^oC in 5% CO_2 incubator. After 3 days of the incubated period, 12.5 µl of 62.5 µg/ml resazurin solution was added to each well, and the plates were then incubated at 37 ^oC for 4 hours. Fluorescence signal was measured using Spectra Max M5 multi-detection microplate reader (Molecular Devices, USA) at the excitation and emission wave lengths of 530 nm and 590 nm. Percent inhibition of cell growth was calculated by the following equation, whereas FU_T and FU_C are the mean fluorescent units from treated and untreated conditions, respectively.

$$\% \text{ Inhibition} = [1-(FU_T/FU_C)] \times 100$$

If any of test samples has $\geq$ 50% inhibition at the final concentration tested of 50 µg/ml, it will be considered as active and the concentration that inhibit cell growth by 50% (IC_{50}) will be included. Dose response curves were plotted from 6 concentrations of 2 flod serially diluted test compounds and the IC_{50} can derived using the SOFTMax pro software (Molecular Devices, USA). Ellipticine and 0.5% DMSO were used as a positive and a negative controls, respectively.

Cytotoxicity against the non-tumer cell line of test samples was performed using Green fluorescent protein (GFP) detection (Hunt et al., 1999). Briefly, the GFP-expressing cell line was generated in house by stably transecting the african green monkey kidney cell line (Vero, ATCC CCL-81), with pEGFP-N1 plasmid (Clontech). The cell line was maintained in minimal essential medium supplement with 10% heat-inactivated fetal bovine serum, 2 ml L-glutamine, 1 ml sodium pyruvate, 1.5 mg/ml sodium bicarbonate and 0.8 mg/ml geneticin, at 37 ^{o}C in the humidified incubator with 5% CO_2. The assay was carries out by adding 45 µl of cell suspension at $3.3x10^4$ cell/ml to each well of 384 well plates containing 5 µl of test compounds previously diluted in 0.5% DMSO, and then incubating for 4 days in 37 ^{o}C incubator with 5% CO_2. Fluorescence signals were measured by using Spectra Max M5 microplate reader (Molecular Devices, USA) in the bottom-up reading mode with excitation and emission wave lengths of 485 and 535 nm, respectively. Fluorescence signal at day 4 was subtracted with background fluorescence at day 0. The percentage of cytotoxicity was calculated by the following equation, where FU_T and FU_C represent the fluorescence units of cells treated with test sample and untreated cells, respectively. If any of test samples exhibited % cell growth inhibition of $\leq$ 50 at the final concentration tested of 50 µg/ml, it will be considered as cytotoxic and IC_{50} will be included.

$$\% \text{ cytotoxicity} = [1-(FU_T/FU_C] \times 100$$

IC_{50} values were derived from dose-response curves, using 6 concentration of 2-fold serially diluted samples, by the SOFT Max Pro software (Molecular device). Elliticine and 0.5% DMSO were used as a positive and a negative controls, respectively.

5.2 Determination of Antibacterial activity

The bacterial strain tested in this study was *Streptococcus mutans* ATCC25175, The culture was grown in tryptic soy broth (TSB) (Difco; Sparks, USA) at 37 ^{o}C for 24 h and incubated in CO_2 incubator. Glycerol stock of the bacteria was kept at -80 ^{o}C. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of compounds isolated from *A. myriophylla* were determined using a modified broth microdilution method according to Clinical and Laboratory Standards Institute Guidelines (CLSI, 2009). The compounds were dissolved in 10% dimethyl sulfoxide (DMSO, Merck, Germany) and two-fold dilutions were made. Suspension of *S. mutans* in BHI broth was prepared from the overnight broth culture. The bacterial suspension (180 µl) was mixed with the diluted test agents (20 µl) in 96 wells flat bottom microtiter plate (Corning Life Sciences, USA). The final bacterial cell concentration was approximately 5 x 10^5 cfu/ml. The final concentration of the test agents was ranging from 0.5-1024 µg/ml. Penicillin G and 1% DMSO were used as positive and negative controls, respectively. The microtiter plates were incubated with 5 % CO_2 at 37 ^{o}C for 24 h. The MIC was recorded as the lowest concentration that completely suppressed the visible growth. An aliquot (20 µl) from the broth with no growth was dropped onto BHI agar and incubated with 5% CO_2 at 37 ^{o}C for 48 h. The MBC was defined as the lowest concentration of the test agents completely preventing bacterial growth. All tests were performed in triplicate independent experiments.

CHAPTER 4

RESULTS AND DISCUSSION

Chromatographic separation of the hexane fraction of the ethanol extract of *Albizia myriophylla* wood yielded 6 compounds (**Figure 2**) including HAM1 (a mixture of HAM1A and HAM1B), HAM2, HAM3 (a mixture of HAM3A and HAM3B), and HAM4. The dichloromethane fraction of *A. myriophylla* was separated by extensive column chromatography to get 2 compounds including DAM1 and DAM2. The structures of these compounds were identified on the basis of their spectroscopic data as well as comparison with previously reported data (Wright et al., 1978; Reynolds et al., 1986; Mahidol et al., 1997; Soonthornchareonnon et al., 2004; Herath et al., 2009; Yoon et al., 2011).

1. Structure Determination of Compound HAM1

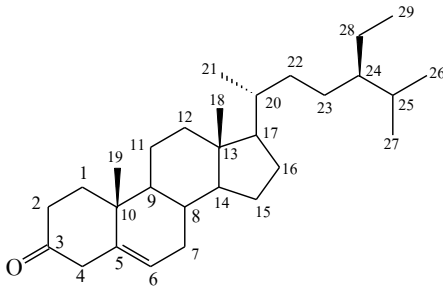
Compound HAM1was obtained as colorless needles. Preliminary comparison of its ^{1}H and ^{13}C NMR spectra (**Figures 3 and 4**) with those of a mixture of β-sitosterol and stigmasterol (Wright et al., 1978) revealed that their NMR spectra were closely similar, including the presence of six tertiary methyl groups, however, instead of secondary alcohol as in a mixture of a β-sitosterol and stigmasterol, a keto carbonyl could be detected at δ_C 211.9 in this compound. All these data suggested that the compound has stigmastane-type skeleton identical to that of a mixture of β-sitosterol and stigmasterol. This was also confirmed by the following evidences.

The ^{1}H NMR spectral data of compound HAM1 (**Figure 3**) exhibited two methyl singlet signals at δ_H 0.99 and 0.55, two methyl doublet signals at δ_H 1.01 (d, *J*=6.3 Hz), and δ_H 0.83 (d, *J*=6.3 Hz), the olefinic proton signal at δ_H 5.35 (d, *J*=8.5 Hz, H-6), and two double doublet signals at δ_H 5.03 (dd, *J*=15.5, 9.0 Hz, H-22), and δ_H 5.12 (dd, *J*=15.5, 9.0 Hz, H-23).

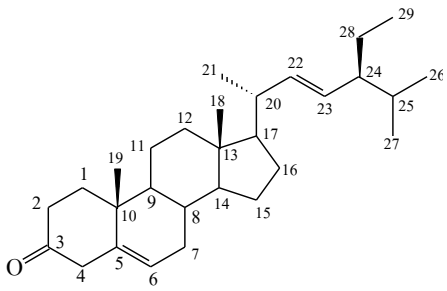
The ^{13}C NMR spectral data (**Figure 4**) together with the DEPT (**Figures 6 and 7**) experiment resolved the presence of six methyls (δ_C 21.36, 21.08, 18.98, 18.88, 12.2, 12.08), eleven methylenes (δ_C 42.8, 39.4, 38.1, 33.8, 31.8, 30.0, 29.1, 26.1, 26.2, 25.3, 21.0), ten methines (δ_C138.0, 129.5, 116.9, 56.03, 55.8, 51.2, 48.8, 40.8, 34.3, 31.8), two of which resonated at δ_C 138 and 129.5 were due to the signals for C-22 and C-23 of stigmasta-5,22-diene-3-one, respectively, and four quaternary carbons. The ratio of the mixture was deduced from the integration value between H-6 and H-22 or H-23 to be 1:1.

In the HMBC spectrum (**Figure 9**) of HAM1, the olefinic proton at δ_H 5.15 (H-6) showed correlations with C-4 (δ_C 42.84), C-7 (δ_C 31.85), and C-10 (δ_C 39.30), suggesting the presence of a double bond between C-5 and C-6.

According to the data mentioned above, compound HAM1 was suggested to be a mixture of β-sitosterone (1A) and stigmasta-5,22-dien-3-one (1B) Its NMR spectral data as compared with those of β-sitosterol and stigmasterol are summarized in **Tables 3 and 4**.



β-sitosterone



stigmasta-5,22-dien-3-one

Table 3 NMR spectral data of HAM1A as compared with β-sitosterol (in $CDCl_3$)

Position	Compound of HAM1A		β-Sitosterol*	
	^{1}H (mult., *J* in Hz)	^{13}C (mult)	^{1}H (mult., *J* in Hz)	^{13}C (mult)
1	-	38.1 (t)	-	37.3 (t)
2	-	30.3 (t)	-	31.6 (d)
3	-	211.9 (s)	-	71.7 (d)
4	-	42.8 (t)	-	42.5 (t)
5	-	139.4 (s)	-	140.8 (s)
6	5.35 (d, 8.5)	116.9 (d)	5.36 (t)	121.6 (d)
7	-	33.8 (t)	-	31.9 (d)

Table 3 (Continued)

Position	Compound of HAM1A		β-Sitosterol*	
	^{1}H (mult., *J* in Hz)	^{13}C (mult)	^{1}H (mult., *J* in Hz)	^{13}C (mult)
8	-	31.8 (d)	-	31.9 (d)
9	-	48.8 (d)	-	50.2 (d)
10	-	36.5 (s)	-	36.5 (s)
11		21.0 (t)		21.1 (t)
12	-	39.4 (t)	-	39.8 (t)
13	-	43.2 (s)	-	42.3 (s)
14	-	56.03 (d)	-	56.7 (d)
15	-	25.3 (t)	-	24.3 (t)
16	-	28.4 (t)	-	28.3 (t)
17	-	55.8 (d)	-	56.1 (d)
18	0.55 (s)	11.9 (q)	0.68 (s)	11.9 (q)
19	0.99 (s)	19.8 (q)	0.79 (s)	19.4 (q)
20	-	36.5 (d)	-	36.2 (d)
21	0.83 (d, 6.3)	18.8 (q)	0.82 (s)	18.8 (q)
22	-	33.8 (t)	-	33.9 (t)
23	-	26.2 (t)	-	26.1 (d)
24	-	45.8 (d)	-	45.9 (d)
25	-	29.1 (d)	-	29.2 (d)
26	-	19.8 (q)	-	19.8 (q)
27	0.89 (s)	18.9 (q)	0.92 (s)	19.0 (q)
28	-	22.9 (d)	-	23.1 (t)
29	0.82	11.9 (q)	0.86	12.3 (q)

* Wright et al., 1978 (in $CDCl_3$ 100 MHz)

Table 4 NMR spectral data of HAM1B as compared with stigmasterol (in $CDCl_3$)

Position	Compound of HAM1B		Stigmasterol*	
	^{1}H (mult., *J* in Hz)	^{13}C (mult)	^{1}H (mult., *J* in Hz)	^{13}C (mult)
1	-	38.1 (t)	-	37.3 (t)
2	-	30.0 (t)	-	31.7 (t)
3	-	211.9 (s)	-	71.8 (d)
4	2.34 (m)	42.8 (t)	-	42.4 (t)
5	-	139.4 (s)	-	140.8 (s)
6	5.35 (d, 8.5)	119 .9 (d)	5.36 (t)	121.7 (d)
7	-	31.85 (t)	-	31.9 (t)
8	-	30.08 (d)	-	31.9 (d)
9	-	48.8 (d)	-	50.2 (d)
10	-	36.5 (s)	-	36.6 (d)
11		21.0 (t)		21.1 (t)
12	-	39.4 (t)	-	39.7 (t)
13	-	43.2 (s)	-	42.4 (s)
14	-	56.03 (d)	-	56.9 (d)
15	-	25.3 (t)	-	24.4 (t)
16	-	29.1 (t)	-	28.9 (t)
17	-	55.8 (d)	-	56.1 (d)
18	0.55 (s)	12.0 (q)	0.68 (s)	12.1 (q)
19	0.99 (s)	19.8 (q)	0.79 (s)	19.4 (q)
20	-	40.8 (d)	-	40.5 (q)
21	0.83 (d, 6.3)	21.3 (q)	0.82 (s)	21.1 (q)
22	5.15 (dd, 15.5, 9 0)	138.0 (d)	5.03 (s)	138.3 (d)
23	5.15 (dd, 15.5, 9 0)	129.5 (d)	5.01 (s)	129.3 (d)
24	-	51.2 (d)	-	51.3 (d)
25	-	34.3 (d)	-	31.9 (d)
26	1.01 (d, 6.3)	21.6 (q)	1.02 (s)	21.3 (q)

Table 4 (Continued)

Position	Compound of HAM1B		Stigmasterol*	
	^{1}H (mult., *J* in Hz)	^{13}C (mult)	^{1}H (mult., *J* in Hz)	^{13}C (mult)
27	0.89 (m)	18.9 (q)	0.92 (m)	19.0 (q)
28	-	26.1 (t)	-	25.4 (t)
29	0.82 (m)	12.4 (q)	0.86 (m)	12.3 (q)

* Wright et al., 1978 (in $CDCl_3$ 100 MHz)

2. Structure Determination of Compound HAM2

Compound HAM2 was obtained as white needle crystals. The compound gave a purple vanillin sulfuric acid test, indicating the triterpene skeleton in this molecule.

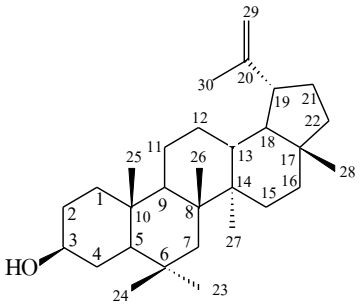
The ^{1}H NMR (**Figure 10**) spectrum exhibited characteristic of lupane-type triterpennoid (Tanaka and Matsunaga et al., 1998), which including seven tertiary methyl groups (δ_H 0.94, 0.73, 0.80, 1.02, 0.92, 0.76; Me-23-Me-28 and Me-30, respectively), one vinyl methyl (δ_H 1.68, Me-30), one terminal methylene δ_H 4.66/4.54 (d, *J*=2.5 Hz, H_2-29), a C-3α oxymethine proton (δ_H 3.18, dd, *J*=10.8, 5.4 Hz, H-3), and the remaining signals due to methylene and methine protons in the high field region (δ_H 0.67-2.38).

The ^{13}C NMR spectrum (**Figue 11**) together with DEPT (**Figures 12 and 13**) experiment resolved the 30 carbon signals as seven methyl (δ_C 14.51, 15.35, 15.94, 16.10, 17.97, 19.28, 27.96), eleven methylene (δ_C 20.89, 25.08, 27.40, 27.41, 29.80, 34.22, 35.54, 38.71, 38.81, 39.97, 109.31), five methine (δ_C 47.96, 48.24, 50.38, 55.24, 78.96), and seven quaternary carbons (δ_C 18.2, 37.13, 38.01, 40.97, 42.80, 42.97, 150.98), thus also supporting the triterpenoid structure. The presence of isopropenyl group was determined from the signals of two olefinic carbons at δ_C 150.98 (C-20) and 109.31 (C-29) and a singlet methyl at δ_C 19.2 (C-30) as well as

the corresponding signals at δ_H 4.66/4.54 (each d, *J*=2.5 Hz, H_2-29) and 1.65 (s, Me-30), respectively, in the ^{1}H NMR spectrum.

Comparison of the ^{1}H and ^{13}C NMR spectral data (**Table 5**) of compound HAM2 with those already reported (Reynolds, 1986), this compound was finally assigned as lupeol.

Lupeol, the most common plant triterpenoid, has previously demonstrated to have interesting therapeutic properties such as antimalarial (Alves et al., 1997), anti-inflammatory (Vasconcelos et al., 2008), and antitumor (Gallo et al., 2009) activities.



lupeol

Table 5 NMR spectral data of HAM2 as compared with lupeol (in $CDCl_3$)

position	Compound HAM2		Lupeol*	
	^{1}H (mult., *J* in Hz)	^{13}C (mult)	^{1}H (mult., *J* in Hz)	^{13}C (mult)
1	0.91 (m)	38.7 (t)	0.91(m)	38.7 (t)
2	1.56 (m)	27.4 (t)	1.56 (m)	27.4 (t)
3	3.18 (dd, 10.8, 5.4)	78.9 (d)	3.19 (dd, 10.8, 5.1)	79.0 (d)
4	-	38.8 (t)	-	38.8 (t)
5	0.67 (m)	55.2 (d)	0.67 (m)	55.3 (d)
6	1.40 /1.55 (m)	18.2 (s)	1.40 /1.55 (m)	18.3 (s)
7	1.40 (m)	34.2 (t)	1.40 (m)	34.2 (t)
8	-	40.7 (s)	-	40.8 (s)
9	1.28 (m)	50.4 (d)	1.28 (m)	50.4 (d)
10	-	37.1 (s)	-	37.1(s)
11	1.22 /1.45 (m)	20.8 (t)	1.22 /1.45 (m)	20.9 (t)
12	1.08 (m)	25.0 (t)	1.08 (m)	25.0 (t)

Table 5 (Continued)

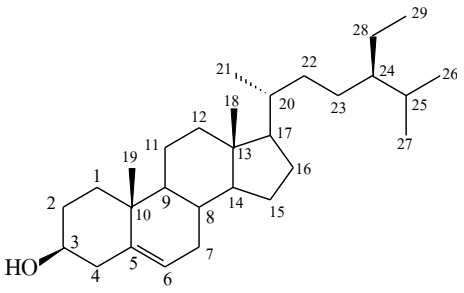
position	Compound HAM2		Lupeol*	
	^{1}H (mult., *J* in Hz)	^{13}C (mult)	^{1}H (mult., *J* in Hz)	^{13}C (mult)
13	1.63 (m)	37.9 (s)	1.63 (m)	38.0 (s)
14	-	42.8 (s)	-	42.8 (s)
15	1.56 (m)	27.4 (t)	1.56 (m)	27.4 (t)
16	1.51 (m)	35.5 (t)	1.51 (m)	35.5 (t)
17	-	42.9 (s)	-	43.0 (s)
18	1.38 (m)	48.2 (d)	1.38 (m)	48.2 (d)
19	2.38 (m)	47.9 (d)	2.38 (m)	47.9 (d)
20	-	150.9 (s)	-	150.9 (s)
21	1.93 (m)	29.8 (t)	1.93 (m)	29.8 (t)
22	1.20 /1.40 (each m)	39.9 (t)	1.20 /1.40 (m)	40.0 (t)
23	0.94 (s)	27.9 (q)	0.97 (s)	28.0 (q)
24	0.73 (s)	15.3 (q)	0.76 (s)	15.4 (q)
25	0.80 (s)	16.1 (q)	0.83 (s)	16.1 (q)
26	1.02 (s)	15.9 (q)	1.03 (s)	16.0 (q)
27	0.92 (s)	14.5 (q)	0.94 (s)	14.5 (q)
28	0.76 (s)	17.9 (q)	0.79 (s)	18.0 (q)
29	4.66 /4.54 (d, 2.5 each m)	109.3 (t)	4.68/4.56 (d, 2.1 each m)	109.3 (t)
30	1.65 (s)	19.2 (q)	1.68 (s)	19.3 (q)

*Reynolds et al., 1986 (in $CDCl_3$ 125 MHz)

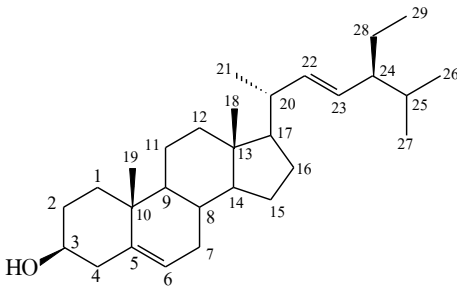
3. Structure Determination of Compound HAM3

Compound HAM3 was obtained as colorless needle crystals. The ^{1}H NMR (**Figure 11**) spectral data were similar to those of compound HAM1, but instead of a keto carbonyl, it showed an extra secondary alcohol suggesting its nature as the 3-hydroxy derivative of HAM1.

In the ^{1}H NMR spectrum (**Figure 5**), the signals including the olefinic proton signal at δ_H 5.35, two double doublet signals at δ_H 5.01 (dd, *J* =15, 8.7 Hz) and δ_H 5.12 (dd, *J* =15, 8.7 Hz), and a desheilded signal at δ_H 3.43 (m) were agree well with those previously reported (Cheenpracha., 2004, Charuwan., 2006, Charoenpakatirathien., 2006) data of the proposed structure. TLC co-spotting of this compound with an authentic sample was further confirmed HAM3 as a mixture of β-sitosterol (HAM3A) and stigmasterol (HAM3B).



β-sitosterol



stigmasterol

4. Structure Determination of Compound HAM4

Compound HAM4 was obtained as yellow needles. A formula of $C_{25}H_{26}O_5$, was deduced from its [M^{+}] ion peak at m/z 406.1775 (calcd for $C_{25}H_{26}O_5$ 406.1819) in the HREIMS (**Figures 17 and 18**). The FT-IR spectrum (**Figure 16**) showed absorption bands for hydroxy (3422 cm^{-1}), conjugated carbonyl (1643 and 1619 cm^{-1}), aromatic ring (1520, 1452, and 1380

cm^{1}), and ether (1239 and 1196 cm^{-1}) functional groups. The UV absorptions (**Figure 15**) at 274, 313, and 367 nm were indicative of a flavanone skeleton (Roussis et al., 1987).

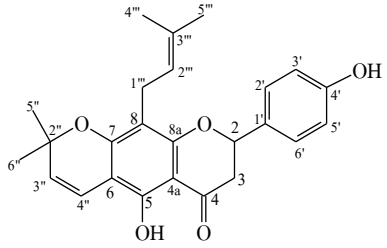
The ^{1}H and ^{13}C NMR spectrum (**Figures 19 and 20**) showed characteristic set of signals at δ_H 5.32 (1H, dd, *J*=12.8, 3.1 Hz, H-2), 2.78 (1H, dd, *J*=17.1, 3.2 Hz, H-3eq), and δ_H 3.03 (1H, dd, *J*=17.1, 12.8 Hz, H-3ax), and at δ_C 78.48 (C-2) and δ_C 43.18 (C-3) of a flavanone skeleton. A low-field singlet at δ_H 12.2 indicated a C-5 OH group hydrogen bonded to a carbonyl carbon at C-4. Aromatic proton signals at δ_H 7.29 (2H, d, *J*=8.2 Hz, H-2' and H-6'), and δ_H 6.85 (2H, d, *J*=8.2 Hz, H-3' and H-5') could be assigned as 1,4-disubstituted aromatic ring B protons, as evidences from the HMBC correlations from H-2' and H-6' to C-2 (δ_C 78.48) and the correlations of H-3' and H-5' to C-1'.

The ^{1}H NMR **(Figure 19)** signals at δ_H 5.49 (1H, d, *J*=10.1 Hz H-3''), δ_H 6.62 (1H, d, *J*=10.1 Hz H-4''), δ_H 1.42 (1H, s, H-5''), and δ_H 1.41 (1H, s, H-6'') showed correlations with the ^{13}C NMR signals at δ_C 125.98, 115.48, 28.39, and 28.29, respectively, and were assigned to a dimethylchromene group. The ^{1}H NMR signals at δ_H 3.19 (2H, d, *J*=7.5 Hz, H-1'''), δ_H 5.14 (1H, tp *J*=7.5 Hz, H-2''') and two singlets at δ_H 1.62 and 1.63 as well as ^{13}C NMR signals at δ_C 17.80, 122.43, 131.10, and 25.8 (2x) were assigned to a dimethylallyl group. The key HMBC correla- tions between H-4''/C-5 (δ_C 156.53) required the placement of a chromene ring at C-6 and C-7 and the correlations of H-1''' with C-8 (δ_C 108.61) and C-8a (δ_C 159.33) indicated a dimethylally group at C-8 position.

^{13}C NMR spectral data **(Figure 20)** together with the DEPT (**Figure 21**) experiment resolved the presence of four methyls (δ_C 28.39, 28.29, 25.80, 17.80) and two methylenes (δ_C 43.18, 17.80), eight methine (δ_C 127.69, 127.6, 125.98, 122.43, 115.60, 115.6, 115.48, 78.48), and eleven quaternary carbons (δ_C 196.50, 159.8, 159.33, 156.53, 155.83, 131.10, 130.98, 115.58, 108.61, 102.79, 78.4). Comparison of the ^{1}H and ^{13}C NMR spectral data (**Table 6**) of HAM4 with those already reported (Soonthornchareonnon et al., 2004), compound HAM4 was finally assigned as lupinifolin.

Lupinifolin was first isolated from root of *Tephrosia lupinifolia* Burch (DC). (Lin, et al 1991) This compound has previously demonstrated to have interesting therapeutic

properties such as anti-inflammatory (Ganapaty et al., 2006), antimalarial (Khaomek et al., 2008), antioxidant and cytotoxic (Soonthornchareonnon et al., 2004) activities.



lupinifolin

Table 6 ^{1}H and ^{13}C NMR spectral data of HAM4 as compared with lupinifolin (in $CDCl_3$)

Position	Compound HAM4			Lupinifolin*	
	^{1}H (mult., *J* in Hz)	^{13}C (mult)	HMBC	^{1}H (mult., *J* in Hz)	^{13}C (mult)
2	5.32 (dd, 3.1, 12.8)	78.4 (d)	C-3, C-4, C-1', C-6'	5.33 (dd, 3.2, 12.8)	78.5 (d)
3ax	3.03 (dd, 12.8, 17.1)	43.1 (t)	C-4, C-8a	3.03 (dd, 12.8, 17.1)	43.2 (t)
3eq	2.78 (dd, 3.2, 17.1)	43.1 (t)	-	2.80 (dd, 3.2, 17.3)	43.2 (t)
4	-	196.5 (s)	-	-	196.5 (s)
4a	-	102.6 (s)	-	-	102.6 (s)
5	-	156.5 (s)	-	-	156.5 (s)
6	-	102.7 (s)	-	-	102.6 (s)
7	-	159.8 (s)	-	-	159.9 (s)
8	-	108.6 (s)	-	-	108.6 (s)
8a	-	159.3 (s)	-	-	159.3 (s)
1'	-	130.9 (s)	-	-	131.1 (s)
2'	7.29 (d, 8.2)	127.6 (d)	C-2, C-3'	7.30 (d, 8.2)	127.7 (d)
3'	6.85 (d, 8.2)	115.6 (d)	C-1', C-4'	6.86 (d, 8.2)	115.5 (d)
4'	-	155.8 (s)	-	-	155.9 (s)
5'	6.85 (d, 8.2)	115.6 (d)	C-1'	6.86 (d, 8.2)	115.5 (d)

Table 6 (Continued)

Position	Compound HAM4			Lupinifolin*	
	^{1}H (mult., *J* in Hz)	^{13}C (mult)	HMBC	^{1}H (mult., *J* in Hz)	^{13}C (mult)
6'	7.29 (d, 8.2)	127.6 (d)	C-4', C-5',C-2	7.30 (d, 8.2)	127.7 (d)
2"	-	78.1 (s)	-	-	78.1 (s)
3"	5.49 (d, 10.1)	125.9 (d)	-	5.49 (d, 10.1)	125.9 (d)
4"	6.62 (d, 10.1)	115.4 (d)	C-5, C-6, C-7	6.62 (d, 10.1)	115.5 (d)
5"	1.42 (s)	28.3 (q)	-	1.43 (s)	28.3 (q)
6"	1.41 (s)	28.2 (q)	C-2", C-3"	1.41 (s)	28.2 (q)
1"'	3.19 (d, 7.6)	17.8 (t)	C-8, C-8a	3.19 (d, 7.6)	17.6 (t)
2"'	5.13 (tp,7.6,1.5)	122.4 (d)	C-8, C-1"'	5.13 (tp,7.6, 1.5)	122.4 (d)
3"'	-	131.1 (s)	-	-	131.0 (s)
4"'	1.62 (s)	25.8 (q)	C-3"', C-5"'	1.63 (s)	25.8 (q)
5"'	1.62 (s)	25.8 (q)	C-2"', C-3"', C-4"'	1.62 (s)	25.8 (q)
5-OH	12.22 (s)	-	C-4, C-5	12.24 (s)	115.5
4"'	1.62 (s)	25.8 (q)	C-3"', C-5"'	1.63 (s)	25.8 (q)
5"'	1.62 (s)	25.8 (q)	C-2"', C-3"', C-4"'	1.62 (s)	25.8 (q)

*Soonthornchareonnon et al., 2004 (in $CDCl_3$ 125 MHz)

5. Structure Determination of Compound DAM1

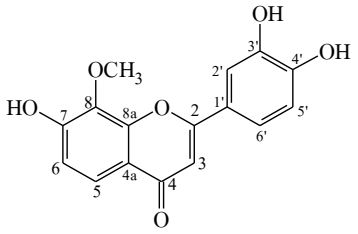
Compound DAM1 was obtained as yellow solid. A formula of $C_{16}H_{12}O_6$ was deduced from its [M^+] ion at m/z 300.0628 (calcd for $C_{16}H_{12}O_6$ 300.0640) in the HREIMS (**Figues 28 and 29**). The FT-IR spectrum (**Figure 27**) showed absorption bands at 3458, 3399, 3318,

3138, 1599, 1581, and 1506 cm^{-1}. The UV (**Figure 26)** absorption in MeOH at 274 and 313 nm suggested compound DAM1 to be flavone derivative (Tyukavkina et al., 1975).

The ^{1}H NMR spectrum (**Figure 30**) showed one methoxyl singlet at δ_H 3.74, a one proton singlet at δ_H 6.87 ascribed to H-3, and showed two *ortho* coupled aromatic doublets at δ_H 7.88 and δ_H 6.91 assigned to H-5 and H-6, respectively, as the former showed HMBC correlations with C-4, C-7, and C-8a and the later with C-4a, C-5, and C-7 (**Figure 38**). It also displayed three aromatic proton signals at δ_H 7.52 (1H, d, *J*=2 Hz), δ_H 6.89 (1H, d, *J*=8,2 Hz), and δ_H 7.42 (1H, dd, *J*= 8.2, 2.0 Hz) assigned to H-2', H-5', and H-6', respectively, characteristic of 3',4'-dihydroxyphenyl.

The ^{13}C NMR spectral data (**Figure 31**) together with the DEPT (**Figures 32 & 33**) experiment resolved the presence of 16 signals due to one methyl (δ_C 60.34), six methine (δ_C 127.82, 122.28, 116.14, 116.57, 116.41, 103.13), and nine quaternary carbons (δ_C 176.51, 164.61, 158.64, 157.89, 149.74, 146.45, 141.21, 123.33, 117.75). All protonated carbons were assigned by HMQC (**Figure 37**), in the ^{13}C NMR spectrum of DAM1 (**Figure 31**), significant flavone signals at δ_C176.51 (C-4) δ_C 127.82 (C-5), 116.10 (C-6), 116.57 (C-2'), 116.41 (C-5'), and 122.28 (C-6') were observed. The methoxy group at δ_H 3.74 was placed at C-8 as it showed HMBC (**Figure 38**) correlation with this carbon at δ_C 141.21 (C-8).

From the foregoing spectral studies, compound DAM1 was finally identified as 8-methoxy-7,3',4'-trihydroxyflavone. This compound was isolated for the first time from *Albizia* species. Its NMR spectral data are summarized in **Table 7**.



8-methoxy-7,3',4'-trihydroxylflavone

Table 7 NMR spectral data of DAM1 (in DMSO-d_6)

Position	Compound DAM1		
	^{1}H (mult., *J* in Hz)	^{13}C (mult)	HMBC
2	-	157.89 (s)	-
3	6.87 (s)	103.13 (d)	C-6', C-4a
4	-	176.51 (s)	-
4a	-	117.75 (s)	-
5	7.88 (d, 8.48)	127.82 (d)	C-4, C-7, C-8a
6	6.91 (d, 8.4)	116.1 (d)	C-5, C-4a, C-7
7	-	164.6 (s)	-
8	-	141.21 (s)	-
8a	-	158.64 (s)	-
1'	-	123.33 (s)	-
2'	7.52 (d, 2.0)	116.57 (d)	C-2, C-3', C-6'
3'	-	146.45 (s)	-
4'	-	149.74 (s)	-
5'	6.89 (d, 8.2)	116.41 (d)	C-3', C-1'
6'	7.42 (dd, 8.2, 2.0)	122.28 (d)	-
8-OCH_3	-	60.34 (s)	C-8

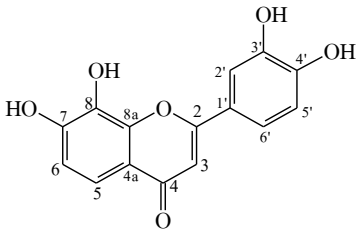
6. Structure Determination of Compound DAM2

Compound DAM2 was obtained as yellow solid. A formula of $C_{15}H_{10}O_6$ was assigned from EIMS spectrum (**Figure 42**), which showed the [M^+] ion at m/z 286. The FT-IR spectrum (**Figure 41**) showed absorption bands at 3435 and 1608 cm^{-1}. The UV spectrum (**Figure 40**) showed absorption in MeOH at 209, 358, 425, and 488 nm.

The ^{1}H NMR spectral data (**Figure 43**) of compound DAM2 was closely correlated to those of compound DAM1, but differed in the absence of signal at δ_H 3.74 of methoxy proton in spectrum of compound DAM1. This observation suggested compound DAM2 to be a flavone derivative of DAM1. The signals of 1,3',4'-trisubstituted aromatic ring at δ_H 7.53 (1H, dd, *J*= 8.2, 2.0 Hz), δ_H 7.67 (1H, d, *J*=2 Hz), and δ_H 6.89 (1H, d, *J*=8,2 Hz) assigned to H-6', H-2', and H-5', respectively, indicated the presence of a 3',4'-dihydroxy B-ring system in flavone. Two *ortho* coupled aromatic doublets at δ_H 7.91 and δ_H 6.91 assigned to H-5 and H-6, respectively, were deduced from the HMBC (**Figure 49**) studies.

The ^{13}C NMR spectral data (**Figure 44**) together with the DEPT (**Figure 45**) experiment resolved the presence of six methine (δ_C 127.51, 121.65, 116.02, 116.25, 102.99, 115.97), and nine quaternary carbons (δ_C 174.43, 164.26, 158.53, 148.66, 146.25, 138.58, 124.39, 121.3, 115.49). All protonated carbons were assigned by HMQC studies (**Figures 47 and 48**), In the ^{13}C NMR spectrum **(Figure 44)**, the important signals of flavone at δ_C 127.51 (C-5), 116.25 (C-6), 116.02 (C-2'), 115.97 (C-5'), and 121.65 (C-6') were observed.

The location of each functional group (hydroxyl and ketone groups) in the molecule was confirmed by HMBC experiment (**Figure 50**), suggesting that these groups are present at similar positions as those in DAM1. Compound DAM2 was thus identified as-7,8,3',4'-tetrahydroxyflavone. Its NMR spectral data are summarized in **Table 8**.



7,8,3',4'-tetrahydroxylflavone

Table 8 ^{1}H NMR and ^{13}C NMR spectral data of DAM2 (in DMSO-d_6)

Position	Compound DAM2		
	^{1}H (mult., *J* in Hz)	^{13}C (mult)	HMBC
2	-	146.25 (s)	-
3	6.87 (s)	102.99 (d)	C-2, C-6'
4	-	174.43 (s)	-
4a	-	115.49 (s)	-
5	7.91 (d, 9.32)	127.51 (d)	C-4, C-7, C-8a
6	6.91 (d, 9.32)	116.25 (d)	C-5, C-4a, C-7
7	-	164.26 (s)	-
8	-	138.58 (s)	-
8a	-	158.53 (s)	-
1'	-	124.39 (s)	-
2'	7.67 (d, 2.0)	116.02 (d)	C-2, C-6'
3'	-	147.55 (s)	-
4'	-	148.66 (s)	-
5'	6.89 (d, 8.5)	115.97 (d)	C-3', C-1'
6'	7.53 (dd, 8.5, 2.0)	121.65 (d)	C-2, C-2', C-4'

7. Determination of Biological Activities

In the search for biologically active constituents of *Albizia myriophylla*, the crude ethanol extract and fractions (hexane, dichloromethane, EtOAc and BuOH) as well as the compounds from this plant species were subjected to in vitro screenings for their antibacterial activity against *Streptococcus mutans* and cytotoxic activity against KB and vero cell lines.

7.1 Cytotoxic Activity

The compounds including HAM1 [β-sitosterone and stigmasta-5, 22-dien-3-one], HAM2 (lupeol), HAM4 (lupinifolin), DAM1 (8-methoxy-7,3',4'-trihydroxyflavone), and DAM2 (7,8,3',4'-tetrahydroxyflavone), were subjected to cytotoxicity test against KB and vero cell lines. As can be seen in **Table 9**, lupinifolin was strongly active against KB and vero cell lines with IC_{50} of 4.95 and 1.99 μg/ml, respectively, whereas the others were inactive against either cancer or normal cells when tested at the concentration of 50 μg/ml. Our finding has confirmed the previous report of the cytotoxic activity of lupinifolin isolated from *Myriopteron extensum* against KB cell lines (Soonthornchareonnon et al, 2004). Althrough lupinifolin exhibited higher selectivity to the normal cells than the cancer cells in the present *in vitro* assays, it did not produce any toxic signs or deaths in previous *in vivo* study (Chivapat et al., 2009). Therefore, oral use of lupinifolin for various medicinal purposes could be considered as safe.

Table 9 Cytotoxicity of compounds isolated from *Albizia myriophylla* wood

Test agents	KB cell		vero cell	
	Cytotoxicity (50 μg/ml)	IC_{50} (μg/ml)	Cytotoxicity (50 μg/ml)	IC_{50} (μg/ml)
Mixture of β-sitosterone and stigmasta-5,22-dien-3-one	inactive	ND	ND	ND
Lupeol	inactive	ND	ND	ND
Lupinifolin	active	4.95	active	1.99
8-Methoxy-7,3',4'-trihydroxy-flavone	inactive	ND	ND	ND
7,8,3',4'-Tetrahydroxyflavone	ND	ND	ND	ND
Ellipticine	active	1.96	active	0.38

*Not determined

7.2 Antibacterial Activity

The antibacterial activity of all compounds isolated from *Albizia myriophylla* wood except for β-sitosterol/stigmasterol mixture against *Streptococcus mutans* ATCC 25175 was performed using broth microdilution method. All compounds exhibited antibacterial activity against *S. mutans* with MICs and MBCs raining from 1-256 μg/ml, and 2-256 μg/ml respectively (**Table 10**). Among the isolated compounds, lupinifolin was found to be the most potent with MIC and MBC of 1 and 2 μg/ml, respectively. The antibacterial activity of *Albizia myriophylla* against *S. mutans* has been shown in previous studies. In particular, in clinical study, the mouth wash of *A. myriophylla* significantly reduce *mutans streptococci* counts in saliva of school children (Cholticha et al., 2006). In this study, we found that *A. myriophylla* components including three flavonoids, a triterpenoid, and two steroids have an antibacterial effect on *S. mutans* that associated with dental plaque formation and caries development. This result reveals the correlation between scientific evidence and the ethnomedical use of this plant against dental caries. Although lupinifolin has been reported in several plants of Leguminosae including the root and aerial part of *Tephrosia lupinifolia* (Smalberger et al., 1977), the root of *Derris laxiflora* (Lin et al., 1991), the stem of *Derris reticulata* (Mathidol et al., 1997), and the root of *Euchresta formosana* (Matsuura et al., 1995), there is no record in the genus *Albizia*. To best of our knowledge, this is the first report of lupinifolin isolated from *A. myriophylla* wood and the antibacterial activity of this compound against cariogenic *S. mutans*. Considering the strong anti-*S. mutans* activity of lipinifolin, this compound may have potential for further development as natural anti-cariogenic agents. Further research is necessary to establish the antibacterial mechanisms of action of this compound against *S. mutans* or other cariogenic bacterial strains.

Table 10 Anti-*Streptococcus mutans* activity of crude extract, fractions, and pure compounds from *A. myriophylla*

Crude extract/Fractions/Pure compounds	*Streptococcus mutans* ATCC 25175	
	MIC (μg/ ml)	MBC (μg/ ml)
Ethanol	512	1024
Hexane	256	512
Dichloromethane	1024	1024
Ethyl acetate	>1024	>1024
Butanol	1024	1024
Lupeol	128	256
Mixture of β-sitosterone and stigmasta-5,22-dien-3-one	128	256
Lupinifolin	1	2
8-Methoxy-7,3',4'-trihydroxyflavone	128	256
7,8,3',4'-Tetrahydroxyflavone	64	128
Penicillin G*	0.015	0.015

*Positive standard

CHAPTER V

CONCLUSION

The present study reported the phytochemical components from the wood of *Albizia myriophylla* (Leguminosae-Mimosoideae). Biological activity studies, including antibacterial activity against *Streptococcus mutans* ATCC 25175 and cytotoxicity against oral cavity cancer (KB) cell line, of some isolated compounds were also established. Various compounds including a flavanone lupinifolin, a triterpenoid lupeol, and two sets of sterol namely a mixture of β-sitosterone and stigmasta-5,22-dien-3-one and a mixture of β-sitosterols and stigmasterol, were isolated from the hexane fraction of this plant species. While those from the dichloromethane fraction of this species were two rare flavones, 8-methoxy-7,3′,4′-trihydroxyflavone and 7,8,3′,4′-tetrahydroxyflavone. The structures of these compounds were identified on the basis of their spectroscopic data as well as comparison with previously reported data. All the isolated compounds except for the mixture of β-sitosterol and stigmasterol were evaluated for their antibacterial and anticancer activities. All the tested compounds were effective against *S. mutans* ATCC 25175 with MIC and MBC values ranging from 1-256 and 2-256 μg/ml, respectively. Lupinifolin displayed the highest activity with MIC and MBC values of 1 and 2 μg/ml, respectively. In addition, lupinifolin also exhibited promising anticancer activity against KB cell with IC_{50} of 4.9 μg/ml while the others showed no activity against the cancer cell line tested. To the best of our knowledge, this is the first report of lupinifolin isolated from *A. myriophylla* wood and the antibacterial activity of this compound against cariogenic *S. mutans*. Regarding the strong anti *S. mutans* activity of lupinifolin, this flavanone may have potential for further development as natural anti-cariogenic agent. Further research is necessary to establish the antibacterial mechanisms of action of this compound against *S. mutans* or other cariogenic bacterial strains.

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APPENDICES

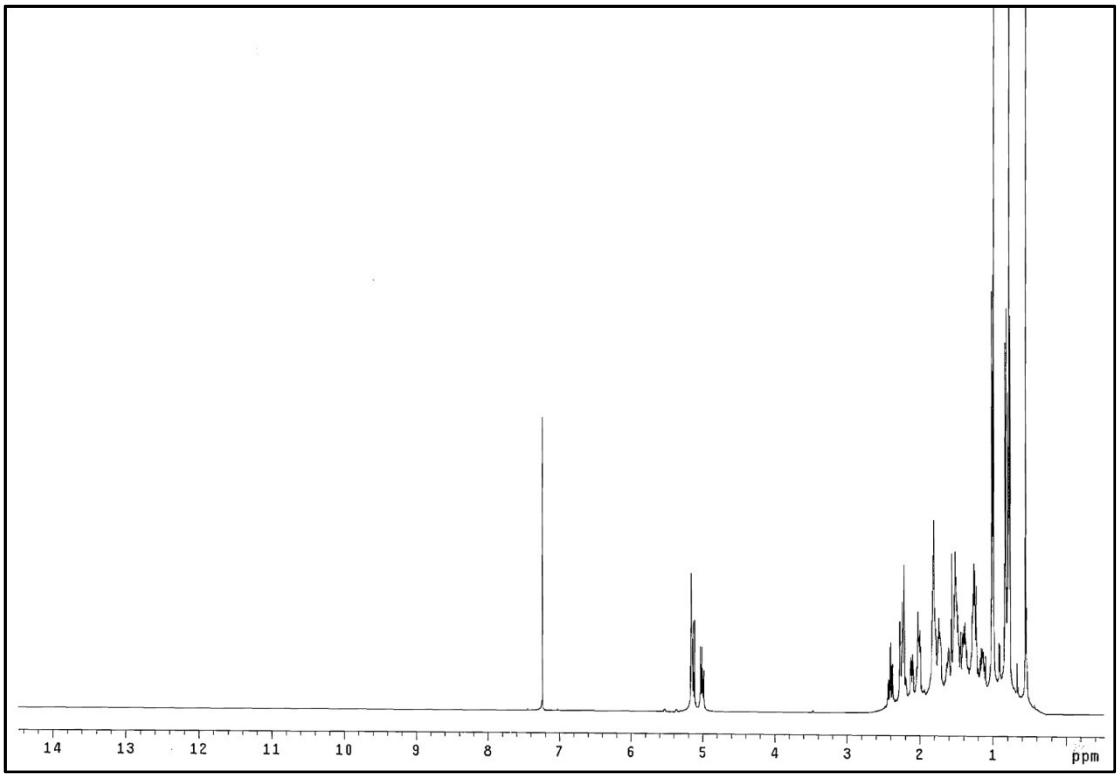

Figure 3 ^{1}H NMR (500 MHz) spectrum of compound HAM1 ($CDCl_3$)

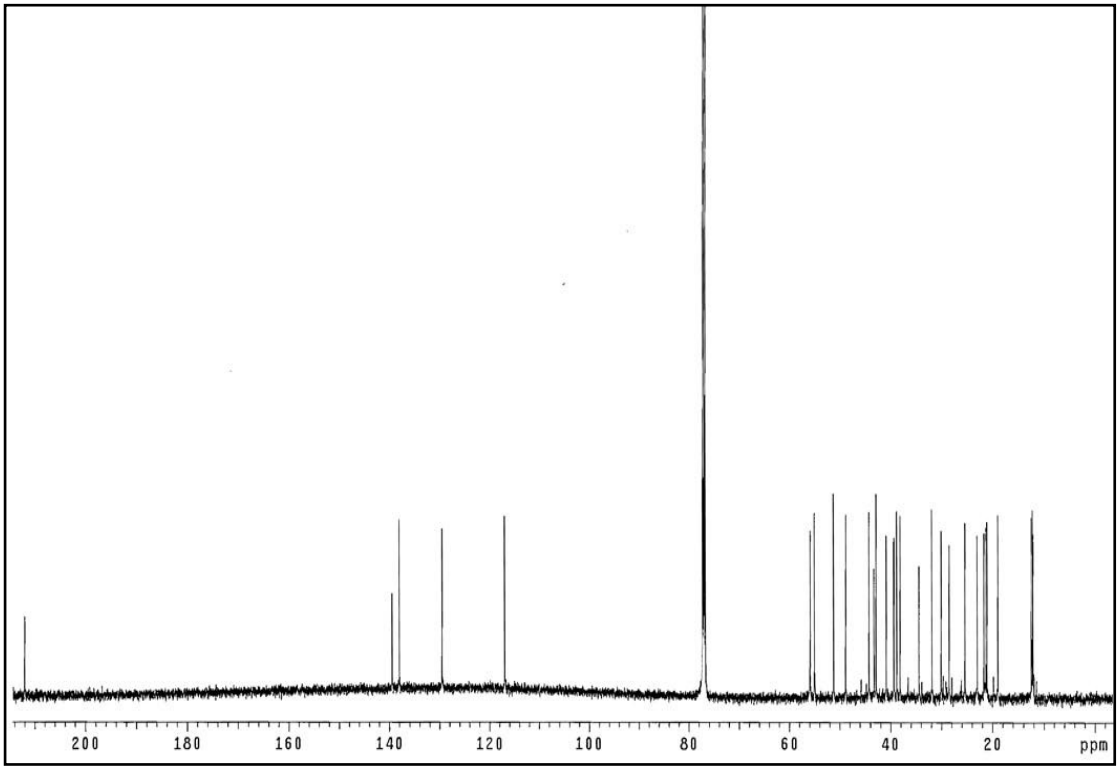

Figure 4 ^{13}C NMR (125 MHz) spectrum of compound HAM1 ($CDCl_3$)

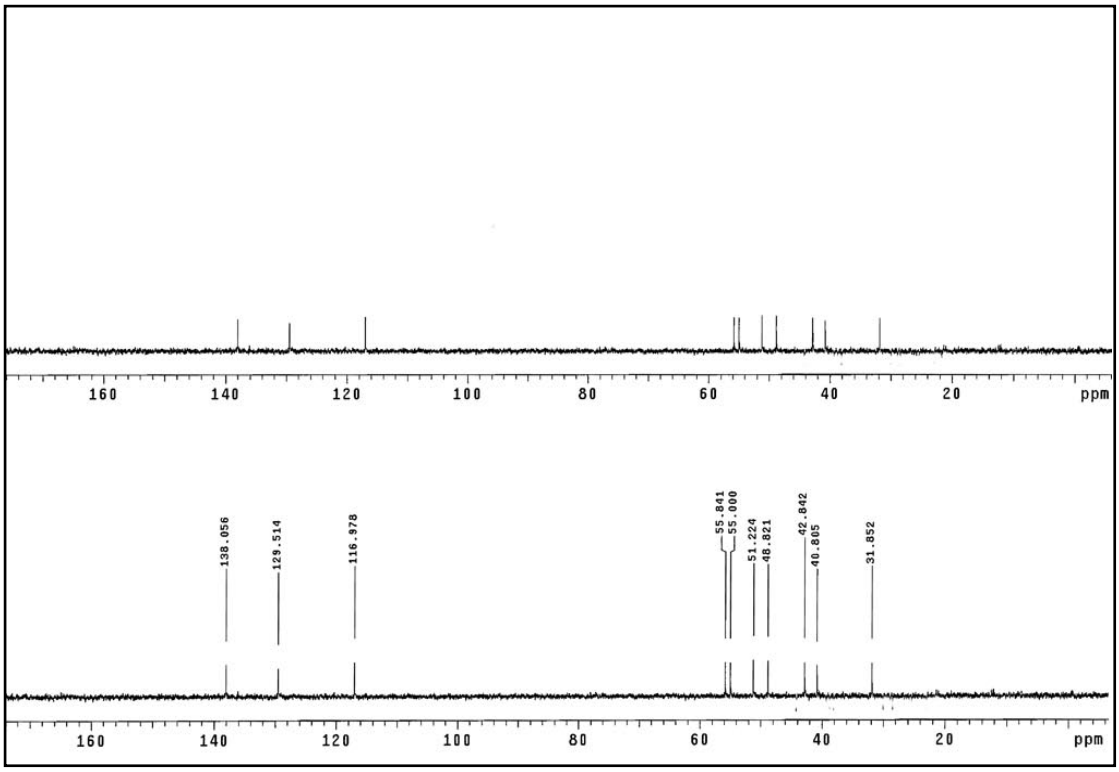

Figure 5 DEPT 90 spectrum of compound HAM1 ($CDCl_3$)

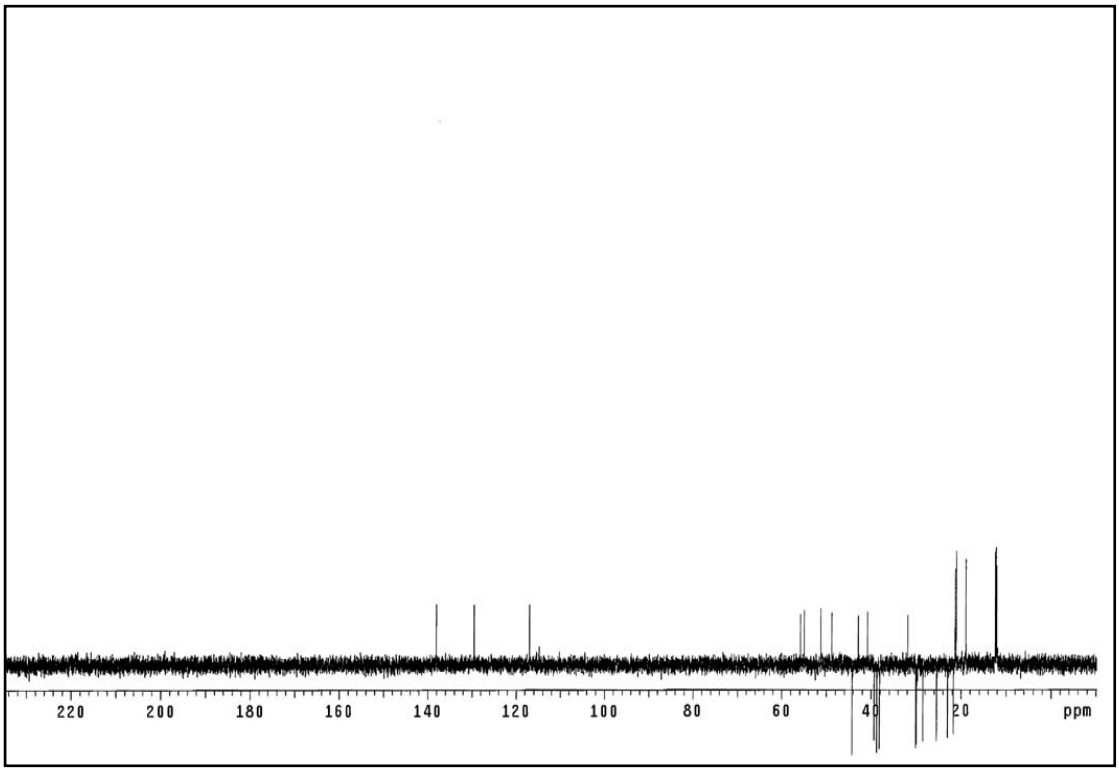

Figure 6 DEPT 135 spectrum of compound HAM1 ($CDCl_3$)

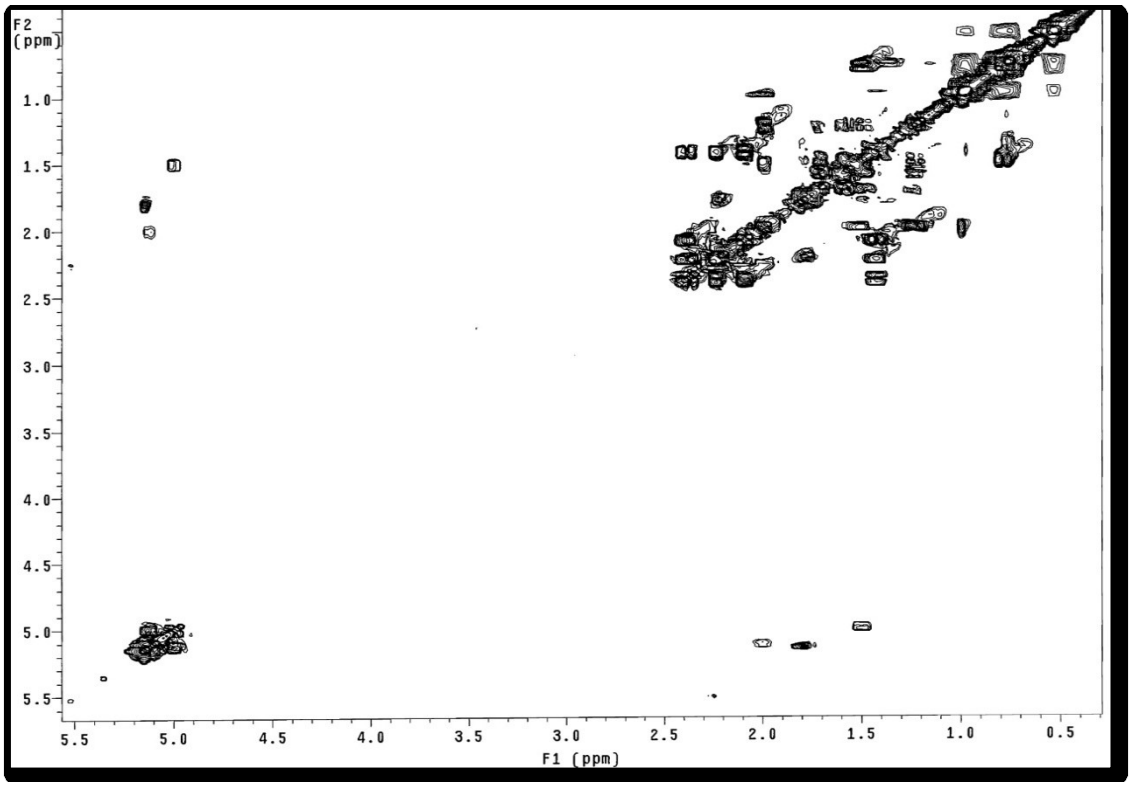

Figure 7 COSY spectrum of compound HAM1 ($CDCl_3$)

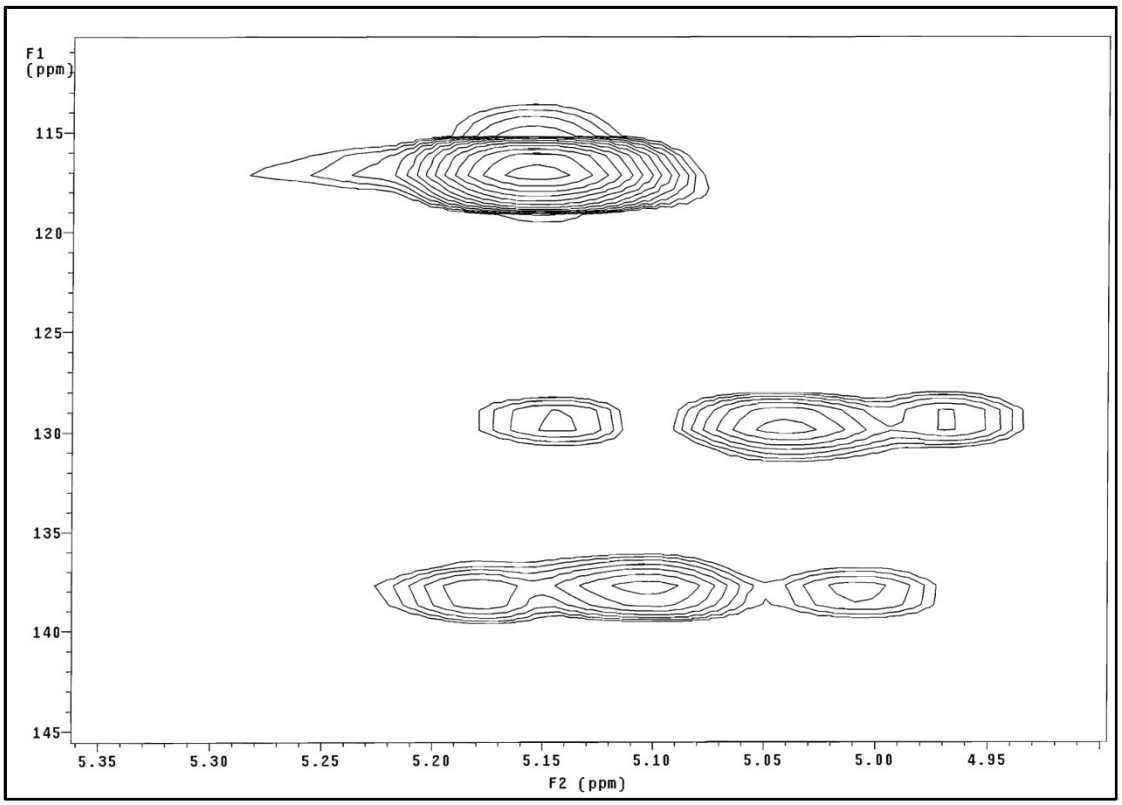

Figure 8 HMQC spectrum of compound HAM1 ($CDCl_3$)

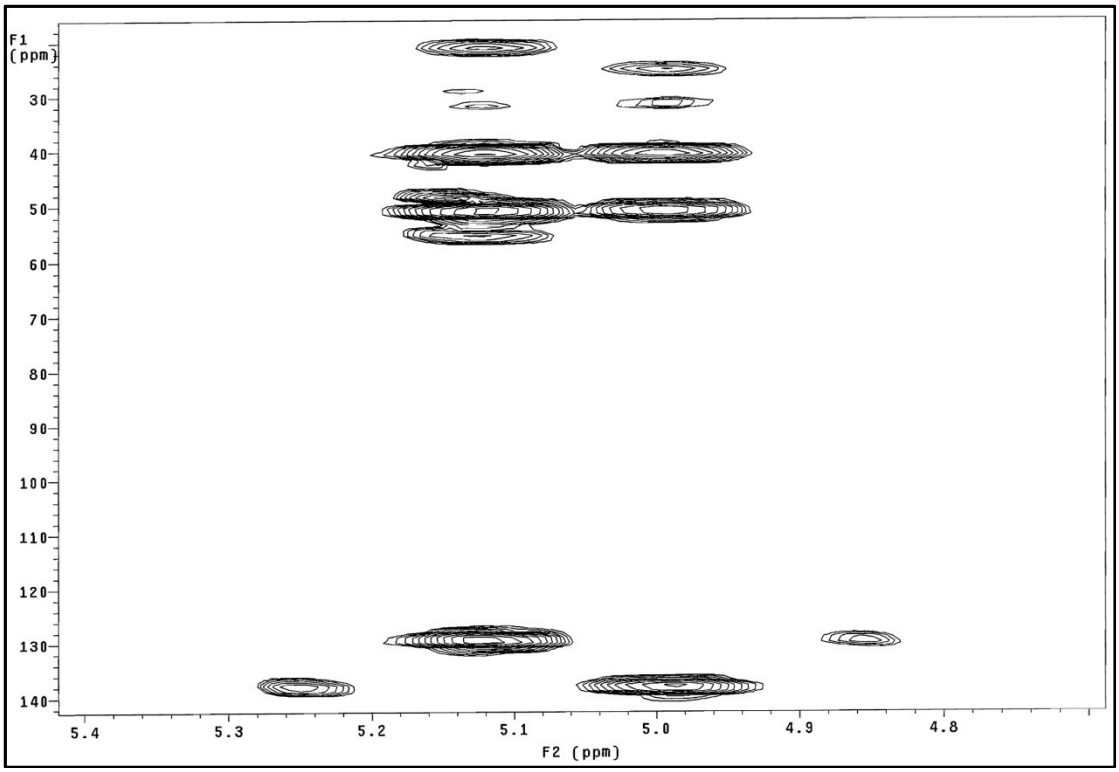

Figure 9 HMBC spectrum of compound HAM1 ($CDCl_3$)

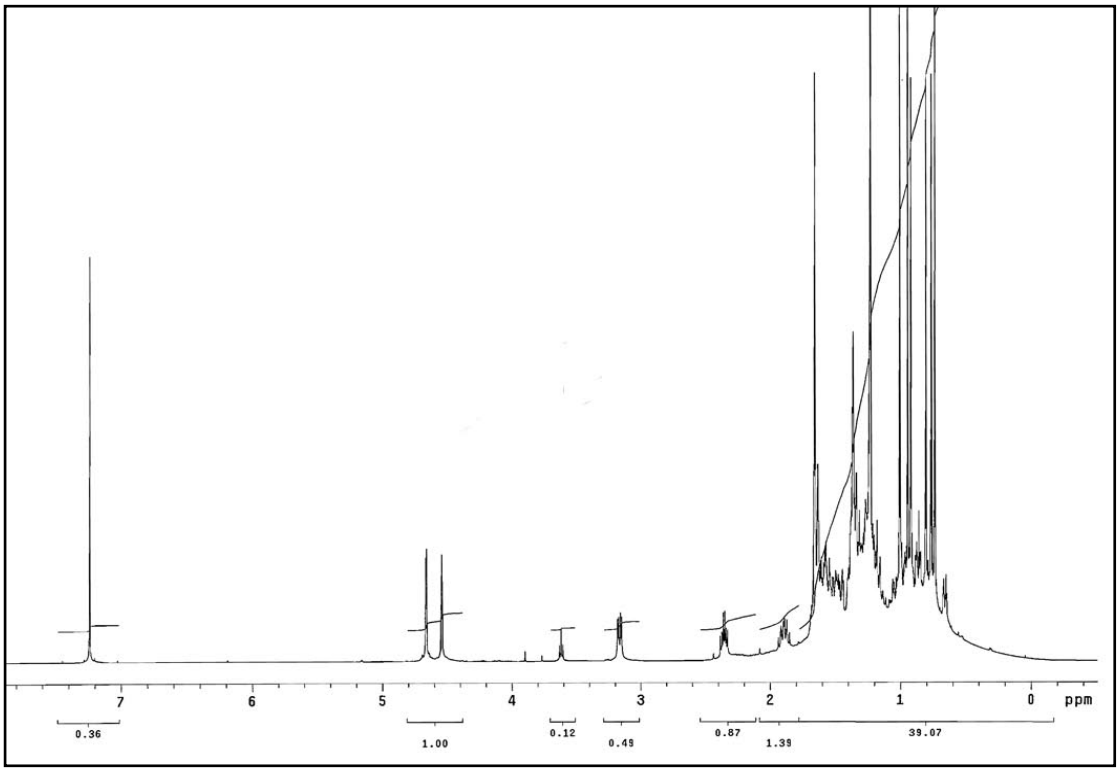

Figure 10 ^{1}H NMR (500 MHz) spectrum of compound HAM2 ($CDCl_3$)

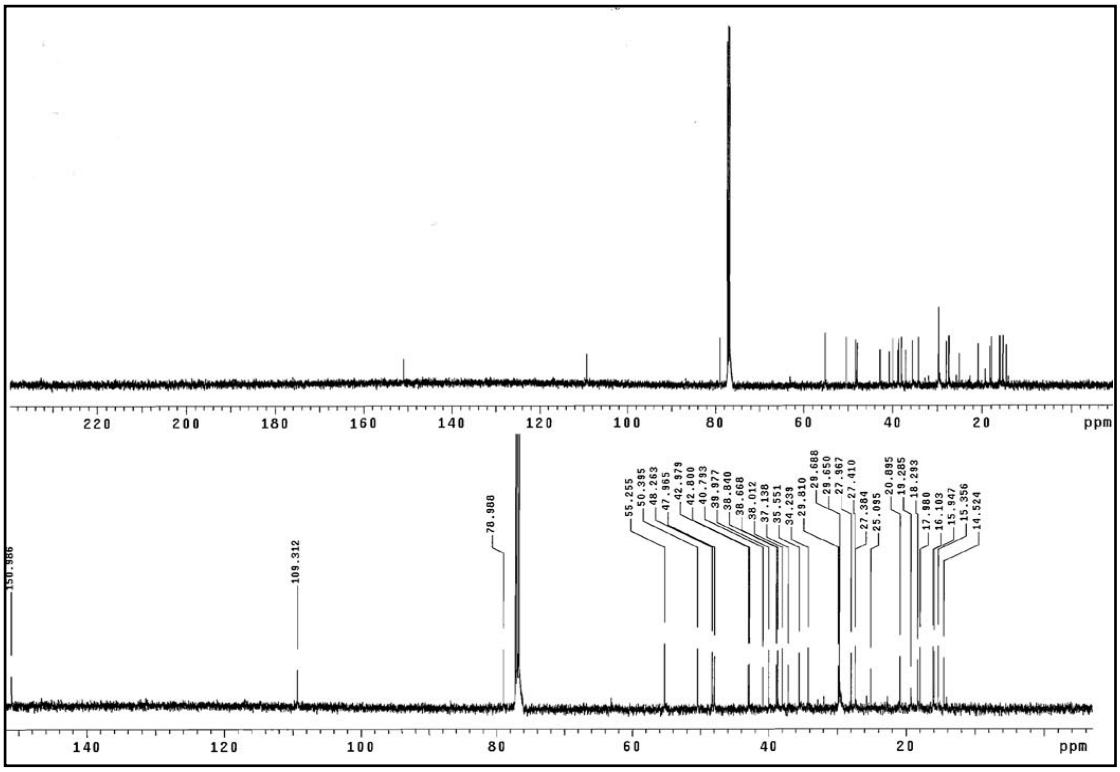

Figure 11 ^{13}C NMR (125 MHz) spectrum of compound HAM2 ($CDCl_3$)

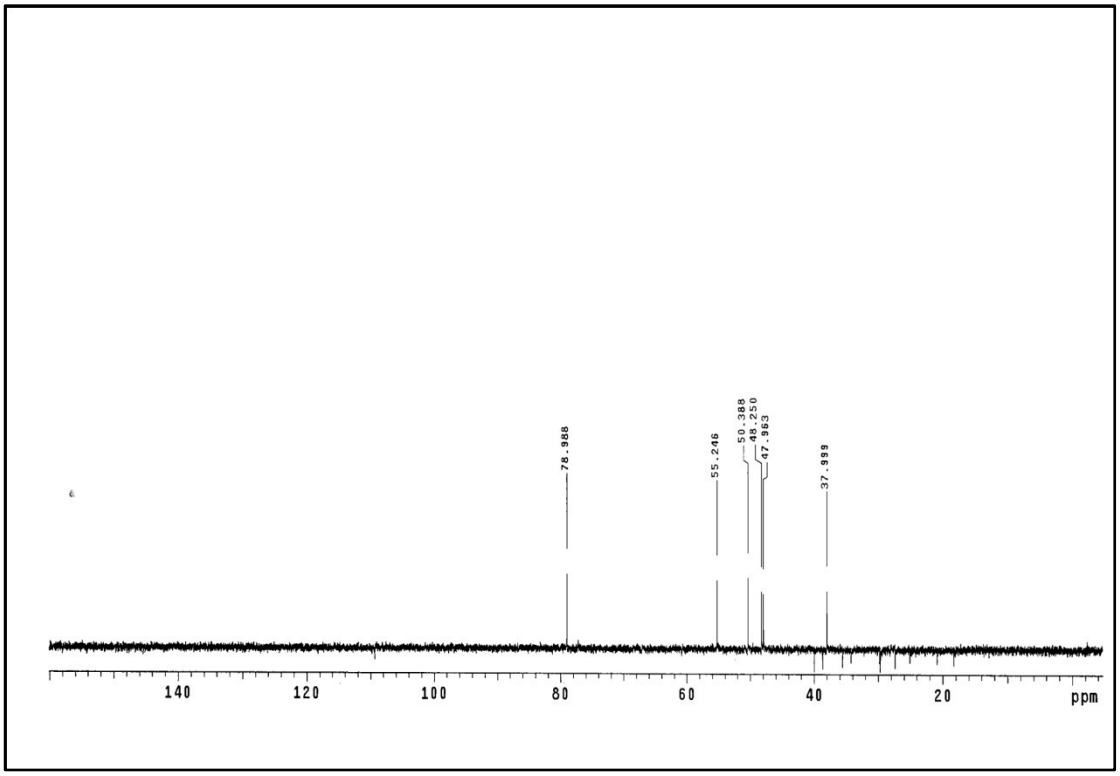

Figure 12 DEPT 90 spectrum of compound HAM2 ($CDCl_3$)

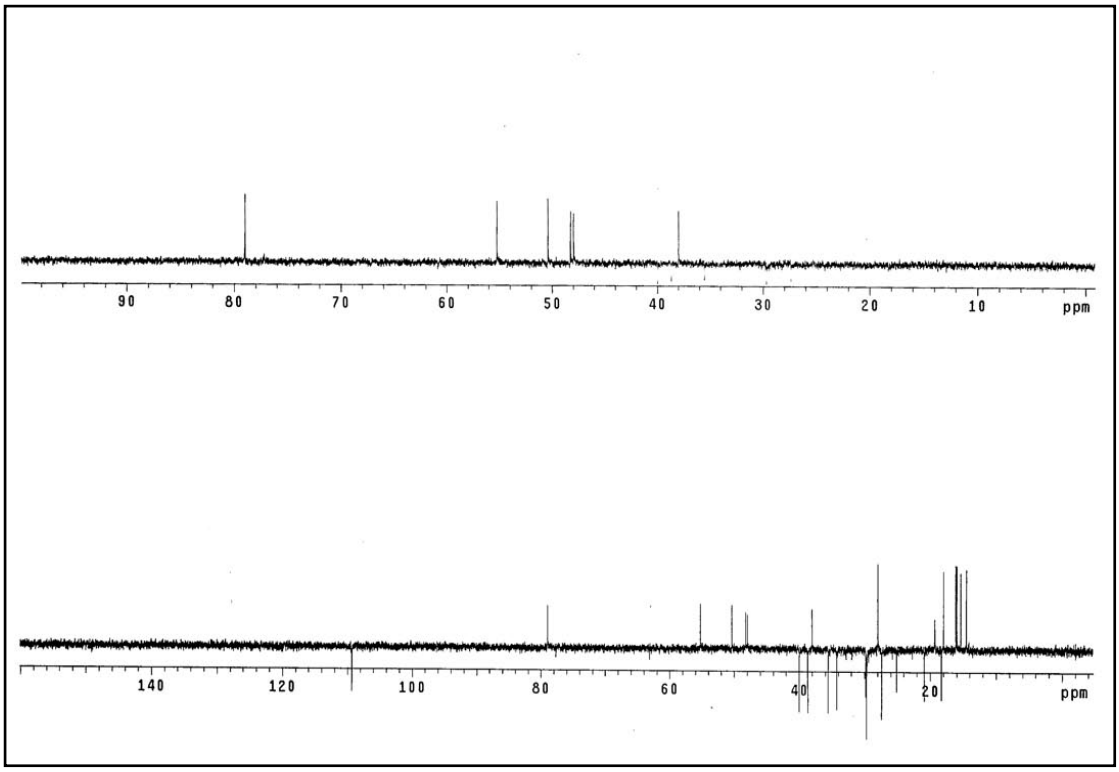

Figure 13 DEPT 135 spectrum of compound HAM2 ($CDCl_3$)

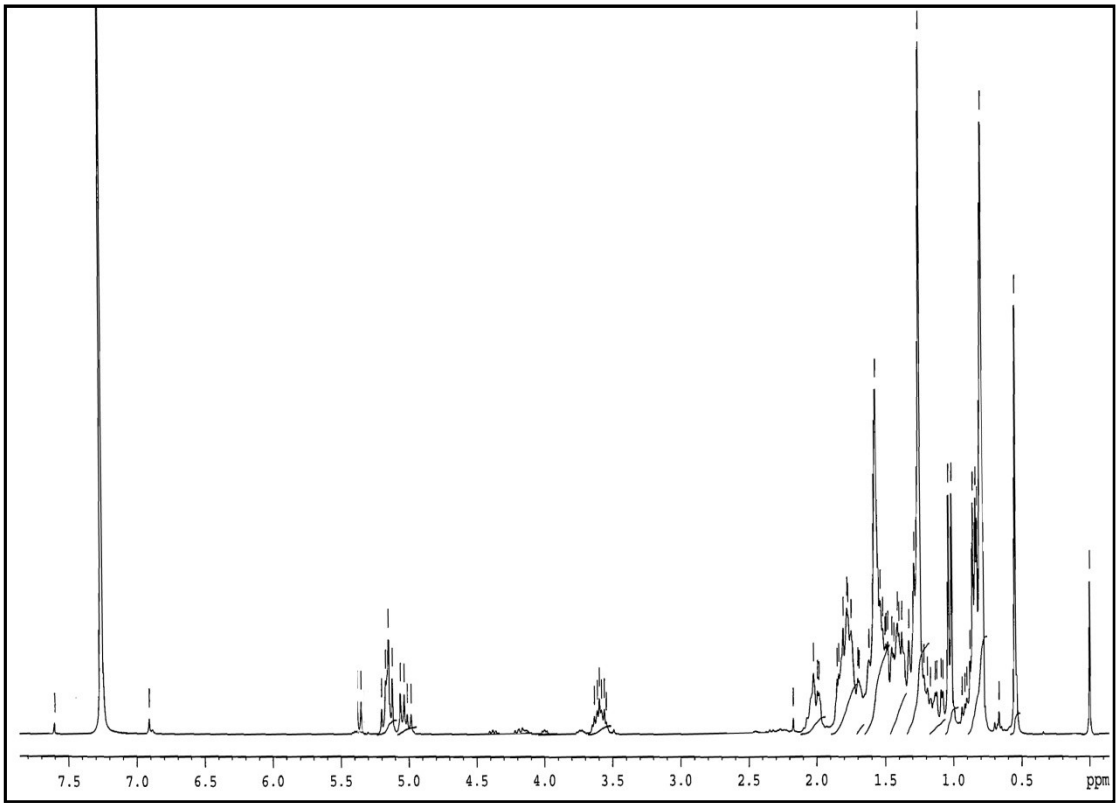

Figure 14 ^{1}H NMR (300 MHz) spectrum of compound HAM3 ($CDCl_3$)

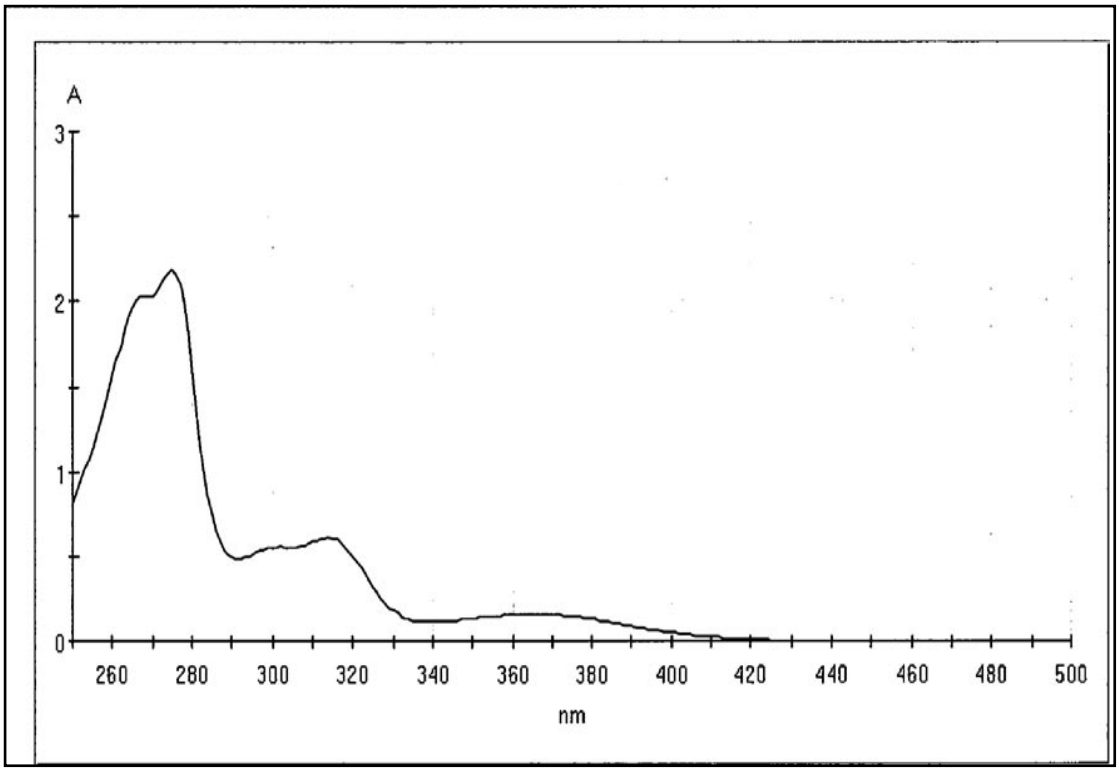

Figure 15 UV spectrum of compound HAM4

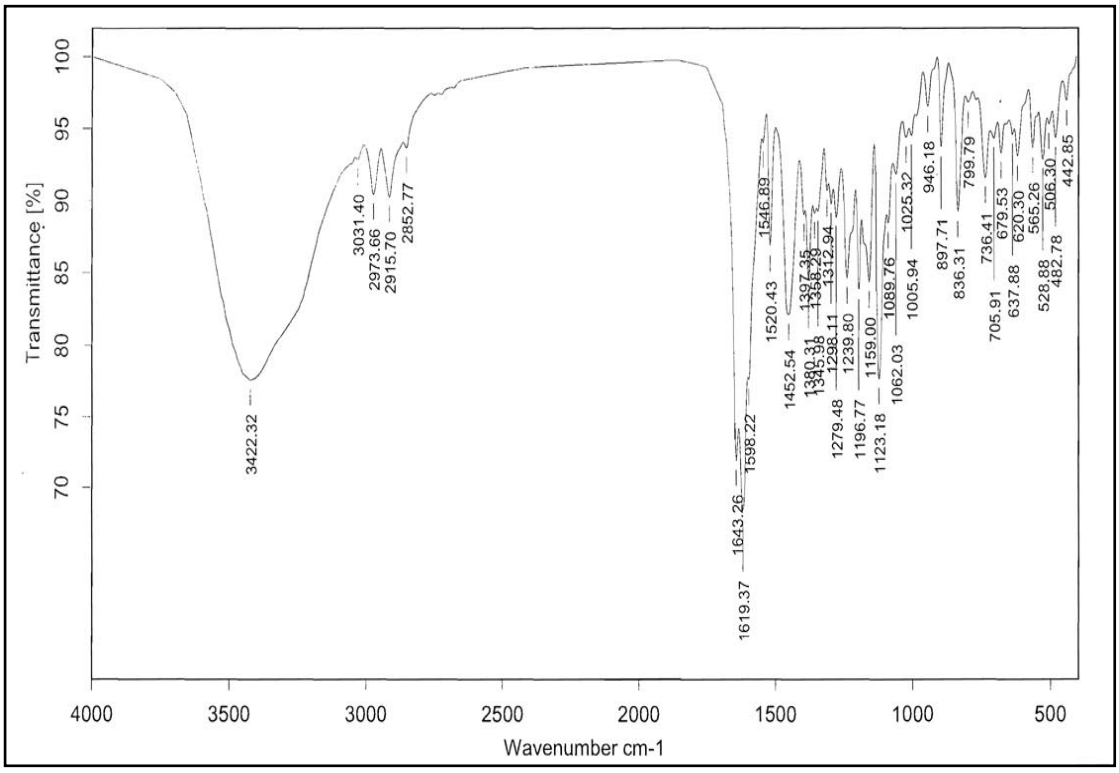

Figure 16 FT-IR spectrum of compound HAM4

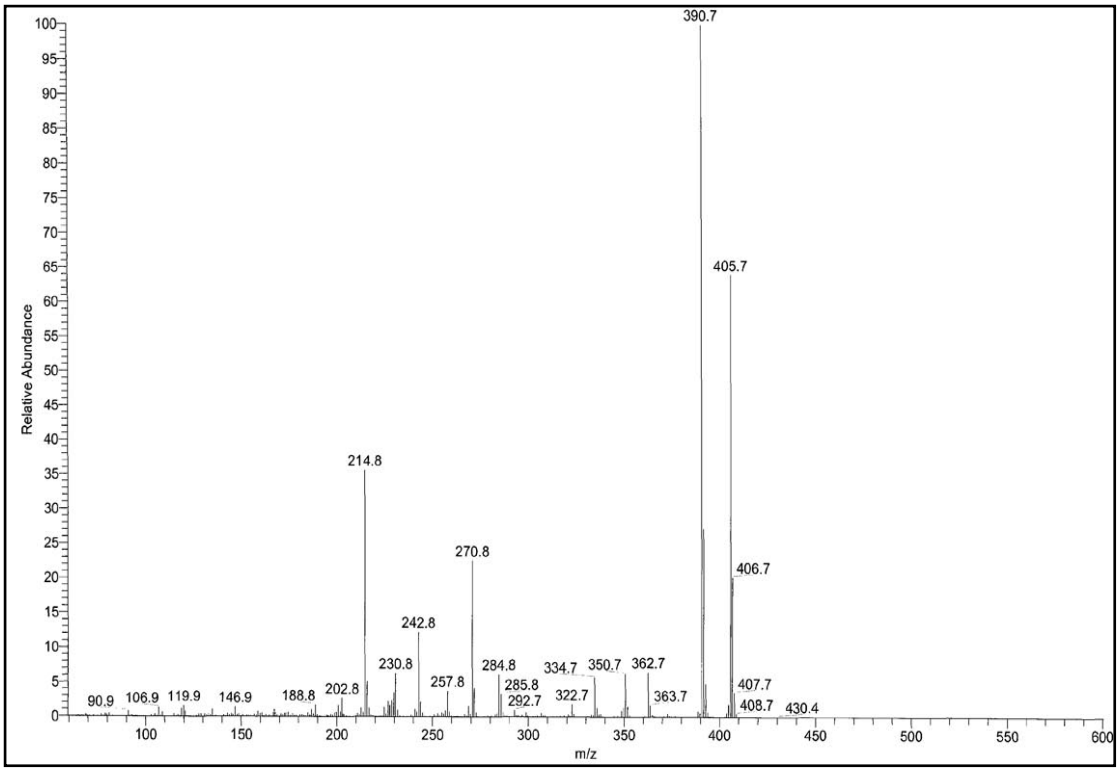

Figure 17 EIMS spectrum of compound HAM4

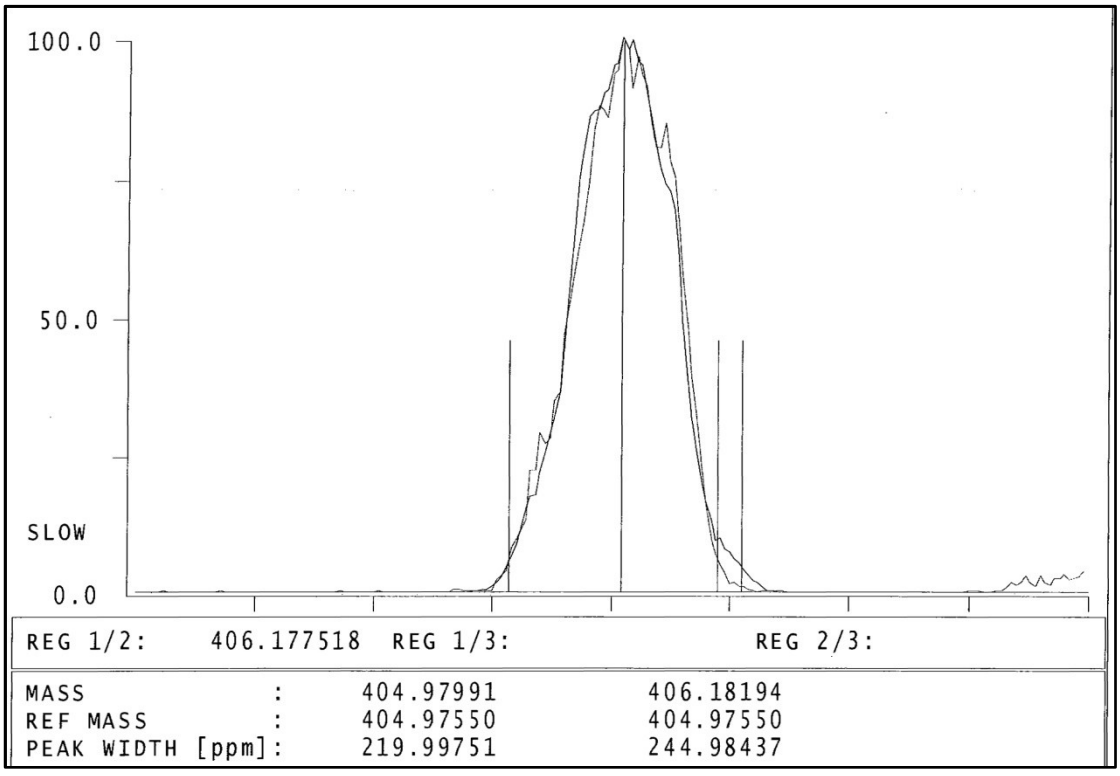

Figure 18 HREIMS spectrum of compound HAM4

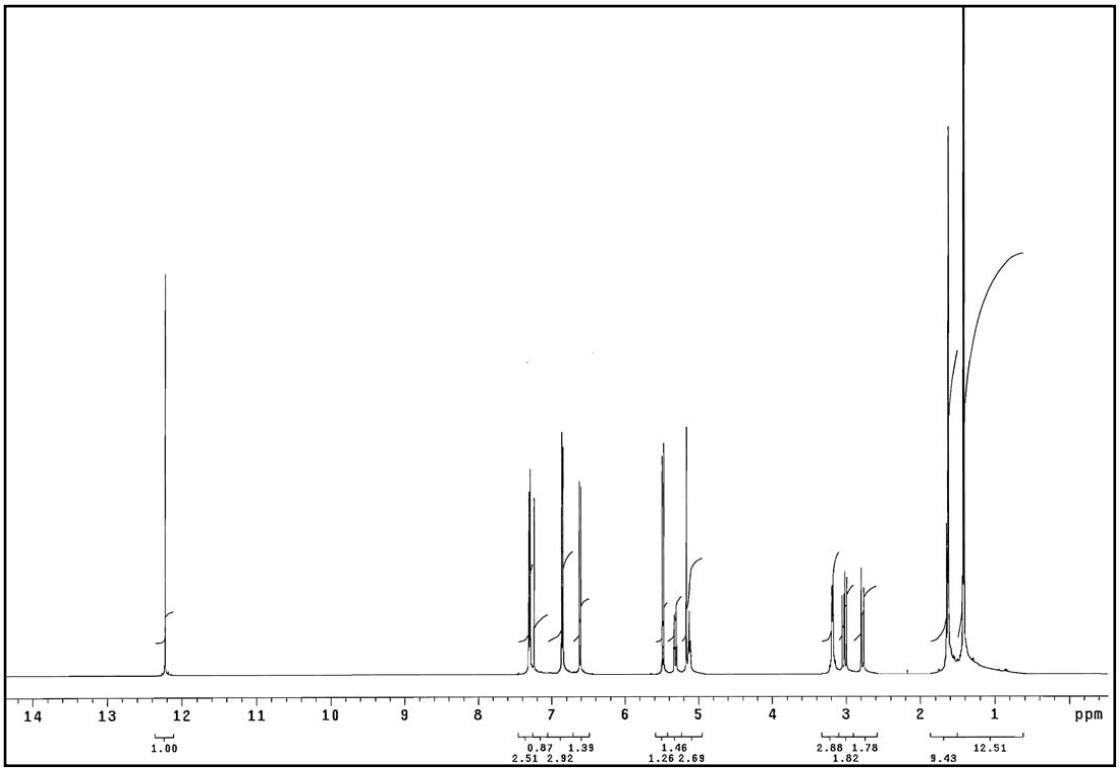

Figure 19 ^{1}H NMR (500 HMZ) spectrum of compound HAM4 ($CDCl_3$)

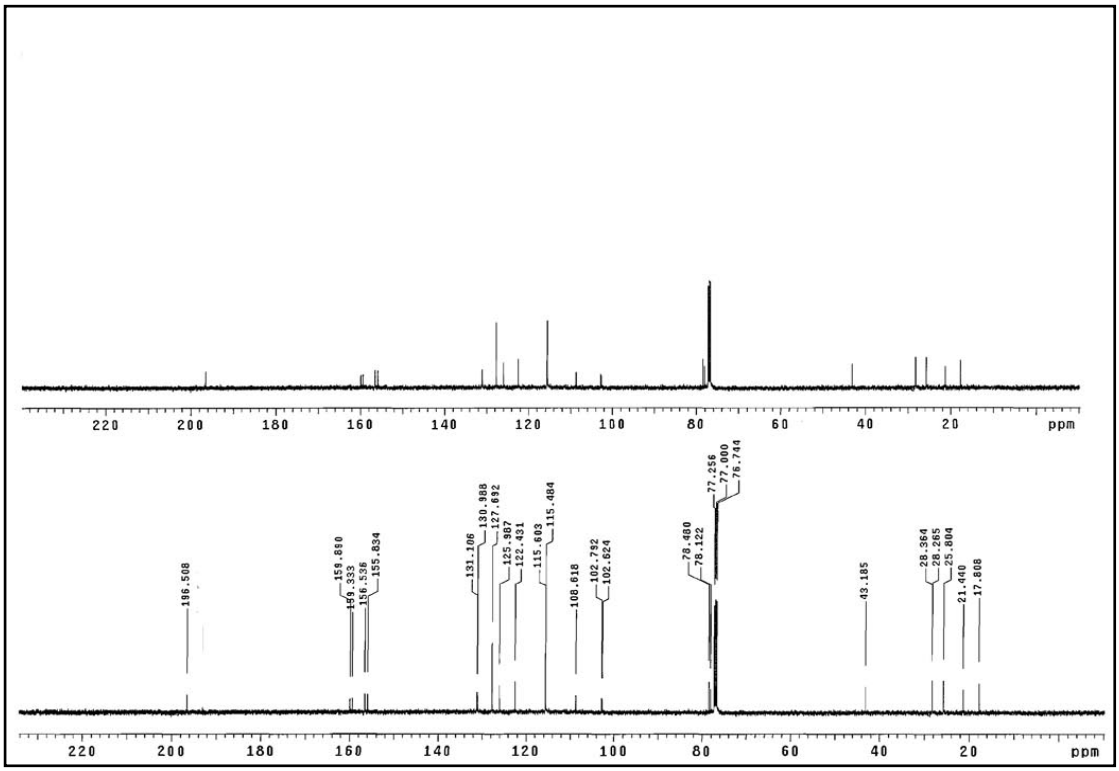

Figure 20 ^{13}C NMR (500 MHz) spectrum of compound HAM4 ($CDCl_3$)

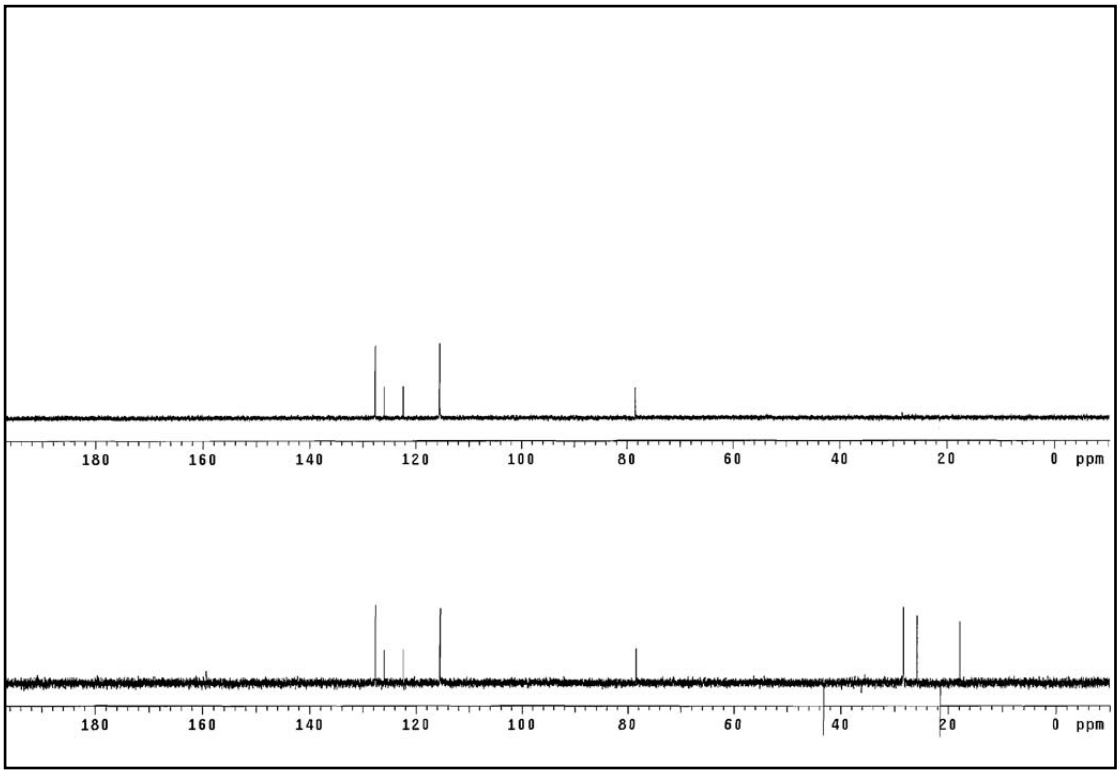

Figure 21 DEPT 90 and 135 spectrum of compound HAM4 ($CDCl_3$)

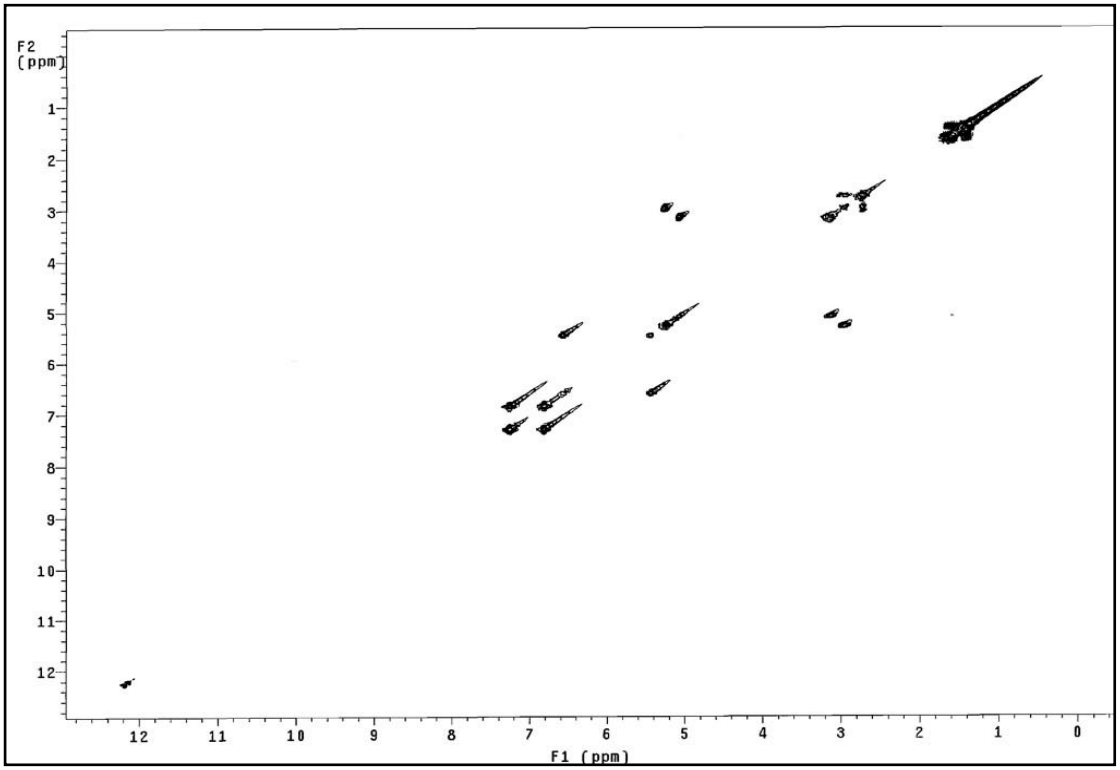

Figure 22 COSY spectrum of compound HAM4 $CDCl_3$)

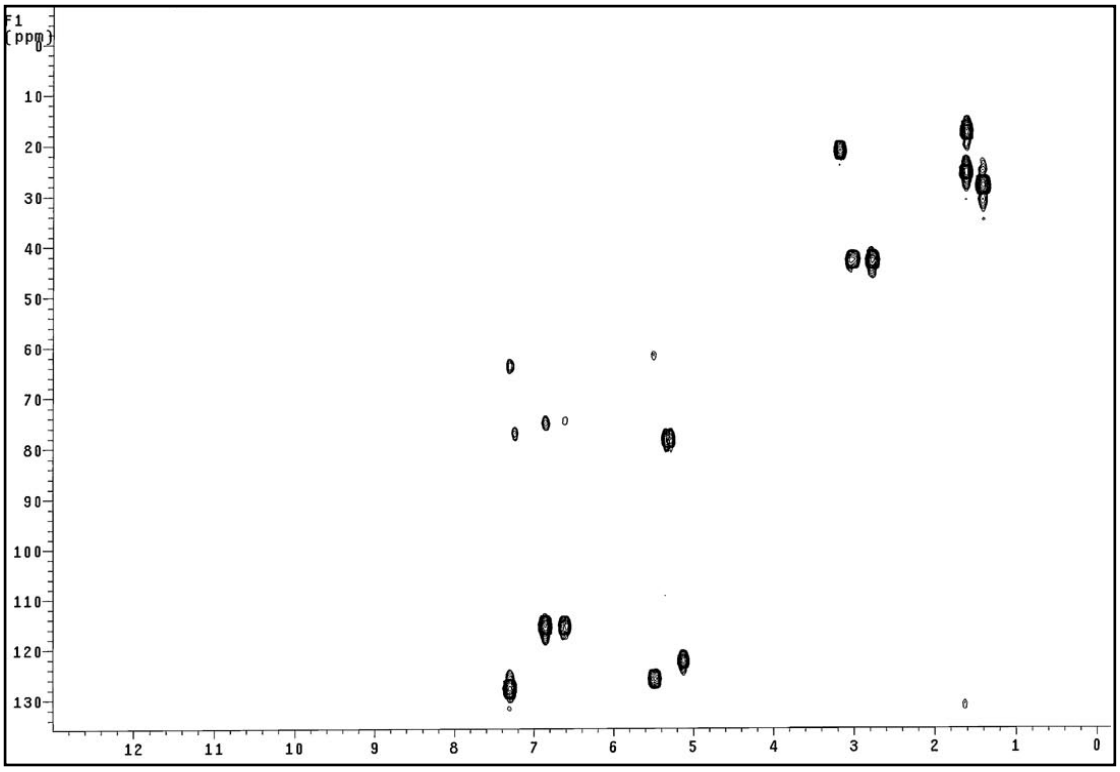

Figure 23 HMQC spectrum of compound HAM4 ($CDCl_3$)

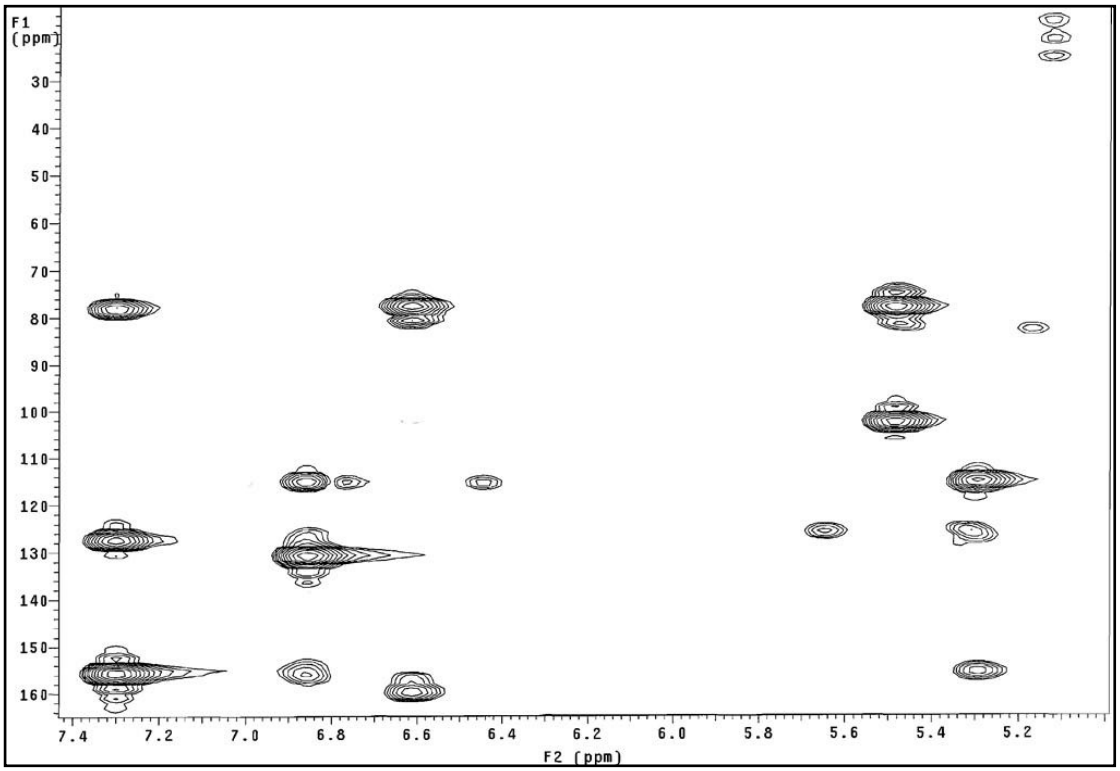

Figure 24 HMBC spectrum of compound HAM4 ($CDCl_3$)

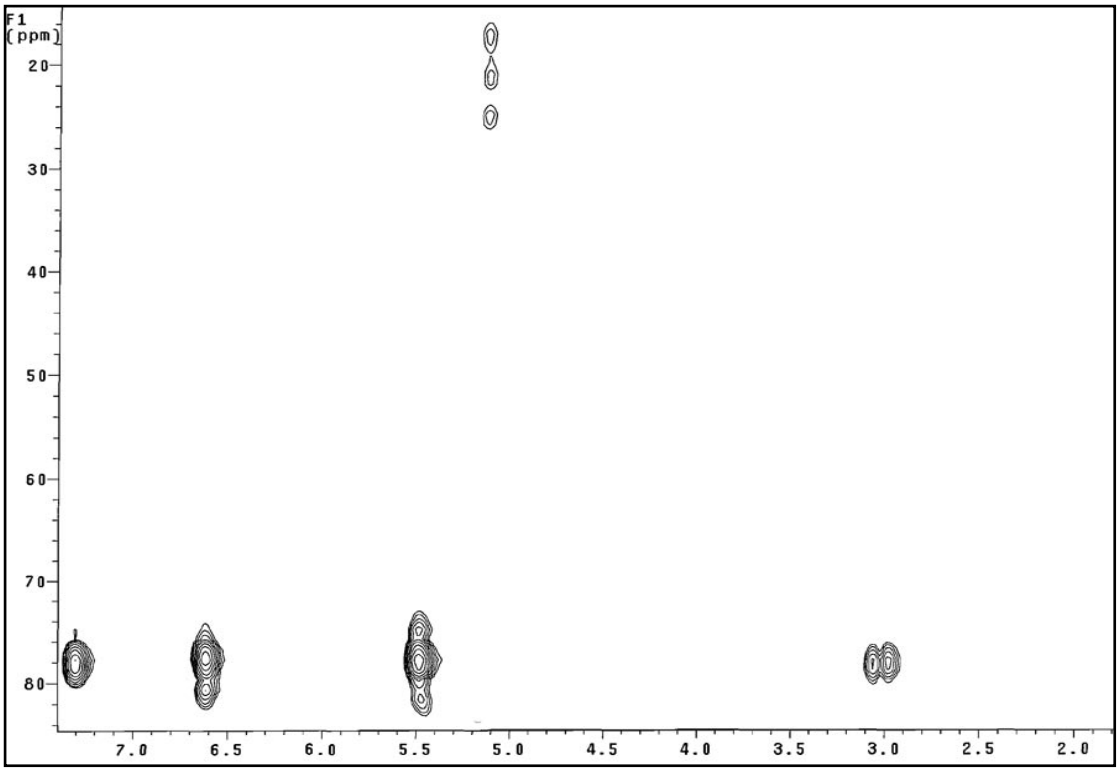

Figure 25 HMBC spectrum of compound HAM4 ($CDCl_3$)

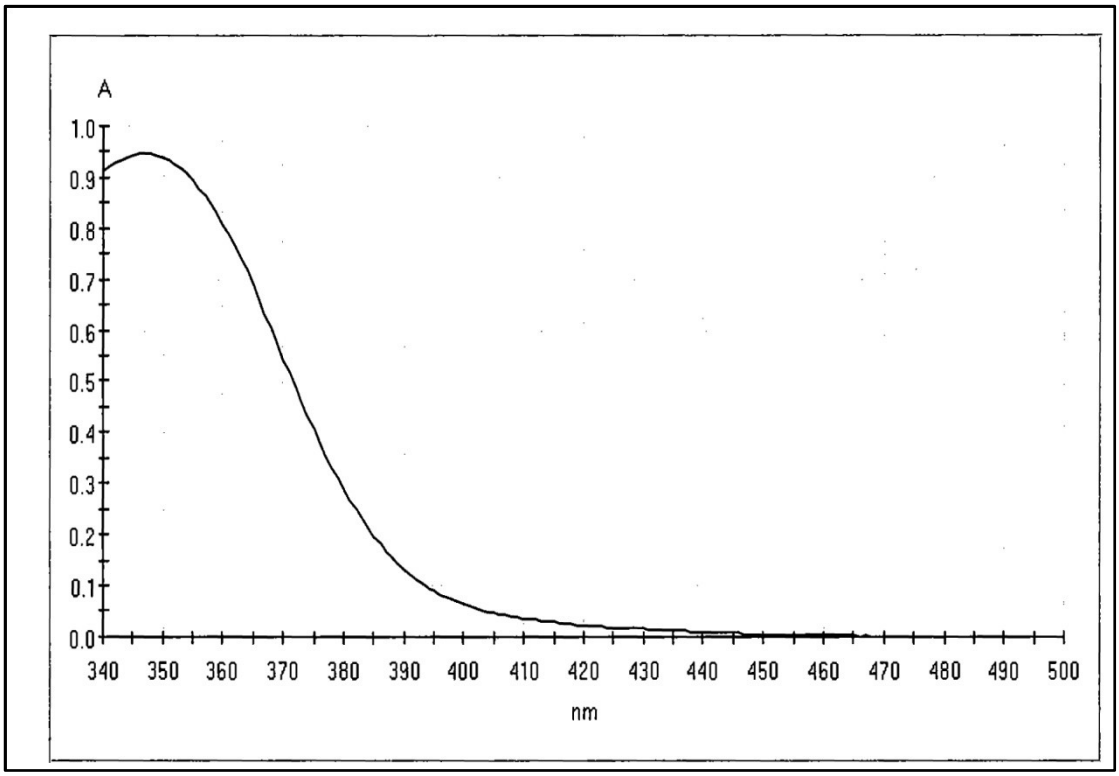

Figure 26 UV spectrum of compound DAM1

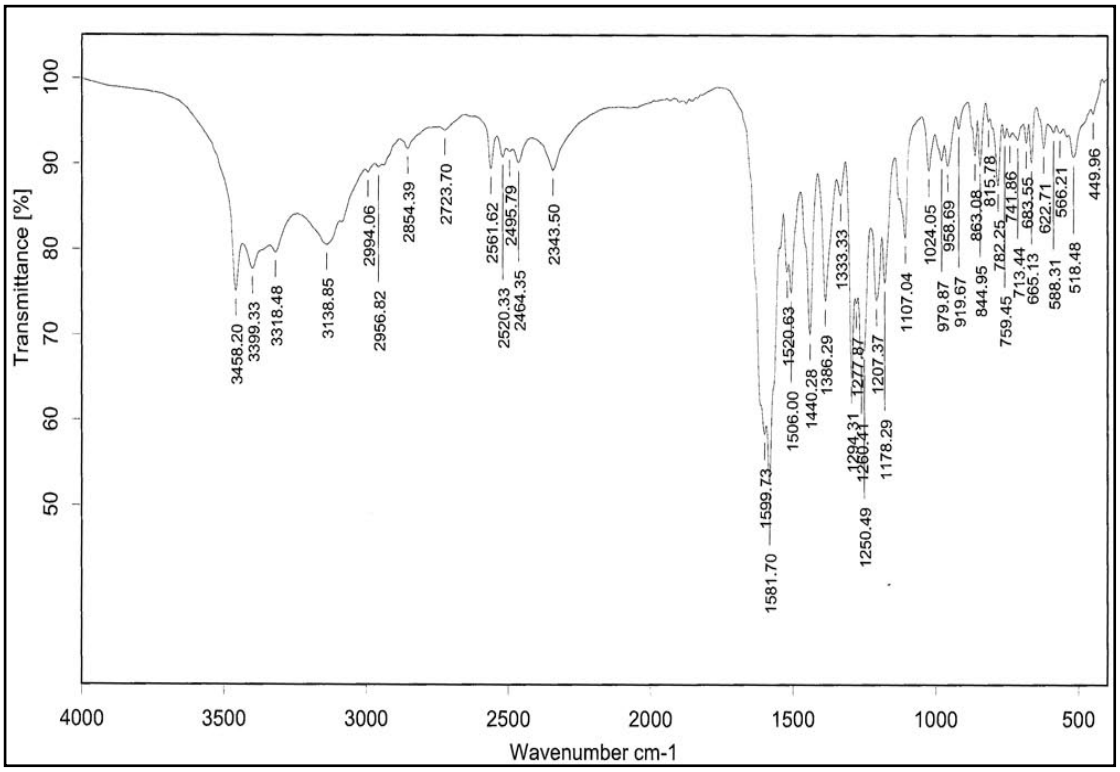

Figure 27 FT-IR spectrum of compound DAM1

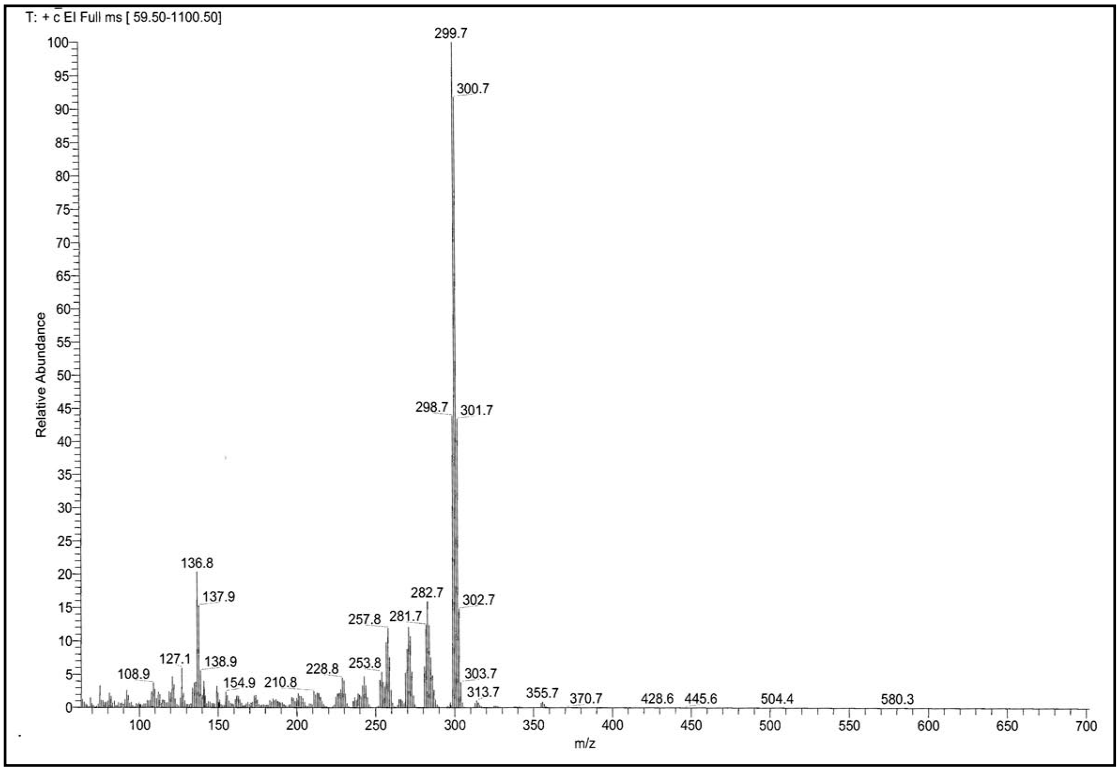

Figure 28 EIMS spectrum of compound DAM1

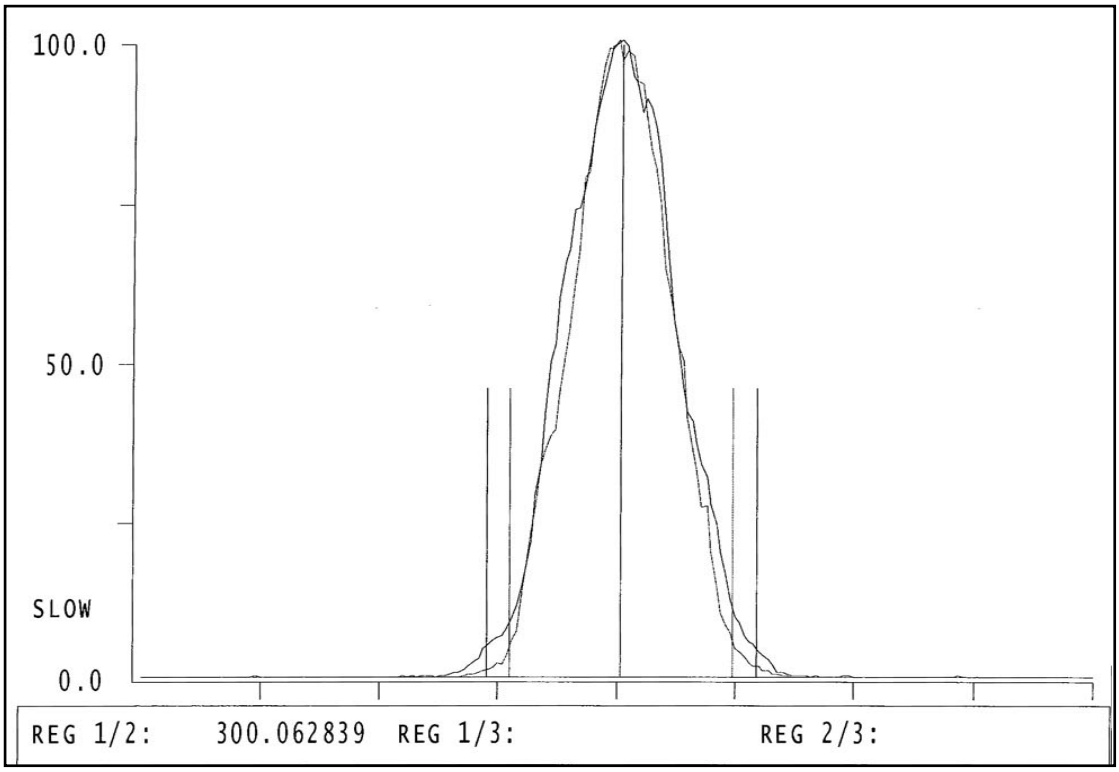

Figure 29 HREIMS spectrum of compound DAM1

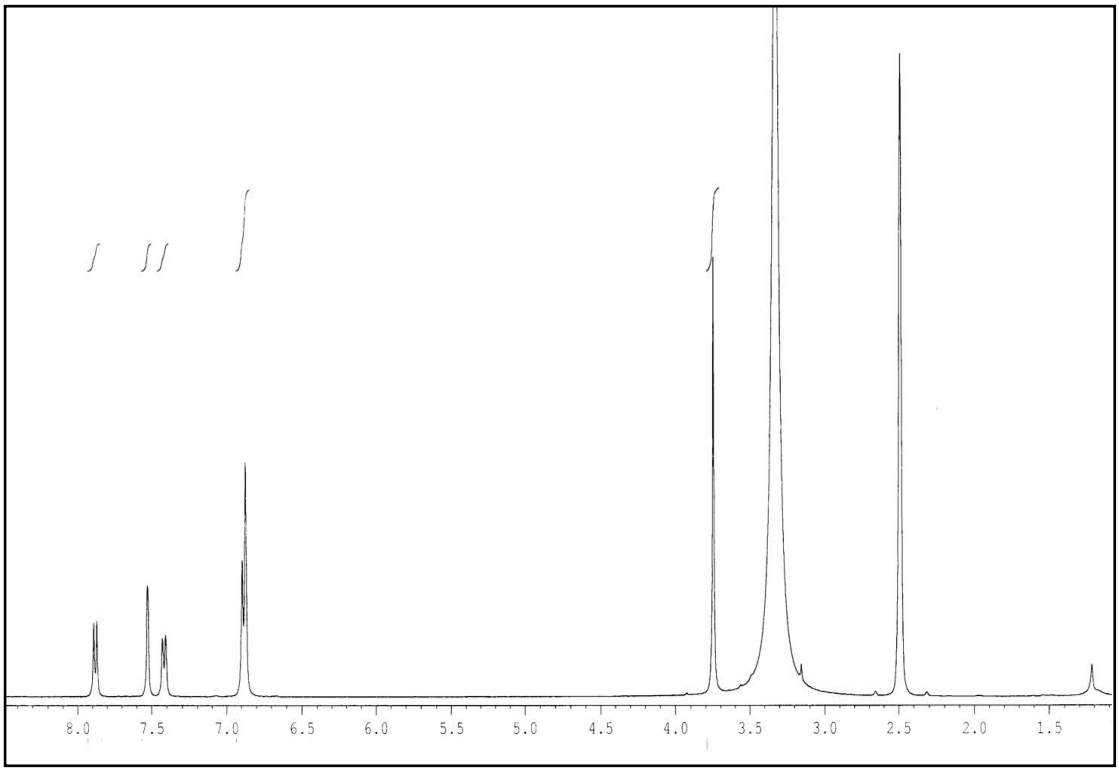

Figure 30 ^{1}H NMR (400 MHz) spectrum of compound DAM1 (DMSO-d_6)

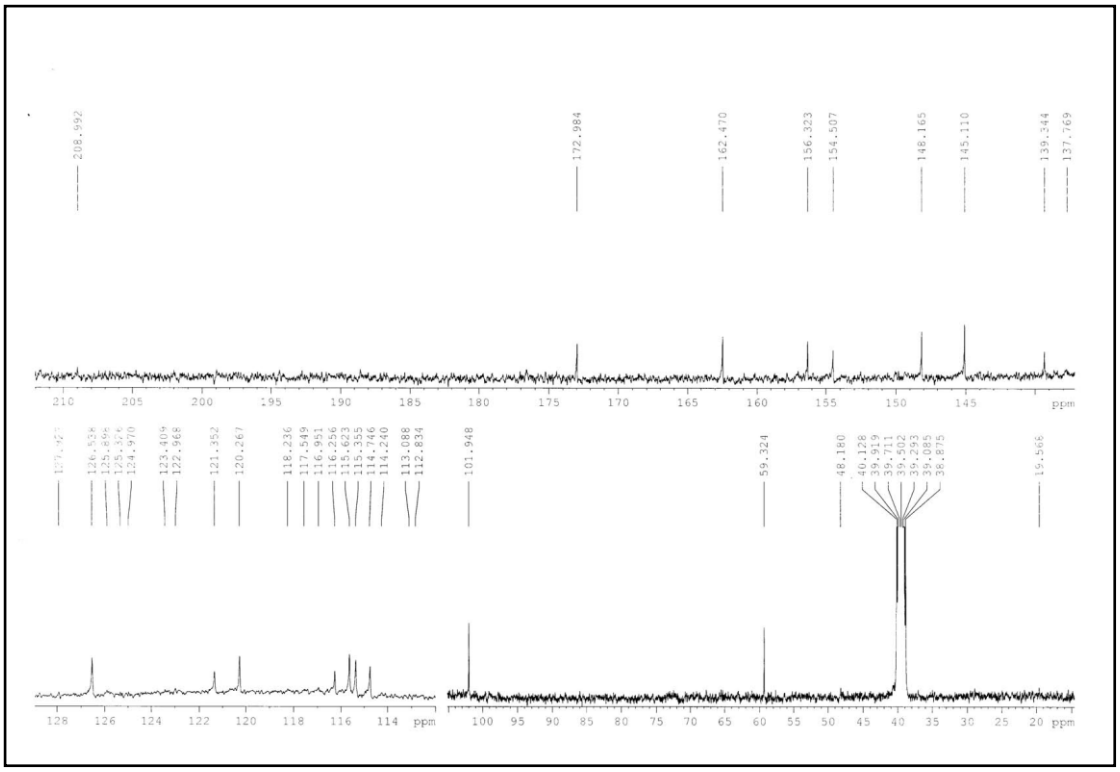

Figure 31 ^{13}C NMR (100 MHz) spectrum of compound DAM1 (DMSO-d_6)

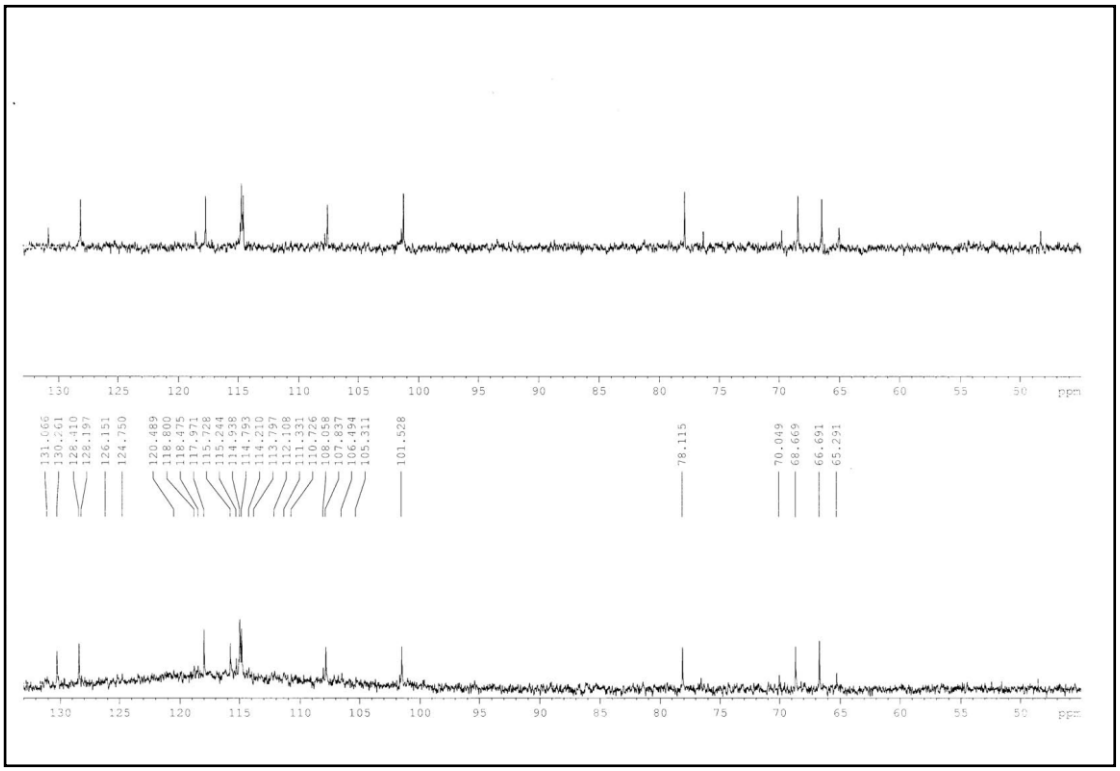

Figure 32 DEPT 90 spectrum of compound DAM1 (DMSO-d_6)

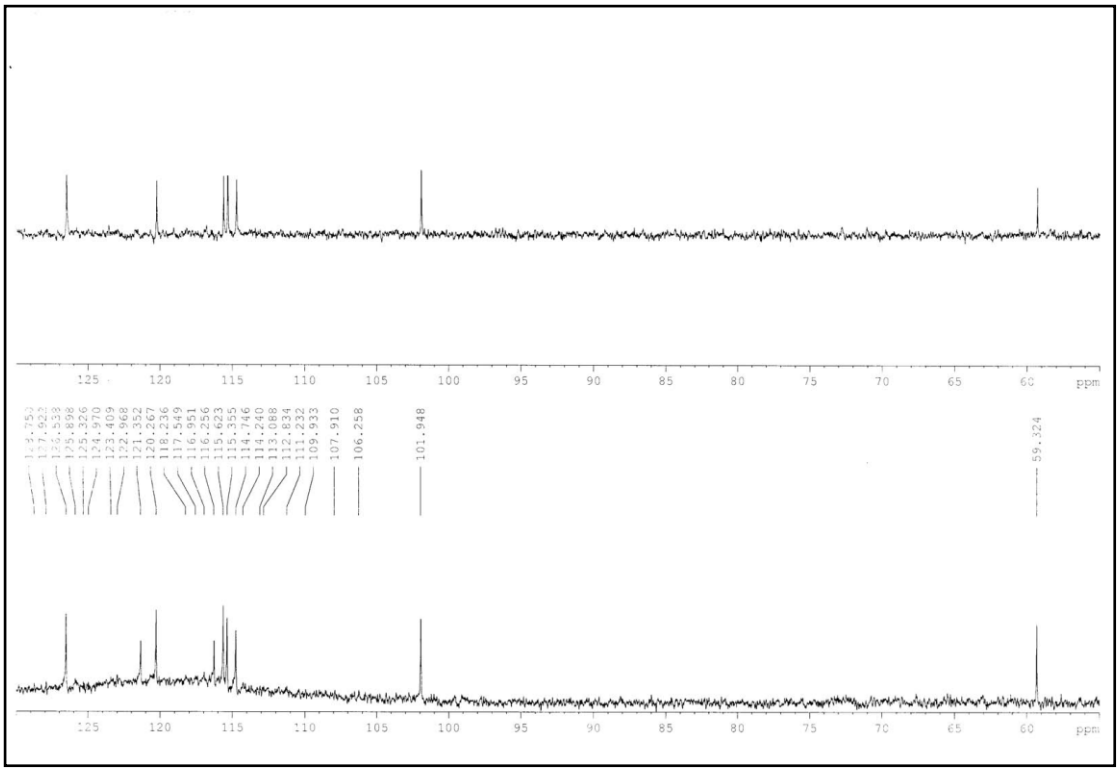

Figure 33 DEPT 135 spectrum of compound DAM1 (DMSO-d_6)

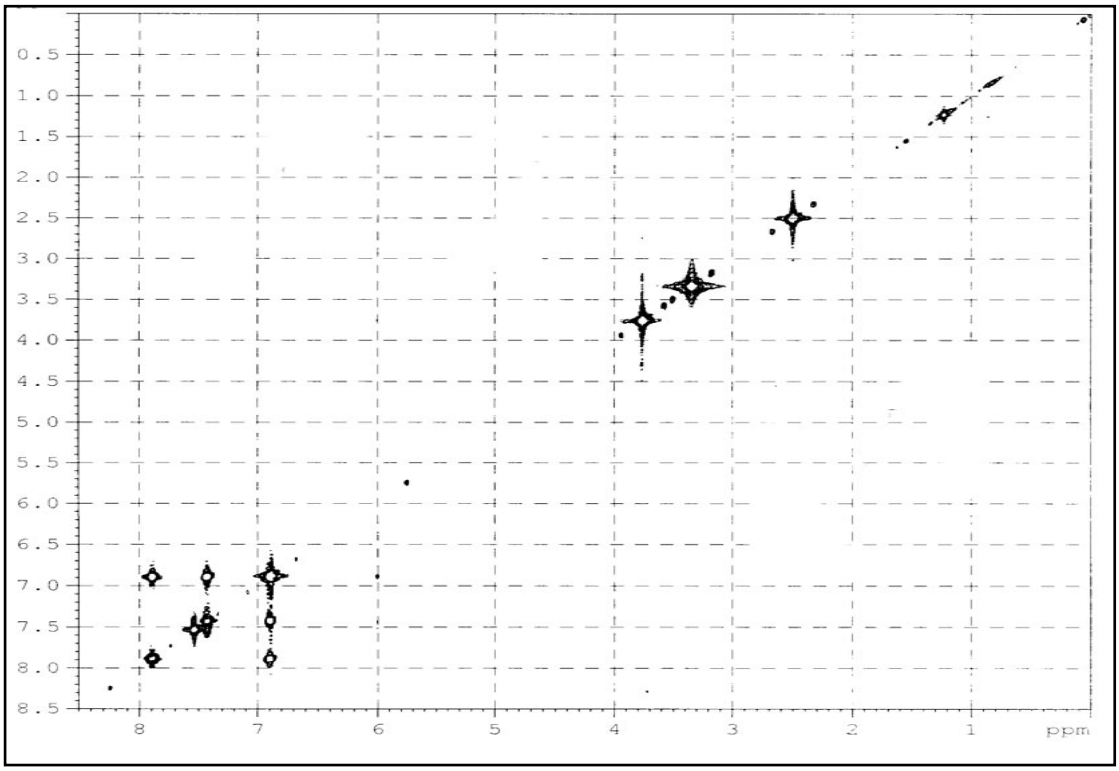

Figure 34 COSY spectrum of compound DAM1 (DMSO-d_6)

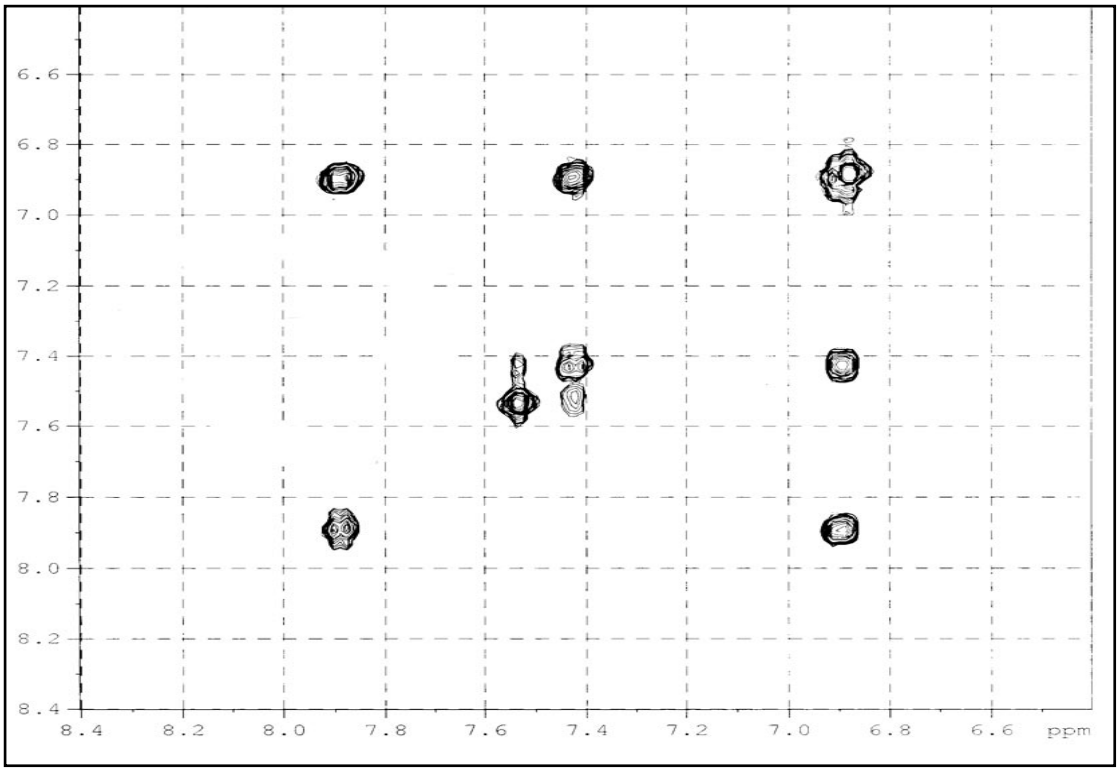

Figure 35 COSY spectrum of compound DAM1 (DMSO-d_6)

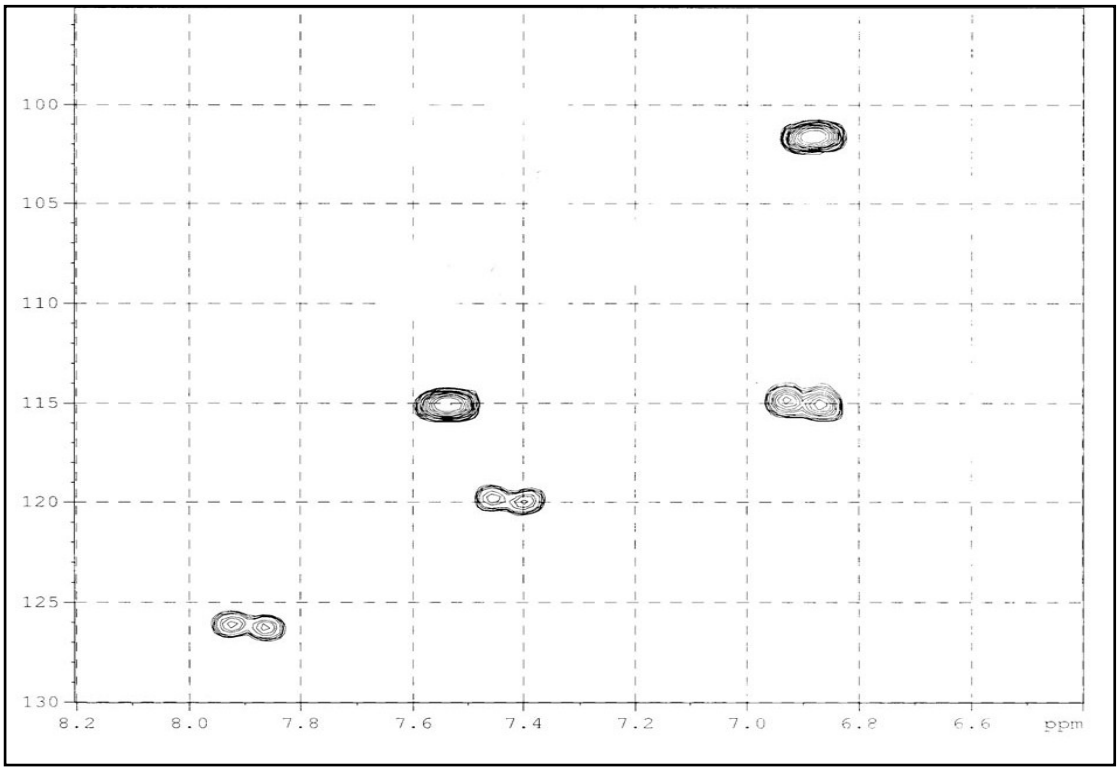

Figure 36 HMQC spectrum of compound DAM1 (DMSO-d_6)

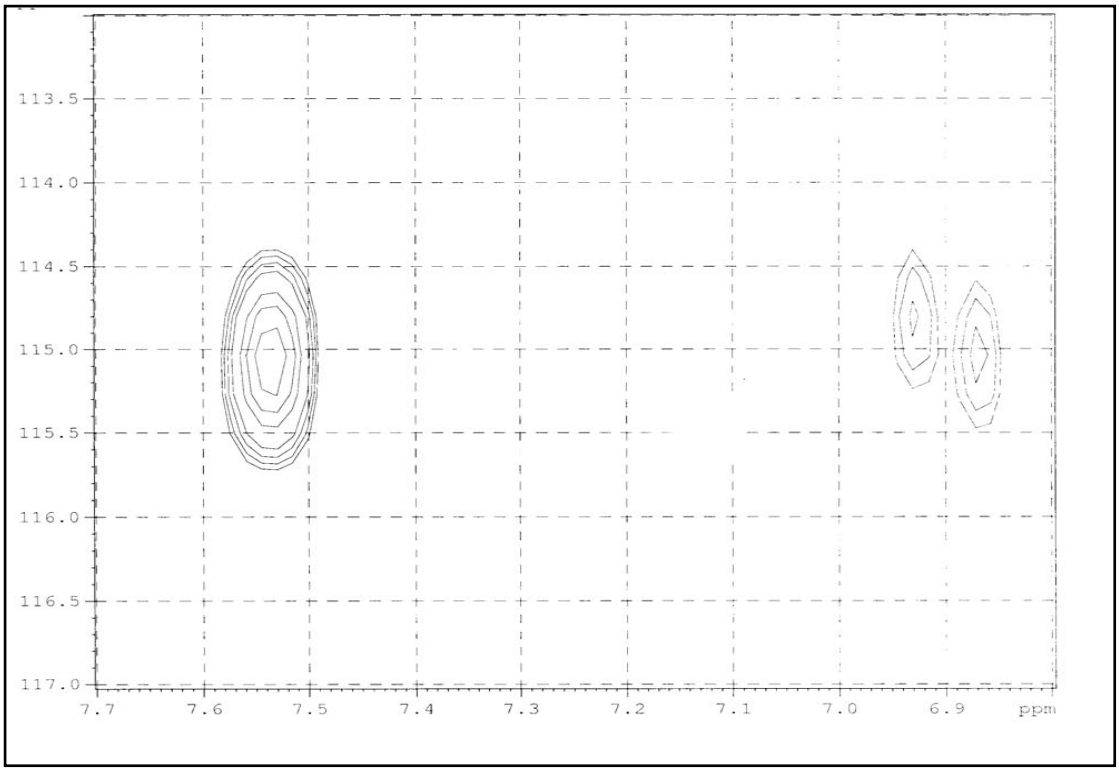

Figure 37 HMQC spectrum of compound DAM1 (DMSO-d_6)

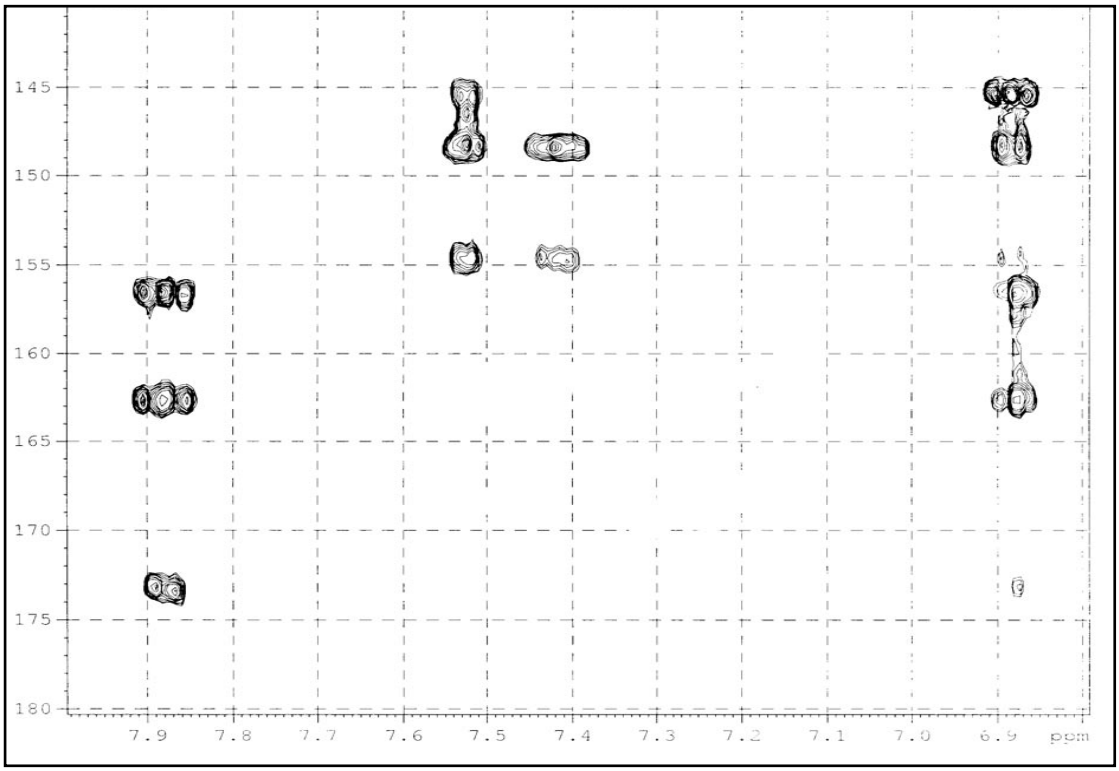

Figure 38 HMBC spectrum of compound DAM1 (DMSO-d_6)

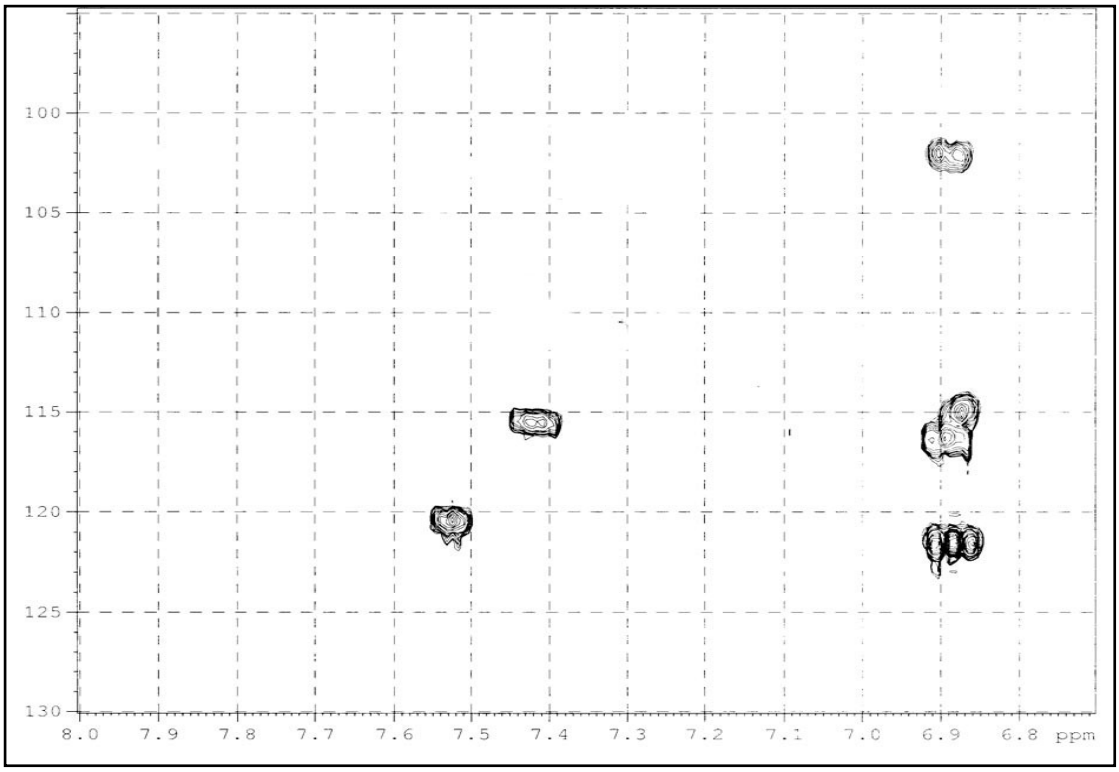

Figure 39 HMBC spectrum of compound DAM1 (DMSO-d_6)

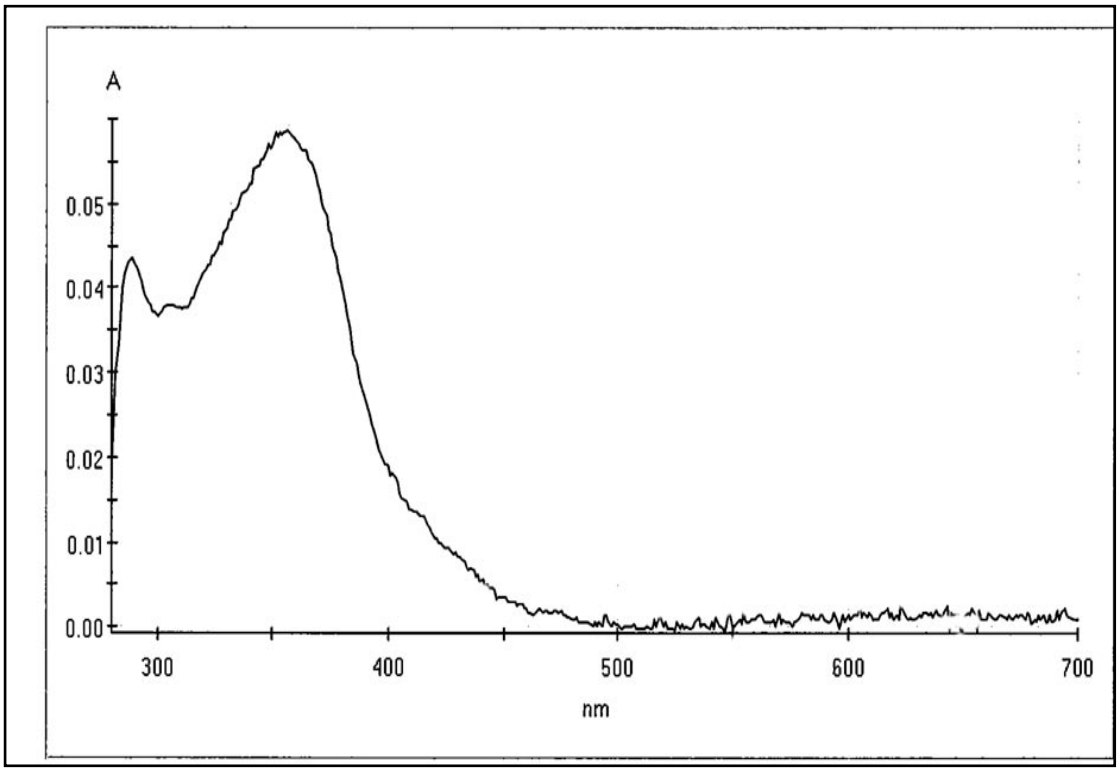

Figure 40 UV spectrum of compound DAM2

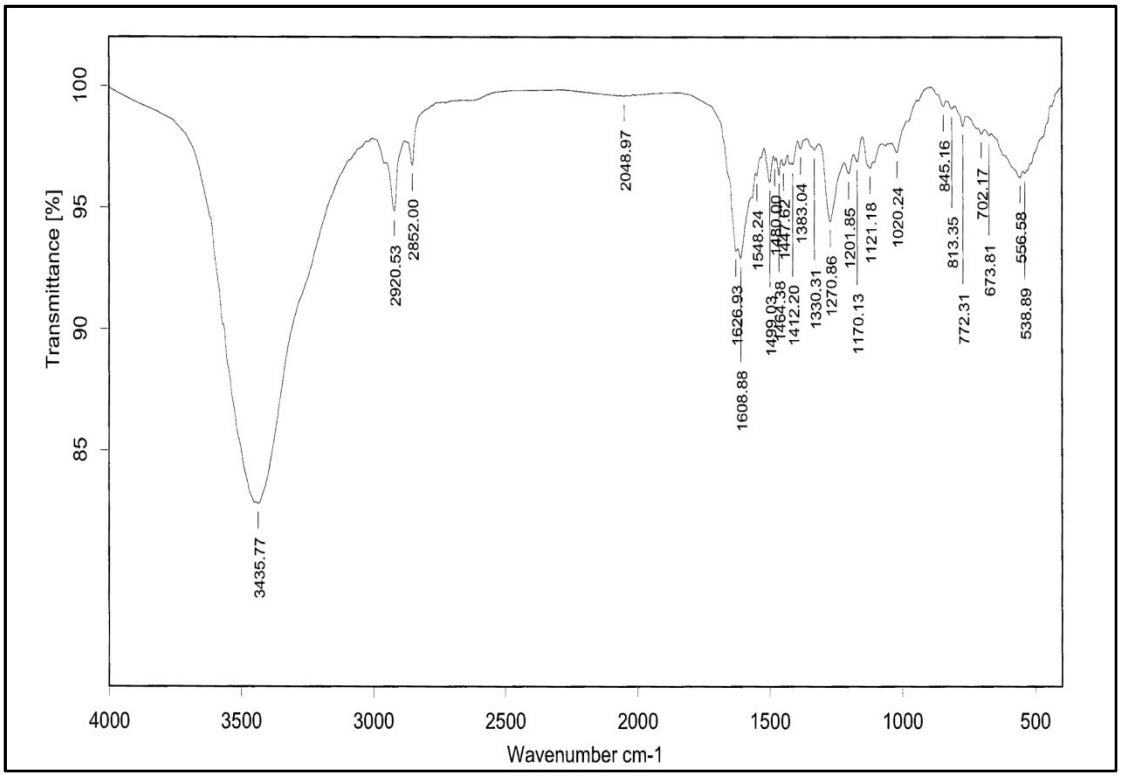

Figure 41 FT-IR spectrum of compound DAM2

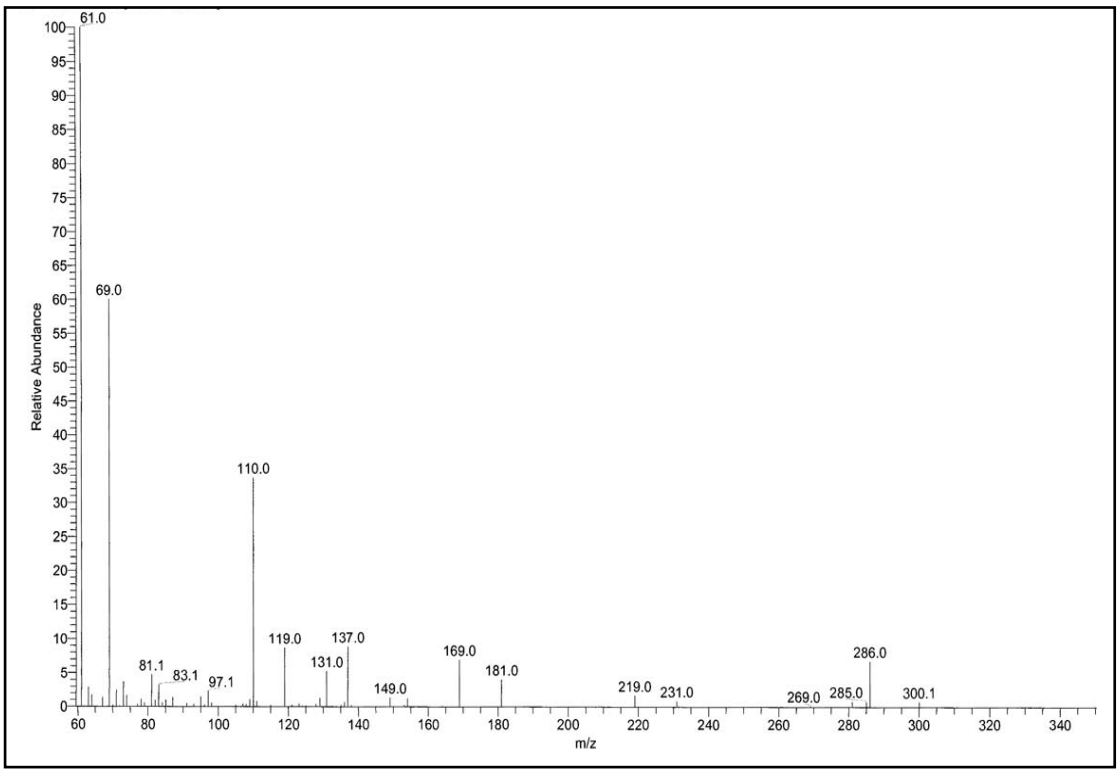

Figure 42 EIMS spectrum of compound DAM2

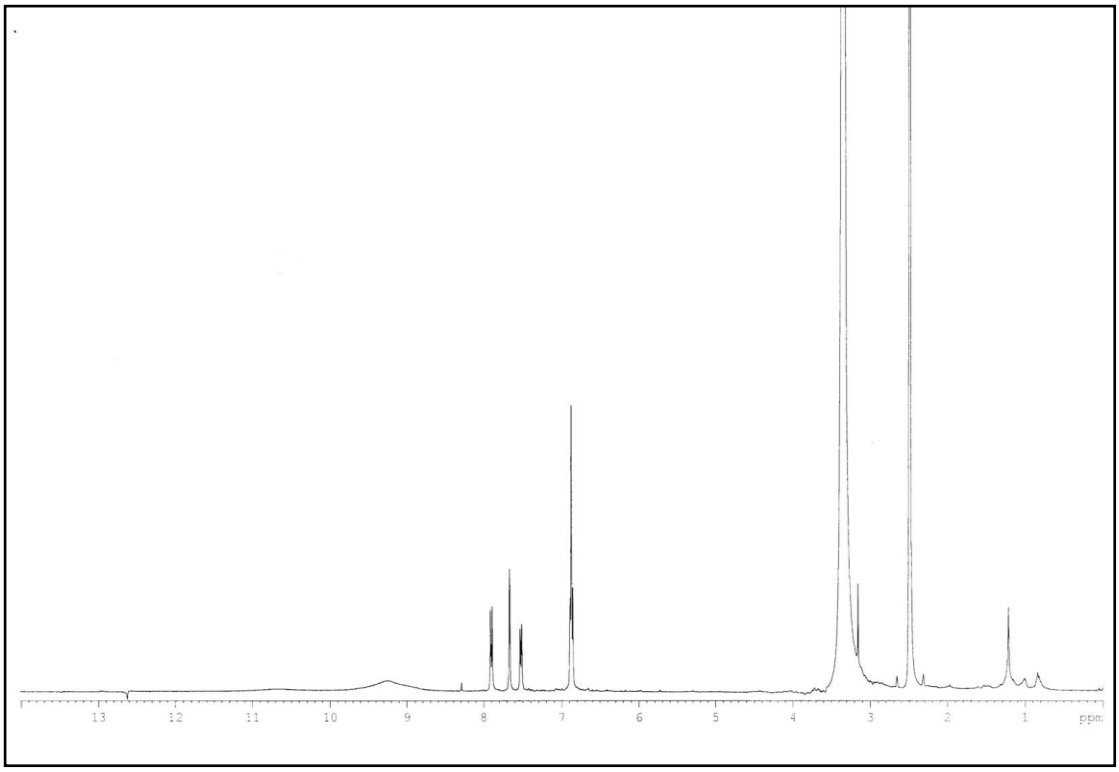

Figure 43 ^{1}H NMR (400 MHz) spectrum of compound DAM2 (DMSO-d_6)

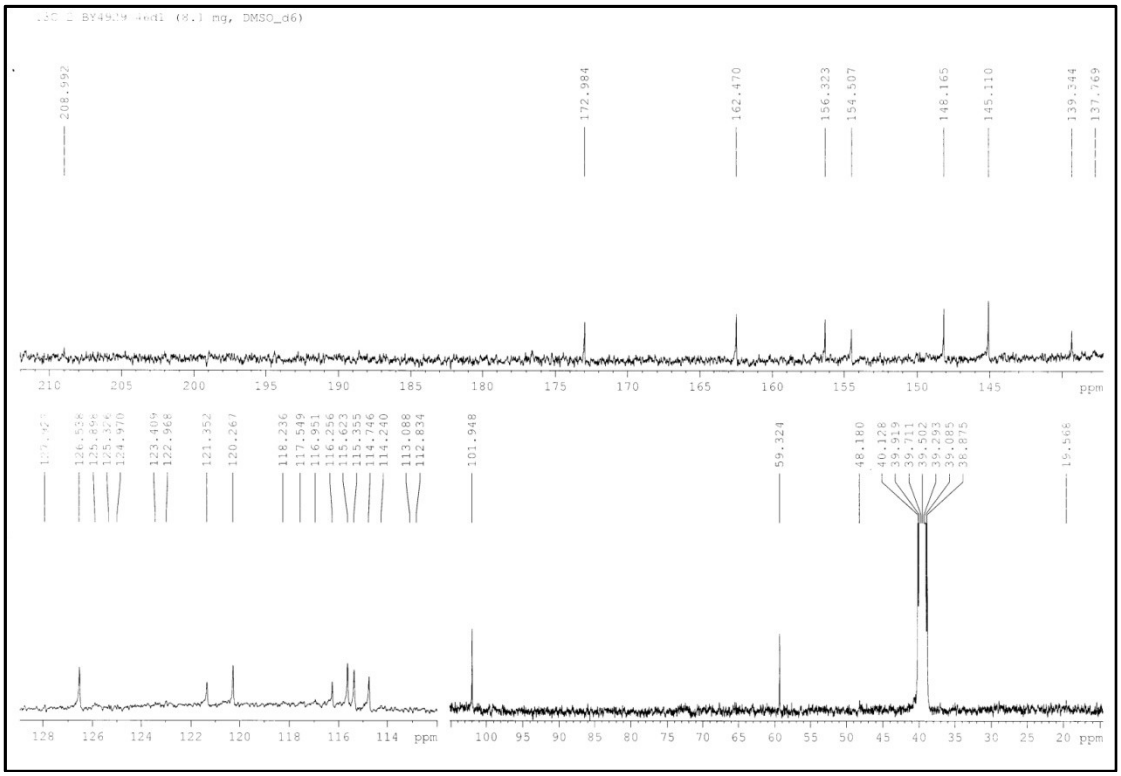

Figure 44 ^{13}C NMR spectrum of compound DAM2 (DMSO-d_6)

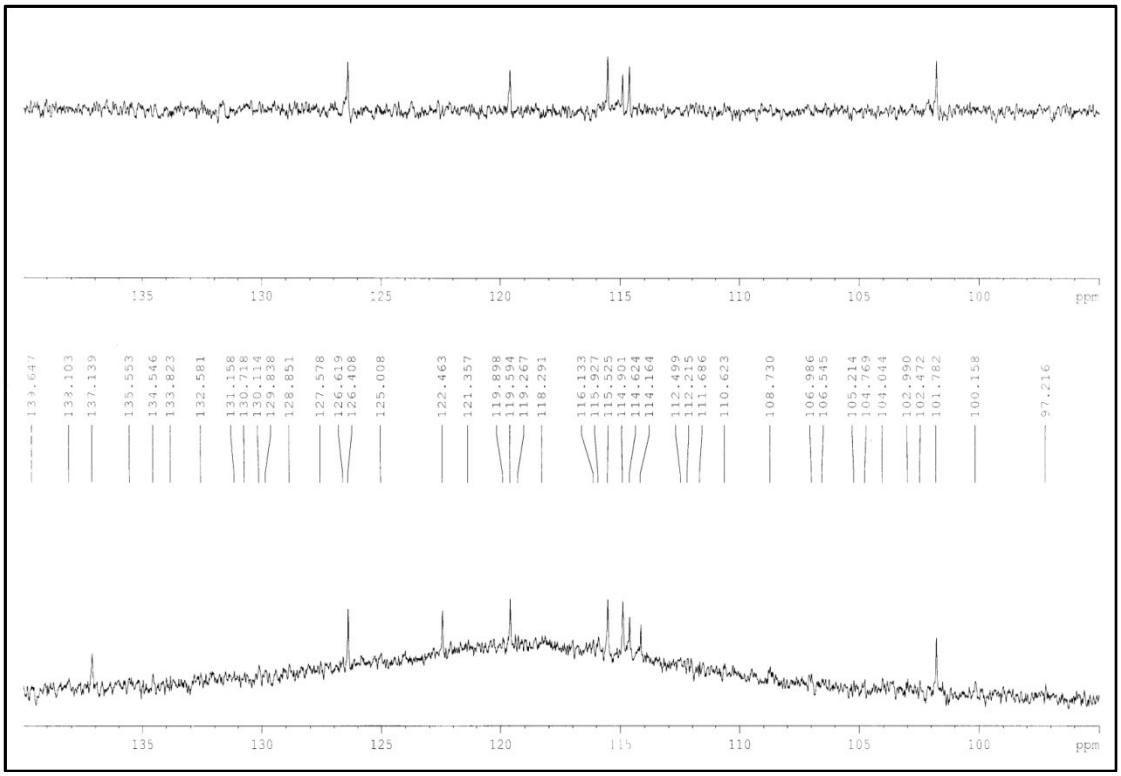

Figure 45 DEPT 135 spectrum of compound DAM2 (DMSO-d_6)

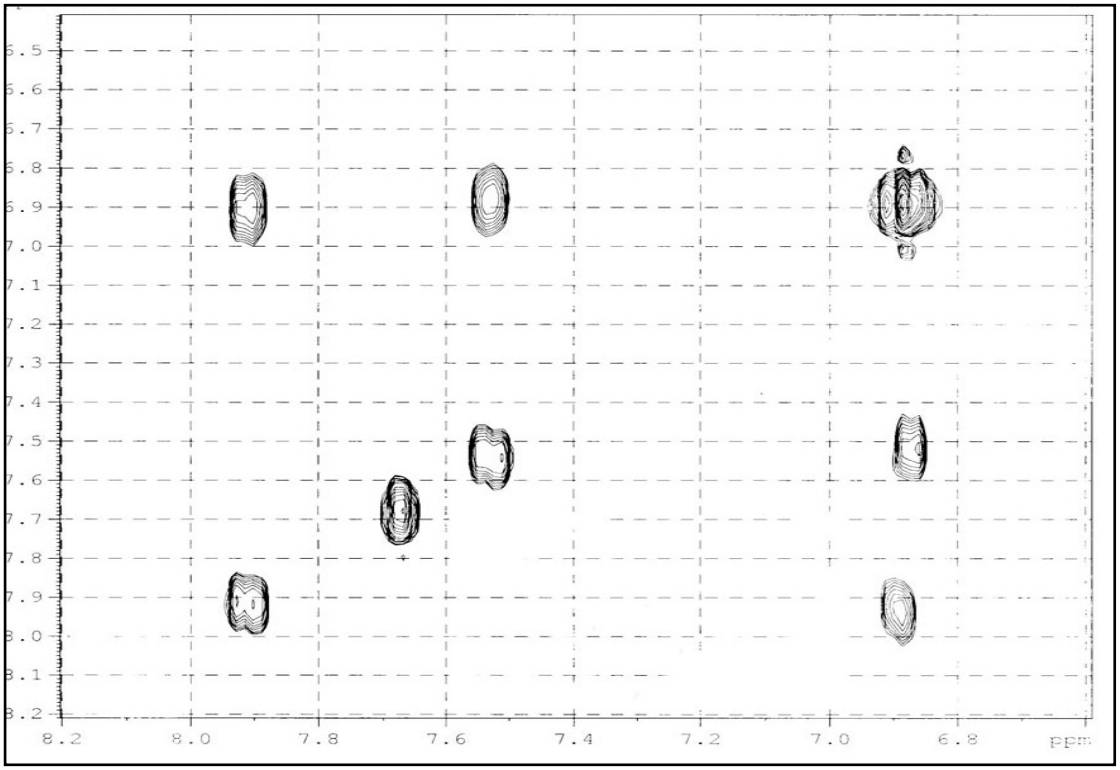

Figure 46 COSY spectrum of compound DAM2 (DMSO-d_6)

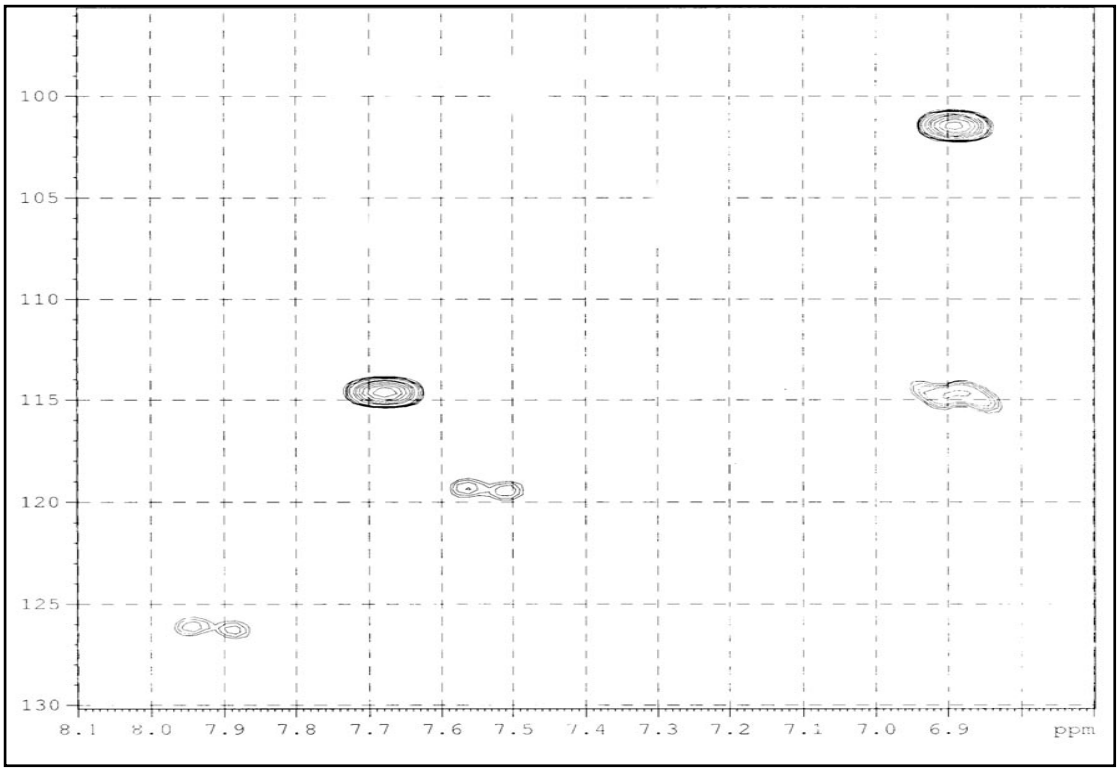

Figure 47 HMQC spectrum of compound DAM2 (DMSO-d_6)

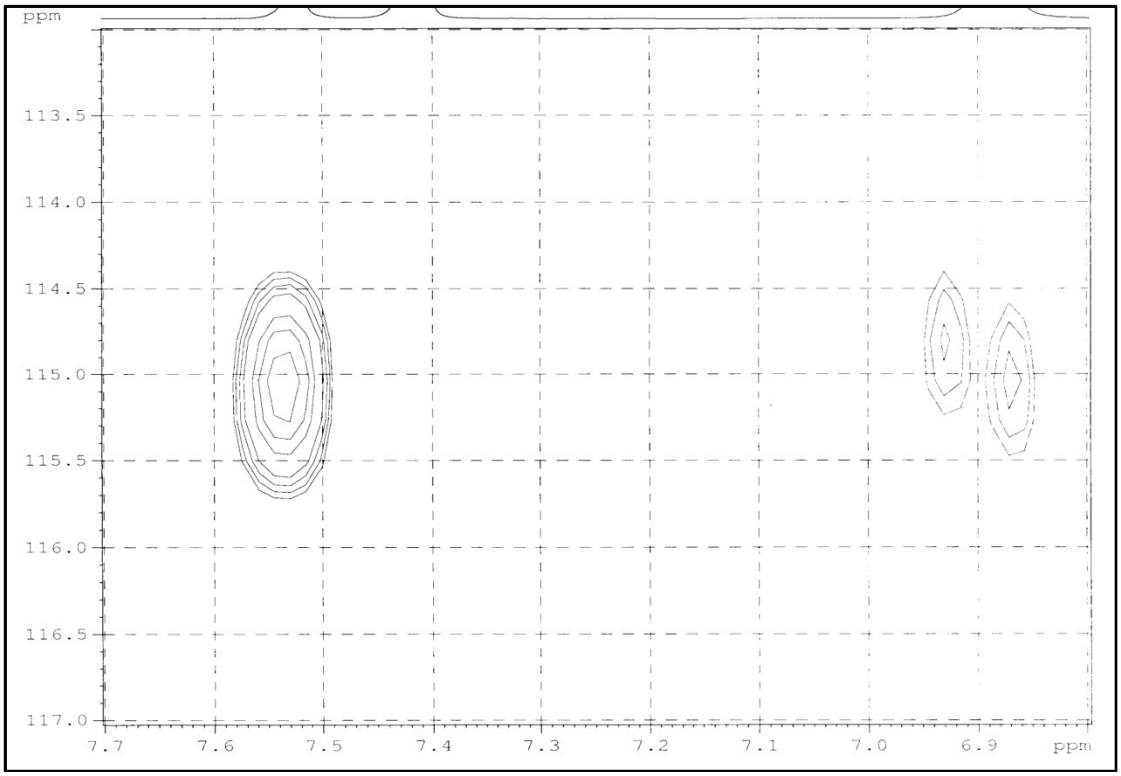

Figure 48 HMQC spectrum of compound DAM2 (DMSO-d_6)

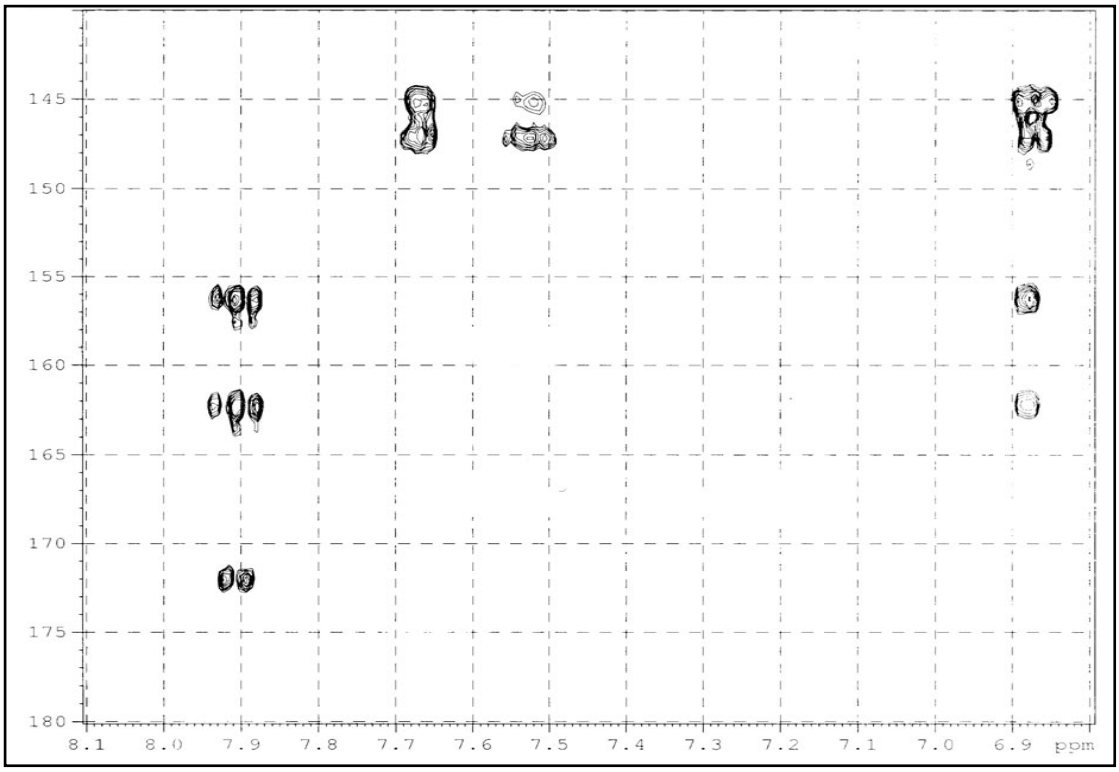

Figure 49 HMBC spectrum of compound DAM2 (DMSO-d_6)

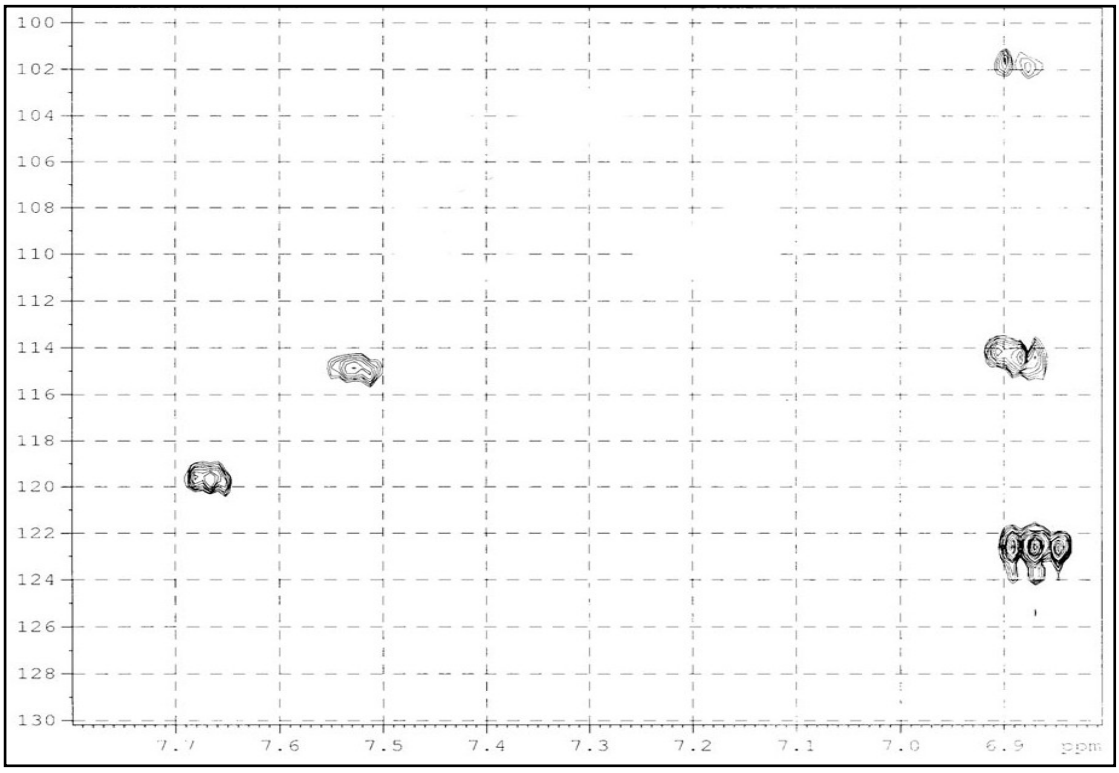

Figure 50 HMBC spectrum of compound DAM2 (DMSO-d_6)

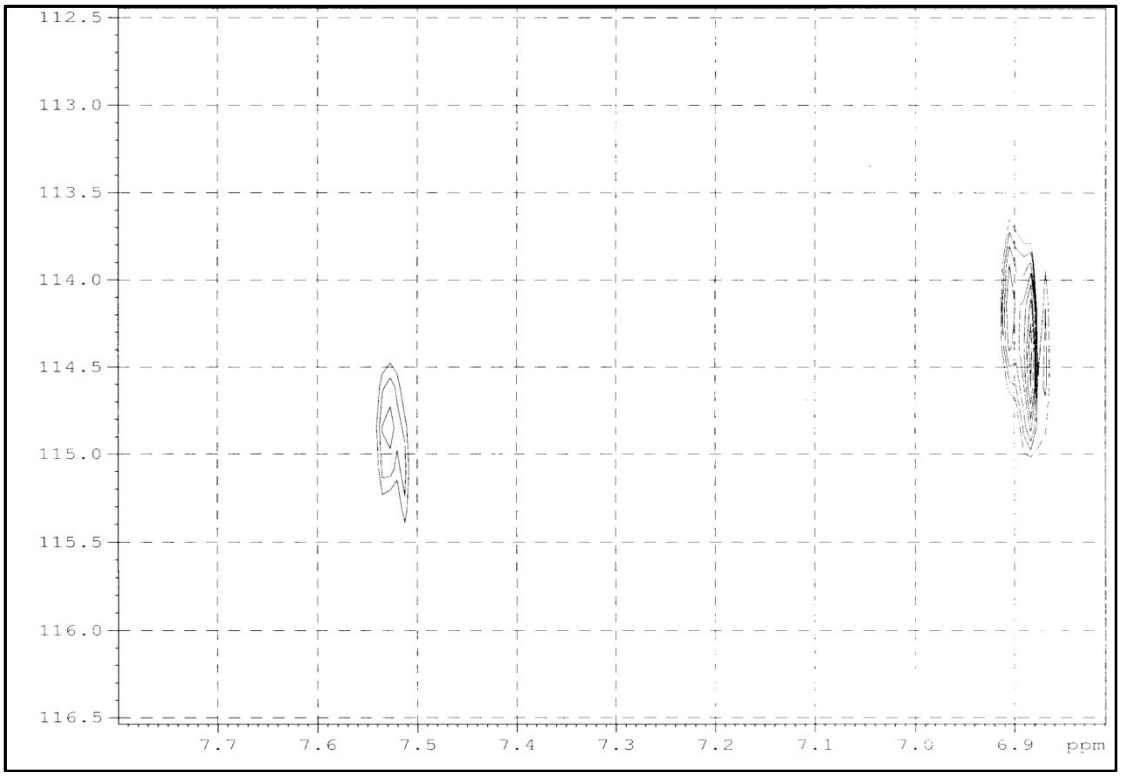

Figure 51 HMBC spectrum of compound DAM2 (DMSO-d_6)

VITAE

Name: Mr.Sonesay Thammavong

Student ID: 5411420004

Educational Attainment

Degree	Name of Institution	Year of Graduation
Bachelor (Pharmaceutical Sciences)	University of Health Science Lao PDR	2010

Scholarship Awards during Enrollments

Faculty of Traditional Thai Medicine, Prince of Songkla University, Thailand and Faculty of Pharmaceutical Sciences, University of Health Science, Lao PDR

Work-position and Address

Faculty of Pharmaceutical Sciences, University of Health Science Lao PDR

List of Publication and Proceeding

Joycharat N, **Thammavong S,** Limsuwan S, Homlaead S, Voravuthikunchai SP, Yingyongnarongkul BE, Dej-Adisai S, Subhadhirasakul S. 2013. Antibacterial substances from *Albizia myriophylla* wood against cariogenic *Streptococcus mutans*. *Arch Pharm Res.* (In press)