

องค์ประกอบทางเคมีของเนื้อไม้ต้นสาทร



นางสาว มนัสนันท์ ชาติสัมปັນน์

ศูนย์วิทยทรัพยากร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเกสัชศาสตรมหาบัณฑิต

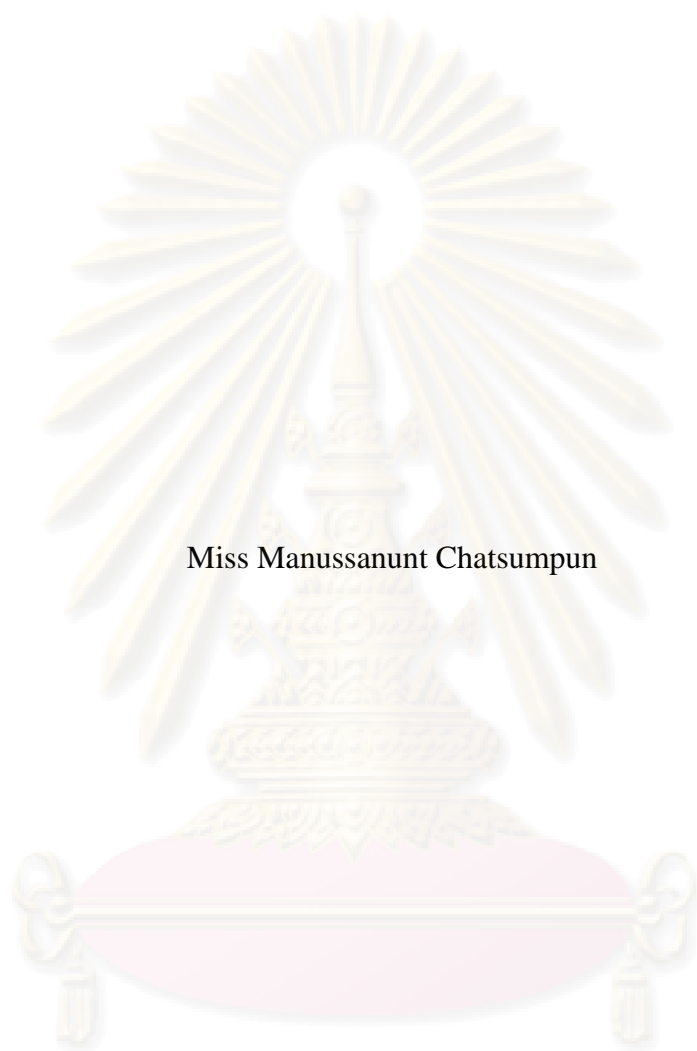
สาขาวิชาเภสัชเวท ภาควิชาเภสัชเวท

คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2551

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

CHEMICAL CONSTITUENTS OF *MILLETTIA LEUCANTHA* STEMWOOD



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A Thesis Submitted in Partial Fulfillment of the Requirements  
for the Degree of Master of Science in Pharmacy Program in Pharmacognosy

Department of Pharmacognosy  
Faculty of Pharmaceutical Sciences  
Chulalongkorn University

Academic Year 2008

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Thesis Title                      CHEMICAL CONSTITUENTS OF *MILLETTIA LEUCANTHA*  
STEMWOOD  
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มนัสนันท์ ซาคิสัมปັນน์ : องค์ประกอบทางเคมีของเนื้อไม้ต้นสาทร. (CHEMICAL CONSTITUENTS OF *MILLETTIA LEUCANTHA* STEMWOOD) อ. ที่ปริกษาวิทยานิพนธ์หลัก : รศ.ดร. กิตติศักดิ์ ลิขิตวิทยาวุฒิ, อ. ที่ปริกษาวิทยานิพนธ์ร่วม : ผศ.ดร. บุญชู ศรีตุลาภรณ์, 152 หน้า.

การศึกษาทางพฤกษเคมีของเนื้อไม้สาทร สามารถแยกสารบริสุทธิ์ 6 ชนิด ได้แก่ cycloeucaleanol, maackiain, 4-hydroxy-3-methoxybenzoic acid, syringic acid, balanocarpol และ diptoindonesin D นอกจากนี้ยังแยกได้สารผสมระหว่าง  $\beta$ -sitosterol กับ stigmasterol และสารผสมระหว่าง 7-oxositosterol กับ 7-oxostigmasterol การพิสูจน์โครงสร้างทางเคมีของสารที่แยกได้นี้อาศัยการวิเคราะห์สเปกตรัมของ UV, MS, NMR ร่วมกับการเปรียบเทียบข้อมูลของสารที่รายงานมาแล้ว และได้ทดสอบฤทธิ์ในการจับสารอนุมูลอิสระของสารที่แยกได้ พบว่า syringic acid มีฤทธิ์ปานกลางในการจับสารอนุมูลอิสระ

## ศูนย์วิทยทรัพยากร จุฬาลงกรณ์มหาวิทยาลัย

ภาควิชา.....เภสัชเวท..... ลายมือชื่อนิสิต..... Wissara Oritsart  
 สาขาวิชา.....เภสัชเวท..... ลายมือชื่ออ.ที่ปริกษาวิทยานิพนธ์หลัก..... Man  
 ปีการศึกษา.....2551..... ลายมือชื่ออ.ที่ปริกษาวิทยานิพนธ์ร่วม..... Boonchoo Sritulanab

## 4976589933 : MAJOR PHARMACOGNOSY

KEY WORD: *MILLETTIA LEUCANTHA*

MANUSSANUNT CHATSUMPUN : CHEMICAL CONSTITUENTS OF *MILLETTIA LEUCANTHA* STEMWOOD. THESIS PRINCIPAL ADVISOR : ASSOC. PROF. KITTISAK LIKHITWITAYAWUID, Ph.D., THESIS COADVISOR : ASST. PROF. BOONCHOO SRITULARAK, Ph.D., 152 pp.

Phytochemical study of the stemwood of *Millettia leucantha* Kurz led to the isolation of six pure compounds, including cycloeucaleanol, maackiain, 4-hydroxy-3-methoxybenzoic acid, syringic acid, balanocarpol and diptoindonesin D. In addition, a mixture of  $\beta$ -sitosterol and stigmasterol and a mixture of 7-oxositosterol and 7-oxostigmasterol were identified. The identification and structure determination of the isolated compounds were achieved by analysis of their spectroscopic data (UV, MS, NMR) in comparison with previously reported data. Evaluation of the free radical scavenging activity of the isolated compounds showed moderate activity for syringic acid.

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## Acknowledgements

The author would like to express her deepest gratitude to her thesis advisor, Associate Professor Dr. Kittisak Likhitwitayawuid of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for his valuable advice, useful guidance, endless support, concern, patience and encouragement throughout the course of this study.

The author wishes to express her sincere thanks to Assistant Professor Dr. Boochoo Sritularak of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, her thesis co-advisor, for his helpful advice, constant help and kindness.

The author wishes to express her thanks to the members of her thesis committee for their critical perusal and useful advice.

The author would like to thank the Graduate School of Chulalongkorn University for granting partial financial support to conduct this investigation.

The author would also like to thank all staff members of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for supplying chemicals and facilities.

The author is grateful to all students of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for unforgettable friendship and kindness.

Finally, the author wishes to express her infinite gratitude to her family for their love, understanding and encouragements.

ศูนย์วิทยทรัพยากร  
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## ABBREVIATIONS

$\alpha$	= Alpha
Acetone- $d_6$	= Deuterated acetone
$\beta$	= Beta
br	= Broad (for NMR spectra)
C	= Concentration
$^{\circ}\text{C}$	= Degree Celsius
$\text{CDCl}_3$	= Deuterated chloroform
$\text{CH}_2\text{Cl}_2$	= Dichloromethane
$^{13}\text{C}$ NMR	= Carbon-13 Nuclear Magnetic Resonance
cm	= Centimeter
1-D	= One dimensional (for NMR spectra)
2-D	= Two dimensional (for NMR spectra)
d	= Doublet (for NMR spectra)
dd	= Doublet of doublets (for NMR spectra)
DEPT	= Distortionless Enhancement by Polarization Transfer
$\delta$	= Chemical shift
EIMS	= Electron Impact Mass Spectrometry
ESIMS	= Electrospray Ionization Mass Spectrometry
EtOAc	= Ethyl acetate
FCC	= Flash Column Chromatography
g	= Gram
GF	= Gel Filtration Chromatography
$^1\text{H}$ -NMR	= Proton Nuclear Magnetic Resonance
HMBC	= $^1\text{H}$ -detected Heteronuclear Multiple Bond Correlation
HMQC	= $^1\text{H}$ -detected Heteronuclear Multiple Quantum Coherence
HPLC	= High Pressure Liquid Chromatography
Hz	= Hertz
IR	= Infrared
$\text{IC}_{50}$	= Concentration showing 50% inhibition
$J$	= Coupling constant
Kg	= Kilogram



L	=	Liter
$\mu\text{l}$	=	microliter
$\lambda_{\text{max}}$	=	Wavelength at maximal absorption
$\epsilon$	=	Molar absorptivity
$\text{M}^+$	=	Molecular ion
m	=	Multiplet (for NMR spectra)
MeOH	=	Methanol
mg	=	Milligram
$\mu\text{g}$	=	Microgram
MHz	=	Mega Hertz
ml	=	Milliliter
mm	=	Millimeter
MLPC	=	Medium Pressure Liquid Chromatography
$m/z$	=	Mass to charge ratio
MS	=	Mass spectrum
MW	=	Molecular weight
nm	=	Nanometer
NMR	=	Nuclear Magnetic Resonance
ppm	=	Part per million
s	=	Singlet (for NMR spectra)
spp.	=	Species
t	=	Triplet (for NMR spectra)
TLC	=	Thin Layer Chromatography
UV-VIS	=	Ultraviolet and Visible spectrophotometry
VLC	=	Vacuum Liquid Column Chromatography
$\nu_{\text{max}}$	=	Wave number at maximal absorption
$[\alpha]_{\text{D}}^{20}$	=	Specific rotation at 20°C and Sodium D line (589 nm)

## CHAPTER I

### INTRODUCTION

Plants of the genus *Millettia* are trees, shrubs, and climbers. They are members of Leguminosae family. Leaves are odd-pinnate with 3-10 pairs of opposite leaflets, often with tiny points (stipels) at base of leaflet stalks. Flowers are white or pink, in branched clusters (cymes) at leaf axils, with top petal curved sharply backwards. Calyx is wider than long with 5 short teeth, the 2 upper ones often united. Ten stamens are fused into a single sheath, sometimes with 1 stamen free from others, ovary without stalk. Pods are flattened, not veined or swollen over seeds, eventually splitting into 2 sections, but often not until after falling from the tree (Gardner *et al.*, 2000).

The species of *Millettia* in Thailand according to Smitinand (2001) are as follows.

<i>Millettia atropurpurea</i> Wall.	= <i>Collerya atropurpurea</i> (Wall.) Schott
<i>M. brandisiana</i> Kurz	กระพี้จั่น Kra phi chan, จั่น Phi chan, (General); ปี้จั่น Pi chan (Northern).
<i>M. caerulea</i> Baker	ปัวเปาะเตี๊ยะ Pua-po-do (Karen-Mae Hong Son); ผักเขี้ยวว้าว Phak yiao wua (Nakhon Sawan, Northern); หางไหลแดง Hang lai daeng (Kanchanaburi).
<i>M. decipiens</i> Prain	ปารี Pari (Malay-Narathiwat).
<i>M. extensa</i> Benth. ( <i>M. auriculata</i> Bak. var. <i>extensa</i> Benth)	ก้ำวเครีอ Kao khrua, กวาวเครีอ Kwao khrua (Chiang Mai); ตานครบ Tan krop (Lampang).
<i>M. glaucescens</i> Kurz	ยาดดา Yada (Malay-Narathiwat); หิยน้ำ Yi nam (Peninsular).

<i>M. kangensis</i> Craib	กระเจาะ Kra cho, ขะเจาะ Kha cho, ขะเจาะน้ำ Kha chon am (Chiang Mai).
<i>M. kityana</i> Craib	เครือข้าวเย็น Khrua khao yen, ลางเย็น Lang yen, ฮางจืด Hang chute, ฮางเย็น Hang yen (Northern).
<i>M. latifolia</i> Dunn	ขะเจาะ Kha cho (General).
<i>M. leucantha</i> Kurz var. <i>leucantha</i>	กะเขาะ Kaso (Central); กระเจาะ Kra cho, ขะเจาะ Khra cho (Northern); กระพีเขากวาย Kra phi khao khwai (Prachuap Kgori khan); ขะแมบ Kha maep, คำแมบ Kham maep (Chiang Mai).
<i>M. leucantha</i> Kurz var. <i>buteoides</i> (Gagnep.) P.K.Loc ( <i>M. buteoides</i> Gagnep.var. <i>siamensis</i> Craib, <i>M. pendula</i> Benth)	กระเจี๊ยะ Kra cho, ขะเจี๊ยะ Kha cho (Lampang); กระท่อน Kra thon (Phetchabun, Phisanulok); ไม้กระทงน้ำ ผัก Mai kra thong nam phak (Loei); สะท่อน Sa sathon (Saraburi); สาทร Sa thon (Ubon Ratchathani).
<i>M. macrostachya</i> Collett & Hemsl. var. <i>macrostachya</i>	ขะเจาะน้ำ Kha cho nam (Chiang Mai)
<i>M. macrostachya</i> Collett & Hemsl. var. <i>tecta</i>	ขะเจาะหลวง Kha cho luang, ขะเจาะใหญ่ Kha cho yai (Narathiwat).
<i>M. pachycarpa</i> Beth. Kurz	เถาะ Ke-tha (Karen-Chiang Mai); เครือไหล Kruea lai (Chiang Mai)
<i>M. peguensis</i> Ali ( <i>M. ovalifolia</i> Kurz)	ตอหี To-hi (Karen-Kanchanaburi).
<i>M. pulchra</i> Benth. Kurz	จันพอ Chan pho (Northern).

<i>M. racemosa</i> (Roxb.) Benth.	= <i>Endosamara racemosa</i> (Roxb.) R.Geesink
<i>M. sericea</i> (Vent.) Benth.	จ๋นไค้ชะ Cha-nai-kho, ปาตุ Pa-tu (Malay-Narathiwat); นอเราะ No-ro (Malay-Yala, Pattani); ยิมแมก๊ะ Yim- mae-ko (Malay-Yala); อ้อยสามสวน Oi sam suan (Nong Khai).
<i>M. thorelii</i> Gagnep.	= <i>Derris thorelii</i> Craib
<i>M. utilis</i> Dunn	สะท่อนน้ำผัก Sathon nam phak (Loei).
<i>M. xylocarpa</i> Miq. var. <i>tecta</i> ( <i>M. hemsleyana</i> Prain, <i>M.</i> <i>pubinervis</i> Kurz)	กะเจ๊ะ Ka che, ขะเจาะ Kho cho (General); คะแมด Kha maet (Chiang Mai); จักจัน Chakkachan (Loei); ฝึ้ง Phi phong (Phrae); ยะดา Ya-da (Malay-Yal); ไยยี Yai-yi (Karen-Mae Hong son); สาร Sa thon, หยี่น้ำ yin am (Pattai, Yala).

*Millettia leucantha* Kurz has a local name as ka so, kra cho, sa thon. It is a deciduous tree to 20 m, with 3 pairs of leaflets, 5-12 cm, abruptly tipped, completely smooth, stalks  $\pm$  4 mm with persistent narrow stipels. Flowers are  $\pm$  1.2 cm, white, smooth petals. Pods are 4-10 x 2 cm, woody, widest near top, rough with many lenticles, rounded edges (Gardner *et al.*, 2000).

A previous study on the ethanol extract of the stem bark of *M. leucantha* revealed the presence of flavones and chalcones. Some of these compounds possessed moderate cytotoxic activity, moderate anti-herpes simplex virus activity and significant anti-inflammatory effect inhibiting both cyclooxygenases-1 and -2 (Phrutivorapongkul *et al.*, 2003). This chemical investigation is focused on the constituents of the stemwood of *M. leucantha*.

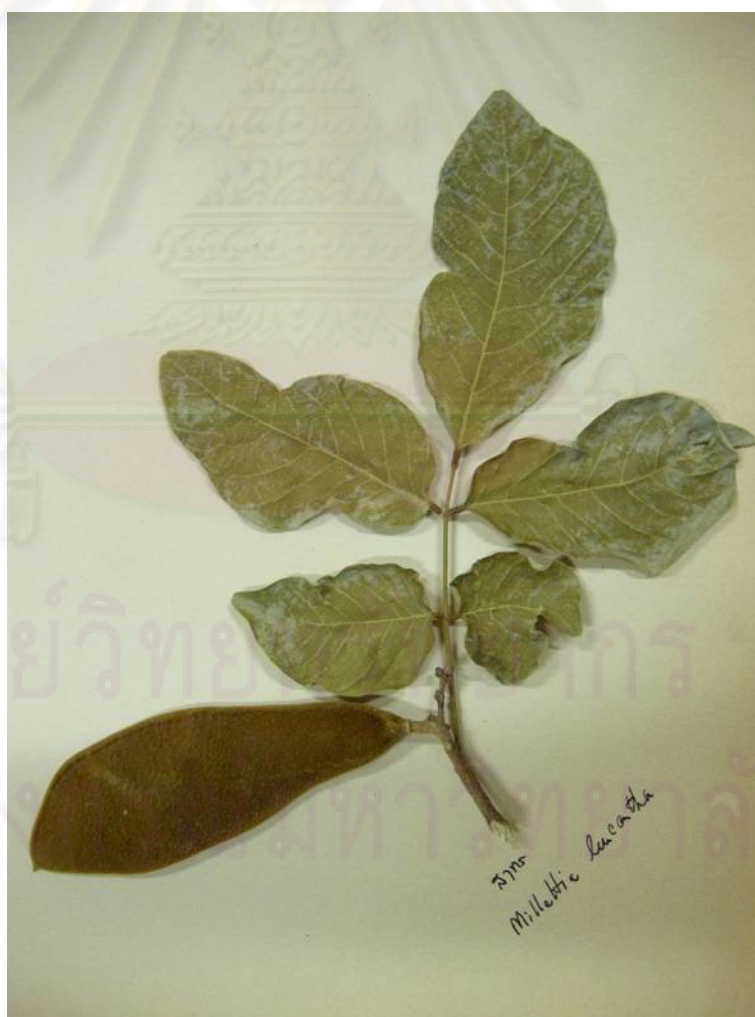
The main objectives in this study are as follows.

1. Isolation and purification of compounds from *M. leucantha* stemwood.
2. Determination of the chemical structure of each isolated compound.

3. Evaluation of each isolated compound for its free radical scavenging activity.



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**Figure 1** *Millettia leucantha* Kurz



## CHAPTER II

### HISTORICAL

#### 1. Chemical constituents of *Millettia* spp.

A number of chemical constituents isolated from the genus *Millettia* can be classified as flavonoids of various types. In addition, other classes of natural compounds such as terpenoids, coumarins and miscellaneous substances have been found (Table 1).

**Table 1 Distribution of chemical constituents in the genus *Millettia*.**

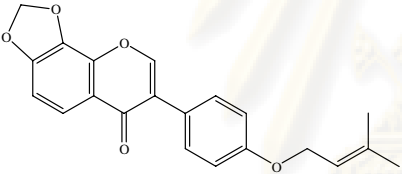
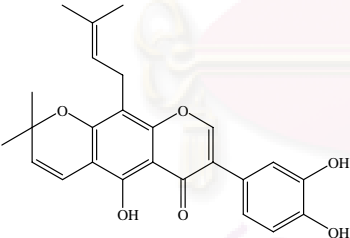
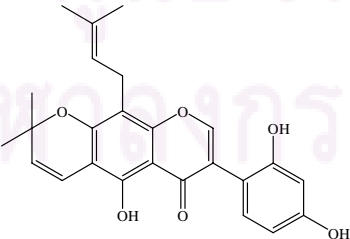
Plant and compound	Category	Plant part	Reference
<b><i>Millettia auriculata</i></b> Auricularin [1] 	Isoflavone	Root	Rao, Prasad and Ganapaty, 1992
Auriculasin [2] 	Isoflavone	Leaf	Minhaj <i>et al.</i> , 1976
Auriculatin [3] 	Isoflavone	Root	Shabbir <i>et al.</i> , 1968

Table 1 (continued)

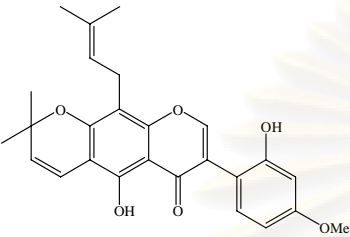
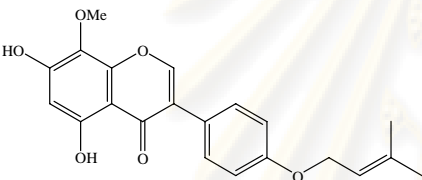
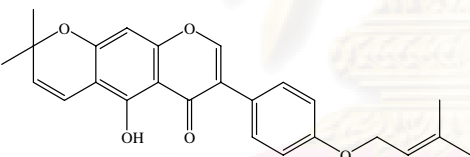
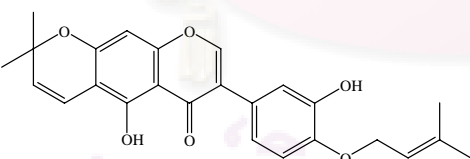
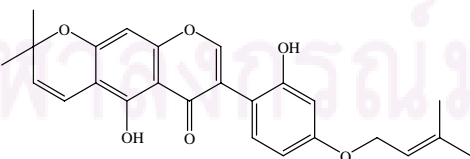
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia auriculata</i></b></p> <p><b>Auriculin [4]</b></p> 	Isoflavone	Root	Shabbir and Zaman, 1970
<p><b>Aurmillone [5]</b></p> 	Isoflavone	Seed	Raju and Srimannarayana, 1978
<p><b>2'-Deoxyisoauriculatin [6]</b></p> 	Isoflavone	Root	Rao, Prasad and Ganapaty, 1992
<p><b>Isoauriculasin [7]</b></p> 	Isoflavone	Leaf	Minhaj <i>et al.</i> , 1976
<p><b>Isoauriculatin [8]</b></p> 	Isoflavone	Root	Shabbir and Zaman, 1970

Table 1 (continued)

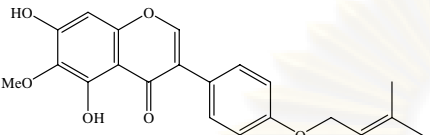
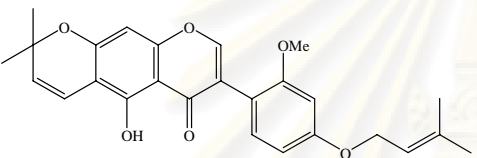
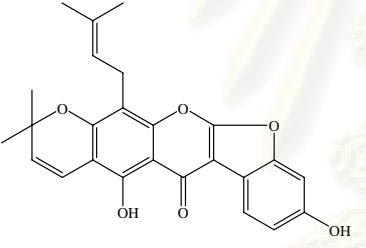
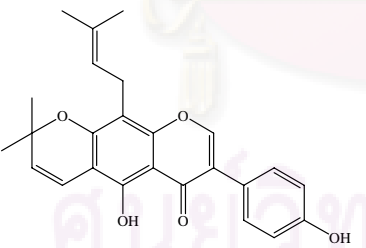
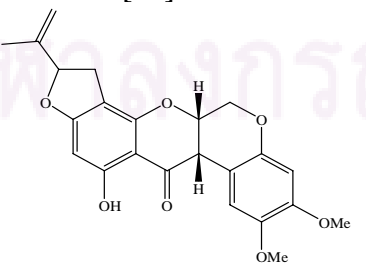
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia auriculata</i></b></p> <p>Isoaurmillone [9]</p> 	Isoflavone	Pod	Gupta <i>et al.</i> , 1983
<p>2'-<i>O</i>-Methyisoauriculatin [10]</p> 	Isoflavone	Root	Rao, Prasad and Ganapaty, 1992
<p>Millettin [11]</p> 	Isoflavone	Root	Rao, Prasad and Ganapaty, 1992
<p>Scandenone [12]</p> 	Isoflavone	Root	Rao, Prasad and Ganapaty, 1992
<p>Sumatrol [13]</p> 	Rotenoid	Root, Seed	Rao, Prasad and Ganapaty, 1992

Table 1 (continued)

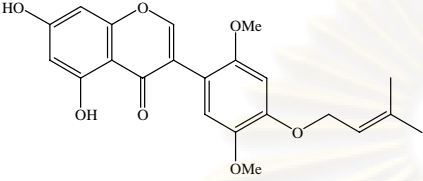
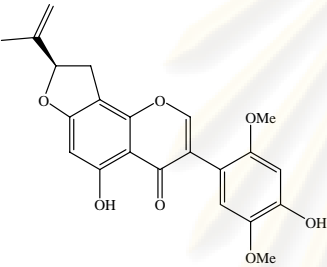
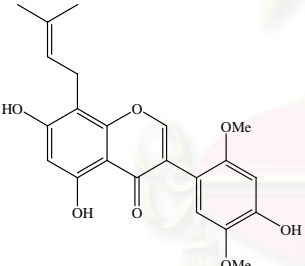
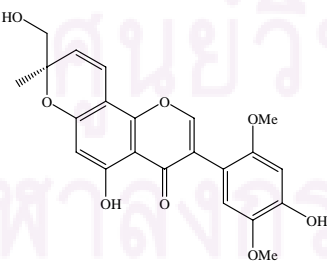
Plant and compound	Category	Plant part	Reference
<b><i>Millettia brandisiana</i></b>			
Brandisianin A [14] 	Isoflavone	Leaf	Kikuchi <i>et al.</i> , 2007
Brandisianin B [15] 	Isoflavone	Leaf	Kikuchi <i>et al.</i> , 2007
Brandisianin C [16] 	Isoflavone	Leaf	Kikuchi <i>et al.</i> , 2007
Brandisianin D [17] 	Isoflavone	Leaf	Kikuchi <i>et al.</i> , 2007

Table 1 (continued)

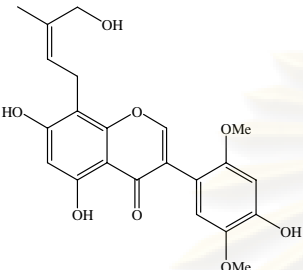
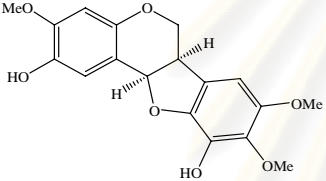
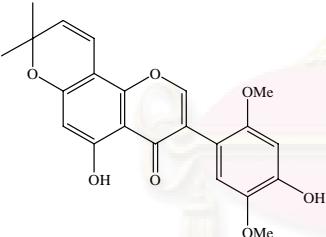
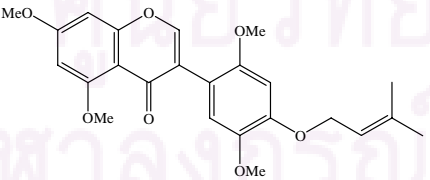
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia brandisiana</i></b></p> <p><b>Brandisianin E [18]</b></p> 	Isoflavone	Leaf	Kikuchi <i>et al.</i> , 2007
<p><b>Brandisianin F [19]</b></p> 	Pterocarpan	Leaf	Kikuchi <i>et al.</i> , 2007
<p><b>4'-Demethyltoxicarol isoflavone [20]</b></p> 	Isoflavone	Leaf	Kikuchi <i>et al.</i> , 2007
<p><b>4'-<math>\gamma,\gamma</math>-Dimethylallyloxy-5,7,2',5'-tetramethoxyisoflavone [21]</b></p> 	Isoflavone	Leaf	Pancharoen <i>et al.</i> , 2008

Table 1 (continued)

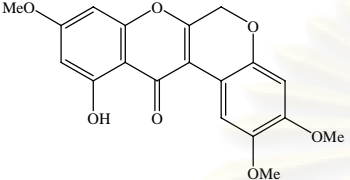
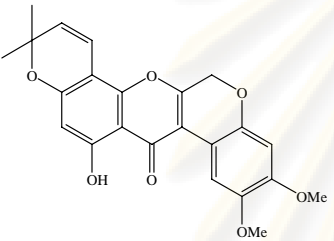
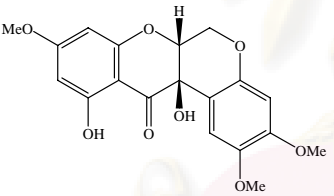
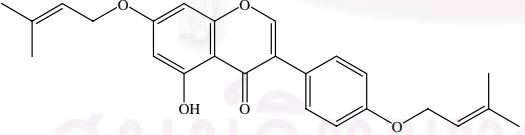
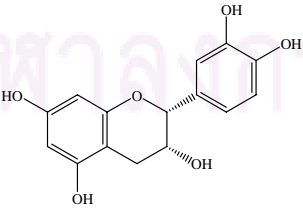
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia brandisiana</i></b></p> <p>6a,12a-Dehydrosermundone [22]</p> 	Rotenoid	Leaf	Pancharoen <i>et al.</i> , 2008
<p>6a,12a-Dehydro-<math>\alpha</math>-toxicarol [23]</p> 	Rotenoid	Leaf	Pancharoen <i>et al.</i> , 2008
<p>6-Deoxyclitoriactal [24]</p> 	Rotenoid	Leaf	Pancharoen <i>et al.</i> , 2008
<p>7,4'-Di-<i>O</i>-prenylgenistein [25]</p> 	Isoflavone	Leaf	Pancharoen <i>et al.</i> , 2008
<p>(-)-Epicatechin [26]</p> 	Flavan	Leaf	Kikuchi <i>et al.</i> , 2007



Table 1 (continued)

Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia brandisiana</i></b></p> <p>6-Hydroxy-6a,12a-dehydro-<math>\alpha</math>-toxicarol [27]</p>	Rotenoid	Leaf	Pancharoen <i>et al.</i> , 2008
<p>12a-Hydroxy-<math>\alpha</math>-toxicarol [28]</p>	Rotenoid	Leaf	Pancharoen <i>et al.</i> , 2008
<p>Naringenin [29]</p>	Flavanone	Leaf	Kikuchi <i>et al.</i> , 2007
<p>Olibergin A [30]</p>	Isoflavone	Leaf	Kikuchi <i>et al.</i> , 2007
<p>Robustigenin [31]</p>	Isoflavone	Leaf	Pancharoen <i>et al.</i> , 2008

Table 1 (continued)

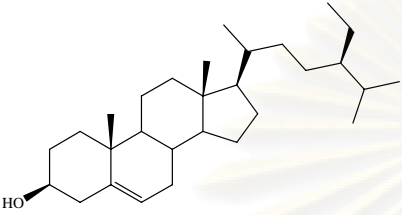
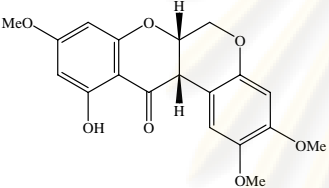
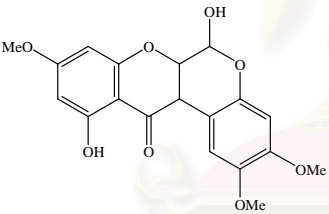

Plant and compound	Category	Plant part	Reference
<p><i>Millettia brandisiana</i></p> <p><math>\beta</math>-Sitosterol [32]</p> 	Steroid	Leaf	Pancharoen <i>et al.</i> , 2008
<p>Sermundone [33]</p> 	Rotenoid	Leaf	Pancharoen <i>et al.</i> , 2008
<p>Stemonal [34]</p> 	Rotenoid	Leaf	Pancharoen <i>et al.</i> , 2008
<p><math>\alpha</math>-Toxicarol [35]</p> 	Rotenoid	Leaf	Pancharoen <i>et al.</i> , 2008

Table 1 (continued)

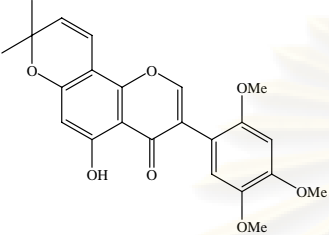
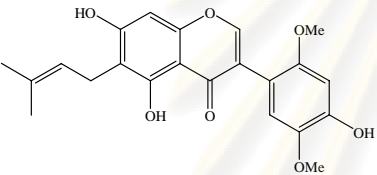
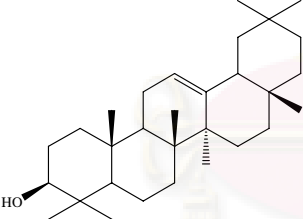
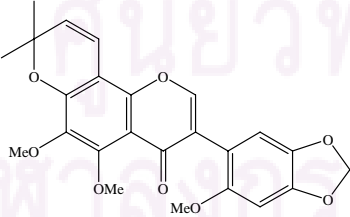
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia brandisiana</i></b></p> <p>Toxicarol isoflavone [36]</p> 	Isoflavone	Leaf	Pancharoen <i>et al.</i> , 2008
<p>Viridiflorin [37]</p> 	Isoflavone	Leaf	Kikuchi <i>et al.</i> , 2007, Pancharoen <i>et al.</i> , 2008
<p><b><i>Millettia conraui</i></b></p> <p><math>\beta</math>-amyrin [38]</p> 	Triterpenoid	Stem bark	Tchinda <i>et al.</i> , 2007
<p>Conrauinone A [39]</p> 	Isoflavone	Stem bark	Fuendjiep <i>et al.</i> , 1998a

Table 1 (continued)

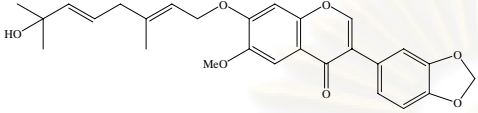
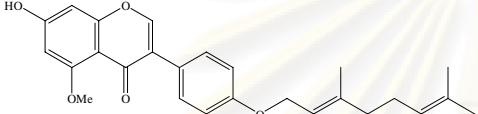
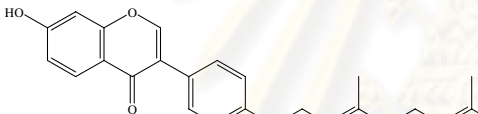
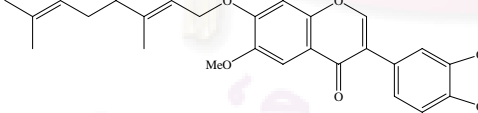
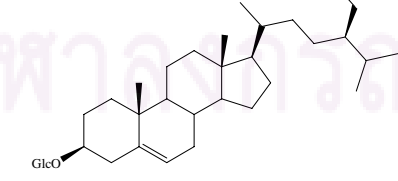
Plant and compound	Category	Plant part	Reference
<b><i>Millettia conraui</i></b>			
Conrauinone B [40] 	Isoflavone	Stem bark	Fuendjiep <i>et al.</i> , 1998a
Conrauinone C [41] 	Isoflavone	Stem bark	Fuendjiep <i>et al.</i> , 1998b
Conrauinone D [42] 	Isoflavone	Stem bark	Fuendjiep <i>et al.</i> , 1998b
n-Docosanol [43]	Alcohol	Stem bark	Tchinda <i>et al.</i> , 2007
7- <i>O</i> -Geranyl-6-methoxypseudo- baptigenin [44] 	Isoflavone	Stem bark	Tchinda <i>et al.</i> , 2007
3- <i>O</i> -β-D-Glucopyranosyl sitosterol [45] 	Steroid	Stem bark	Tchinda <i>et al.</i> , 2007

Table 1 (continued)

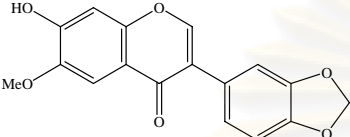
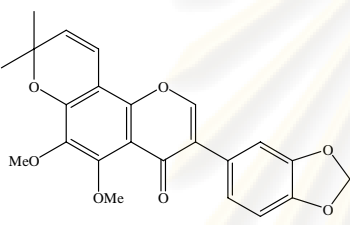
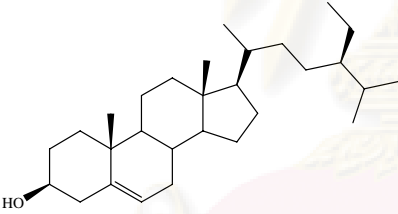
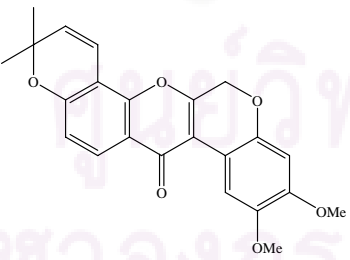
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia conraui</i></b></p> <p>7-Hydroxy-6-methoxy-3',4'-methylenedioxyisoflavone [46]</p> 	Isoflavone	Stem bark	Fuendjiep <i>et al.</i> , 1998b
<p>5-Methoxydurmillone [47]</p> 	Isoflavone	Stem bark	Fuendjiep <i>et al.</i> , 1998a
<p>Sitosterol [32]</p> 	Steroid	Stem bark	Tchinda <i>et al.</i> , 2007
<p><b><i>Millettia duchesnei</i></b></p> <p>6a,12a-Dehydrodeguelin [48]</p> 	Rotenoid	Twig	Ngandeu <i>et al.</i> , 2008

Table 1 (continued)

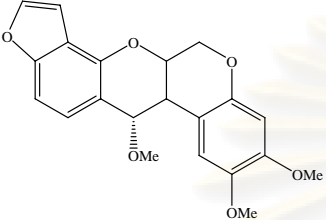
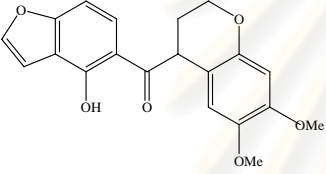
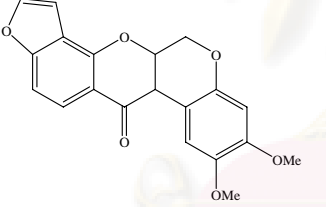
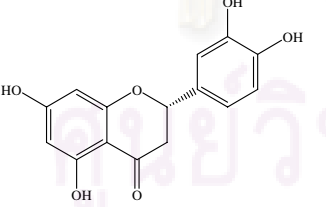
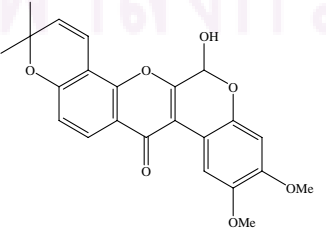
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia duchesnei</i></b></p> <p>12-Deoxo-12<math>\alpha</math>-methoxyelliptone [49]</p> 	Rotenoid	Twig	Ngandeu <i>et al.</i> , 2008
<p>Elliptol [50]</p> 	Rotenoid	Twig	Ngandeu <i>et al.</i> , 2008
<p>Elliptone [51]</p> 	Rotenoid	Twig	Ngandeu <i>et al.</i> , 2008
<p>Eriodictyol [52]</p> 	Flavanone	Twig	Ngandeu <i>et al.</i> , 2008
<p>6-Hydroxy-6a,12a-dehydrodeguelin [53]</p> 	Rotenoid	Twig	Ngandeu <i>et al.</i> , 2008

Table 1 (continued)

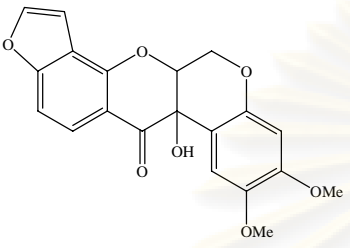
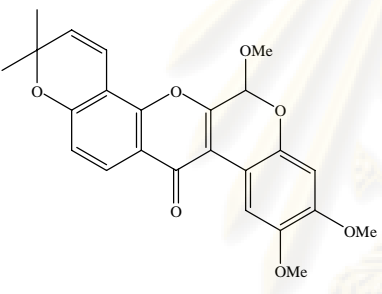
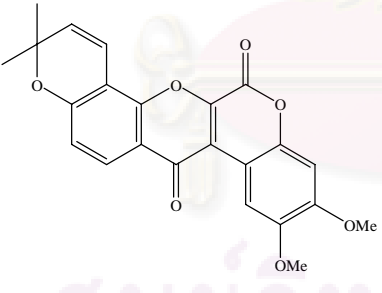
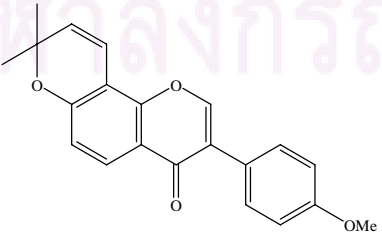
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia duchesnei</i></b></p> <p>12a-Hydroxyelliptone [54]</p>  <p>The structure shows a coumarin core with a furan ring fused at the 2-position. At the 3-position, there is a 2-hydroxy-2-(3,4-dimethoxyphenyl)ethyl side chain. The furan ring has a double bond between the 2 and 3 positions.</p>	Rotenoid	Twig	Ngandeu <i>et al.</i> , 2008
<p>6-Methoxy-6a,12a-dehydrodeguelin [55]</p>  <p>The structure is a rotenoid with a coumarin core and a furan ring fused at the 2-position. It features a 2-(3,4,5-trimethoxyphenyl)ethyl side chain at the 3-position and a methoxy group at the 6-position of the coumarin ring.</p>	Rotenoid	Twig	Ngandeu <i>et al.</i> , 2008
<p>6-Oxo-6a,12a-dehydrodeguelin [56]</p>  <p>The structure is similar to 6-methoxy-6a,12a-dehydrodeguelin, but instead of a methoxy group at the 6-position, it has a carbonyl group (=O).</p>	Rotenoid	Twig	Ngandeu <i>et al.</i> , 2008
<p><b><i>Millettia dura</i></b></p> <p>Calopogonium isoflavone A [57]</p>  <p>The structure is an isoflavone with a coumarin core and a furan ring fused at the 2-position. It has a 4-methoxyphenyl group attached to the 3-position of the coumarin ring.</p>	Isoflavone	Stem bark	Yenesew, Midiwo and Waterman, 1996

Table 1 (continued)

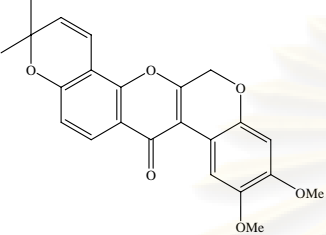
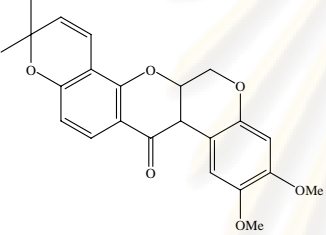
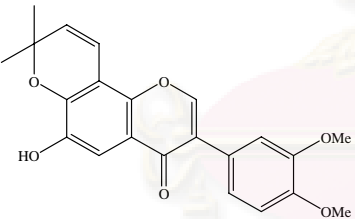
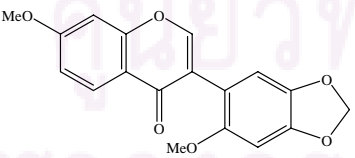
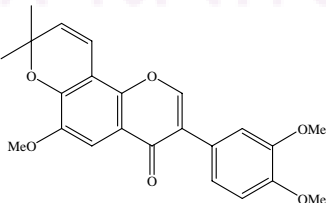
Plant and compound	Category	Plant part	Reference
<p><i>Millettia dura</i></p> <p>6a,12a-Dehydrodeguelin [48]</p> 	Rotenoid	Seed	Ollis, Rhodes and Sutherland, 1967
<p>Deguelin [58]</p> 	Rotenoid	Seed	Dagne, Mammo and Bekele, 1991
<p>6-Demethyldurallone [59]</p> 	Isoflavone	Seed pod	Yenesew, Midiwo and Waterman, 1996
<p>7,2'-Dimethoxy-4',5'-methylene-dioxyisoflavone [60]</p> 	Isoflavone	Stem bark, Root bark	Dagne, Mammo and Bekele, 1991
<p>Durallone [61]</p> 	Isoflavone	Seed pod	Yenesew, Midiwo and Waterman, 1996



Table 1 (continued)

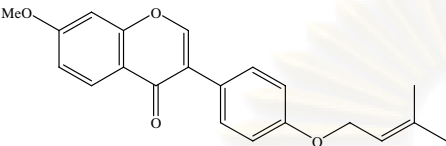
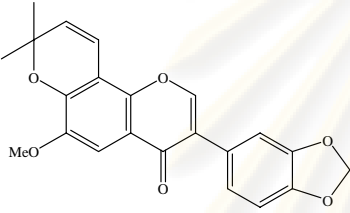
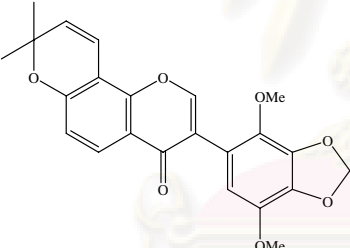
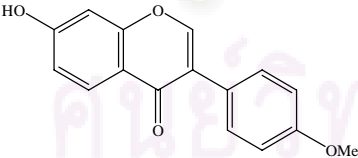
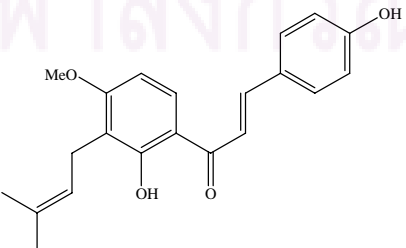
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia dura</i></b></p> <p>Durlettone [62]</p> 	Isoflavone	Seed	Ollis, Rhodes and Sutherland, 1967
<p>Durmillone [63]</p> 	Isoflavone	Seed, Stem bark	Ollis, Rhodes and Sutherland, 1967 Yenesew, Midiwo and Waterman, 1996
<p>Ferrugone [64]</p> 	Isoflavone	Seed pod	Yenesew, Midiwo and Waterman, 1997
<p>Formononetin [65]</p> 	Isoflavone	Seed pod	Yenesew, Midiwo and Waterman, 1997
<p>4-Hydroxyderricin [66]</p> 	Chalcone	Stem bark, Root bark	Dagne, Mammo and Bekele, 1991

Table 1 (continued)

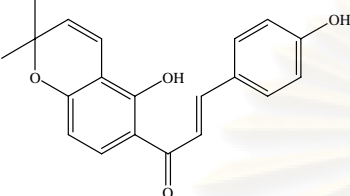
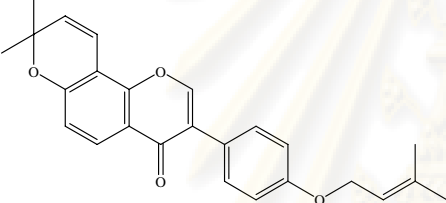
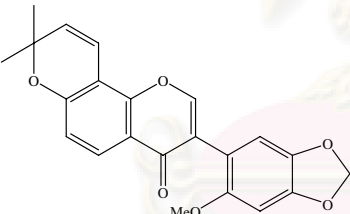
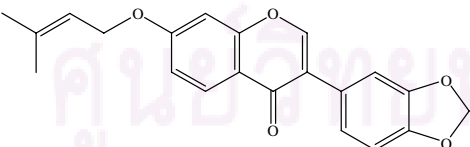
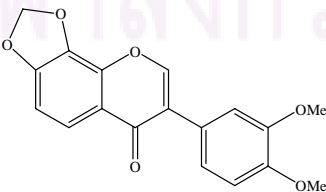
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia dura</i></b></p> <p>4-Hydroxylonchocarpin [67]</p> 	Chalcone	Stem bark, Root bark	Dagne, Mammo and Bekele, 1991
<p>Isoerythrin-A-4'-(3-methylbut-2-enyl) ether [68]</p> 	Isoflavone	Seed pod	Yenesew, Midiwo and Waterman, 1996
<p>Jamaicin [69]</p> 	Isoflavone	Seed pod	Yenesew, Midiwo and Waterman, 1997
<p>Maximaisoflavone B [70]</p> 	Isoflavone	Stem bark	Dagne, Mammo and Bekele, 1991
<p>Maximaisoflavone D [71]</p> 	Isoflavone	Stem bark	Yenesew, Midiwo and Waterman, 1996

Table 1 (continued)

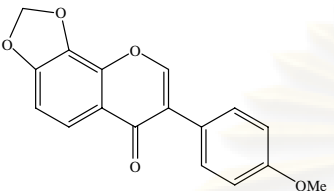
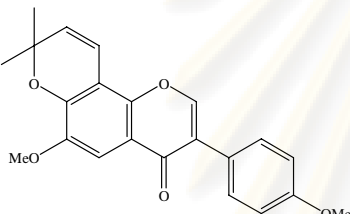
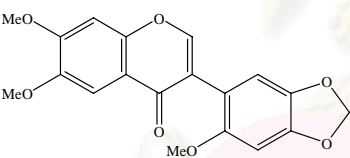
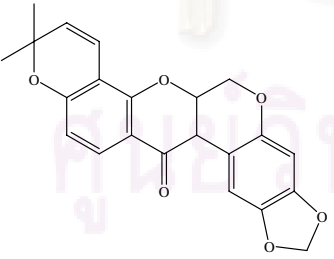
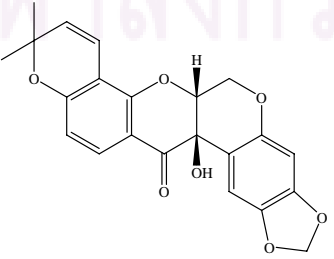
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia dura</i></b></p> <p>Maximaisoflavone H [72]</p> 	Isoflavone	Stem bark	Yenesew, Midiwo and Waterman, 1996
<p>6-Methoxycalopogonium isoflavone A [73]</p> 	Isoflavone	Seed pod	Yenesew, Midiwo and Waterman, 1997
<p>Milldurone [74]</p> 	Isoflavone	Seed	Ollis, Rhodes and Sutherland, 1967
<p>Millettone [75]</p> 	Rotenoid	Seed	Ollis, Rhodes and Sutherland, 1967
<p>Millettosin [76]</p> 	Rotenoid	Seed	Ollis, Rhodes and Sutherland, 1967

Table 1 (continued)

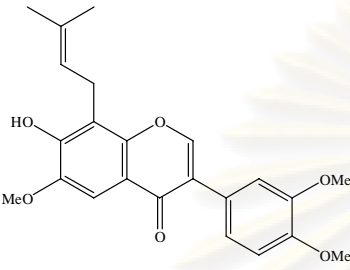
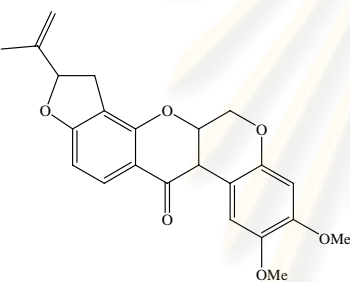
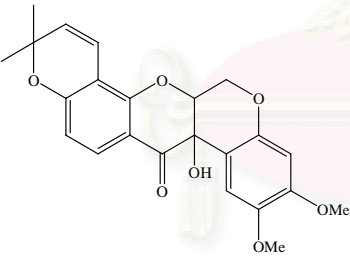
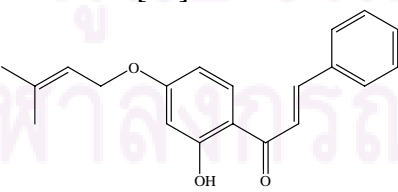
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia dura</i></b></p> <p>Predurrallone [77]</p> 	Isoflavone	Seed pod	Yenesew, Midiwo and Waterman, 1996
<p>Rotenone [78]</p> 	Rotenoid	Seed	Ollis, Rhodes and Sutherland, 1967
<p>Tephrosin [79]</p> 	Rotenoid	Seed, Seed pod	Ollis, Rhodes and Sutherland, 1967 Yenesew, Midiwo and Waterman, 1997
<p><b><i>Millettia erythrocalyx</i></b></p> <p>Derricidin [80]</p> 	Chalcone	Stem bark, Root	Sritularak <i>et al.</i> , 2002a Sritularak <i>et al.</i> , 2002b

Table 1 (continued)

Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia erythrocalyx</i></b></p> <p>6,3'-Dimethoxy-[2'',3'':7,8]-furanoflavone [81]</p>	Flavone	Pod	Sritularak and Likhitwitayawuid, 2006
<p>3',5'-Dimethoxy-[2'',3'' : 7,8]-furanoflavone [82]</p>	Flavone	Leaf	Likhitwitayawuid <i>et al.</i> , 2005
<p>2,5-Dimethoxy-4-hydroxy-[2'',3'':7,8]-furanoflavan [83]</p>	Flavan	Root	Sritularak <i>et al.</i> , 2002b
<p>2',3-Dihydroxy-4-methoxy-4'-<math>\gamma,\gamma</math>-dimethylallyloxychalcone [84]</p>	Chalcone	Pod	Sritularak and Likhitwitayawuid, 2006
<p>7-<math>\gamma,\gamma</math>-Dimethylallyloxyflavanone [85]</p>	Flavanone	Stem bark	Sritularak <i>et al.</i> , 2002a

Table 1 (continued)

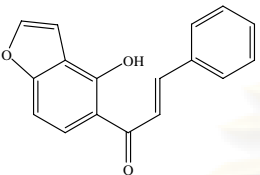
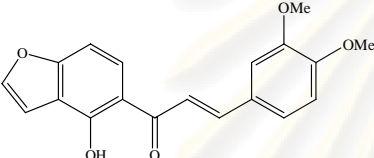
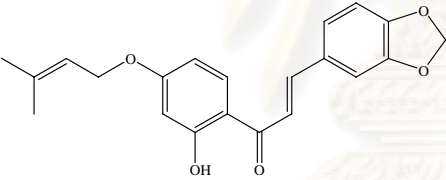
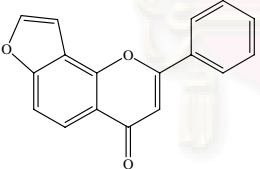
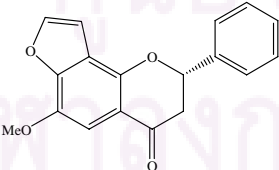
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia erythrocalyx</i></b></p> <p>1-(4-Hydroxy-5-benzofuranyl)-3-phenyl-2-propen-1-one [86]</p> 	Chalcone	Root	Sritularak <i>et al.</i> , 2002b
<p>2'-Hydroxy-3,4-dimethoxy-[2'',3'':4',3']-furanochalcone [87]</p> 	Chalcone	Pod	Sritularak and Likhitwitayawuid, 2006
<p>2'-Hydroxy-3,4-methylenedioxy-4'-<math>\gamma</math>, <math>\gamma</math>-dimethylallyloxychalcone [88]</p> 	Chalcone	Stem bark, Pod	Sritularak <i>et al.</i> , 2002a Sritularak and Likhitwitayawuid, 2006
<p>Lanceolatin B [89]</p> 	Flavone	Root	Sritularak <i>et al.</i> , 2002b
<p>6-Methoxy-[2'',3'':7,8]-furanoflavanone [90]</p> 	Flavanone	Root	Sritularak <i>et al.</i> , 2002b

Table 1 (continued)

Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia erythrocalyx</i></b></p> <p>3',4'-Methylenedioxy-[2'',3'':7,8]-furanoflavonol [91]</p>	Flavonol	Pod	Sritularak and Likhitwitayawuid, 2006
<p>3,4-Methylenedioxy-2',4'-dimethoxy-chalcone [92]</p>	Chalcone	Root	Sritularak <i>et al.</i> , 2002b
<p>3',4'-Methylenedioxy-6,7-dimethoxyflavone or milletenin C [93]</p>	Flavone	Stem bark, Leaf	Sritularak <i>et al.</i> , 2002a Likhitwitayawuid <i>et al.</i> , 2005
<p>3',4'-Methylenedioxy-7-methoxyflavone [94]</p>	Flavone	Stem bark, Leaf	Sritularak <i>et al.</i> , 2002a Likhitwitayawuid <i>et al.</i> , 2005
<p>Milletenone [95]</p>	Chalcone	Stem bark, Root	Sritularak <i>et al.</i> , 2002a Sritularak <i>et al.</i> , 2002b
<p>Millettocalyxin A [96]</p>	Flavone	Stem bark	Sritularak <i>et al.</i> , 2002a

Table 1 (continued)

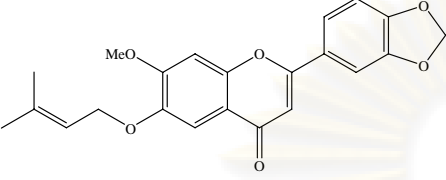
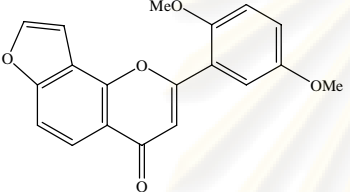
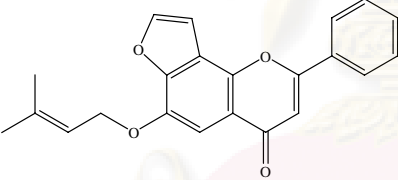
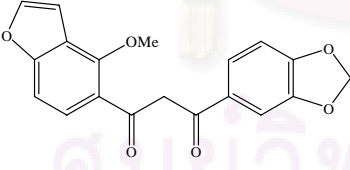
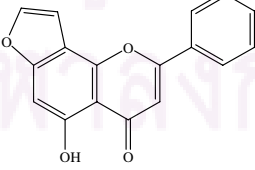
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia erythrocalyx</i></b></p> <p>Millettocalyxin B [97]</p> 	Flavone	Stem bark	Sritularak <i>et al.</i> , 2002a
<p>Millettocalyxin C [98]</p> 	Flavone	Stem bark, Pod	Sritularak <i>et al.</i> , 2002a Sritularak and Likhitwitayawuid, 2006
<p>Ovalifolin [99]</p> 	Flavone	Stem bark, Pod	Sritularak <i>et al.</i> , 2002a Sritularak and Likhitwitayawuid, 2006
<p>Ovalitenone [100]</p> 	Chalcone	Root	Sritularak <i>et al.</i> , 2002b
<p>Pongaglabol [101]</p> 	Flavone	Root	Sritularak <i>et al.</i> , 2002b



Table 1 (continued)

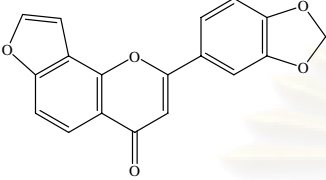
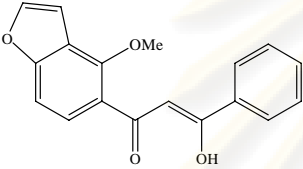
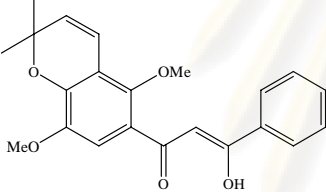
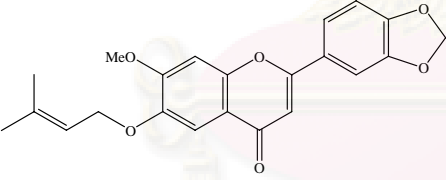
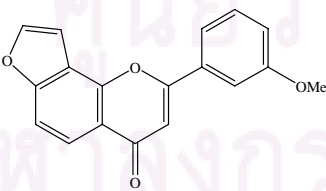
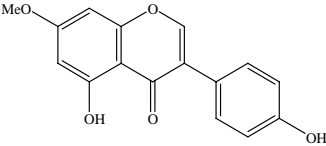
Plant and compound	Category	Plant part	Reference
<b><i>Millettia erythrocalyx</i></b>			
Pongaglabrone [102] 	Flavone	Stem bark, Leaf	Sritularak <i>et al.</i> , 2002a Likhitwitayawuid <i>et al.</i> , 2005
Pongamol [103] 	Chalcone	Root	Sritularak <i>et al.</i> , 2002b
Ponganone I [104] 	Chalcone	Stem bark, Root	Sritularak <i>et al.</i> , 2002a Sritularak <i>et al.</i> , 2002b
Ponganone V [105] 	Flavanone	Root	Sritularak <i>et al.</i> , 2002b
Pongol methyl ether [106] 	Flavone	Stem bark, Pod	Sritularak <i>et al.</i> , 2002a Sritularak and Likhitwitayawuid, 2006
Prunetin [107] 	Isoflavone	Stem bark	Sritularak <i>et al.</i> , 2002a

Table 1 (continued)

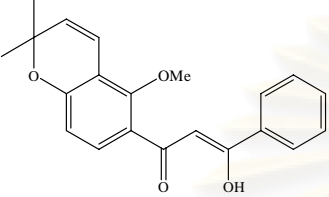
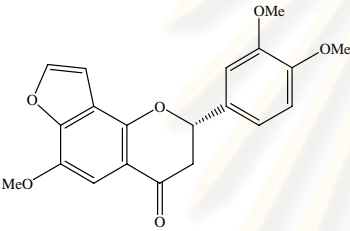
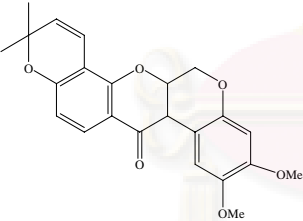
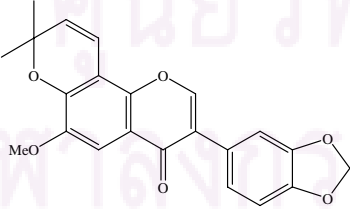
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia erythrocalyx</i></b></p> <p>Purpurenone [108]</p>  <p>(-)-(2<i>S</i>)-6,3',4'-Trimethoxy-[2'',3'':7,8]-furanoflavanone [109]</p> 	<p>Chalcone</p> <p>Flavanone</p>	<p>Root</p> <p>Pod</p>	<p>Sritularak <i>et al.</i>, 2002b</p> <p>Sritularak and Likhitwitayawuid, 2006</p>
<p><b><i>Millettia ferruginea</i></b></p> <p>Deguelin [58]</p>  <p>Durmillone [63]</p> 	<p>Rotenoid</p> <p>Isoflavone</p>	<p>Seed</p> <p>Seed</p>	<p>Hight and Hight, 1967</p> <p>Hight and Hight, 1967</p>



Table 1 (continued)

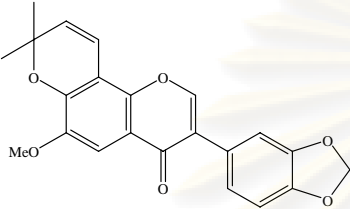
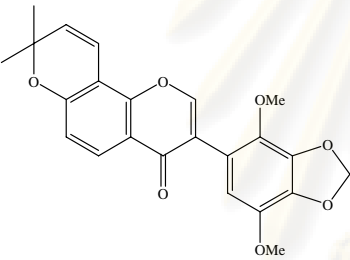
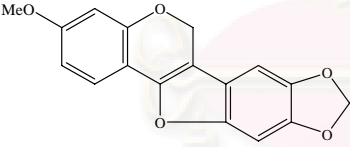
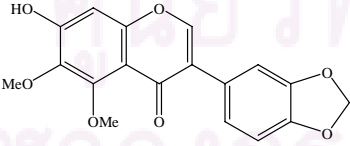
Plant and compound	Category	Plant part	Reference
<b><i>Millettia ferruginea</i> subsp.</b>			
<b><i>darassana</i></b>			
Durmillone [63] 	Isoflavone	Seed pod,	Dagne, Bekele and Waterman, 1989
Ferrugone [64] 	Isoflavone	Stem bark,	Dagne, Bekele and Waterman, 1989
Flemichapparin B [111] 	Pterocarpene	Stem bark	Dagne, Bekele and Waterman, 1989
7-Hydroxy-5,6-dimethoxy-3',4'-methylenedioxyisoflavone [112] 	Isoflavone	Stem bark	Dagne, Bekele and Waterman, 1989

Table 1 (continued)

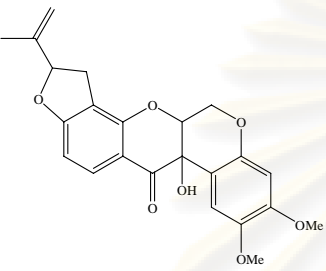
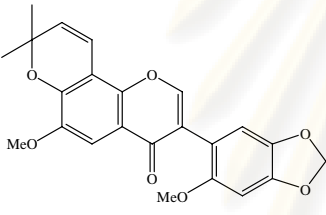
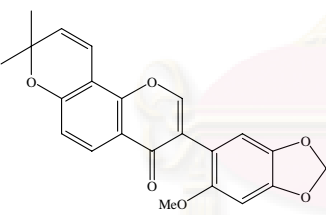
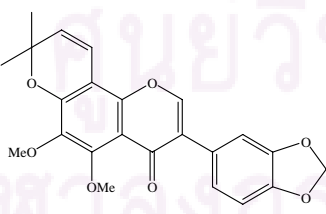
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia ferruginea</i> subsp. <i>darassana</i></b></p> <p>12a-Hydroxyrotenone [113]</p> 	Rotenoid	Seed	Dagne and Bekele, 1990
<p>Ichthynone [114]</p> 	Isoflavone	Stem bark	Dagne, Bekele and Waterman, 1989
<p>Jamaicin [69]</p> 	Isoflavone	Stem bark	Dagne, Bekele and Waterman, 1989
<p>5-Methoxydurmillone [47]</p> 	Isoflavone	Stem bark	Dagne, Bekele and Waterman, 1989 Dagne and Bekele, 1990

Table 1 (continued)

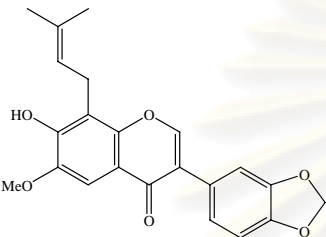
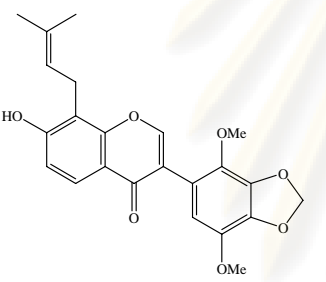
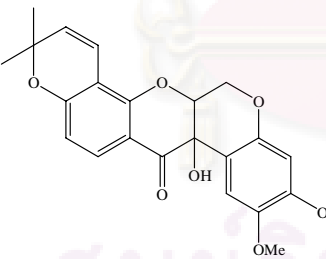
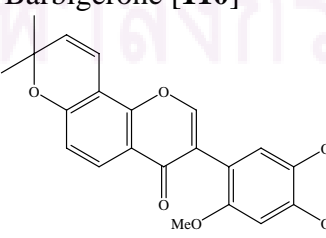
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia ferruginea</i> subsp. <i>darassana</i></b></p> <p>Predurmillone [115]</p>  <p>Prefurrugone [116]</p>  <p>Tephrosin [79]</p> 	<p>Isoflavone</p> <p>Isoflavone</p> <p>Rotenoid</p>	<p>Seed</p> <p>Seed</p> <p>Seed</p>	<p>Dagne and Bekele, 1990</p> <p>Dagne and Bekele, 1990</p> <p>Dagne and Bekele, 1990</p>
<p><b><i>Millettia ferruginea</i> subsp. <i>ferruginea</i></b></p> <p>Barbigerone [110]</p> 	<p>Isoflavone</p>	<p>Seed</p>	<p>Dagne and Bekele, 1990</p>

Table 1 (continued)

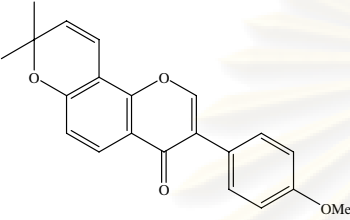
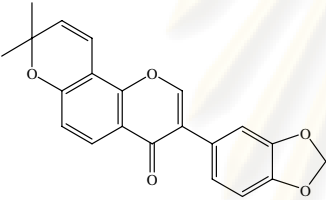
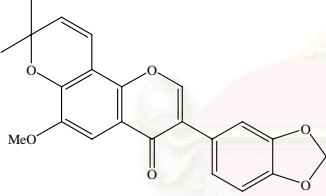
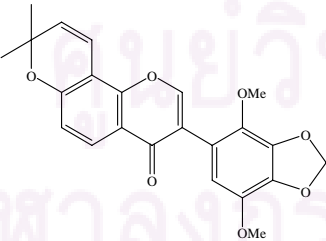
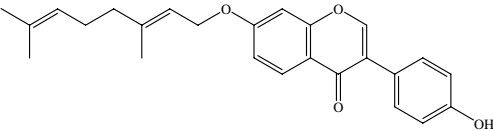
Plant and compound	Category	Plant part	Reference
<b><i>Millettia ferruginea</i> subsp.</b>			
<b><i>ferruginea</i></b>			
Calopogonium isoflavone A [57] 	Isoflavone	Seed	Dagne and Bekele, 1990
Calopogonium isoflavone B [117] 	Isoflavone	Stem bark	Dagne and Bekele, 1990
Durmillone [63] 	Isoflavone	Seed	Dagne and Bekele, 1990
Ferrugone [64] 	Isoflavone	Seed, Root bark	Dagne and Bekele, 1990 Dagne <i>et al.</i> , 1990
7- <i>O</i> -Geranylformononetin [118] 	Isoflavone	Root bark	Dagne <i>et al.</i> , 1990

Table 1 (continued)

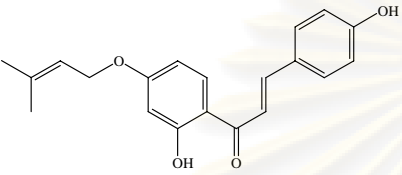
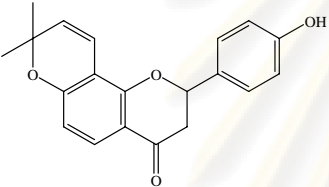
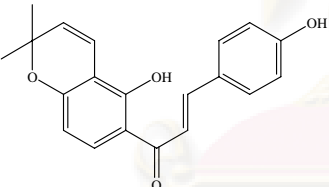
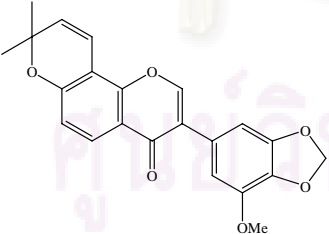
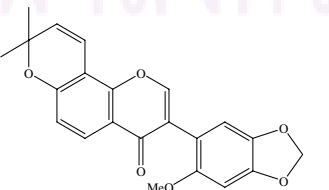
Plant and compound	Category	Plant part	Reference
<b><i>Millettia ferruginea</i> subsp. <i>ferruginea</i></b>			
4'- <i>O</i> -Geranylisoliquiritigenin [119] 	Chalcone	Root bark	Dagne <i>et al.</i> , 1990
4'-Hydroxyisolonchocarpin [120] 	Flavanone	Stem bark	Dagne, Bekele and Waterman, 1989
4-Hydroxylonchocarpin [67] 	Chalcone	Stem bark	Dagne, Bekele and Waterman, 1989
Isojamaicin [121] 	Isoflavone	Stem bark	Dagne, Bekele and Waterman, 1989
Jamaicin [69] 	Isoflavone	Stem bark	Dagne, Bekele and Waterman, 1989



Table 1 (continued)

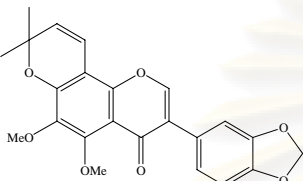
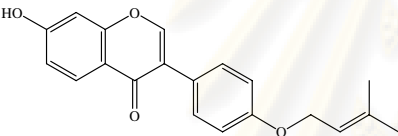
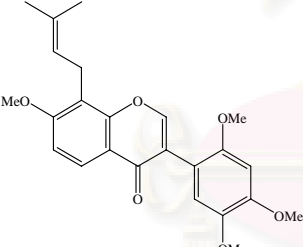
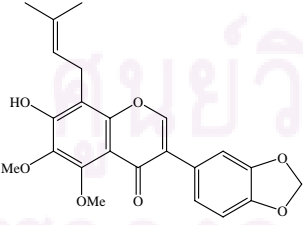
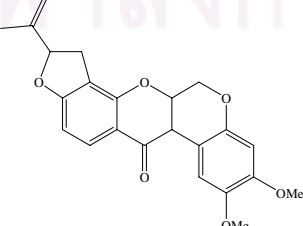
Plant and compound	Category	Plant part	Reference
<b><i>Millettia ferruginea</i> subsp.</b>			
<b><i>ferruginea</i></b>			
5-Methoxydurmillone [47] 	Isoflavone	Stem bark,	Dagne, Bekele and Waterman, 1989
		Root bark	Dagne <i>et al.</i> , 1990
Nordurlettone [122] 	Isoflavone	Seed	Dagne <i>et al.</i> , 1990
Prebarbigerone [123] 	Isoflavone	Seed	Dagne and Bekele, 1990
Pre-5-methoxydurmillone [124] 	Isoflavone	Root bark	Dagne and Bekele, 1990
Rotenone [78] 	Rotenoid	Seed	Dagne and Bekele, 1990

Table 1 (continued)

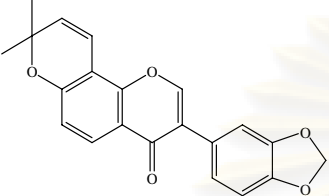
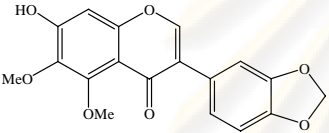
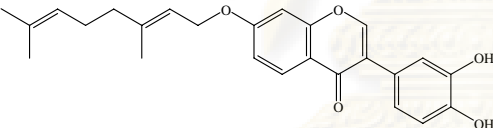
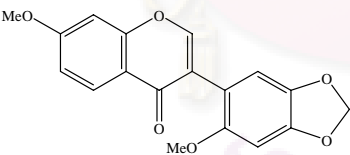
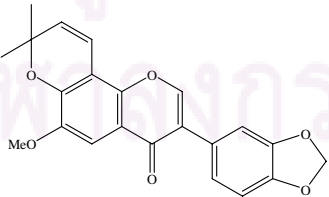
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia griffoniana</i></b></p> <p>Calopogonium isoflavone B [117]</p> 	Isoflavone	Root	Yankep, Fomum and Dagne, 1997
		bark,	
		Seed	Ngamga <i>et al.</i> , 2005a
<p>Dipterixine [125]</p> 	Isoflavone	Seed	Ngamga <i>et al.</i> , 2005b
<p>3',4'-Dihydroxy-7-O-[(E)-3,7-dimethyl-2,6-octadienyl]-isoflavone [126]</p> 	Isoflavone	Root bark	Yankep <i>et al.</i> , 1998
<p>7,2'-Dimethoxy-4',5'-methylene-dioxyflavone [60]</p> 	Isoflavone	Root	Yankep, Fomum and Dagne, 1997
		bark,	
		Seed	Ngamga <i>et al.</i> , 2005a
<p>Durmillone [63]</p> 	Isoflavone	Root bark	Yankep, Fomum and Dagne, 1997

Table 1 (continued)

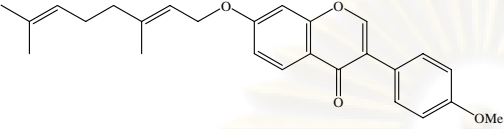
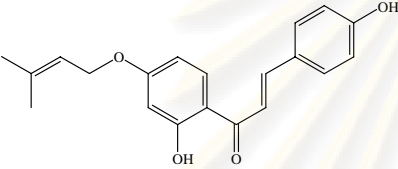
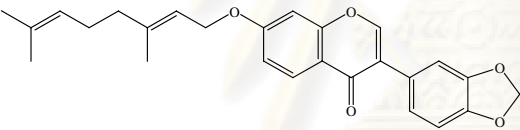
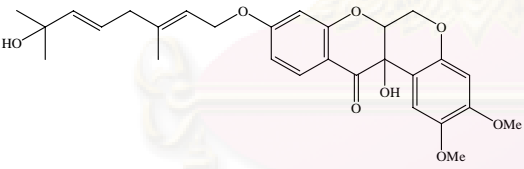
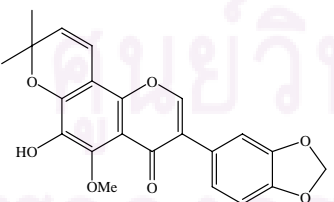
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia griffoniana</i></b></p> <p>7-<i>O</i>-Geranylformononetin [118]</p> 	Isoflavone	Root bark	Yankep, Fomum and Dagne, 1997
<p>4'-<i>O</i>-Geranylisoliquiritigenin [119]</p> 	Chalcone	Root bark	Yankep, Fomum and Dagne, 1997
<p>7-<i>O</i>-Geranylpseudobaptigenin [127]</p> 	Isoflavone	Root bark	Yankep, Fomum and Dagne, 1997
<p>Griffonianone A [128]</p> 	Rotenoid	Root bark	Yankep <i>et al.</i> , 2001
<p>Griffonianone B [129]</p> 	Isoflavone	Root bark	Yankep <i>et al.</i> , 2001

Table 1 (continued)

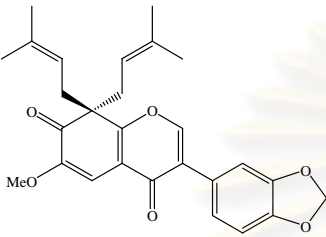
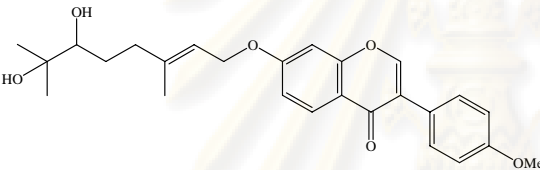
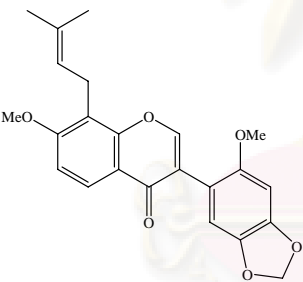
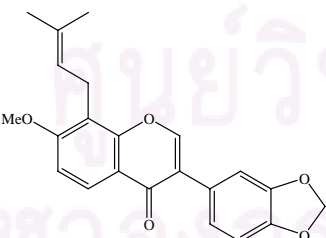
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia griffoniana</i></b></p> <p>Griffonianone C [130]</p>  <p>The structure of Griffonianone C is a complex isoflavone. It features a central chromone core with a methoxy group (MeO) at the 7-position. At the 3-position, there is a side chain consisting of a propyl group with a methyl branch and a terminal isopropenyl group. At the 4-position, there is a side chain consisting of a propyl group with a methyl branch and a terminal isopropenyl group. At the 6-position, there is a side chain consisting of a propyl group with a methyl branch and a terminal isopropenyl group. At the 8-position, there is a side chain consisting of a propyl group with a methyl branch and a terminal isopropenyl group.</p>	Isoflavone	Root bark	Yankep <i>et al.</i> , 2001
<p>Griffonianone D [131]</p>  <p>The structure of Griffonianone D is a complex isoflavone. It features a central chromone core with a methoxy group (OMe) at the 7-position. At the 3-position, there is a side chain consisting of a propyl group with a methyl branch and a terminal isopropenyl group. At the 4-position, there is a side chain consisting of a propyl group with a methyl branch and a terminal isopropenyl group. At the 6-position, there is a side chain consisting of a propyl group with a methyl branch and a terminal isopropenyl group. At the 8-position, there is a side chain consisting of a propyl group with a methyl branch and a terminal isopropenyl group.</p>	Isoflavone	Root bark	Yankep <i>et al.</i> , 2003
<p>Griffonianone E [132]</p>  <p>The structure of Griffonianone E is a complex isoflavone. It features a central chromone core with a methoxy group (MeO) at the 7-position. At the 3-position, there is a side chain consisting of a propyl group with a methyl branch and a terminal isopropenyl group. At the 4-position, there is a side chain consisting of a propyl group with a methyl branch and a terminal isopropenyl group. At the 6-position, there is a side chain consisting of a propyl group with a methyl branch and a terminal isopropenyl group. At the 8-position, there is a side chain consisting of a propyl group with a methyl branch and a terminal isopropenyl group.</p>	Isoflavone	Seed	Ngamga <i>et al.</i> , 2005a
<p>Griffonianone F [133]</p>  <p>The structure of Griffonianone F is a complex isoflavone. It features a central chromone core with a methoxy group (MeO) at the 7-position. At the 3-position, there is a side chain consisting of a propyl group with a methyl branch and a terminal isopropenyl group. At the 4-position, there is a side chain consisting of a propyl group with a methyl branch and a terminal isopropenyl group. At the 6-position, there is a side chain consisting of a propyl group with a methyl branch and a terminal isopropenyl group. At the 8-position, there is a side chain consisting of a propyl group with a methyl branch and a terminal isopropenyl group.</p>	Isoflavone	Seed	Ngamga <i>et al.</i> , 2005b

Table 1 (continued)

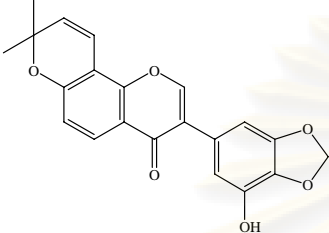
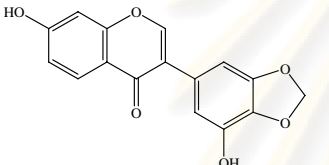
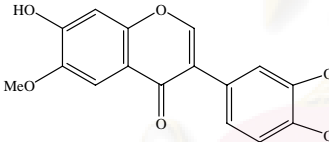
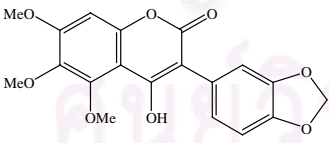
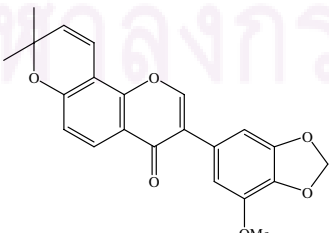
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia griffoniana</i></b></p> <p>Griffonianone G [134]</p> 	Isoflavone	Seed	Ngamga <i>et al.</i> , 2005b
<p>Griffonianone H [135]</p> 	Isoflavone	Seed	Ngamga <i>et al.</i> , 2005b
<p>7-Hydroxy-6-methoxy-3',4'-methyleneedioxyisoflavone [46]</p> 	Isoflavone	Root bark	Yankep <i>et al.</i> , 2001
<p>4-Hydroxy-5,6,7-trimethoxy-3-(3,4-methylenedioxy) phenylcoumarin [136]</p> 	Coumarin	Root bark	Yankep <i>et al.</i> , 1998
<p>Isojamaicin [121]</p> 	Isoflavone	Seed	Ngamga <i>et al.</i> , 2005b

Table 1 (continued)

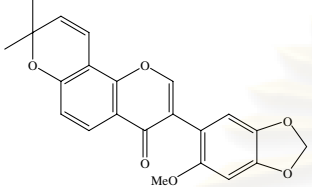
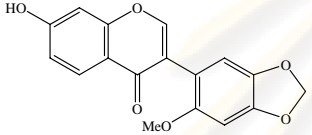
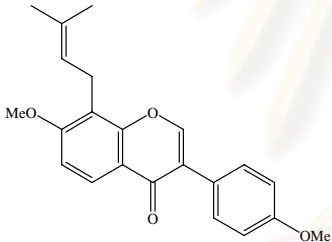
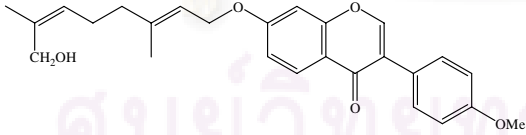
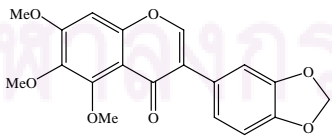
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia griffoniana</i></b></p> <p><b>Jamaicin [69]</b></p> 	Isoflavone	Root bark	Yankep, Fomum and Dagne, 1997
<p><b>Maximaisoflavne G [137]</b></p> 	Isoflavone	Root bark	Yankep <i>et al.</i> , 2001
<p><b>7-Methoxyebenosin [138]</b></p> 	Isoflavone	Seed	Ngamga <i>et al.</i> , 2005a
<p><b>4'-Methoxy-7-O-[(E)-3-methyl-7-hydroxymethyl-2,6-octadienyl] isoflavone [139]</b></p> 	Isoflavone	Root bark	Yankep <i>et al.</i> , 2001
<p><b>Odorantin [140]</b></p> 	Isoflavone	Root bark	Yankep, Fomum and Dagne, 1997

Table 1 (continued)

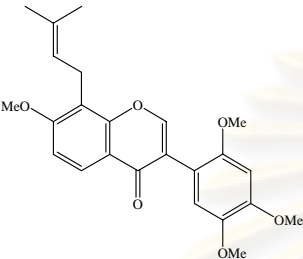
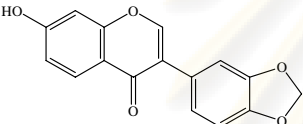
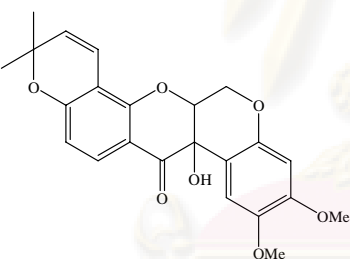
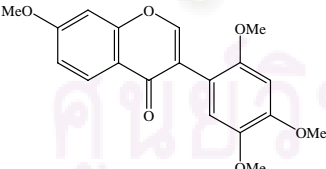
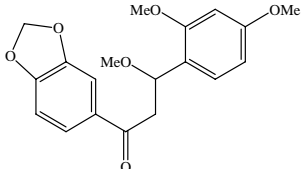
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia griffoniana</i></b></p> <p>Prebarbigerone [123]</p> 	Isoflavone	Seed	Ngamga <i>et al.</i> , 2005b
<p>Pseudobaptigenin [141]</p> 	Isoflavone	Seed	Ngamga <i>et al.</i> , 2005b
<p>Tephrosin [79]</p> 	Rotenoid	Seed	Ngamga <i>et al.</i> , 2005b
<p>7,2',4',5'-Tetramethoxyisoflavone [142]</p> 	Isoflavone	Seed	Ngamga <i>et al.</i> , 2005b
<p><b><i>Millettia hemsleyana</i></b></p> <p>Dihydroisomilletenone methyl ether [143]</p> 	Chalcone	Stem bark	Mahmoud and Waterman, 1985

Table 1 (continued)

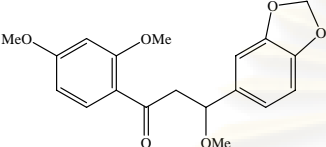
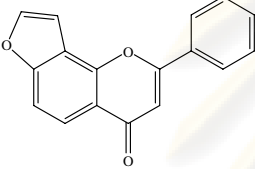
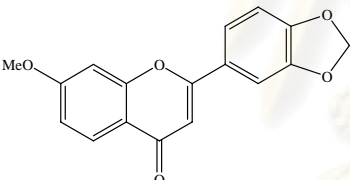
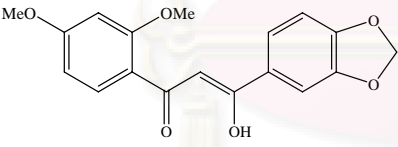
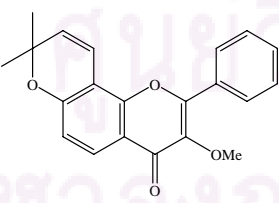
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia hemsleyana</i></b></p> <p>Dihydromilletinone methyl ether [144]</p> 	Chalcone	Stem bark	Mahmoud and Waterman, 1985
<p>Lanceolatin B [89]</p> 	Flavone	Stem bark	Mahmoud and Waterman, 1985
<p>3',4'-Methylenedioxy-7-methoxy-flavone [94]</p> 	Flavone	Stem bark	Mahmoud and Waterman, 1985
<p>Milletinone [95]</p> 	Chalcone	Stem bark	Mahmoud and Waterman, 1985
<p>Pongaflavone [145]</p> 	Flavone	Stem bark	Mahmoud and Waterman, 1985



Table 1 (continued)

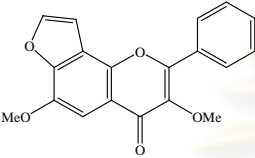
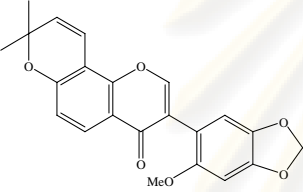
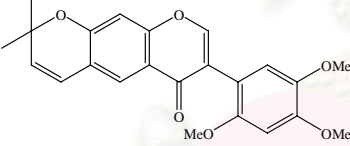
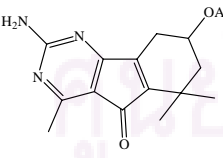
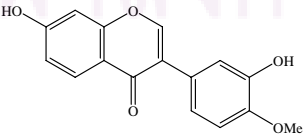
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia ichthyochtona</i></b></p> <p>3,6-Dimethoxyfurano[4'',5'':8,7] flavone [146]</p> 	Flavone	Leaf	Kamperdick <i>et al.</i> , 1998
<p>Jamaicin [69]</p> 	Isoflavone	Leaf	Kamperdick <i>et al.</i> , 1998
<p>2',4',5'-Trimethoxy-2'',2''-dimethylpyrano[5'',6'':6,7] isoflavone [147]</p> 	Isoflavone	Leaf	Kamperdick <i>et al.</i> , 1998
<p><b><i>Millettia laurentii</i></b></p> <p><i>O</i>-Acetylmillaurine [148]</p> 	Alkaloid	Seed	Ngamga <i>et al.</i> , 1993
<p>Calycosin [149]</p> 	Isoflavone	Wood	Kamnaing <i>et al.</i> , 1999

Table 1 (continued)

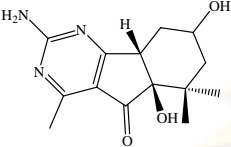
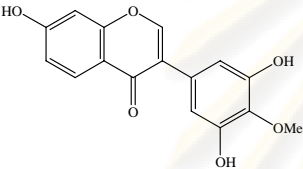
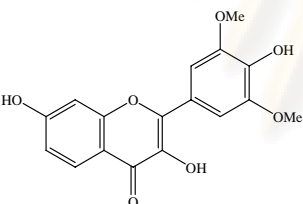
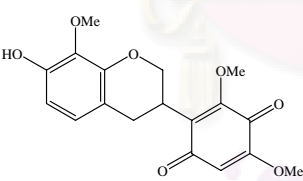
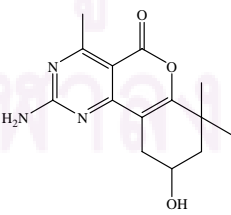
Plant and compound	Category	Plant part	Reference
<b><i>Millettia laurentii</i></b>			
5a,9a-dihydro-5a-hydroxymyllaurine [150]	Alkaloid	Seed	Ngamga <i>et al.</i> , 1994
			
Glyricidin [151]	Isoflavone	Wood	Kamnaing <i>et al.</i> , 1999
			
Laurentinol [152]	Flavonol	Wood	Kamnaing <i>et al.</i> , 1999
			
Laurentiquinone [153]	Isoflavan	Wood	Kamnaing <i>et al.</i> , 1999
			
Millettonine [154]	Alkaloid	Stem bark	Kamnaing <i>et al.</i> , 1994
			

Table 1 (continued)

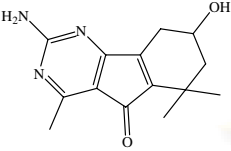
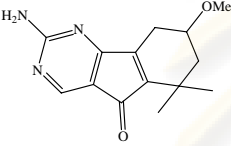
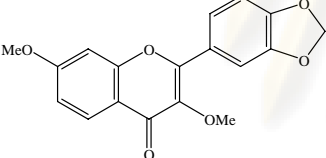
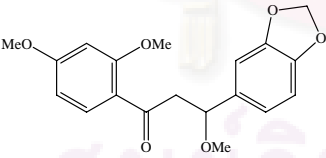
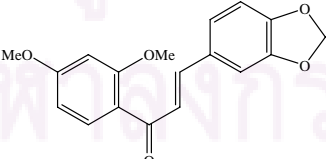
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia laurentii</i></b></p> <p>Millaurine [155]</p> 	Alkaloid	Seed	Ngamga <i>et al.</i> , 1993
<p>Millaurine A [156]</p> 	Alkaloid	Seed	Ngamga <i>et al.</i> , 2007
<p><b><i>Millettia leucantha</i></b></p> <p>Desmethoxykanugin [157]</p> 	Flavone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
<p>Dihydromillettinone methyl ether [144]</p> 	Chalcone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
<p>2',4'-Dimethoxy-3,4-methylene-dioxychalcone [158]</p> 	Chalcone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003

Table 1 (continued)

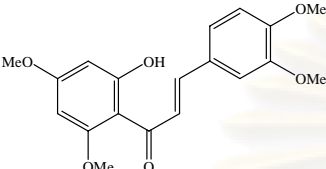
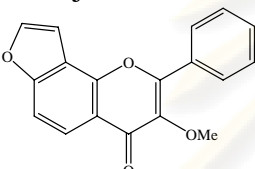
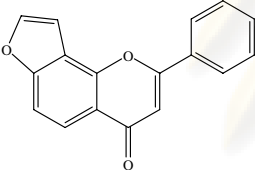
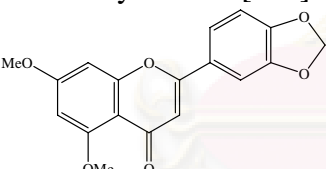
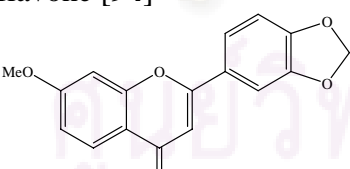
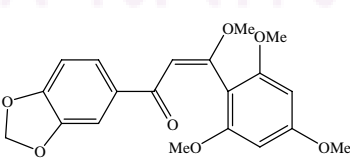
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia leucantha</i></b></p> <p>2'-Hydroxy-3,4,4',6'-tetramethoxy-chalcone [159]</p> 	Chalcone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
<p>Karanjin [160]</p> 	Flavone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
<p>Lanceolatin B [89]</p> 	Flavone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
<p>3',4'-methyleneedioxy-5,7-dimethoxyflavone [161]</p> 	Flavone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
<p>3',4'-Methyleneedioxy-7-methoxy-flavone [94]</p> 	Flavone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
<p>2,4,6,β -tetramethoxy-3',4'-methylenedioxychalcone [162]</p> 	Chalcone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003

Table 1 (continued)

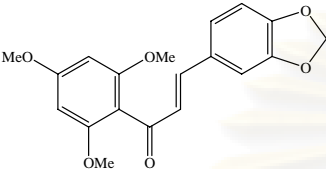
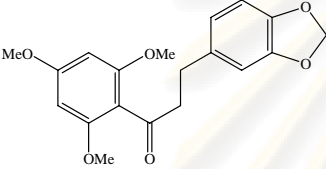
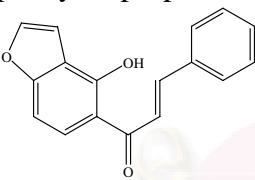
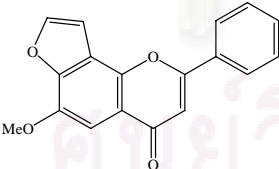
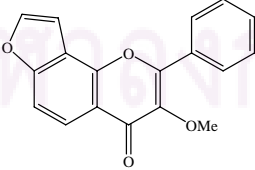
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia leucantha</i></b></p> <p>2',4',6'-Trimethoxy-3,4-methylene-dioxychalcone [163]</p> 	Chalcone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
<p>2',4',6'-Trimethoxy-3,4-methylene-dioxydihydrochalcone [164]</p> 	Chalcone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
<p><b><i>Millettia ovalifolia</i></b></p> <p>1-(4-Hydroxy-5-benzofuranyl)-3-phenyl-2-propen-1-one [86]</p> 	Chalcone	Root	Saxena <i>et al.</i> , 1987
<p>Kanjone [165]</p> 	Flavone	Seed	Gupta and Krishnamurti, 1976a
<p>Karanjin [160]</p> 	Flavone	Seed	Gupta and Krishnamurti, 1976a

Table 1 (continued)

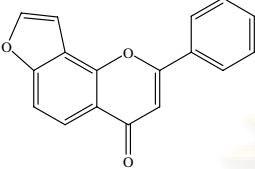
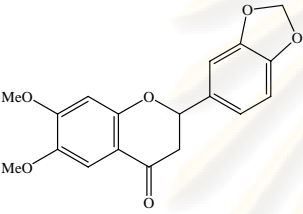
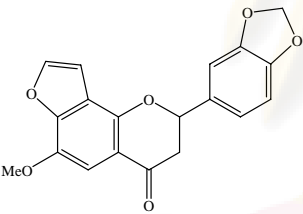
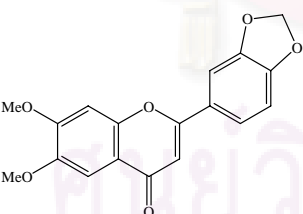
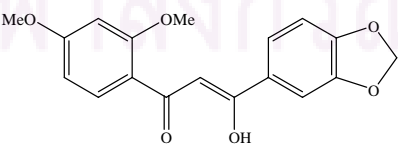
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia ovalifolia</i></b></p> <p>Lanceolatin B [89]</p> 	Flavone	Seed	Gupta and Krishnamurti, 1976a
<p>Milletenin A [166]</p> 	Flavanone	Leaf	Khan and Zaman, 1974
<p>Milletenin B [167]</p> 	Flavanone	Leaf	Khan and Zaman, 1974
<p>Milletenin C [93]</p> 	Flavone	Leaf	Khan and Zaman, 1974
<p>Milletenone [95]</p> 	Chalcone	Leaf	Khan and Zaman, 1974

Table 1 (continued)

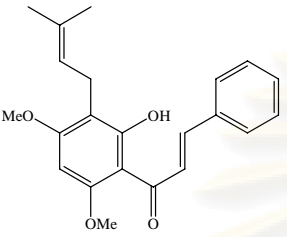
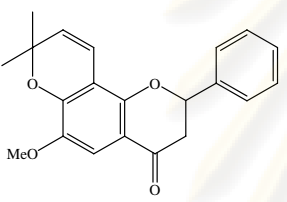
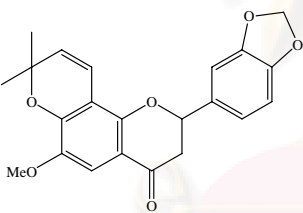
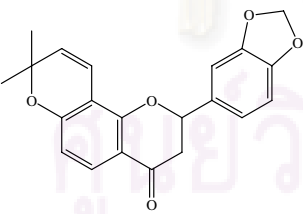
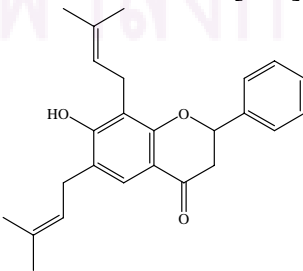
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia ovalifolia</i></b></p> <p>Ovalichalkone [168]</p> 	Chalcone	Seed	Gupta and Krishnamurti, 1977a
<p>Ovalichromene [169]</p> 	Flavanone	Seed	Gupta and Krishnamurti, 1976b
<p>Ovalichromene A [170]</p> 	Flavanone	Seed	Gupta and Krishnamurti, 1976c
<p>Ovalichromene B [171]</p> 	Flavanone	Seed	Gupta and Krishnamurti, 1976c
<p>Ovaliflavanone A [172]</p> 	Flavanone	Seed	Gupta and Krishnamurti, 1976a

Table 1 (continued)

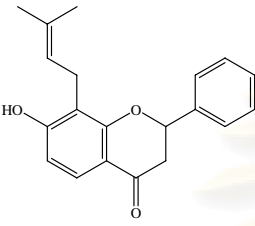
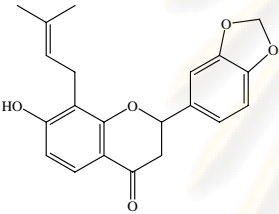
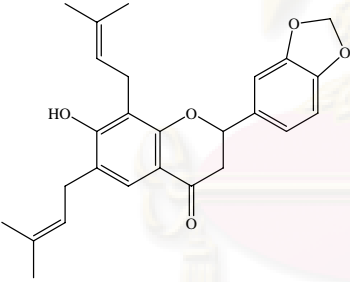
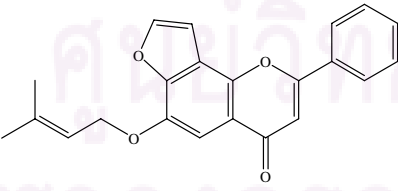
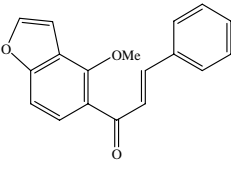
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia ovalifolia</i></b></p> <p>Ovaliflavanone B [173]</p> 	Flavanone	Seed	Gupta and Krishnamurti, 1976a
<p>Ovaliflavanone C [174]</p> 	Flavanone	Seed	Islam, Gupta and Krishnamurti, 1980
<p>Ovaliflavanone D [175]</p> 	Flavanone	Seed	Islam, Gupta and Krishnamurti, 1980
<p>Ovalifolin [99]</p> 	Flavone	Leaf	Khan and Zaman, 1974
<p>Ovalitenin A [176]</p> 	Chalcone	Seed	Gupta and Krishnamurti, 1977



Table 1 (continued)

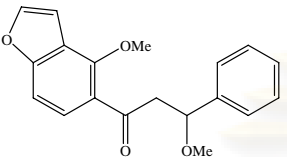
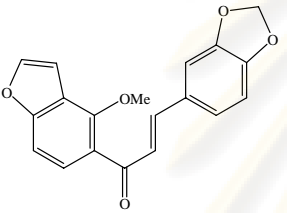
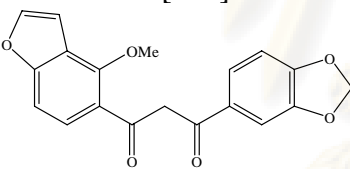
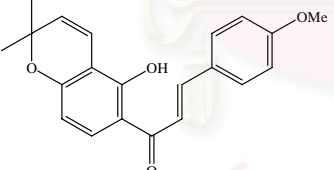
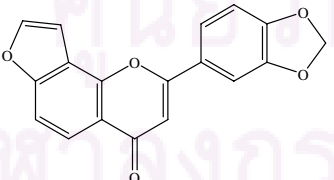
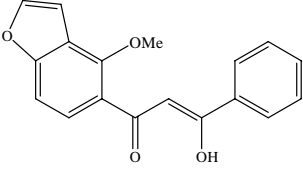
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia ovalifolia</i></b></p> <p>Ovalitenin B [177]</p> 	Chalcone	Seed	Gupta and Krishnamurti, 1977
<p>Ovalitenin C [178]</p> 	Chalcone	Seed	Islam, Gupta and Krishnamurti, 1980
<p>Ovalitenone [100]</p> 	Chalcone	Seed	Gupta and Krishnamurti, 1977
<p>Pongachalcone I [179]</p> 	Chalcone	Seed	Gupta and Krishnamurti, 1976c
<p>Pongaglabrone [102]</p> 	Flavone	Seed	Gupta and Krishnamurti, 1976a
<p>Pongamol [103]</p> 	Chalcone	Seed	Gupta and Krishnamurti, 1976b

Table 1 (continued)

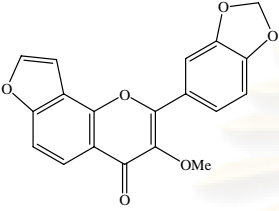
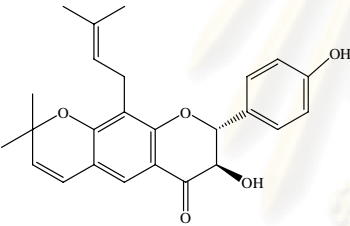
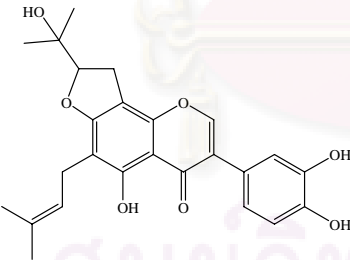
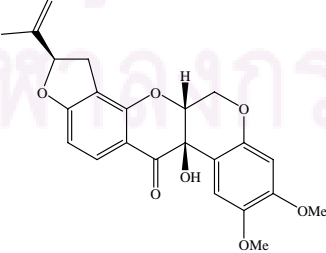
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia ovalifolia</i></b></p> <p>Pongapin [180]</p> 	Flavone	Seed	Gupta and Krishnamurti, 1976b
<p><b><i>Millettia pachycarpa</i></b></p> <p>(2<i>R</i>,3<i>R</i>)-5,4'-Dihydroxy-8-Prenyl-6'',6''-dimethylpyranol[2'',3'':7,6]-dihydroflavonol [181]</p> 	Flavonol	Aerial part	Singhal <i>et al.</i> , 1980
<p>Furowanin [182]</p> 	Flavone	Leaf	Ito <i>et al.</i> , 2006
<p><i>cis</i>-12a-Hydroxyrotenone [183]</p> 	Rotenoid	Root	Singhal <i>et al.</i> , 1980

Table 1 (continued)

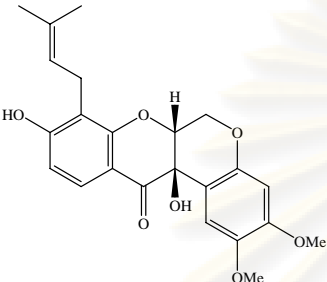
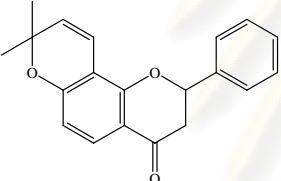
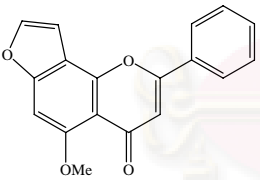
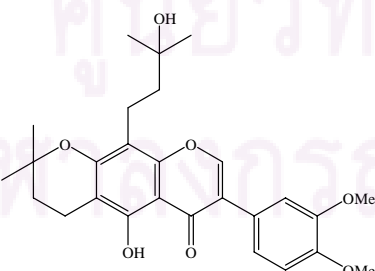
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia pachycarpa</i></b></p> <p><i>cis</i>-12a-Hydroxyrot-2'-enoic acid [184]</p> 	Rotenoid	Root	Singhal <i>et al.</i> , 1980
<p>Isolonchocarpin [185]</p> 	Flavanone	Root	Shao <i>et al.</i> , 2001a
<p>5-Methoxy furo[8,7:4'',5'']flavone [186]</p> 	Flavone	Root	Lu <i>et al.</i> , 1999
<p><i>Millettia</i> isoflavone 7A [187]</p> 	Isoflavone	Leaf	Singhal <i>et al.</i> , 1981

Table 1 (continued)

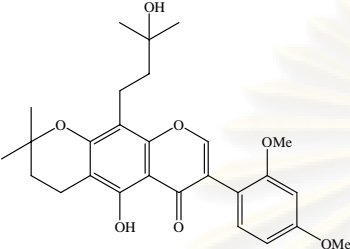
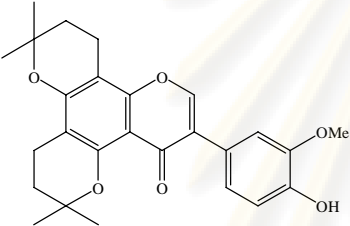
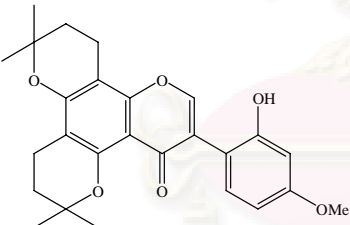
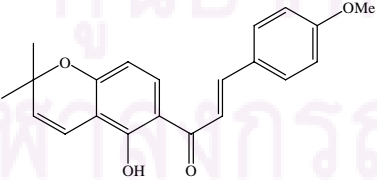
Plant and compound	Category	Plant part	Reference
<b><i>Millettia pachycarpa</i></b>			
Millettia isoflavone 7B [188] 	Isoflavone	Leaf	Singhal <i>et al.</i> , 1981
Millettia isoflavone 10B [189] 	Isoflavone	Leaf	Singhal <i>et al.</i> , 1981
Millettia isoflavone 11A [190] 	Isoflavone	Leaf	Singhal <i>et al.</i> , 1981
Millettia pachycarpa pyranochalcone [191] 	Chalcone	Seed	Singhal <i>et al.</i> , 1983

Table 1 (continued)

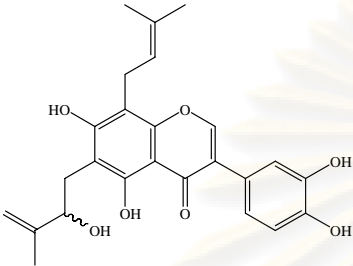
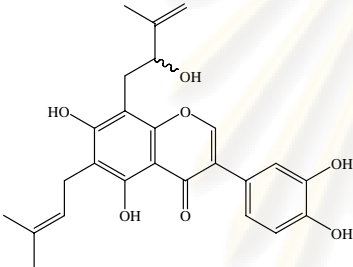
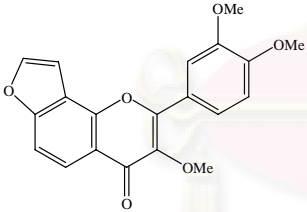
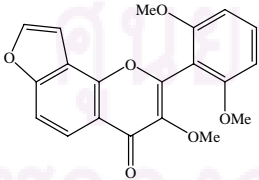
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia pachycarpa</i></b></p> <p>Millewanin G [192]</p> 	Flavone	Leaf	Ito <i>et al.</i> , 2006
<p>Millewanin H [193]</p> 	Flavone	Leaf	Ito <i>et al.</i> , 2006
<p>Pachycarin A [194]</p> 	Flavone	Root	Chen <i>et al.</i> , 1999
<p>Pachycarin B [195]</p> 	Flavone	Root	Chen <i>et al.</i> , 1999

Table 1 (continued)

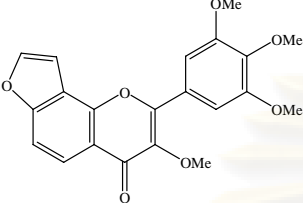
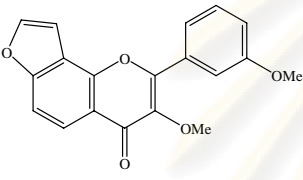
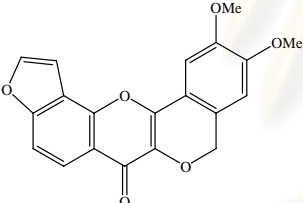
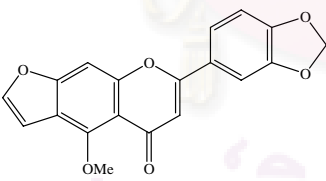
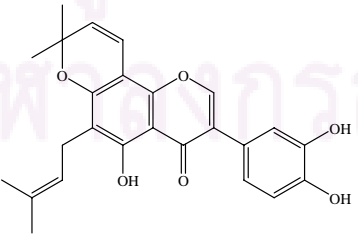
Plant and compound	Category	Plant part	Reference
<b><i>Millettia pachycarpa</i></b>			
Pachycarin C [196] 	Flavone	Root	Shao <i>et al.</i> , 2001b
Pachycarin D [197] 	Flavone	Root	Shao <i>et al.</i> , 2001b
Pachycarin E [198] 	Flavone	Root	Shao <i>et al.</i> , 2001b
Pinnatin [199] 	Flavone	Root	Shao <i>et al.</i> , 2001b
Pomiferin [200] 	Isoflavone	Seed	Singhal <i>et al.</i> , 1983

Table 1 (continued)

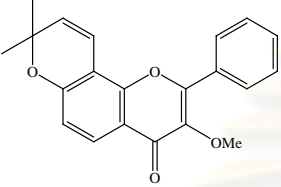
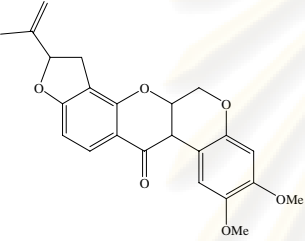
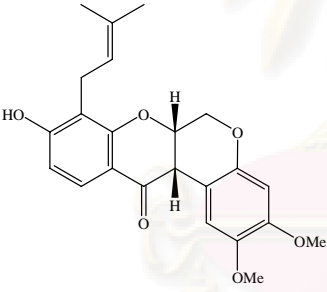
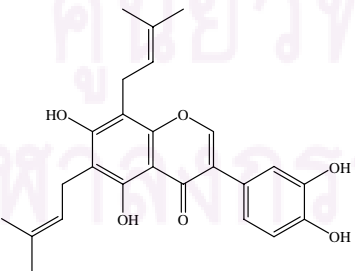
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia pachycarpa</i></b></p> <p>Pongaflavone [145]</p> 	Flavone	Root	Shao <i>et al.</i> , 2001a
<p>Rotenone [78]</p> 	Rotenoid	Root	Singhal <i>et al.</i> , 1982
<p>Rot-2'-enonic acid [201]</p> 	Rotenoid	Root	Singhal <i>et al.</i> , 1982
<p>5,7,3',4'-Tetrahydroxy-6,8-diprenylisoflavone [202]</p> 	Isoflavone	Aerial part	Singhal <i>et al.</i> , 1980

Table 1 (continued)

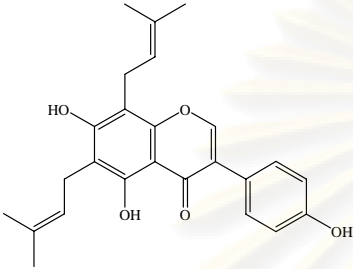
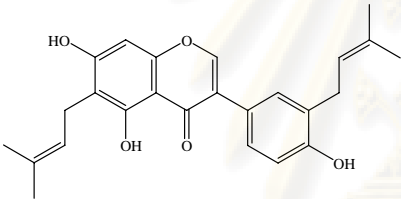
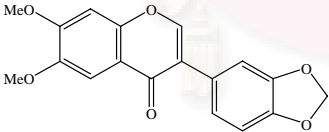
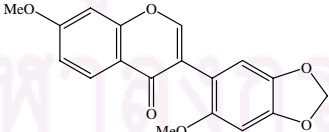
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia pachycarpa</i></b></p> <p>5,7,4'-Trihydroxy-6,8-diprenyl- isoflavone [203]</p>  <p>5,7,4'-Trihydroxy-6, 3'-diprenyl- isoflavone [204]</p> 	Isoflavone	Aerial part	Singhal <i>et al.</i> , 1980
<p><b><i>Millettia peguensis</i></b></p> <p>6,7-Dimethoxy-3',4'-methylene- dioxyisoflavone [205]</p>  <p>7,2'-Dimethoxy-4',5'-methylene- dioxyisoflavone [206]</p> 	Isoflavone	Root	Kapingu, <i>et al.</i> , 2006



Table 1 (continued)

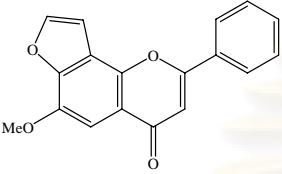
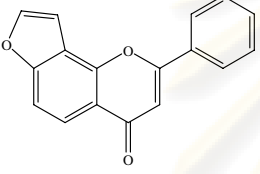
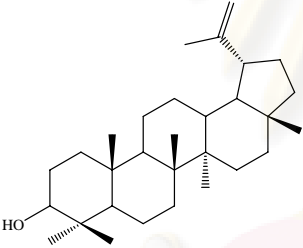
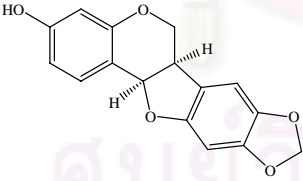
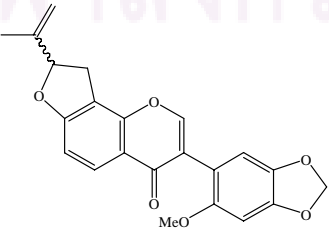
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia peguensis</i></b></p> <p><b>Kanjone [165]</b></p> 	Flavone	Stem bark	Ganapaty <i>et al.</i> , 1998
<p><b>Lanceolatin B [89]</b></p> 	Flavone	Stem bark	Ganapaty <i>et al.</i> , 1998
<p><b>Lupeol [207]</b></p> 	Triterpenoid	Root	Kapingu, <i>et al.</i> , 2006
<p><b>Maackiain [208]</b></p> 	Pterocarpan	Root	Kapingu, <i>et al.</i> , 2006
<p><b>2'-Methoxy-4',5'-methylenedioxy-7,8-[2-(1-methylethnyl)furo] isoflavone [209]</b></p> 	Isoflavone	Root	Kapingu, <i>et al.</i> , 2006

Table 1 (continued)

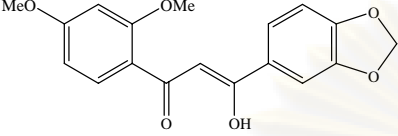
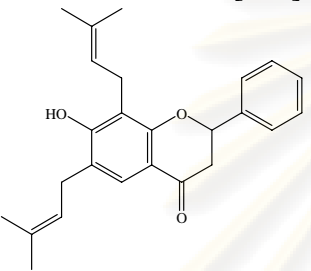
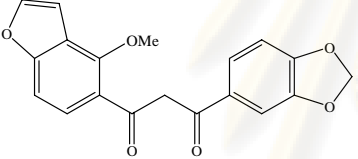
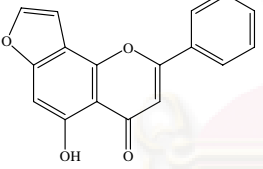
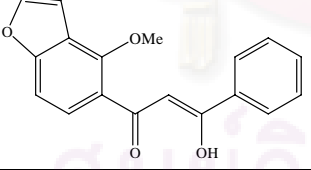
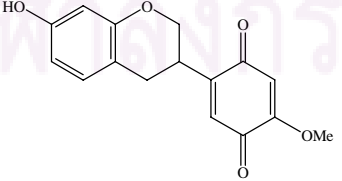
Plant and compound	Category	Plant part	Reference
<b><i>Millettia peguensis</i></b>			
<p>Milletenone [95]</p> 	Chalcone	Stem bark	Ganapaty <i>et al.</i> , 1998
<p>Ovaliflavanone A [172]</p> 	Flavanone	Stem bark	Ganapaty <i>et al.</i> , 1998
<p>Ovalitenone [100]</p> 	Chalcone	Stem Bark	Ganapaty <i>et al.</i> , 1998
<p>Pongaglabol [101]</p> 	Flavone	Leaf	Ganapaty <i>et al.</i> , 1998
<p>Pongamol [103]</p> 	Chalcone	Leaf	Ganapaty <i>et al.</i> , 1998
<b><i>Millettia pendura</i></b>			
<p>Claussequinine [210]</p> 	Isoflavan	Heart-wood	Hayashi <i>et al.</i> , 1978

Table 1 (continued)

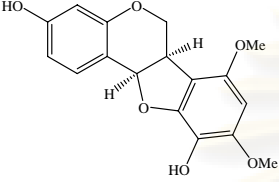
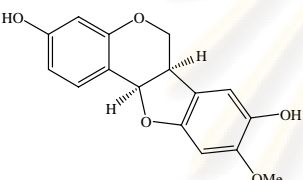
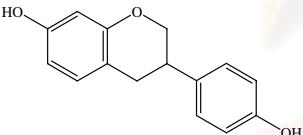
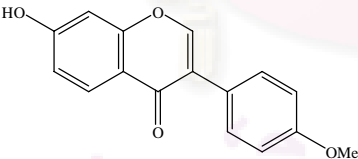
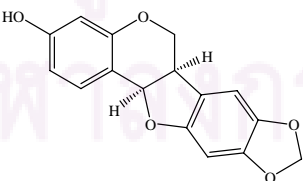
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia pendura</i></b></p> <p>3,10-Dihydroxy-7,9-dimethoxy-pterocarpan [211]</p> 	Pterocapan	Timber	Takahashi <i>et al.</i> , 2006
<p>3,8- Dihydroxy-9-methoxy-pterocarpan [212]</p> 	Pterocapan	Timber	Takahashi <i>et al.</i> , 2006
<p>Equol [213]</p> 	Isoflavan	Heart-wood	Hayashi <i>et al.</i> , 1978
<p>Formononetin [65]</p> 	Isoflavone	Timber	Takahashi <i>et al.</i> , 2006
<p>Maackiain [208]</p> 	Pterocarpan	Heart-wood	Hayashi <i>et al.</i> , 1978

Table 1 (continued)

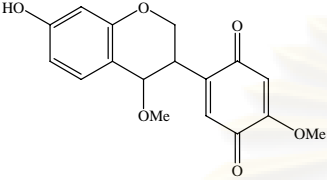
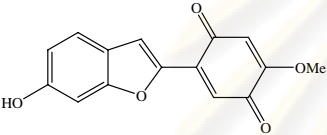
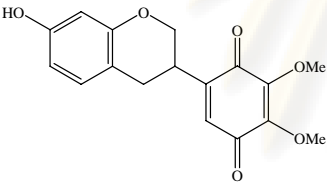
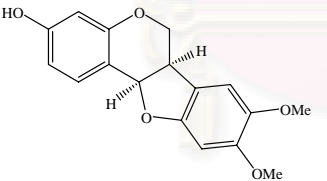
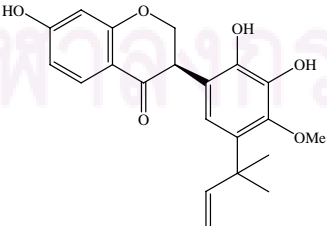
Plant and compound	Category	Plant part	Reference
<b><i>Millettia pendura</i></b>			
Millettitone A [214] 	Isoflavan	Timber	Takahashi <i>et al.</i> , 2006
Millettitone B [215] 	Arylbenzo- furan	Timber	Takahashi <i>et al.</i> , 2006
Pendulone [216] 	Isoflavan	Heart- wood	Hayashi <i>et al.</i> , 1978
Secundiflorol I [217] 	Pterocarpan	Timber	Takahashi <i>et al.</i> , 2006
<b><i>Millettia pervilleana</i></b>			
3'-Demethylpervilleanone [218] 	Isoflavanone	Root bark	Galeffi <i>et al.</i> , 1997

Table 1 (continued)

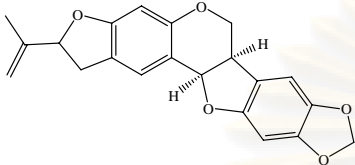
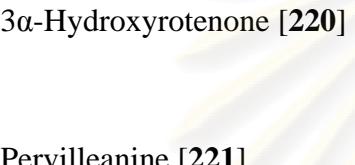
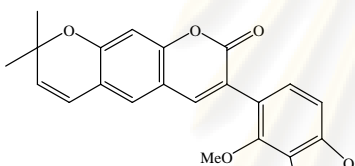
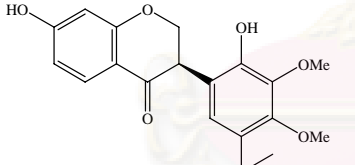
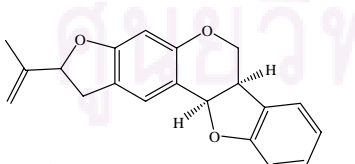
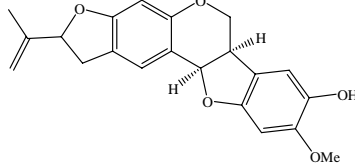
Plant and compound	Category	Plant part	Reference
<b><i>Millettia pervilleana</i></b>			
Emoroidocarpan [219] 	Pterocarpan	Root bark	Palazzino <i>et al.</i> , 2003
3 $\alpha$ -Hydroxyrotenone [220] 	Rotenoid	Root bark	Palazzino <i>et al.</i> , 2003
Pervilleanine [221] 	Coumarin	Root bark	Palazzino <i>et al.</i> , 2003
Pervilleanone [222] 	Isoflavanone	Root bark	Galeffi <i>et al.</i> , 1997
Pervilline [223] 	Pterocarpan	Root bark	Palazzino <i>et al.</i> , 2003
Pervillinine [224] 	Pterocarpan	Root bark	Palazzino <i>et al.</i> , 2003

Table 1 (continued)

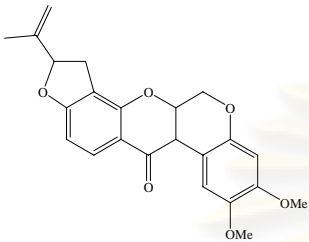
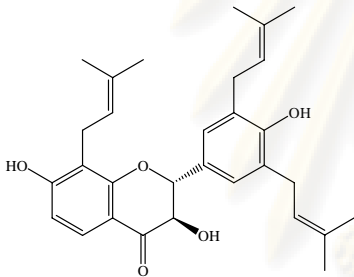
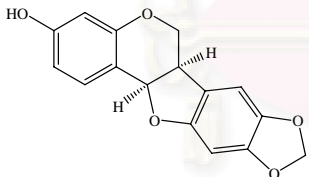
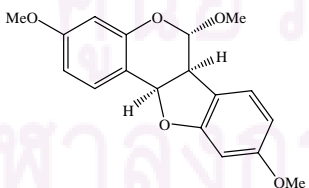
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia pervilleana</i></b></p> <p>Rotenone [78]</p> 	Rotenoid	Root bark	Palazzino <i>et al.</i> , 2003
<p><b><i>Millettia pulchra</i></b></p> <p>7,4'-Dihydroxy-8,3',5'-triprenyl-dihydroflavanol [225]</p> 	Flavanonol	Aerial part	Baruah <i>et al.</i> , 1984
<p>Maackiain [208]</p> 	Pterocarpan	Aerial part	Baruah <i>et al.</i> , 1984
<p>6<math>\alpha</math>-Methoxyhomopterocarpin [226]</p> 	Pterocarpan	Aerial part	Baruah <i>et al.</i> , 1984

Table 1 (continued)

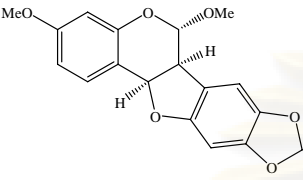
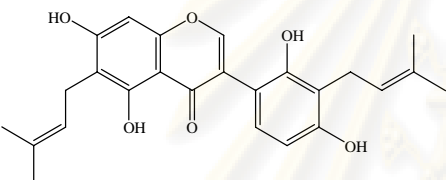
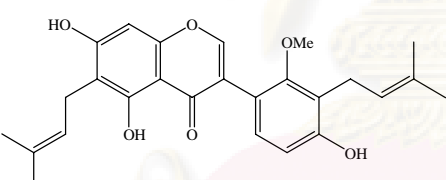
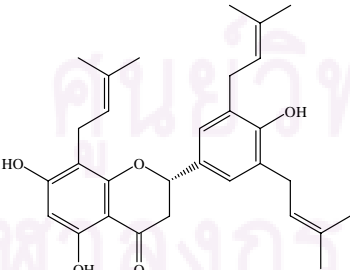
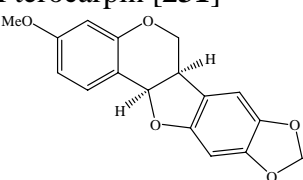
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia pulchra</i></b></p> <p>6<math>\alpha</math>-Methoxypterocarpin [227]</p> 	Pterocarpan	Aerial part	Baruah <i>et al.</i> , 1984
<p>5,7,2',4'-Tetrahydroxy-6,3'-diprenylisoflavone [228]</p> 	Isoflavone	Aerial part	Baruah <i>et al.</i> , 1984
<p>5,7,4'-Trihydroxy-2'-methoxy-6,3'-diprenylisoflavone [229]</p> 	Isoflavone	Aerial part	Baruah <i>et al.</i> , 1984
<p>5,7,4'-Trihydroxy-8,3',5'-triprenylflavanone [230]</p> 	Flavanone	Aerial part	Baruah <i>et al.</i> , 1984
<p>Pterocarpin [231]</p> 	Pterocarpan	Aerial part	Baruah <i>et al.</i> , 1984

Table 1 (continued)

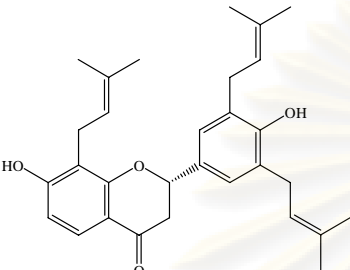
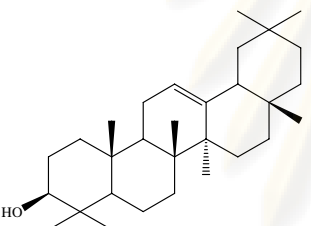
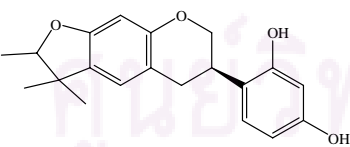
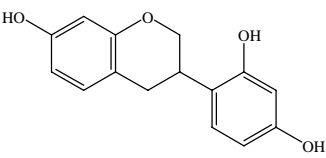
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia pulchra</i></b></p> <p>Sophoranone [232]</p> 	Flavanone	Aerial part	Baruah <i>et al.</i> , 1984
<p><b><i>Millettia racemosa</i></b></p> <p><math>\beta</math>-amyrin [38]</p> 	Triterpenoid	Stem	Rao and Krupadanam, 1994
<p>Behenic acid [233]</p> <p><math>\text{H}_3\text{C}(\text{CH}_2)_{20}\text{COOH}</math></p>	Fatty acid	Stem	Rao and Krupadanam, 1994
<p>(+)-Cyclomillinol [234]</p> 	Isoflavan	Stem	Kumar, Krupadanam and Srimannarayana, 1989
<p>Demethylvestitol [235]</p> 	Isoflavan	Stem	Rao and Krupadanam, 1996



Table 1 (continued)

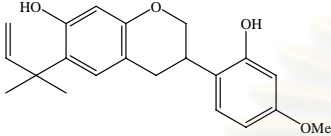
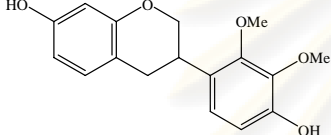
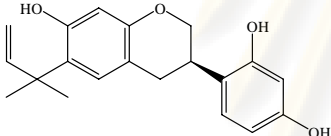
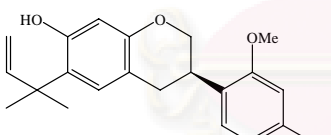
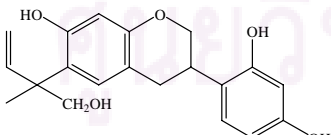
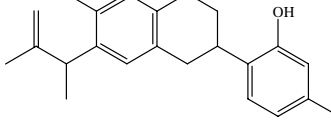
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia racemosa</i></b></p> <p>(-)-Isomillinol B [236]</p> 	Isoflavan	Stem	Rao and Krupadanam, 1994
<p>Laxifloran [237]</p> 	Isoflavan	Stem	Rao and Krupadanam, 1994
<p>(+)-Millinol [238]</p> 	Isoflavan	Stem	Kumar, Krupadanam and Srimannarayana, 1989
<p>(+)-Millinol B [239]</p> 	Isoflavan	Stem	Kumar, Krupadanam and Srimannarayana, 1989
<p>Millinolol [240]</p> 	Isoflavan	Stem	Rao and Krupadanam, 1996
<p>Neomillinolol [241]</p> 	Isoflavan	Stem	Rao and Krupadanam, 1996

Table 1 (continued)

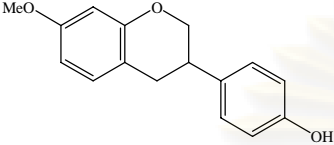
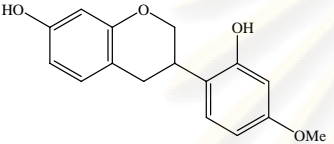
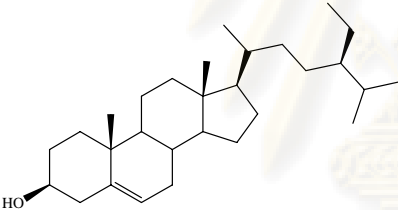
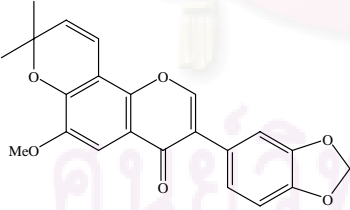
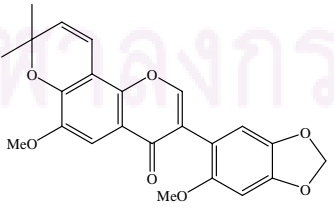
Plant and compound	Category	Plant part	Reference
<b><i>Millettia racemosa</i></b>			
Neovestitol [242] 	Isoflavan	Stem	Rao and Krupadanam, 1996
Vestitol [243] 	Isoflavan	Stem	Rao and Krupadanam, 1994
Sitosterol [32] 	Triterpenoid	Stem	Rao and Krupadanam, 1994
<b><i>Millettia rubiginosa</i></b>			
Durmillone [63] 	Isoflavone	Root	Desai <i>et al.</i> , 1977
Ichthyone [114] 	Isoflavone	Root	Desai <i>et al.</i> , 1977

Table 1 (continued)

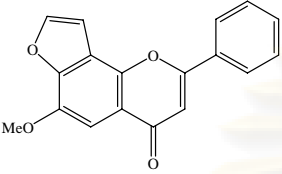
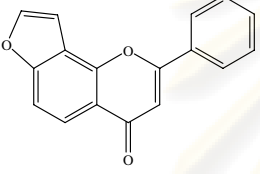
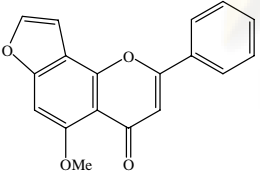
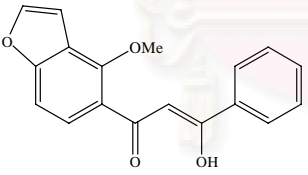
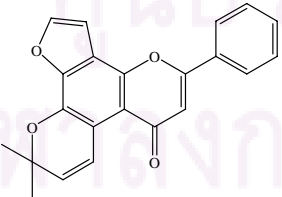
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia sanagana</i></b></p> <p><b>Kanjone [165]</b></p> 	Flavone	Root bark	Mbafor <i>et al.</i> , 1995
<p><b>Lanceolatin B [89]</b></p> 	Flavone	Root bark	Mbafor <i>et al.</i> , 1995
<p><b>5-Methoxyfurano[7,8:4'',5'']flavone [244]</b></p> 	Flavone	Root bark	Mbafor <i>et al.</i> , 1995
<p><b>Pongamol [103]</b></p> 	Chalcone	Root bark	Mbafor <i>et al.</i> , 1995
<p><b>Sanaganone [245]</b></p> 	Flavone	Root bark	Mbafor <i>et al.</i> , 1995

Table 1 (continued)

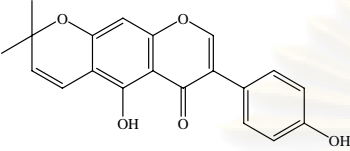
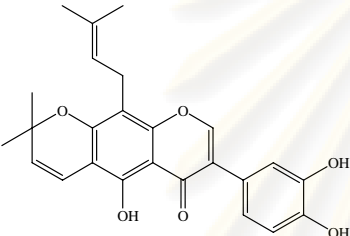
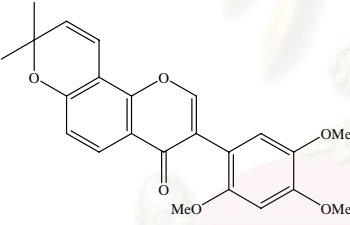
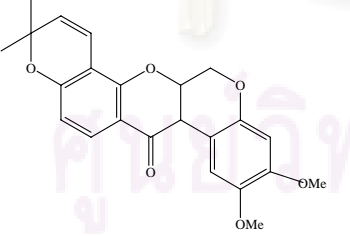
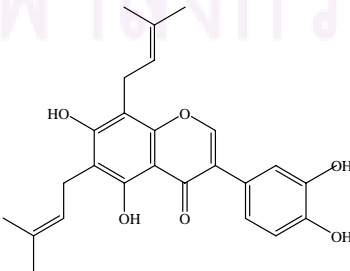
Plant and compound	Category	Plant part	Reference
<b><i>Milletia taiwaniana</i></b>			
Alpinumisoflavone [246] 	Isoflavone	Stem	Ito <i>et al.</i> , 2004
Auriculasin [2] 	Isoflavone	Stem	Ito <i>et al.</i> , 2004
Barbigerone [110] 	Isoflavone	Stem	Ito <i>et al.</i> , 2004
Deguelin [58] 	Rotenoid	Stem	Ito <i>et al.</i> , 2004
6,8-Di- $\gamma,\gamma$ -dimethylallylorobol [247] 	Isoflavone	Leaf	Ito <i>et al.</i> , 2006

Table 1 (continued)

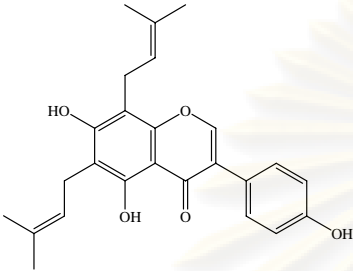
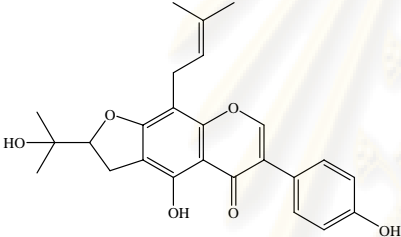
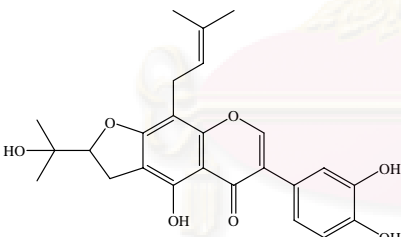
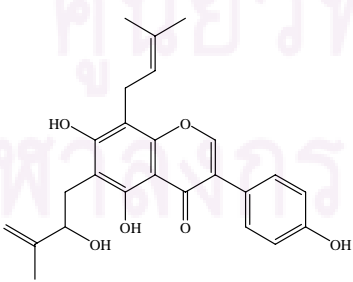
Plant and compound	Category	Plant part	Reference
<b><i>Millettia taiwaniana</i></b>			
8- $\gamma,\gamma$ -Dimethylallylwightenone [248]	Isoflavone	Stem,	Ito <i>et al.</i> , 2004
		Leaf	Ito <i>et al.</i> , 2006
Euchrenone b <sub>10</sub> [249]	Isoflavone	Leaf	Ito <i>et al.</i> , 2006
			
Furowanin A [250]	Isoflavone	Leaf	Ito <i>et al.</i> , 2006
			
Isoerysenegalensein-E [251]	Isoflavone	Leaf	Ito <i>et al.</i> , 2006
			

Table 1 (continued)

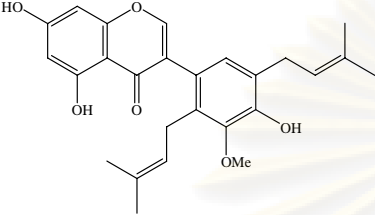
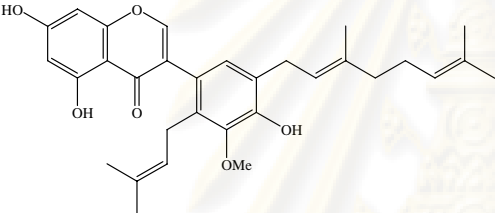
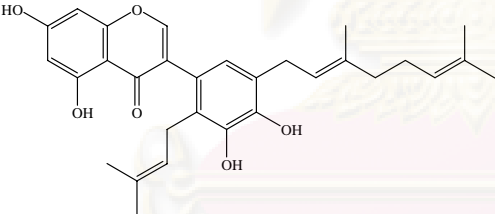
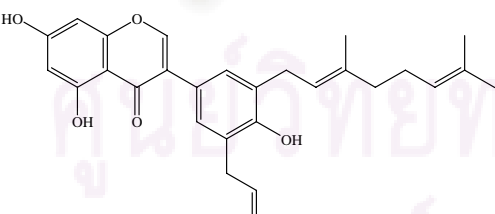
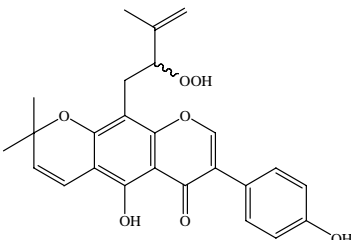
Plant and compound	Category	Plant part	Reference
<b><i>Millettia taiwaniana</i></b>			
<p>Millewanin A [252]</p> 	Isoflavone	Stem	Ito <i>et al.</i> , 2004
<p>Millewanin B [253]</p> 	Isoflavone	Stem	Ito <i>et al.</i> , 2004
<p>Millewanin C [254]</p> 	Isoflavone	Stem	Ito <i>et al.</i> , 2004
<p>Millewanin D [255]</p> 	Isoflavone	Stem	Ito <i>et al.</i> , 2004
<p>Millewanin E [256]</p> 	Isoflavone	Stem	Ito <i>et al.</i> , 2004

Table 1 (continued)

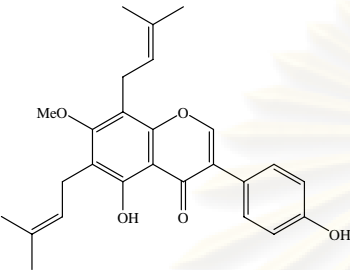
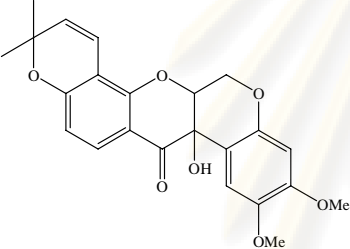
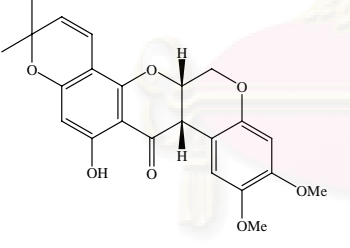
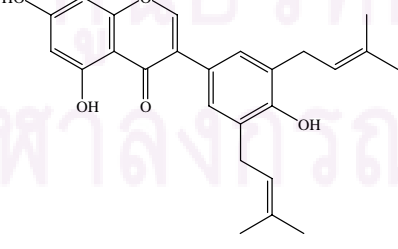
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia taiwaniana</i></b></p> <p>Millewanin F [257]</p> 	Isoflavone	Leaf	Ito <i>et al.</i> , 2006
<p>Tephrosin [79]</p> 	Rotenoid	Stem	Ito <i>et al.</i> , 2004
<p><math>\alpha</math>-Toxicarol [35]</p> 	Rotenoid	Stem	Ito <i>et al.</i> , 2004
<p>5,7,4'-Trihydroxy-3',5'-di-<math>\gamma,\gamma</math>-dimethylallylisoflavone [258]</p> 	Isoflavone	Stem	Ito <i>et al.</i> , 2004

Table 1 (continued)

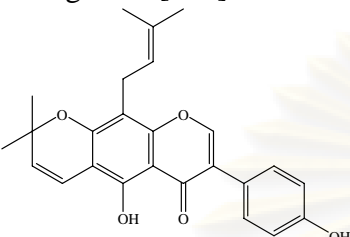
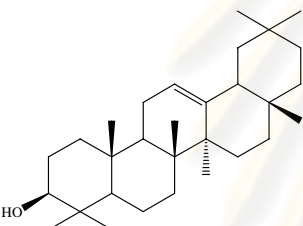
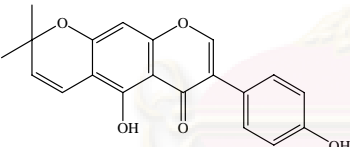
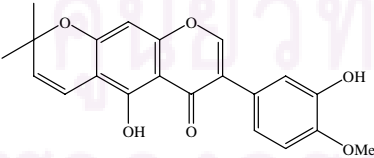
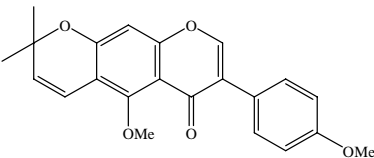
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia taiwaniana</i></b></p> <p>Waragalone [259]</p> 	Isoflavone	Stem, Leaf	Ito <i>et al.</i> , 2004 Ito <i>et al.</i> , 2006
<p><b><i>Millettia thonningii</i></b></p> <p><math>\beta</math>-amyrin [38]</p> 	Triterpenoid	Root bark	Asomaning <i>et al.</i> , 1995
<p>Alpinumisoflavone [246]</p> 	Isoflavone	Seed	Olivares <i>et al.</i> , 1982
<p>3',5-Dihydroxy-4'-methoxy-2'',2''-dimethylpyrano-(5'',6'': 6,7) isoflavone [260]</p> 	Isoflavone	Seed	Olivares <i>et al.</i> , 1982
<p>Dimethylalpinumisoflavone [261]</p> 	Isoflavone	Seed	Olivares <i>et al.</i> , 1982

Table 1 (continued)



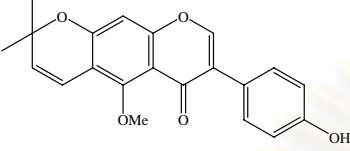
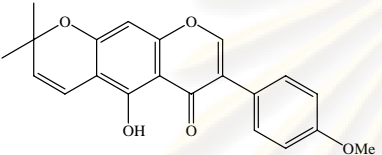
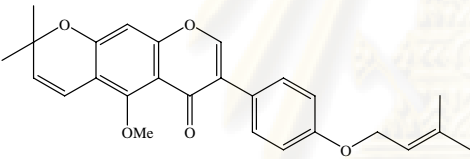
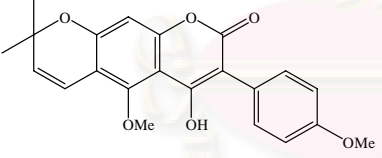
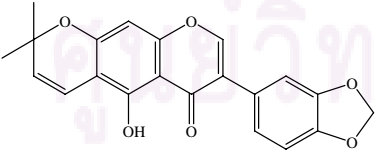
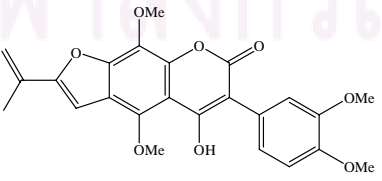
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia thonningii</i></b></p> <p><b>5-<i>O</i>-Methylalpinumisoflavone [262]</b></p> 	Isoflavone	Root	Asomaning <i>et al.</i> , 1999
<p><b>4'-Methylalpinumisoflavone [263]</b></p> 	Isoflavone	Seed	Olivares <i>et al.</i> , 1982
<p><b>5-<i>O</i>-Methyl-4'-<i>O</i>-(3-methyl-2-butenyl)alpinumisoflavone [264]</b></p> 	Isoflavone	Root bark	Asomaning <i>et al.</i> , 1995
<p><b>Robustic acid [265]</b></p> 	Coumarin	Seed	Olivares <i>et al.</i> , 1982
<p><b>Robustone [266]</b></p> 	Isoflavone	Seed	Khalid and Waterman, 1983
<p><b>Thonningine A [267]</b></p> 	Coumarin	Seed	Khalid and Waterman, 1983

Table 1 (continued)

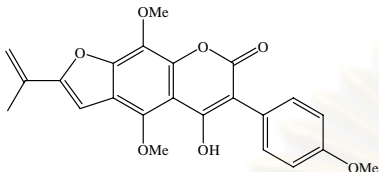
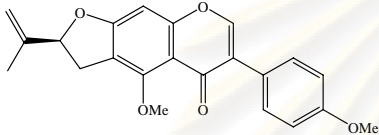
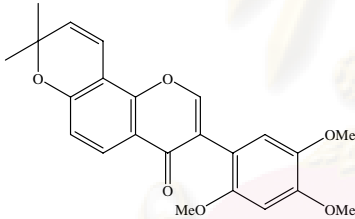
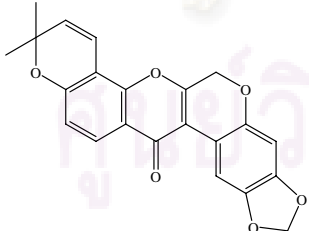
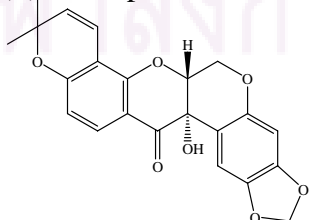
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia thonningii</i></b></p> <p>Thonningine B [268]</p> 	Coumarin	Seed	Khalid and Waterman, 1983
<p>Thonninginisoflavone [269]</p> 	Isoflavone	Root bark	Asomaning <i>et al.</i> , 1995
<p><b><i>Millettia usaramensis</i> subsp. <i>usaramensis</i></b></p> <p>Barbigerone [110]</p> 	Isoflavone	Stem bark	Yenesew, Midiwo and Waterman, 1998 Yenesew <i>et al.</i> , 2003
<p>6a,12a-Dehydromillettone [270]</p> 	Rotenoid	Stem bark	Yenesew <i>et al.</i> , 2003
<p>(+)-12a-Epimillettosin [271]</p> 	Rotenoid	Stem bark	Yenesew, Midiwo and Waterman, 1998

Table 1 (continued)

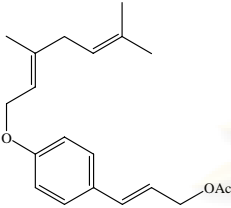
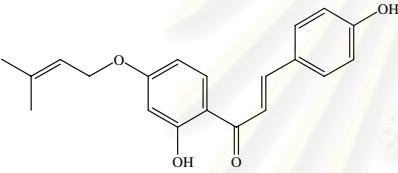
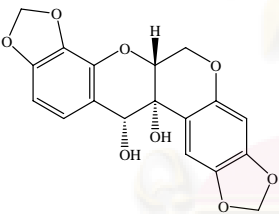
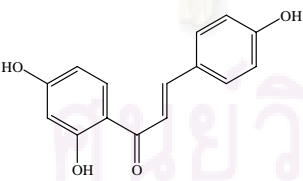
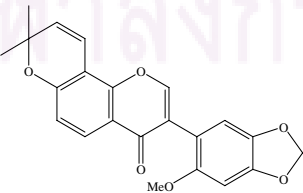
Plant and compound	Category	Plant part	Reference
<b><i>Millettia usaramensis</i> subsp.</b>			
<b><i>usaramensis</i></b>			
4'- <i>O</i> -Geranylcinnylacetate [272] 	Phenyl propanoid	Stem bark	Yenesew, Midiwo and Waterman, 1998
4'- <i>O</i> -Geranylisoliquiritigenin [119] 	Chalcone	Stem bark	Yenesew, Midiwo and Waterman, 1998
(+)-12 $\alpha$ -Hydroxy-12-dihydro- usararotenoid A [273] 	Rotenoid	Stem bark	Yenesew, Midiwo and Waterman, 1998
Isoliquiritigenin [274] 	Chalcone	Stem bark	Yenesew, Midiwo and Waterman, 1998
Jamaicin [69] 	Isoflavone	Stem bark	Yenesew, Midiwo and Waterman, 1998

Table 1 (continued)

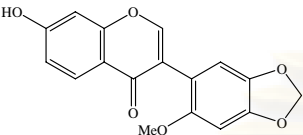
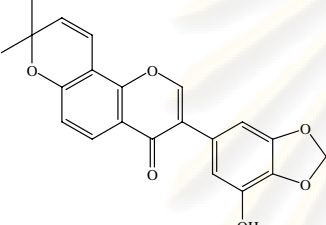
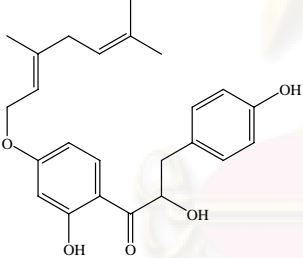
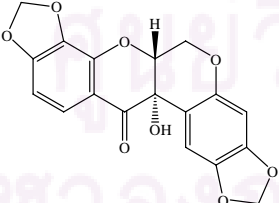
Plant and compound	Category	Plant part	Reference
<b><i>Millettia usaramensis</i> subsp.</b>			
<b><i>usaramensis</i></b>			
Maximaisoflavone G [137] 	Isoflavone	Stem bark	Yenesew, Midiwo and Waterman, 1998
Norisojamaicin [275] 	Isoflavone	Stem bark	Yenesew, Midiwo and Waterman, 1998
$\alpha,4,2'$ -Trihydroxy-4'- <i>O</i> -geranyl- dihydrochalcone [276] 	Chalcone	Stem bark	Yenesew, Midiwo and Waterman, 1998
(+)-Usararotenoid A [277] 	Rotenoid	Stem bark	Yenesew, Midiwo and Waterman, 1998  Yenesew <i>et al.</i> , 2003

Table 1 (continued)

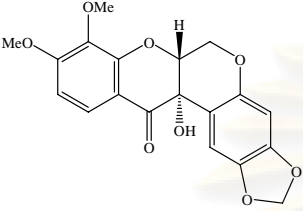
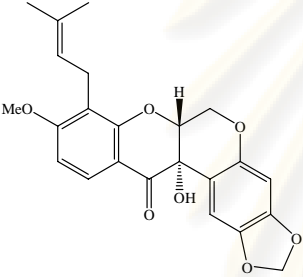
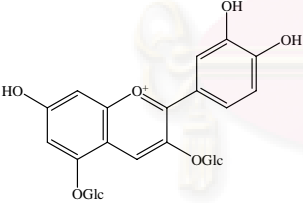
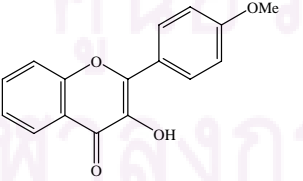
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia usaramensis</i> subsp. <i>usaramensis</i></b></p> <p>(+)-Usararotenoid B [278]</p>  <p>Usararotenoid C [279]</p> 	<p>Rotenoid</p> <p>Rotenoid</p>	<p>Stem bark</p> <p>Stem bark</p>	<p>Yenesew, Midiwo and Waterman, 1998</p> <p>Yenesew <i>et al.</i>, 2003</p>
<p><b><i>Millettia zechiana</i></b></p> <p>Cyanidin-3,5-diglucoside [280]</p>  <p>3-Hydroxy-4'-methoxyflavone [281]</p> 	<p>Flavonoid glycoside</p> <p>Flvonol</p>	<p>Flower</p> <p>Flower</p>	<p>Parvez and Ogbeide, 1990</p> <p>Parvez and Ogbeide, 1990</p>

Table 1 (continued)

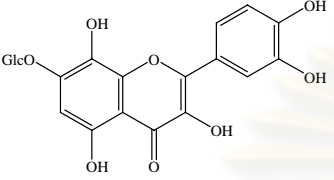
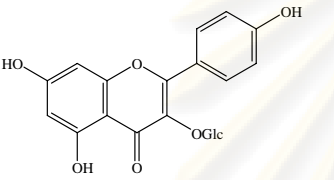
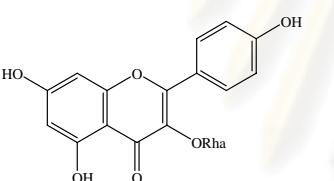
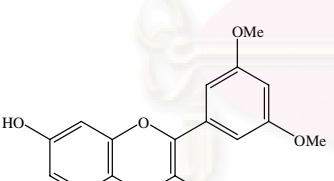
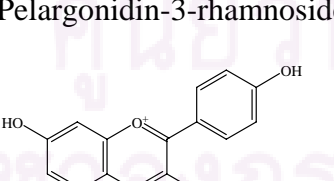
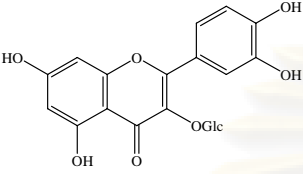
Plant and compound	Category	Plant part	Reference
<p><b><i>Millettia zechiana</i></b></p> <p><b>8-Hydroxyquercetin-7-glucoside [282]</b></p>  <p>The structure shows a flavonoid core with a glucose moiety (GlcO) at position 7 and a hydroxyl group (OH) at position 8. The A-ring has hydroxyl groups at positions 2 and 3, and a carbonyl group at position 4. The C-ring has a hydroxyl group at position 5 and a hydroxyl group at position 6. The D-ring has hydroxyl groups at positions 1 and 3.</p>	Flavonoid glycoside	Flower	Parvez and Ogbeide, 1990
<p><b>Kaempferol-3-glucoside [283]</b></p>  <p>The structure shows a flavonoid core with a glucose moiety (OGlc) at position 3. The A-ring has hydroxyl groups at positions 2 and 3, and a carbonyl group at position 4. The C-ring has a hydroxyl group at position 5 and a hydroxyl group at position 6. The D-ring has a hydroxyl group at position 1 and a hydroxyl group at position 3.</p>	Flavonoid glycoside	Flower	Parvez and Ogbeide, 1990
<p><b>Kaempferol-3-rhamnoside [284]</b></p>  <p>The structure shows a flavonoid core with a rhamnose moiety (ORha) at position 3. The A-ring has hydroxyl groups at positions 2 and 3, and a carbonyl group at position 4. The C-ring has a hydroxyl group at position 5 and a hydroxyl group at position 6. The D-ring has a hydroxyl group at position 1 and a hydroxyl group at position 3.</p>	Flavonoid glycoside	Flower	Parvez and Ogbeide, 1990
<p><b>Malvidin-3,5-diglucoside [285]</b></p>  <p>The structure shows a flavonoid core with two glucose moieties (OGlc) at positions 3 and 5. The A-ring has a hydroxyl group at position 2 and a carbonyl group at position 4. The C-ring has a methoxy group (OMe) at position 5 and a methoxy group at position 6. The D-ring has a methoxy group at position 1 and a hydroxyl group at position 3.</p>	Flavonoid glycoside	Flower	Parvez and Ogbeide, 1990
<p><b>Pelargonidin-3-rhamnoside [286]</b></p>  <p>The structure shows a flavonoid core with a rhamnose moiety (ORha) at position 3. The A-ring has a hydroxyl group at position 2 and a carbonyl group at position 4. The C-ring has a hydroxyl group at position 5 and a hydroxyl group at position 6. The D-ring has a hydroxyl group at position 1 and a hydroxyl group at position 3.</p>	Flavonoid glycoside	Flower	Parvez and Ogbeide, 1990

Table 1 (continued)

Table 1 (continued)

Plant and compound	Category	Plant part	Reference
<p><i>Millettia zechiana</i></p> <p>Quercetin-3-glucoside [287]</p>  <p>The chemical structure shows a flavonoid core (quercetin) with a glucose moiety attached to the 3-position of the C-ring. The A-ring has hydroxyl groups at positions 5 and 7. The C-ring has a carbonyl group at position 4 and a hydroxyl group at position 3. The B-ring has hydroxyl groups at positions 2 and 3.</p>	Flavonoid glycoside	Flower	Parvez and Ogbeide, 1990

ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย

## 2. Traditional uses and biological activities of *Millettia* constituents

Plants of the genus *Millettia* were used in traditional medicine in many countries. In Cameroon, *M. sanagana* and *M. conraui* were used in the treatment of intestinal parasites and cholera in children (Mbafor *et al.*, 1995, Tchinda *et al.*, 2007). Potions made from the root bark of *M. griffoniana* were employed by some village communities of Cameroon as an oral treatment for boils (Yankep *et al.*, 2003). The bark of *M. erythrocalyx* was used for treating stomach pain (Sritularak *et al.*, 2002). Root of *M. puguensis*, together with those of *Suregada zanzivariensis* (Euphorbiaceae), was boiled, and the decoction was used for stomach problems for adults of both sexes in Tanzania (Kapingu *et al.*, 2006). The bark pulp of *M. zechiana*, with sea water and Guinea grains diluted with warm water, was used as a gargle for rhinopharyngeal and bronchial troubles, and the purple leaves were rubbed on painful parts (Parvez and Ogbeide 1990). *M. thonningii* was used throughout the sub-region of west and central Africa in traditional medicine as a laxative, a blood purifier, a dewormer, an analgesic and for the treatment of diarrhea (Asomaning *et al.*, 1995).

Other uses of plants in this genus were insecticidal, piscicidal and molluscicidal activities. In Madagascar, *M. pervilleana* was used as a fish poison (Galeffi *et al.*, 1997). The seed of *M. ichthyochtona* was served as a fishpoison and an insecticide (Kamperdick *et al.*, 1998). Extract and aqueous suspension of finely ground seed of *M. pachycarpa* were reported to possess insecticidal activity when used in sprays against a variety of insects, e.g. houseflies, bean aphids, pentatomids, leaf beetles and cabbage worms (Singhal *et al.*, 1983). The juice from the leaves of *M. thonningii* was reported to be lethal to the *Bulinus* snail, the vector for schistosomiasis (Asomaning *et al.*, 1995).

In view of biological activities of compounds from *Millettia* plants,  $\beta$ -amyryn and 7-*O*-geranyl-6-methoxypseudobaptigenin had significant  $\alpha$ -glucosidase inhibitory activity (Tchinda *et al.*, 2007). From *M. puguensis*, moderate antileishmanial activity was observed for 6,7-dimethoxy-3',4'-methylenedioxy-isoflavone ( $IC_{50} = 32 \mu M$  against *Leishmania infantum*) and moderate cytotoxicity for maackiain ( $IC_{50} = 43 \mu M$  on MRC-5 cells) (Kapingu *et al.*, 2006). Ovalifolin, pongol methyl ether and millettocalyxin A possessed moderate activity against both types of herpes simplex virus (Likhitwitayawuid *et al.*, 2005). Isomillinol-B and vesitol were found to be highly toxic even at 0.1  $\mu g/ml$  to *S. aureus* and *E. coli*. (Rao and Krupadanam, 1994).



## CHAPTER III

### EXPERIMENTAL

#### 1. Source of plant materials

The stemwood of *Millettia leucantha* Kurz was collected from Tungkrui district, Bangkok, Thailand in August 2006. Authentication was performed by comparison with herbarium specimen (BKF No. 18009) at National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment. A voucher specimen (KL 052547) has been deposited at Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

#### 2. General techniques

##### 2.1 Analytical thin-layer chromatography (TLC)

Technique	:	One dimension, ascending
Absorbent	:	Silica gel 60 F <sub>254</sub> (E. Merck) precoated plate
Layer thickness	:	0.2 mm
Distance	:	6 cm
Temperature	:	Laboratory temperature (30-35°C)
Detection	:	1. Ultraviolet light at wavelengths of 254 and 365 nm. 2. Anisaldehyde and heating at 105°C for 10 min.

##### 2.2 Column chromatography

###### 2.2.1 Vacuum liquid column chromatography

Adsorbent	:	Silica gel 60 (No.7734) particle size 0.063-0.200 mm (70-230 mesh ASTM) (E. Merck)
Packing method	:	Dry packing
Sample loading	:	The sample was dissolved in a small amount of organic solvent, mixed with a small quantity of adsorbent, triturated, dried and then placed gently on top of the column.
Detection	:	Fractions were examined by TLC under UV light at the wavelengths of 254 and 365 nm.

### 2.2.2 Flash column chromatography

- Adsorbent : Silica gel 60 (No.9385) particle size 0.040-0.063 nm (70-230 mesh ASTM) (E. Merck)
- Packing method : Wet packing
- Sample loading : The sample was dissolved in a small amount eluent and then applied gently on top of the column.
- Detection : Fractions were examined in the same way as described in section 2.2.1

### 2.2.3 Medium pressure liquid chromatography

- Adsorbent : Silica gel 60 (No.9385) particle size 0.040-0.063 nm (70-230 mesh ASTM) (E. Merck)
- Packing method : Dry packing
- Sample loading : The sample was dissolved in a small amount of organic solvent, mixed with a small quantity of adsorbent, triturated, dried and then placed gently on top of the column.
- Detection : Fractions were examined in the same way as described in section 2.2.1

### 2.2.4 Gel filtration chromatography

- Adsorbent : Sephadex LH 20 (Pharmacia)
- Packing method : Gel filter was suspended in the eluent and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tightly.
- Sample loading : The sample was dissolved in a small amount eluent and then applied gently on top of the column.

### 2.2.5 High pressure liquid chromatography

- Column : Shim-pack Prep-ODS No. 2025820
- Flow rate : 2 ml/min
- Mobile phase : Isocratic 50% methanol in water

Sample preparation	:	The sample was dissolved in small of eluent and filtered through Millipore filter paper before injection.
Injection volume	:	1 ml
Pump	:	LC-8A (Shimadzu)
Detector	:	SPD-10A UV Detector (Shimadzu)
Recorder	:	C-R6A Chromatopac (Shimadzu)
Temperature	:	Room temperature

## 2.3 Spectroscopy

### 2.3.1 Ultraviolet (UV) absorption spectra

UV (in methanol) spectra were obtained on a Shimadzu UV-160A UV/vis spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

### 2.3.2 Mass spectra

Mass spectra were recorded on a Micromass LCT spectrometer or a Thermo-Finnigan Polaris Q mass spectrometer (Department of Chemistry, Faculty of Science, Mahidol University) or a Bruker microTOF mass spectrometer (National Center for Genetic Engineering and Biotechnology).

### 2.3.3 Proton and carbon-13 nuclear magnetic resonance ( $^1\text{H}$ and $^{13}\text{C}$ -NMR) spectra

$^1\text{H}$  NMR (300 MHz) and  $^{13}\text{C}$  NMR (75 MHz) spectra were obtained with a Bruker Avance DPX-300 FT-NMR spectrometer (Faculty of Pharmaceutical Sciences, Chulalongkorn University).

$^1\text{H}$  NMR (500 MHz) and  $^{13}\text{C}$  NMR (125 MHz) spectra were obtained with a JEOL JMN-A 500 NMR spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University).

Solvents for NMR spectra were deuterated chloroform (chloroform-*d*) and deuterated acetone (acetone-*d*<sub>6</sub>). Chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal.

## 2.4 Physical property

### 2.4.1 Optical rotation

Optical rotations were measured on a Perkin Elmer Polarimeter 341 (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

### 2.5 Solvents

All organic solvents employed throughout this work were of commercial grade and were redistilled prior to use.

## 3. Extraction and isolation

### 3.1 Extraction

The dried stemwood (6.9 kg) was chopped, ground and then macerated with methanol (3x10 L) to give, after removal of the solvent, a methanol extract (142 g). The methanol extract was triturated with dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) and ethyl acetate (EtOAc), respectively, to give  $\text{CH}_2\text{Cl}_2$  extract (20 g) and EtOAc extract (11 g).

### 3.2 Separation of $\text{CH}_2\text{Cl}_2$ extract

The  $\text{CH}_2\text{Cl}_2$  extract (10 g) was separated by vacuum liquid column chromatography using a sintered glass filter column of silica gel (No.7734, 250 g). The  $\text{CH}_2\text{Cl}_2$  extract was dissolved in a small amount of  $\text{CH}_2\text{Cl}_2$ , triturated with silica gel (No.7734) and dried under vacuum. Elution was performed in a polarity gradient manner with mixtures of hexane and EtOAc (10:0 to 0:10). The eluates were collected 200 ml per fraction and examined by TLC (silica gel, EtOAc-hexane 6:4) to yield 77 fractions. Another portion of  $\text{CH}_2\text{Cl}_2$  extract (10 g) was separated in the same manner to give 72 fractions. Fractions (149 fractions) with similar chromatographic manner were combined to yield 17 fractions: A (148.9 mg), B (2.34 g), C (2.20 g), D (709.4 mg), E (346.9 mg), F (256.8 mg), G (399.6 mg), H (218.2 mg), I (1.01 g), J (1.17 g), K (1.61 g), L (1.16 g), M (1.23 g), N (1.27 g), O (1.24 g), P (97.4 mg), Q (1.61 g).

#### 3.2.1 Isolation of compound ML1 (cycloeucalenol)

Fraction G (399.6 mg) was fractionated on a silica gel (No. 9385) column. Elution was performed in a polarity gradient manner with mixtures of hexane and EtOAc (10:0 to 0:10). Fractions (62 fractions) showing similar chromatographic

pattern were combined (TLC, silica gel, EtOAc-hexane 4:6) to yield 14 fractions: G1 (6.4 mg), G2 (1.4 mg), G3 (10.4 mg), G4 (33.4 mg), G5 (21.1 mg), G6 (202.8 mg), G7 (7.6 mg), G8 (23.4 mg), G9 (3.5 mg), G10 (5.4 mg), G11 (2.8 mg), G12 (0.5 mg), G13 (5.5 mg), G14 (30 mg).

Fraction G5 (21.1 mg) was purified on a Sephadex LH20 column (acetone) to give compound ML1 as white needles (12 mg,  $R_f$  0.34, silica gel, EtOAc-hexane 2:8). It was identified as cycloeucalenol.

### 3.2.2 Isolation of mixture ML2 (mixture of $\beta$ -sitosterol and stigmasterol)

Repeated column chromatography was performed on fraction G6 (202.8 mg) (silica gel No.9385, gradient elution, hexane-EtOAc 10:0 to 0:10). The eluates (51 fractions) were combined based on their TLC behavior (silica gel, EtOAc-hexane 4:6) to afford 20 fractions: G6A (0.7 mg), G6B (3.2 mg), G6C (6.1 mg), G6D (1.2 mg), G6E (1.3 mg), G6F (1.7 mg), G6G (0.8 mg), G6H (56.2 mg), G6I (69.9 mg), G6J (14.4 mg), G6K (2.1 mg), G6L (2.6 mg), G6M (4.4 g), G6N (14.9 mg), G6O (7 mg), G6P (3 mg), G6Q (5 mg), G6R (0.9 mg), G6S (1.1 mg), G6T (19.2 mg).

Fraction G6H (56.2 mg) was further separated on a Sephadex LH20 column (acetone). Twenty fractions were combined according to their TLC pattern (silica gel, EtOAc-hexane 4:6) to yield 3 fractions: G6H1 (3 mg), G6H2 (18.7 mg), G6H3 (30.9 mg).

Fraction G6H3 (30.9 mg) was repeatedly fractionated on a Sephadex LH20 column (acetone). Thirty four fractions with similar chromatographic pattern were combined (TLC, silica gel, EtOAc-hexane 4:6) to give 6 fractions: G6H3A (0.6 mg.), G6H3B (2.8 mg), G6H3C (14.2 mg), G6H3D (6.4 mg), G6H3E (3.5 mg), G6H3F (0.3 mg).

Fraction G6H3C (14.2 mg) was washed three times with methanol to yield mixture ML2 as a white powder (7.6 mg,  $R_f$  0.43, silica gel, EtOAc-hexane 4:6). It was identified as a mixture of  $\beta$ -sitosterol and stigmasterol.

### 3.2.3 Isolation of mixture ML3 (mixture of 7-oxositosterol and 7-oxostigmasterol)

Fraction J (1.17 g) was further subjected under column chromatography (silica gel, gradient mixtures of hexane-EtOAc 10:0 to 0:10) to yield 49 fractions. Fraction K

(1.61 g) was further separated under the same condition to give 56 fractions. Fractions (105 fractions) with similar TLC patterns (silica gel, EtOAc-hexane 4:6) were combined to afford 26 fractions: JK1 (19.6 mg), JK2 (52.5 mg), JK3 (33.9 mg), JK4 (64 mg), JK5 (17.7 mg), JK6 (174.3 mg), JK7 (80.5 mg), JK8 (127 mg), JK9 (104.7 mg), JK10 (65.2 mg), JK11 (82.3 mg), JK12 (14.9 mg), JK13 (166.7 mg), JK 14 (193.6 mg), JK15 (387 mg), JK16 (73.4 mg), JK17 (190.9 mg), JK18 (32.1 mg), JK19 (95.1 mg), JK20 (291.7 mg), JK21 (23.7 mg), JK22 (77.1 mg), JK23 (48.4 mg), JK24 (23.5 mg), JK25 (50.4 mg), JK 26 (164 mg).

Fraction JK14 (193.6 mg) was fractionated by repeated column chromatography (silica gel, gradient mixture of CH<sub>2</sub>Cl<sub>2</sub>-EtOAc 10:0 to 0:10) to afford compound ML3 as a white powder (19.6 mg, R<sub>f</sub> 0.39, silica gel, EtOAc-CH<sub>2</sub>Cl<sub>2</sub> 2:3). It was identified as a mixture of 7-oxositosterol and 7-oxostigmasterol.

### 3.3 Separation of EtOAc extract

The EtOAc (11 g) was separated into 3 portions. The first portion (3 g) was subjected to medium pressure liquid chromatography (MPLC) (silica gel, gradient mixture of CH<sub>2</sub>Cl<sub>2</sub>-acetone 10:0 to 0:10). The eluates were collected 200 ml per fraction to yield 42 fractions. The second portion (4 g) was separated under the same condition to give 62 fractions. The third portion (4 g) was fractionated under the same condition to afford 68 fractions. Fractions (172 fractions) with similar chromatographic pattern were combined (TLC, silica gel, acetone- CH<sub>2</sub>Cl<sub>2</sub> 1:9) to give 17 fractions: 1 (97.8 mg), 2 (186.2 mg), 3 (80 mg), 4 (167 mg), 5 (47.1 mg), 6 (63.8 mg), 7 (139 mg), 8 (137.6 mg), 9 (251.8 mg), 10 (296.7 mg), 11 (401.6 mg), 12 (17.7 mg), 13 (355.8 mg), 14 (1.14 g), 15 (1.23 g), 16 (1.24 g), 17 (2.54 g).

#### 3.3.1 Isolation of compound ML4 (maackiain)

Fraction 6 (63.8 mg) was purified on a Sephadex LH20 column (acetone) to give 7 mg of compound ML4 as a brown powder (R<sub>f</sub> 0.42, silica gel, acetone- CH<sub>2</sub>Cl<sub>2</sub> 0.6:9.4). It was identified as maackiain.



### 3.3.2 Isolation of compound ML5 (4-hydroxy-3-methoxybenzoic acid) and ML6 (syringic acid)

Fraction 10 (296.7 mg) was fractionated on a Sephadex LH20 column (acetone) to yield 9 fractions: 10A (111.5 mg), 10B (78.2 mg), 10C (79 mg), 10D (7.6 mg), 10E (2.4 mg), 10F (2 mg), 10G (1.2 mg), 10H (1.2 mg), 10I (2.6 mg).

Fraction 10C (79 mg) was separated by flash column chromatography (silica gel, gradient mixture of hexane-EtOAc 10:0 to 0:10) to give compound ML5 as a brown powder (9.7 mg,  $R_f$  0.51, silica gel, EtOAc-CH<sub>2</sub>Cl<sub>2</sub> 7:3) and compound ML6 as a brown powder (12.3 mg,  $R_f$  0.41, silica gel, EtOAc-CH<sub>2</sub>Cl<sub>2</sub> 7:3). ML5 and ML6 were identified as 4-hydroxy-3-methoxybenzoic acid and syringic acid, respectively.

### 3.3.3 Isolation of compound ML7 (balanocarpol)

Fraction 14 (1.14 g) was separated on a flash column (silica gel, gradient mixtures of hexane-EtOAc 10:0 to 0:10). Sixty eight fractions were combined on the basis of their TLC composition (silica gel, EtOAc-hexane 2:8) to yield 15 fractions: 14A (16.7 mg), 14B (14.8 mg), 14C (73.1 mg), 14D (53.1 mg), 14E (78.4 mg), 14F (48.1 mg), 14G (161.3 mg), 14H (75.6 mg), 14I (112.9 mg), 14J (99 mg), 14K (78.2 mg), 14L (23.5 mg), 14M (40 mg), 14N (30.7 mg), 14O (88.3 mg).

Fraction 14G (161.3 mg) was repeatedly fractionated on a flash column (silica gel, gradient mixtures of CH<sub>2</sub>Cl<sub>2</sub>-acetone 10:0 to 0:10). Fifty seven fractions with similar chromatographic pattern (TLC, silica gel, acetone- CH<sub>2</sub>Cl<sub>2</sub> 1:1) were combined to yield 9 fractions: 14G1 (3.9 mg), 14G2 (2 mg), 14G3 (9.2 mg), 14G4 (64.6 mg), 14G5 (3 mg), 14G6 (2.1 mg), 14G7 (10 mg), 14G8 (26.3 mg), 14G9 (18.5 mg).

Fraction 14G4 (64.6 mg) was separated on a Sephadex LH20 column (acetone). Fractions (64 fractions) were combined according to their TLC pattern (silica gel, acetone- CH<sub>2</sub>Cl<sub>2</sub> 1:1) to give 8 fractions: 14G4A (0.1 mg), 14G4B (0.7 mg), 14G4C (0.2 mg), 14G4D (0.9 mg), 14G4E (3.5 mg), 14G4F (40.9 mg), 14G4G (5.6 mg), 14G4H (2.5 mg).

Fraction 14G4F (40.9 mg) was further separated by repeated Sephadex LH20 (MeOH) column chromatography. Forty three fractions were combined based on their TLC behavior (silica gel, acetone- CH<sub>2</sub>Cl<sub>2</sub> 1:1) to afford 12 fractions: I (0.5 mg), II (0.3 mg), III (0.2 mg), IV (0.7 mg), V (0.3 mg), VI (2 mg), VII (25.8 mg), VIII (5.1 mg), IX (4 mg), X (0.9 mg), XI (1.8 mg), XII (16.5 mg).

Purification of fraction VII (25.8 mg) by RP18 HPLC (Shim-pack Prep-ODS) with 50% methanol in water as eluent and UV-VIS detection ( $\lambda$  254 nm.) gave compound ML7 as a brown powder (4.6 mg,  $R_f$  0.34, silica gel, acetone-  $\text{CH}_2\text{Cl}_2$  1:1). It was identified as balanocarpol.

### 3.3.4 Isolation of compound ML8 (diptoindonesin D)

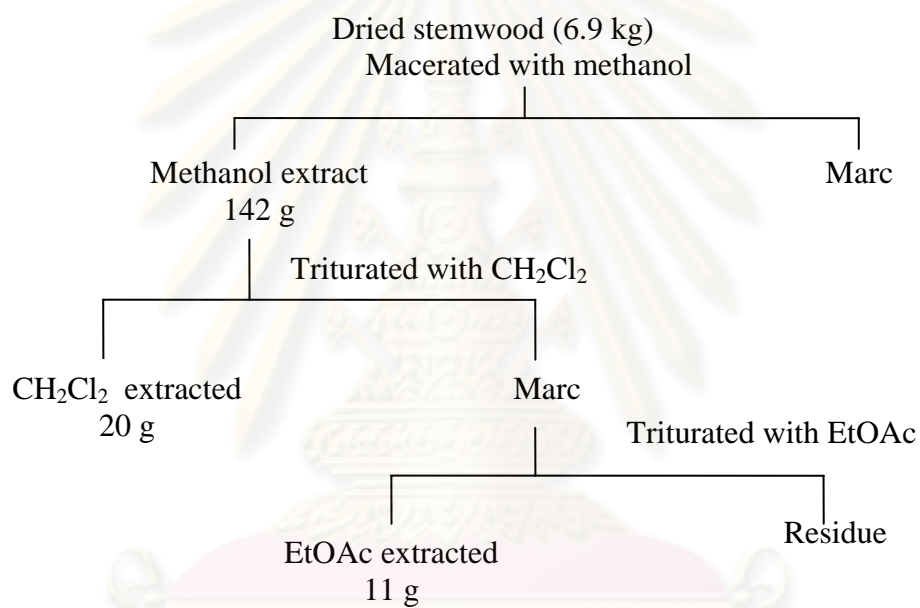
Fraction 15 (1.23 g) was subjected to flash column chromatography (silica gel). The elution was performed in a polarity manner with mixtures of  $\text{CH}_2\text{Cl}_2$ -methanol (10:0 to 0:10). Fractions (55 fractions) with similar TLC pattern (silica gel, methanol- $\text{CH}_2\text{Cl}_2$  1:9) were combined to give 13 fractions: 15A (27.7 mg), 15B (12.9 mg), 15C (54.7 mg), 15D (49.2 mg), 15E (76.7 mg), 15F (63.8 mg), 15G (170.6 mg), 15H (237.4 mg), 15I (111.7 mg), 15J (104.8 mg), 15K (47.8 mg), 15L (85.3 mg), 15M (55.1 mg).

Fraction 15H (237.4 mg) was purified on a Sephadex LH20 (MeOH) column to yield compound ML8 as a brown powder (4 mg,  $R_f$  0.17, silica gel, methanol- $\text{CH}_2\text{Cl}_2$  1:9). It was identified as diptoindonesin D.

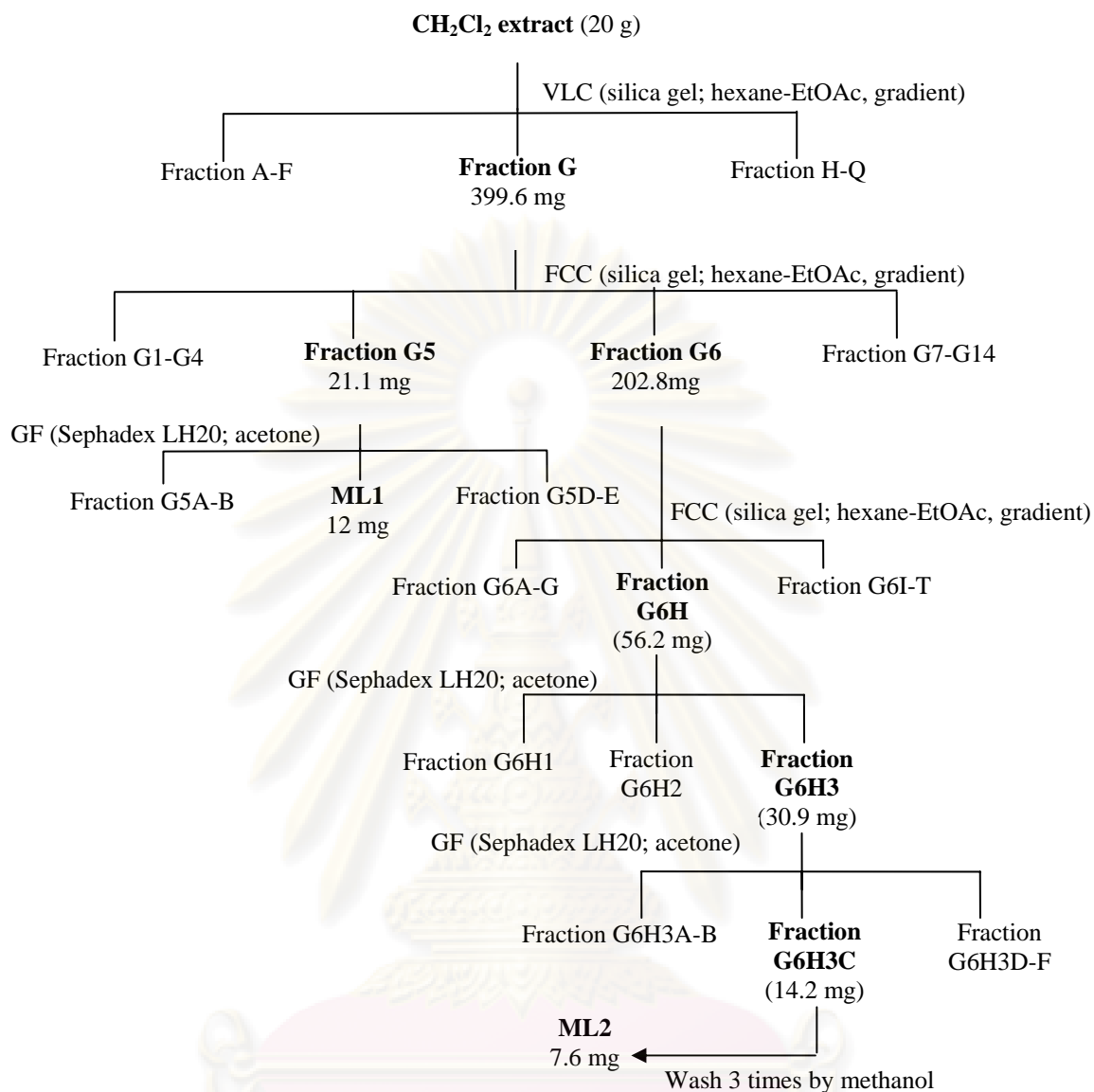


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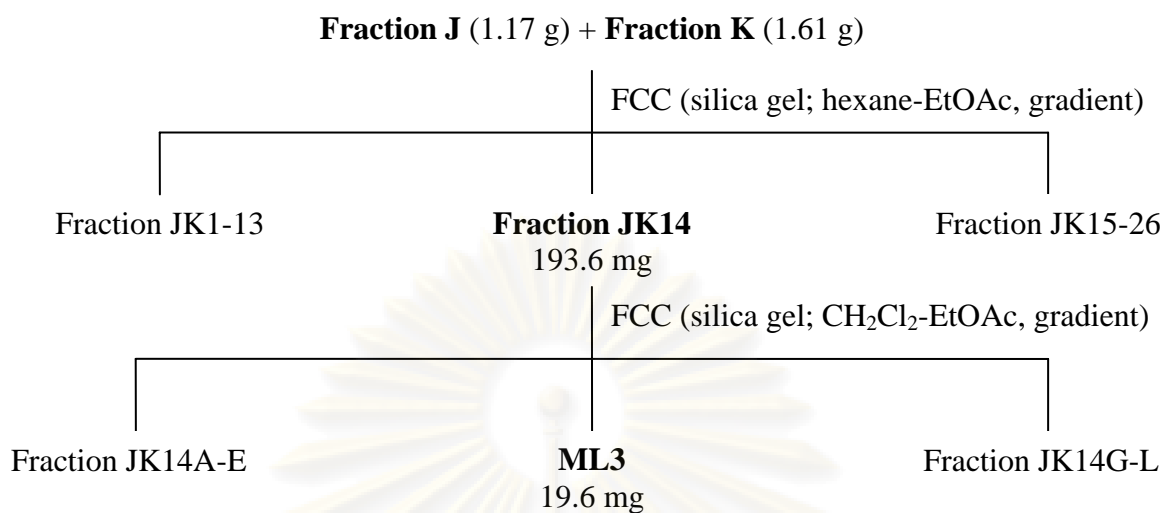


**Scheme 1** Extraction of *Millettia leucantha* stemwood

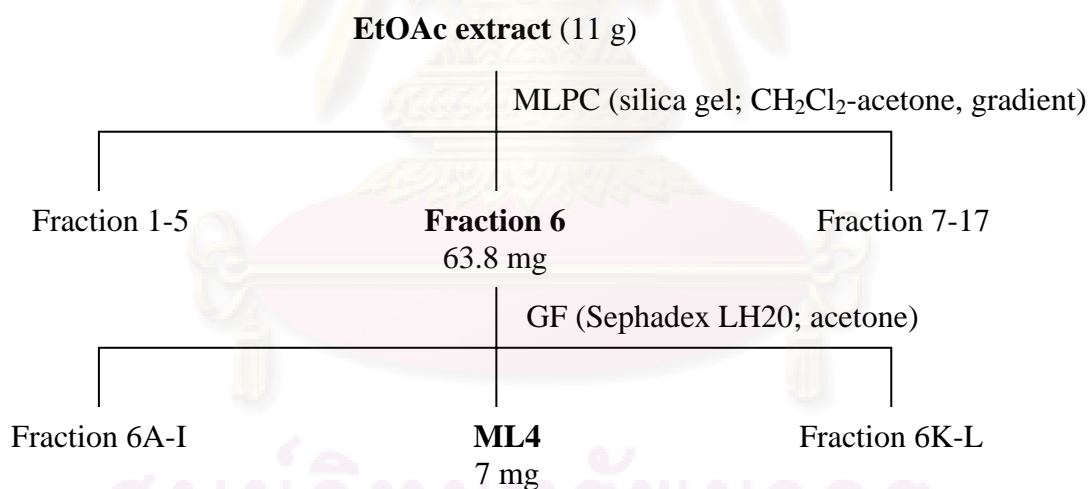


**Scheme 2** Separation of the CH<sub>2</sub>Cl<sub>2</sub> extract of *Millettia leucantha*

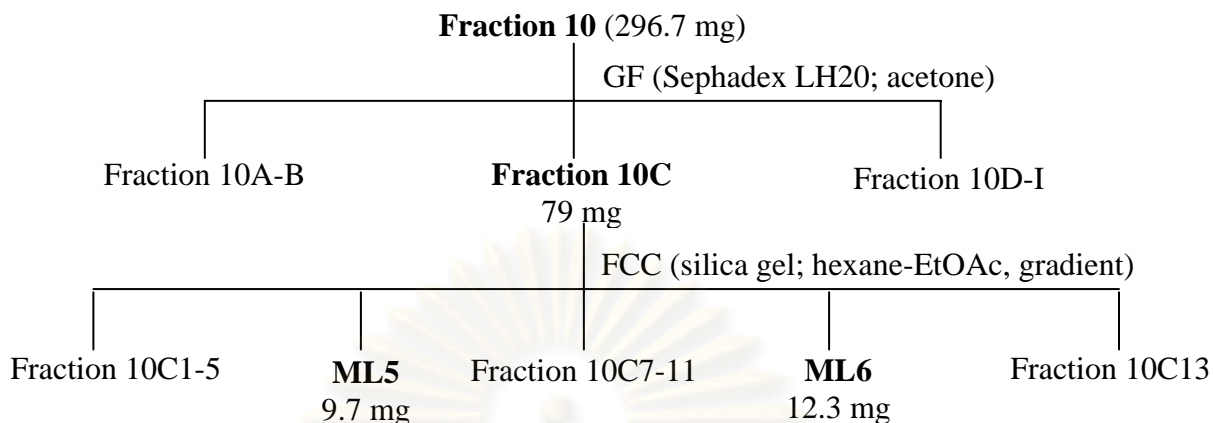
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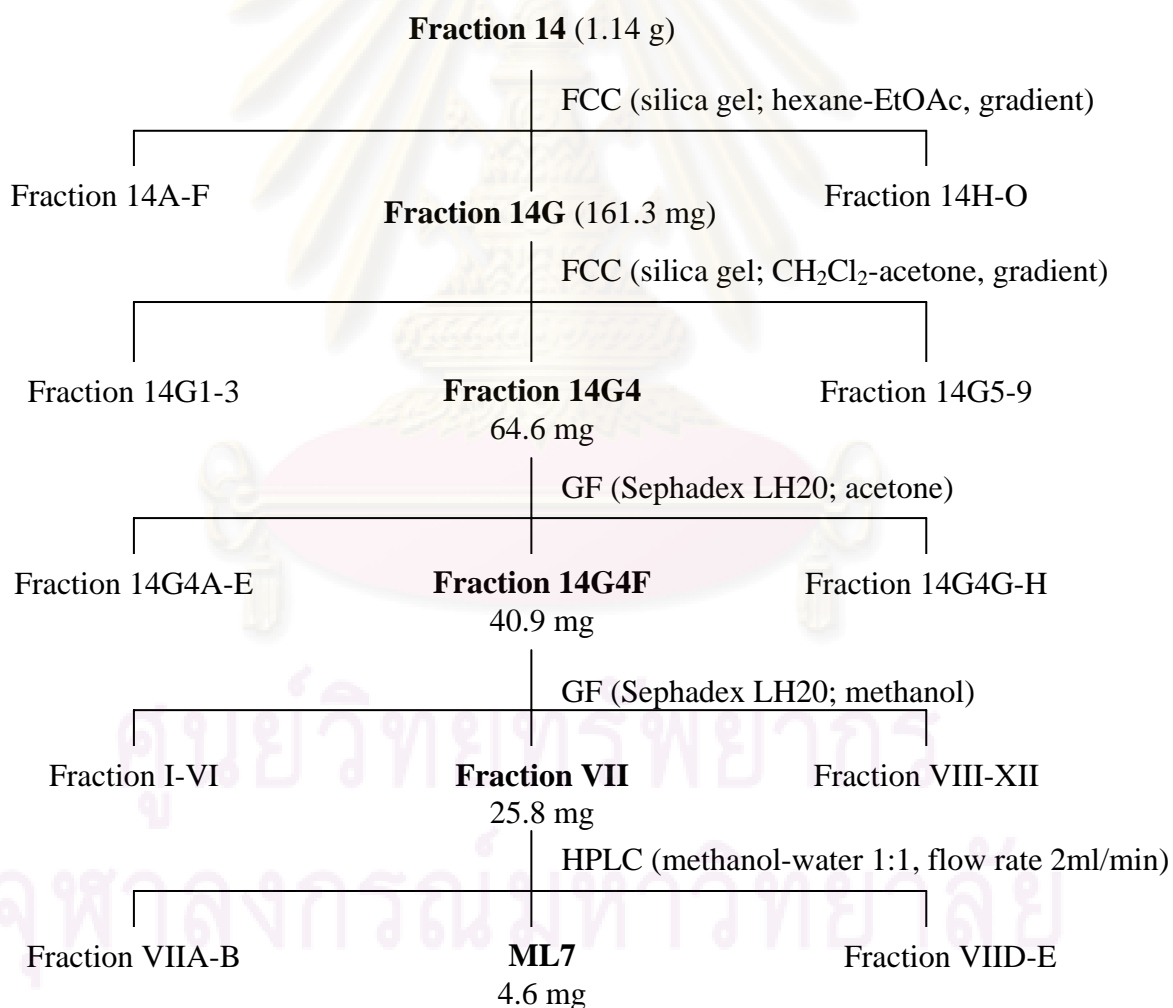
**Scheme 3** Separation of fraction J and K of the  $\text{CH}_2\text{Cl}_2$  extract



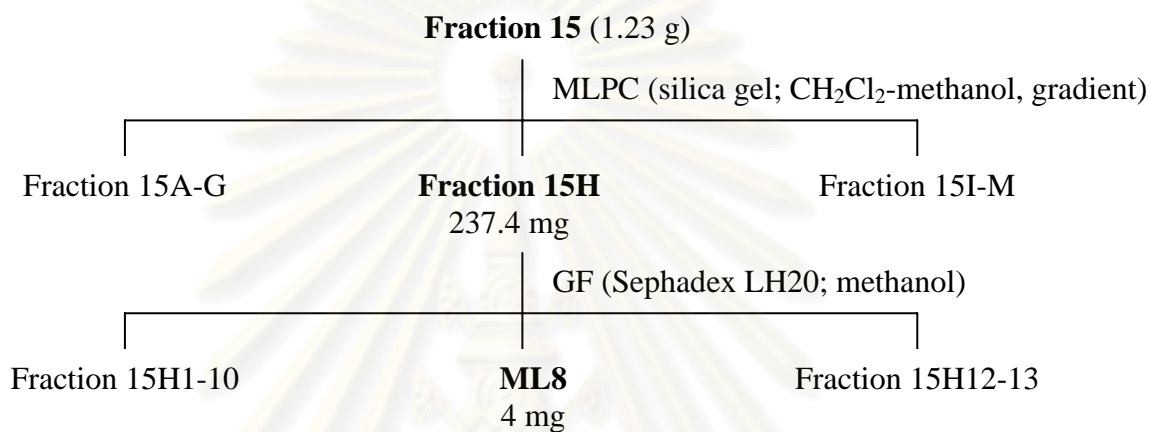
**Scheme 4** Separation of the EtOAc extract of *Millettia leucantha*



**Scheme 5** Separation of fraction 10 of EtOAc extract



**Scheme 6** Separation of fraction 14 of EtOAc extract



**Scheme 7** Separation of fraction 15 of EtOAc extract

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#### 4. Physical and spectral data of isolated compounds

##### 4.1 Compound ML1 (cycloeucaleanol)

Compound ML1 was obtained as white needles, soluble in  $\text{CH}_2\text{Cl}_2$  (21.1 mg,  $3.06 \times 10^{-4}$  % based on dried weight of stemwood).

- EIMS** :  $m/z$  (% relative intensity); **Figure 2**  
 426 (10), 408 (24), 393 (45), 189 (20), 163 (24), 161 (42), 147 (53),  
 145 (48), 135 (30), 133 (53), 123 (23), 121 (44), 119 (58), 109 (41),  
 107 (45), 105 (79), 95 (44), 81 (52)
- $^1\text{H NMR}$**  :  $\delta$  ppm, 300 MHz, in  $\text{CDCl}_3$ ; see **Figure 3**
- $^{13}\text{C NMR}$**  :  $\delta$  ppm, 75 MHz, in  $\text{CDCl}_3$ ; see **Table 2, Figure 4**

##### 4.2 Mixture ML2 (mixture of $\beta$ -sitosterol and stigmasterol)

Mixture ML2 was obtained as a white powder, soluble in  $\text{CH}_2\text{Cl}_2$  (7.6 mg,  $2.06 \times 10^{-4}$  % based on dried weight of stemwood).

- $^1\text{H NMR}$**  :  $\delta$  ppm, 300 MHz, in  $\text{CDCl}_3$ ; see **Figure 6**
- $^{13}\text{C NMR}$**  :  $\delta$  ppm, 75 MHz, in  $\text{CDCl}_3$ ; see **Table 3, Figure 7**

##### 4.3 Mixture ML3 (mixture of 7-oxositosterol and 7-oxostigmasterol)

Mixture ML3 was obtained as a white powder, soluble in  $\text{CH}_2\text{Cl}_2$  (19.6 mg,  $1.10 \times 10^{-4}$  % based on dried weight of stemwood).

- $^1\text{H NMR}$**  :  $\delta$  ppm, 300 MHz, in  $\text{CDCl}_3$ ; see **Figure 8**
- $^{13}\text{C NMR}$**  :  $\delta$  ppm, 75 MHz, in  $\text{CDCl}_3$ ; see **Table 4, Figure 9**

##### 4.4 Compound ML4 (maackiain)

Compound ML4 was obtained as a brown powder, soluble in  $\text{CH}_2\text{Cl}_2$  (7 mg,  $1.01 \times 10^{-4}$  % based on dried weight of stemwood).

- EIMS** :  $m/z$  (% relative intensity); **Figure 12**  
 284 (42), 267 (17), 175 (12), 162 (16)
- $[\alpha]_D^{20}$**  :  $-255^\circ$  ( $c$  0.02; MeOH)
- UV** :  $\lambda_{\text{max}}$  nm ( $\log \epsilon$ ), in methanol; **Figure 11**  
 227.5 (5.06), 286.5 (4.77), 311 (4.88)
- $^1\text{H NMR}$**  :  $\delta$  ppm, 300 MHz, in  $\text{CDCl}_3$ ; see **Table 5, Figure 13**
- $^{13}\text{C NMR}$**  :  $\delta$  ppm, 75 MHz, in  $\text{CDCl}_3$ ; see **Table 5, Figure 14**

#### 4.5 Compound ML5 (4-hydroxy-3-methoxybenzoic acid)

Compound ML5 was obtained as a brown powder, soluble in acetone (9.7 mg,  $1.41 \times 10^{-4}\%$  based on dried weight of stemwood).

- EIMS** :  $m/z$  (% relative intensity); **Figure 16**  
168 (100), 153 (68), 151 (13), 125 (27), 97 (43)
- UV** :  $\lambda_{\max}$  nm (log  $\epsilon$ ), in methanol; **Figure 15**  
258 (3.95), 290 (3.72)
- $^1\text{H}$  NMR** :  $\delta$  ppm, 300 MHz, in Acetone- $d_6$ ; see **Table 6, Figure 17**
- $^{13}\text{C}$  NMR** :  $\delta$  ppm, 75 MHz, in Acetone- $d_6$ ; see **Table 6, Figure 18**

#### 4.6 Compound ML6 (syringic acid)

Compound ML6 was obtained as a brown powder, soluble in acetone (12.3 mg,  $1.78 \times 10^{-4}\%$  based on dried weight of stemwood).

- EIMS** :  $m/z$  (% relative intensity); **Figure 20**  
198 (100), 183 (36), 149 (12), 127 (18), 109 (23), 81 (10), 65 (12)
- UV** :  $\lambda_{\max}$  nm (log  $\epsilon$ ), in methanol; **Figure 19**  
273 (3.97)
- $^1\text{H}$  NMR** :  $\delta$  ppm, 300 MHz, in Acetone- $d_6$ ; see **Table 7, Figure 21**
- $^{13}\text{C}$  NMR** :  $\delta$  ppm, 75 MHz, in Acetone- $d_6$ ; see **Table 7, Figure 22**

#### 4.7 Compound ML7 (balanocarpol)

Compound ML7 was obtained as a brown powder, soluble in acetone (4.6 mg,  $6.67 \times 10^{-5}\%$  based on dried weight of stemwood).

- EIMS** :  $m/z$  (% relative intensity); **Figure 23**  
470 (2), 452 (25), 376 (3), 348 (8), 107 (9), 94 (19)
- $[\alpha]_D^{20}$**  :  $-15^\circ$  ( $c$  0.033; MeOH)
- UV** :  $\lambda_{\max}$  nm (log  $\epsilon$ ), in methanol; **Figure 24**  
226.6 (4.33), 284 (3.78)
- $^1\text{H}$  NMR** :  $\delta$  ppm, 300 MHz, in Acetone- $d_6$ ; see **Table 8, Figure 25**
- $^{13}\text{C}$  NMR** :  $\delta$  ppm, 75 MHz, in Acetone- $d_6$ ; see **Table 8, Figure 26**

#### 4.8 Compound ML8 (diptoindonesin D)

Compound ML8 was obtained as a brown powder, soluble in acetone (4 mg,  $5.80 \times 10^{-5}$  % based on dried weight of stemwood).

<b>ESIMS</b>	: $[M+H]^+$ $m/z$ 379.37; <b>Figure 29</b>
$[\alpha]_D^{20}$	: +100 ° ( $c$ 0.035; MeOH)
<b>UV</b>	: $\lambda_{max}$ nm (log $\epsilon$ ), in methanol; <b>Figure 28</b> 226 (4.07), 372 (3.60)
<b><math>^1H</math> NMR</b>	: $\delta$ ppm, 500 MHz, in Acetone- $d_6$ ; see <b>Table 9, Figure 30</b>
<b><math>^{13}C</math> NMR</b>	: $\delta$ ppm, 125 MHz, in Acetone- $d_6$ ; see <b>Table 9, Figure 31</b>

### 5. Determination of free radical scavenging activity

#### 5.1 TLC screening assay (Takao *et al.*, 1994)

The samples were spotted and developed on TLC plate with suitable developing solvent. After drying, the TLC plate was sprayed with 0.2% solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol. After 30 min, active compounds appeared as yellow spots on the purple background.

#### 5.2 Free radical scavenging activity assay (Braca *et al.*, 2002)

##### 5.2.1 Preparation of test sample

The test compound (0.5 mg) was dissolved in 1 ml methanol (or suitable solvent) and diluted with methanol until a suitable range of concentration (mg/ml) was obtained. The concentration was expressed as  $\mu M$  in final concentration. For example, ML1 (MW 426) at 0.5 mg/1ml was equal to 1173  $\mu M$  ( $0.5 \text{ mg}/1\text{ml} \times 426$ ). For each well, 20  $\mu l$  of test solution was added to the reaction mixture to furnish the total volume of 200  $\mu l$ . The final concentration was calculated by the formula below.

$$N_1 V_1 = N_2 V_2$$

$N_1$  = Beginning concentration ( $\mu M$ )

$V_1$  = Beginning volume ( $\mu l$ )

$N_2$  = Final concentration ( $\mu M$ )

$V_2$  = Final volume ( $\mu l$ )

Thus, the final concentration of ML1 solution =  $1173 \mu M \times 20 \mu l / 200 \mu l$   
= 117.3  $\mu M$



### 5.2.2 Preparation of DPPH solution (100 $\mu$ M)

DPPH (2 mg) was dissolved in 100 ml of methanol, and the solution was stirred for 30 min.

### 5.2.3 Measurement of activity

The test sample (20  $\mu$ l) was added to 180  $\mu$ l DPPH solution (100  $\mu$ M) in 96-well plate. The solution mixture was incubated at 37°C for 30 min and then the absorbance of each well was measured at 510 nm. The DPPH solution (180  $\mu$ l) mixed with methanol (20  $\mu$ l) was used as negative control and quercetin as a reference compound.

### 5.2.4 Calculation of percent inhibition of DPPH free radical scavenging activity

The percentage of DPPH reduction was calculated as follows.

$$\% \text{ DPPH reduction} = (A-B) \times 100 / A$$

A = The absorbance of DPPH solution after incubation at 510 nm

B = The absorbance of the reaction mixture after incubation at 510 nm

For IC<sub>50</sub> evaluation of pure compounds, a graph showing concentration versus % DPPH reduction was plotted. The IC<sub>50</sub> was calculated from the graph.

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## CHAPTER IV

### RESULTS AND DISCUSSION

The dried powdered of *Millettia leucantha* stemwood (6.9 kg) was macerated with methanol. The methanol extract (142 g) was triturated with CH<sub>2</sub>Cl<sub>2</sub> and EtOAc, respectively. The CH<sub>2</sub>Cl<sub>2</sub> extract (20 g) was separated using several chromatographic techniques to afford a pure compound (ML1) and two mixtures (ML2 and ML3). The EtOAc extract (11 g) was separated using several chromatographic techniques to yield six pure compounds (ML4-ML8). The structure determinations of all isolates were carried out by interpretation of their UV, MS and NMR data, and further confirmed by comparison with literature values.

#### 1. Structure determination of isolated compounds

##### 1.1 Structure determination of compound ML1

Compound ML1 was obtained as white needles. The EI mass spectrum (Figure 2) showed a molecular ion [M<sup>+</sup>] at  $m/z$  426, corresponding to C<sub>30</sub>H<sub>50</sub>O.

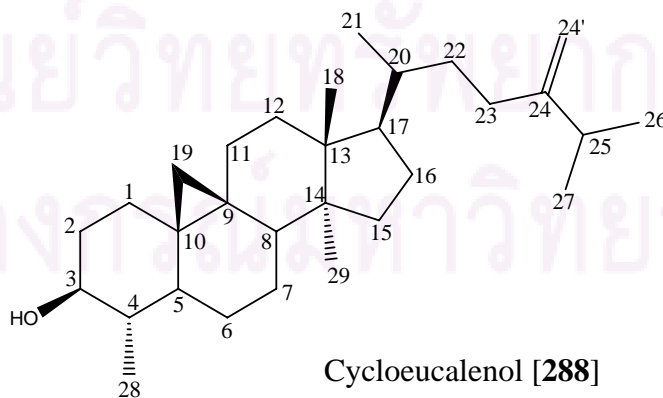
The <sup>1</sup>H NMR spectrum (Figure 3) of compound ML1 showed signals at δ 4.69 (1H, br s) and 4.64 (1H, br s) assignable to H-24', 3.19 (1H, m) due to H-3, and 0.37 (1H, d,  $J=3.6$ ) and 0.12 (1H, d,  $J=3.9$ ) assignable to H-19.

The <sup>13</sup>C NMR, DEPT 90 and DEPT 135 spectra (Figures 4-5 and Table 2) exhibited 30 carbon signals, corresponding to 6 methyls, 12 methylenes, 7 methines and 5 quaternary carbons. Through comparison of its <sup>1</sup>H and <sup>13</sup>C NMR data, and MS data with reported values (Kikuchi *et al.*, 1986), it was identified as cycloeucalenol [288].

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**Table 2** NMR Spectral data of compound ML1 and cycloeucalenol

Carbon position	Chemical shift (ppm)	
	ML1	Cycloeucalenol
1	30.8	30.88
2	34.8	34.90
3	76.6	76.64
4	44.6	44.69
5	43.3	43.43
6	24.7	24.73
7	25.1	25.22
8	46.8	46.90
9	23.5	23.64
10	29.5	29.65
11	27.2	27.08
12	32.9	32.99
13	45.3	45.45
14	48.9	49.00
15	35.3	35.42
16	28.1	28.18
17	52.2	52.31
18	17.8	17.84
19	27.0	27.28
20	36.1	36.21
21	18.3	18.42
22	35.0	35.13
23	31.3	31.41
24	156.9	156.89
25	33.8	33.90
26	22.0	22.06
27	21.9	21.94
28	14.4	14.46
29	19.1	19.21
24'	105.9	106.05

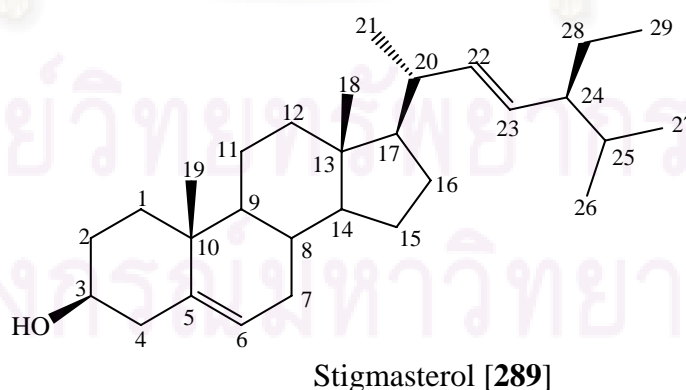
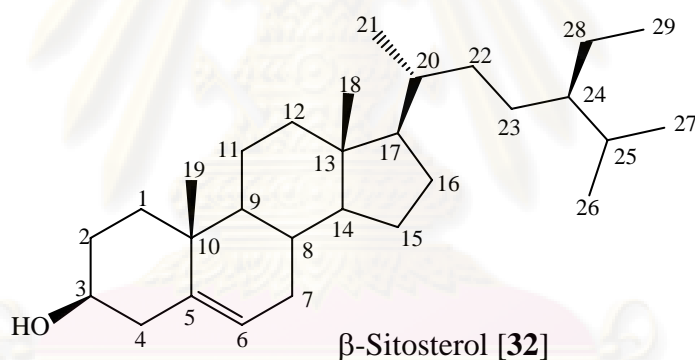


## 1.2 Structure determination of mixture ML2

Mixture ML2 was isolated as a white powder. Anisaldehyde TS test gave a purple color, indicative of steroidal or triterpenoid skeleton. It was identified as a mixture of  $\beta$ -sitosterol [32] and stigmasterol [289] through comparison of its  $^1\text{H}$  and  $^{13}\text{C}$  NMR data with previous reported values (Wright *et al.*, 1978).

The  $^1\text{H}$  NMR spectrum (Figure 6) of ML2 showed signals at  $\delta$  5.33 (0.7H, d,  $J = 4.8\text{Hz}$ ) due to H-6 of  $\beta$ -sitosterol and stigmasterol and 5.14 (0.26H, dd,  $J = 15, 8.4\text{Hz}$ ) and 5.02 (0.26H, dd,  $J = 15, 8.4\text{Hz}$ ) due to H-22 and H-23 of stigmasterol. The integration of H-6, H-22 and H-23 were approximate in the ratio of 0.7:0.26:0.26. Therefore, it could be calculated that ML2 was a mixture of  $\beta$ -sitosterol and stigmasterol in the ratio of 2:1.

The  $^{13}\text{C}$  NMR spectrum (Figure 7 and Table 3) exhibited 42 signals. Comparison of these data with  $^{13}\text{C}$  NMR data of  $\beta$ -sitosterol and stigmasterol (Wright *et al.*, 1978) is shown in Table 3



**Table 3** NMR Spectral data of mixture ML2,  $\beta$ -sitosterol and stigmasterol  
(CDCl<sub>3</sub>)

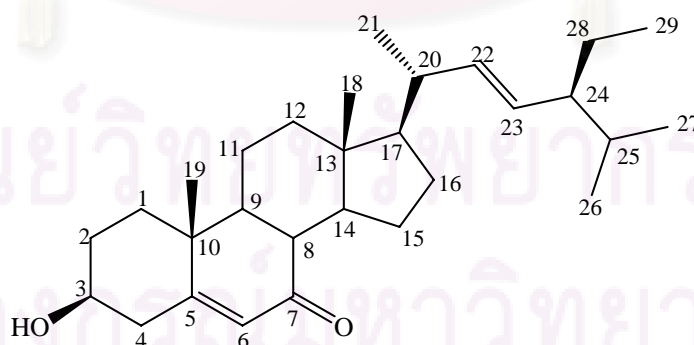
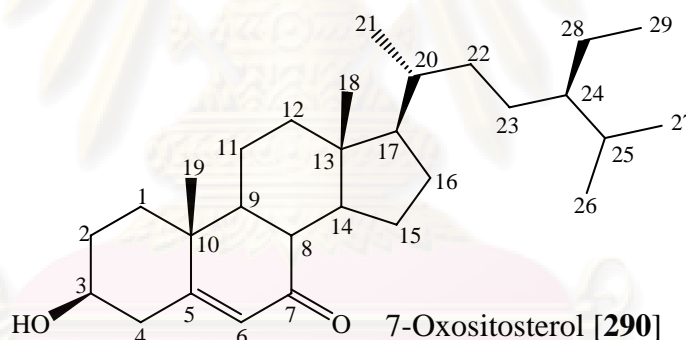
Carbon position	Chemical shift (ppm)		
	$\beta$ -sitosterol	stigmasterol	ML2
1	37.31	37.31	37.3
2	31.57	31.69	31.7
3	71.69	71.81	71.8
4	42.25	42.35	42.3
5	140.76	140.80	140.8
6	121.59	121.69	121.7
7	31.92	31.94	31.9
8	31.92	31.94	31.9
9	50.17	50.20	50.2
10	36.51	36.56	36.5
11	21.11	21.11	21.1
12	39.81	39.74	39.8, 39.7
13	42.33	42.35	42.3
14	56.79	56.91	56.8, 56.9
15	24.32	24.39	24.3, 24.4
16	28.26	28.96	28.2, 28.9
17	56.11	56.06	56.1, 56.0
18	11.87	12.07	11.9, 12.1
19	19.40	19.42	19.4
20	36.17	40.54	36.2
21	18.82	21.11	19.0, 21.1
22	33.95	138.37	34.0, 138.3
23	26.13	129.32	26.1, 129.3
24	45.85	51.29	45.9, 51.2
25	29.18	31.94	29.2, 31.9
26	19.84	21.26	19.8, 21.2
27	19.07	19.02	19.0
28	23.09	25.44	23.1, 25.4
29	12.32	12.27	12.2

### 1.3 Structure determination of mixture ML3

Mixture ML3 was isolated as a white powder. Anisaldehyde TS test gave a purple color, indicative of steroidal or triterpenoid skeleton. It was identified as a mixture of 7-oxositosterol [290] and 7-oxostigmasterol [291] through comparison of its  $^1\text{H}$  and  $^{13}\text{C}$  NMR data with previous reported values (Greca *et al.*, 1990, Aliotta *et al.*, 1991).

The  $^1\text{H}$  NMR spectrum (Figure 8) of compound ML3 showed signals at  $\delta$  5.67 (0.9H, s) due to H-6 of 7-oxositosterol and 7-oxostigmasterol, 5.15 (0.22H, dd,  $J = 15, 8.4\text{Hz}$ ) and 5.00 (0.22H, dd,  $J = 15, 8.4\text{Hz}$ ) due to H-22 and H-23 of 7-oxostigmasterol and 3.65 (1H, m) due to H-3. The integration of H-6, H-22 and H-23 were approximate in the ratio of 0.9:0.2:0.2. Therefore, it could be calculated that ML3 was a mixture of 7-oxositosterol and 7-oxostigmasterol in the ratio 4:1.

The  $^{13}\text{C}$  NMR spectrum (Figure 9 and Table 4) exhibited 33 signals. Comparison of these data with  $^{13}\text{C}$  NMR data of 7-oxositosterol and 7-oxostigmasterol (Greca *et al.*, 1990, Aliotta *et al.*, 1991) is shown in Table 4



7-Oxostigmasterol [291]

**Table 4** NMR Spectral data of mixture ML3, 7-oxositosterol and 7-oxostigmasterol (CDCl<sub>3</sub>)

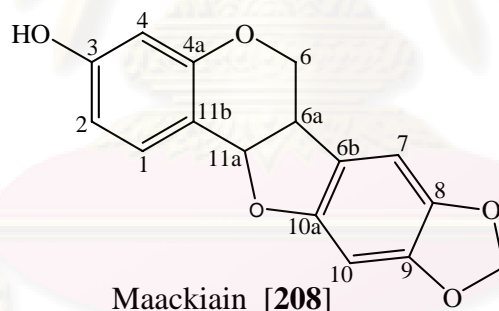
Carbon position	Chemical shift (ppm)		
	7-oxositosterol	7-oxostigmasterol	ML3
1	36.62	36.58	36.5
2	31.18	31.24	31.2
3	70.52	70.45	70.5
4	41.79	41.70	41.8
5	169.35	169.22	165.1
6	126.13	126.04	126.1
7	204.21	204.15	202.3
8	45.39	45.22	45.4
9	50.02	50.18	50.0
10	38.24	38.12	38.3
11	21.20	21.28	21.2
12	39.68	39.74	38.7
13	41.79	41.84	41.8
14	49.93	49.87	50.0
15	26.30	26.38	26.3
16	28.53	28.46	28.5
17	54.69	54.55	54.7
18	11.87	11.96	12.0
19	17.28	17.28	17.3
20	36.06	40.33	36.1, 40.2
21	18.90	21.29	18.9, 21.4
22	33.93	138.26	33.9, 138.1
23	26.07	129.30	26.1, 129.1
24	45.80	51.21	45.8, 51.2
25	29.11	31.80	29.1, 31.9
26	19.77	21.16	19.8, 21.0
27	19.01	19.20	19.0
28	23.04	25.58	23.0, 25.6
29	11.95	12.36	12.0

#### 1.4 Structure determination of Compound ML4

Compound ML4 was obtained as a brown powder. The UV spectrum (Figure 11) showed absorption at 227.5, 286.5 and 311 nm. The EI mass spectrum (Figure 12) displayed a molecular ion peak at  $m/z$  284, consistent with the formula  $C_{16}H_{12}O_5$ .

The  $^1H$  NMR spectrum (Figure 13 and Table 5) showed aromatic protons at  $\delta$  7.34 (1H, d,  $J = 8.4$  Hz) for H-1, 6.70 (1H, s) for H-7, 6.52 (1H, dd,  $J = 8.4, 2.1$  Hz) for H-2, 6.41 (1H, s) for H-10 and 6.39 (1H, d,  $J = 2.1$  Hz) for H-4. The signal at 5.88 (2H, d,  $J = 8.1$  Hz) represented methylene protons of O-CH<sub>2</sub>-O. Other proton signals appeared at  $\delta$  5.45 (1H, d,  $J = 6.9$  Hz) for H-11a, 4.20 (1H, dd,  $J = 11.1, 5.1$  Hz) for H-6 $\alpha$ , 3.62 (1H, t,  $J = 11.1$  Hz) for H-6 $\beta$  and 3.46 (1H, m) for H-6a.

The  $^{13}C$  NMR and DEPT spectra (Figure 14 and Table 5) exhibited 16 signals that were 2 methylenes, 7 methines and 7 quaternary carbons. Through comparison of these data with previously published data, compound ML4 was identified as maackiain [208] (Chaudhuri *et al.*, 1995).



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**Table 5** NMR Spectral data of compound ML4 (CDCl<sub>3</sub>) and maackiain (CDCl<sub>3</sub>)

Position	ML4		Maackiain	
	$\delta$ H (mult., <i>J</i> in Hz)	$\delta$ C	$\delta$ H (mult., <i>J</i> in Hz)	$\delta$ C
1	7.34 (d, 8.4)	132.1	7.35 (d,8.5)	132.1
2	6.52 (dd, 8.4, 2.1)	109.8	6.54 (dd, 8.5, 2.5)	109.8
3	-	157.1	-	157.1
4	6.39 d (2.1)	103.7	6.40 (d, 2.5)	103.6
4a	-	156.6	-	156.6
6	6 $\alpha$ 4.20 (dd, 11.1, 5.1) 6 $\beta$ 3.62 (t, 11.1)	66.5	6 $\alpha$ 4.21 (dd, 11, 5) 6 $\beta$ 3.64 (t, 11.0)	66.4
6a	3.46 (m)	40.2	3.45 (ddd, 11, 7, 5)	40.1
6b	-	117.9	-	117.9
7	6.70 (s)	104.7	6.72 (s)	104.7
8	-	141.7	-	141.7
9	-	148.2	-	148.1
10	6.41 (s)	93.8	6.43 (s)	93.8
10a	-	154.2	-	154.2
11a	5.45 (d, 6.9)	78.5	5.47 (d,7)	78.5
11b	-	112.6	-	112.5
OCH <sub>2</sub> O	5.88 (d, 8.1)	101.3	5.91 (d, 14)	101.3

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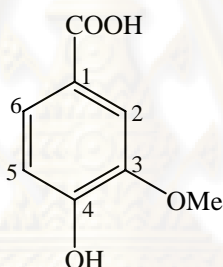
### 1.5 Structure determination of compound ML5

Compound ML5 was obtained as a brown powder. The UV spectrum (Figure 15) showed absorption at 258 and 290 nm. It showed a molecular ion peak at  $m/z$  168 in the EIMS (Figure 16), corresponding to the molecular formula  $C_8H_8O_4$ .

The  $^1H$  NMR spectrum (Figure 17 and Table 6) showed aromatic protons at  $\delta$  7.58 (1H, dd,  $J = 8.4, 1.5$  Hz) for H-6, 7.55 (1H, s) for H-2 and 6.89 (1H, d,  $J = 8.1$  Hz) for H-5. A methoxy signal showed at  $\delta$  3.89 (3H, s).

The  $^{13}C$  NMR spectrum (Figure 18 and Table 6) displayed 8 signals. A ketone carbon was found at  $\delta$  167.5, aromatic carbons were at  $\delta$  152.0, 148.1, 124.8, 122.9, 115.5 and 113.5 and a methoxy peak was at  $\delta$  56.3.

This compound was identified as 4-hydroxy-3-methoxybenzoic acid [**292**] by comparison of the above data with earlier reported values (Pouchert and Behnke, 1993)



4-Hydroxy-3-methoxybenzoic acid [**292**]

**Table 6** NMR Spectral data of compound ML5 (acetone- $d_6$ ) and 4-hydroxy-3-methoxybenzoic acid ( $CDCl_3$ +DMSO- $d_6$ )

Position	Compound ML5		4-hydroxy-3-methoxybenzoic acid
	$\delta H$ (mult., $J$ in Hz)	$\delta C$	$\delta C$
1	-	122.9	121.74
2	7.55 (s)	113.5	112.62
3	-	152.0	150.95
4	-	148.1	147.02
5	6.89 (d, 8.1)	115.5	114.84
6	7.58 (dd, 8.1, 1.5)	124.8	123.56
COOH	-	167.5	167.46
OMe	3.89 (s)	56.3	55.55

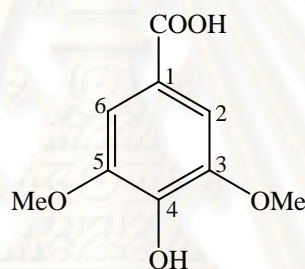
### 1.6 Structure determination of compound ML6

Compound ML6 was obtained as a brown powder. The UV spectrum (Figure 19) showed absorption at 273 nm. The molecular weight should be 198 (C<sub>9</sub>H<sub>10</sub>O<sub>5</sub>) as shown by the molecular ion peak at  $m/z$  198 in the EIMS (Figure 20).

The <sup>1</sup>H NMR spectrum (Figure 21 and Table 7) exhibited aromatic protons at  $\delta$  7.32 (2 x 1H, s) representing H-2 and 6. A methoxy signal showed at  $\delta$  3.87 (2 x 3H, s).

The <sup>13</sup>C NMR spectrum (Figure 22 and Table 7) showed 7 signals. A ketone carbon appeared at  $\delta$  167.52, aromatic carbons were at  $\delta$  148.3, 141.6, 121.5 and 108.1 and a methoxy peak was at  $\delta$  56.6.

Through comparison of these data with reported values, compound ML6 was identified as syringic acid [293] (Abbas *et al.*, 2007).



Syringic acid [293]

**Table 7** NMR Spectral data of compound ML6 (acetone-*d*<sub>6</sub>) and syringic acid (CD<sub>3</sub>OD)

Position	ML6		Syringic acid	
	$\delta$ H (mult., J in Hz)	$\delta$ C	$\delta$ H (mult., J in Hz)	$\delta$ C
1	-	121.5	-	121.9
2	7.32 (s)	108.1	7.35 (s)	108.4
3	-	148.3	-	148.9
4	-	141.6	-	141.8
5	-	148.3	-	148.9
6	7.32 (s)	108.1	7.35 (s)	108.4
COOH	-	167.5	-	170.0
OMe x 2	3.87 (s)	56.6	3.90 (s)	56.8

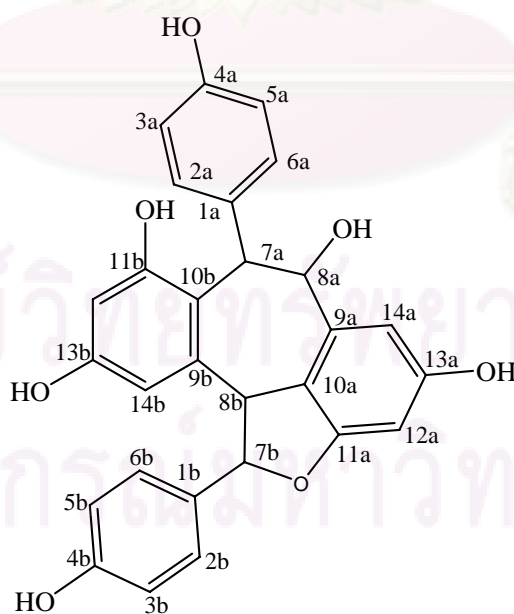
### 1.7 Structure determination of Compound ML7

Compound ML7, a brown powder, was analyzed for  $C_{28}H_{22}O_7$  from its molecular ion peak at  $m/z$  470 in the EIMS (Figure 23). The UV spectrum (Figure 24) showed absorption at 226.6 and 284 nm.

The  $^1H$  NMR spectrum (Figure 25 and Table 8) showed aromatic protons at  $\delta$  7.48 (2H, d,  $J = 8.4$  Hz) for H-2b and 6b, 6.94 (2H, d,  $J = 8.4$  Hz) for H-3b and 5b, 6.74 (2H, d,  $J = 8.4$  Hz) for H-2a and 6a, 6.42 (2H, d,  $J = 8.4$  Hz) for H-3a and 5a, 6.25 (1H, br s) for H-12b, 6.20 (1H, br s) for H-14b, 6.08 (1H, br s) for H-14a and 5.95 (1H, br s) for H-12a. The signal appearing at  $\delta$  5.69 (1H, d, 9.6), 5.39 (1H, br s), 5.15 (1H, d, 9.3) and 4.89 were due to H-7b, 8a, 8b and 7a, respectively.

The  $^{13}C$  NMR and DEPT spectra (Figure 26 and Table 8) displayed 28 carbons indicating 16 methines and 12 quaternary carbons. The compound was identified as balanocarpol [294] by analysis of the above data in comparison with earlier reported data (Tanaka *et al.*, 2000).

From HMQC spectrum (Figure 27), the proton at  $\delta$  5.95 correlated with the carbon at  $\delta$  95.1 of position 12a. The proton at  $\delta$  6.90 correlated with the carbon at  $\delta$  104.4 of position 14a. The proton at  $\delta$  6.25 correlated with the carbon at  $\delta$  102.0 of position 12b. The proton at  $\delta$  6.20 correlated with the carbon at  $\delta$  106.8 of position 14b. These findings are different from those earlier reported (Tanaka *et al.*, 2000).



Balanocarpol [294]

**Table 8** NMR Spectral data of compound ML7 (acetone- $d_6$ ) and balanocarpol (acetone- $d_6$ )

position	ML7		Balanocarpol	
	$\delta$ H (mult., <i>J</i> in Hz)	$\delta$ C	$\delta$ H (mult., <i>J</i> in Hz)	$\delta$ C
1a	-	133.5	-	133.5
2a, 6a	6.74 (d, 8.4)	131.5	6.75 (d, 8.3)	131.5
3a, 5a	6.42 (d, 8.4)	114.2	6.42 (d, 8.3)	114.2
4a	-	155.8	-	155.8
7a	4.89 (br s)	50.3	4.90 (br s)	50.3
8a	5.39 (br s)	73.2	5.40 (br s)	73.2
9a	-	140.8	-	140.8
10a	-	113.8	-	113.8
11a	-	159.7	-	159.7
12a	5.95 (br s)	95.1	6.20 (br s)	95.1
13a	-	159.2	-	159.2
14a	6.09 (br s)	104.4	6.26 (br s)	104.4
1b	-	133.7	-	133.7
2b, 6b	7.49 (d, 8.4)	130.5	7.50 (d, 8.3)	130.5
3b, 5b	6.94 (d, 8.4)	116.5	6.95 (d, 8.3)	116.4
4b	-	158.6	-	158.6
7b	5.69 (d, 9.3)	93.5	5.69 (d, 9.3)	93.5
8b	5.15 (d, 9.3)	52.3	5.16 (d, 9.3)	52.3
9b	-	142.9	-	142.8
10b	-	120.5	-	120.4
11b	-	157.4	-	157.4
12b	6.25 (br s)	102.0	6.09 (br s)	102.0
13b	-	156.9	-	156.9
14b	6.20 (br s)	106.8	5.96 (br s)	106.8

### 1.8 Structure determination of Compound ML8

Compound ML8 was obtained as a brown powder. The UV spectrum (Figure 28) showed absorption at 226 and 372 nm. It showed a  $[M+H]^+$  peak at  $m/z$  379 in the TOF MS (Figure 29), corresponding to the molecular formula  $C_{21}H_{14}O_7$ .

The  $^1H$  NMR spectrum (Figure 30 and Table 9) exhibited aromatic protons at  $\delta$  6.92 (1H, d,  $J = 2.5$  Hz) for H-14b, 6.90 (1H, d,  $J = 2.5$  Hz) for H-14a, 6.79 (1H, d,  $J = 2.5$  Hz) for H-12a, 6.68 (1H, d,  $J = 8$  Hz) for H-2b and 6b, 6.63 (1H, d,  $J = 9$  Hz) for H-3b and 5b and 6.38 (1H, d,  $J = 2.5$  Hz) for H-12b. The signals at  $\delta$  5.88 (1H, s) was assigned to H-7b. The sharp singlet at  $\delta$  13.69 indicated a chelated OH group of C-11b.

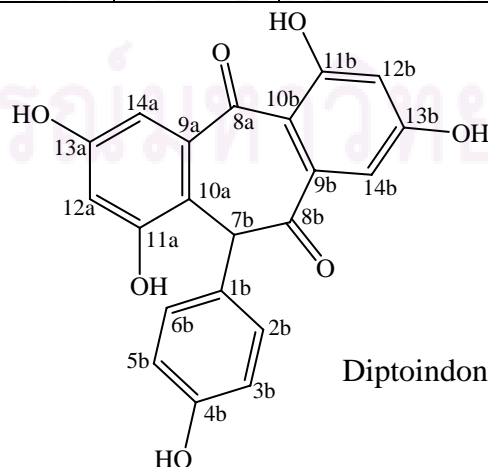
The  $^{13}C$  NMR and DEPT data (Figures 31-32 and Table 9) presented 21 carbons, consisting of 9 methines and 12 quaternary carbons. The HMBC spectrum (Figures 34-43) showed correlation of the long-range coupled  $^1H$  and  $^{13}C$  nuclei. The proton at position 12a showed long range correlations with C-11a, C-13a and C-14a. The methine proton at position 14a showed long range correlations with C-8a, C-10a, C-12a and C-13a. The protons at positions 2b and 6b displayed long range correlations with C-4b, C-2b, C-6b and C-7b. The protons at positions 3b and 5b presented long range correlations with C-1b, C-3b and C-5b. The methine proton at position 7b showed long range correlations with C-9a, C-10a, C-11a, C-1b, C-2b, C-3b, C-5b, C-6b, C-8b and C-9b. The proton at position 12b showed long range correlations with C-10b, C-11b, C-13b and C-14b. The methine proton at position 14b showed long range correlations with C-8b, C-10b, C-12b and C-13b. The H-bonded hydroxyl proton at position 11b showed long range correlations with C-10b, C-11b and C-12b.

Through comparison of these data with reported values, compound ML9 was identified as diptoindonesin D [295] (Sahidin *et al.*, 2005).

The data in Table 9 suggest that revisions for  $^1H$  and  $^{13}C$  assignments for positions 2b, 6b, 3b and 5b are needed, based on the HMQC data (Figure 33), and HMBC correlation (Figure 34) from H-2b and H-6b to C-7.

**Table 9** NMR Spectral data of compound ML8 (acetone- $d_6$ ) and diptoindonesin D (acetone- $d_6$ )

position	ML8		Diptoindonesin D	
	$\delta$ H (mult., <i>J</i> in Hz)	$\delta$ C	$\delta$ H (mult., <i>J</i> in Hz)	$\delta$ C
8a	-	197.4	-	197.1
9a	-	142.8	-	142.3
10a	-	111.2	-	110.7
11a	-	156.8	-	156.6
12a	6.79 (d, 2.5)	107.5	6.8 (d, 2.6)	107
13a	-	158.8	-	158.4
14a	6.90 (d, 2.5)	110.2	6.9 (d, 2.6)	109.8
1b	-	130.5	-	130.0
2b, 6b	6.68 (d, 9)	128.9	6.63 (d, 8.8)	128.5
3b, 5b	6.63 (d, 9)	116.2	6.68 (d, 8.8)	115.8
4b	-	157.0	-	156.4
7b	5.88 (s)	55.2	5.89 (br s)	54.8
8b	-	196.7	-	196.3
9b	-	139.6	-	139.2
10b	-	111.7	-	111.3
11b	-	167.3	-	166.8
12b	6.38 (d, 2.5)	107.0	6.39 (d, 2.6)	106.6
13b	-	165.2	-	164.5
14b	6.92 (d, 2.5)	113.1	6.92 (d, 2.6)	112.5
11b-OH	13.69 (s)		13.68 (s)	



Diptoindonesin D [295]



## 2. Free radical scavenging activity

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals. Radicals derived from oxygen represent the most important class of radical species generated in living systems, including superoxide ( $O_2^{\cdot-}$ ), peroxy ( $ROO^{\cdot}$ ), alkoxy ( $RO^{\cdot}$ ), hydroxyl ( $HO^{\cdot}$ ), nitric oxide ( $NO^{\cdot}$ ) and hydrogen peroxide ( $H_2O_2$ ). Oxygen free radicals or reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are products of normal cellular metabolism. ROS and RNS are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems. Beneficial effects of ROS occur at low/moderate concentrations and involve physiological roles in cellular responses to noxia, as for example in defense against infectious agents. The harmful effect of free radicals causing potential biological damage is termed oxidative stress and nitrosative stress. This occurs in biological systems when there is an overproduction of ROS/RNS on one side and a deficiency of enzymatic and non-enzymatic antioxidants. The excess ROS can damage cellular lipids, proteins, or DNA inhibiting their normal function (Valko *et al.*, 2007, Pietta 2000).

Defense mechanisms against free radical-induced oxidative stress involve: (i) preventative mechanisms, (ii) repair mechanisms, (iii) physical defences, and (iv) antioxidant defences. The mechanisms of antioxidant action can include (1) suppressing ROS formation by inhibition of enzymes or chelating trace elements involved in free radical production; (2) scavenging ROS; and (3) upregulating or protecting antioxidant defenses (Valko *et al.*, 2007, Pietta 2000).

By TLC screening assay, the MeOH extract of *M. leucantha* presented free radical scavenging activity. Isolated compounds were first test at 50  $\mu$ g/ml. Compounds exhibiting more than 50% inhibition were further evaluated for  $IC_{50}$  values. Quercetin was employed as positive control. The results are summarized in Table 10.



**Table 10** Percentage of DPPH reduction by isolated compounds from *M. leucantha*

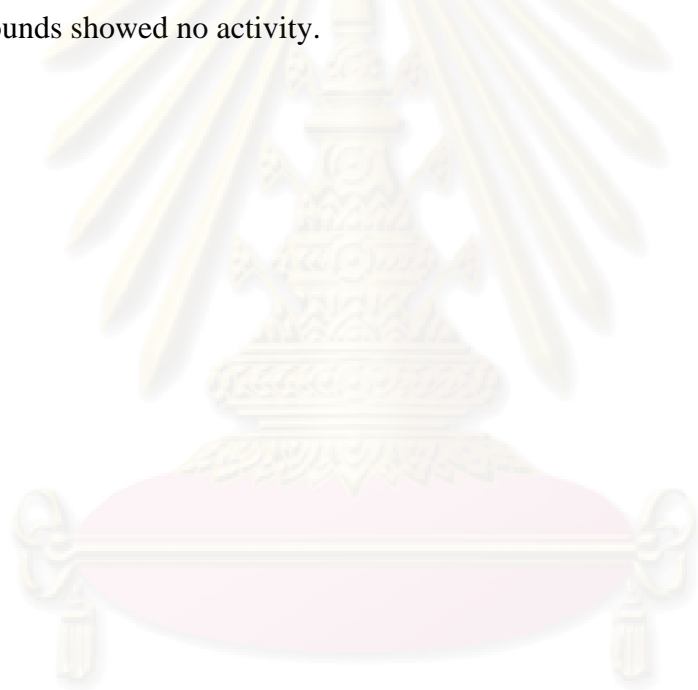
Compounds	% DPPH reduction at 50 µg/ml	IC <sub>50</sub> (µM)
ML1	1.66	-
ML2	2.07	-
ML3	2.50	-
ML4	11.56	-
ML5	33.08	-
ML6 [293]	93.79	16.94
ML7	40.28	-
ML8	16.52	-
Quercetin	93.94	6.07

From Table 10, six pure compounds and two mixtures were tested for free radical scavenging activity. It was found that syringic acid [293] showed moderate activity against DPPH free radical as compared with quercetin.

## CHAPTER V

### CONCLUSION

In this study, six known compounds were isolated from stemwood of *Millettia leucantha* Kurz (Leguminosae). These compounds were identified as cycloeucalenol [288], maackiain [208], 4-hydroxy-3-methoxybenzoic acid [292], syringic acid [293], balanocarpol [294] and diptoindonesin D [295]. In addition, a mixture of  $\beta$ -sitosterol [32] and stigmasterol [289], and a mixture of 7-oxositosterol [290] and 7-oxostigmasterol [291] were identified. The isolated compounds were tested for free radical scavenging activity. Syringic acid [293] showed moderate activity, and the other compounds showed no activity.



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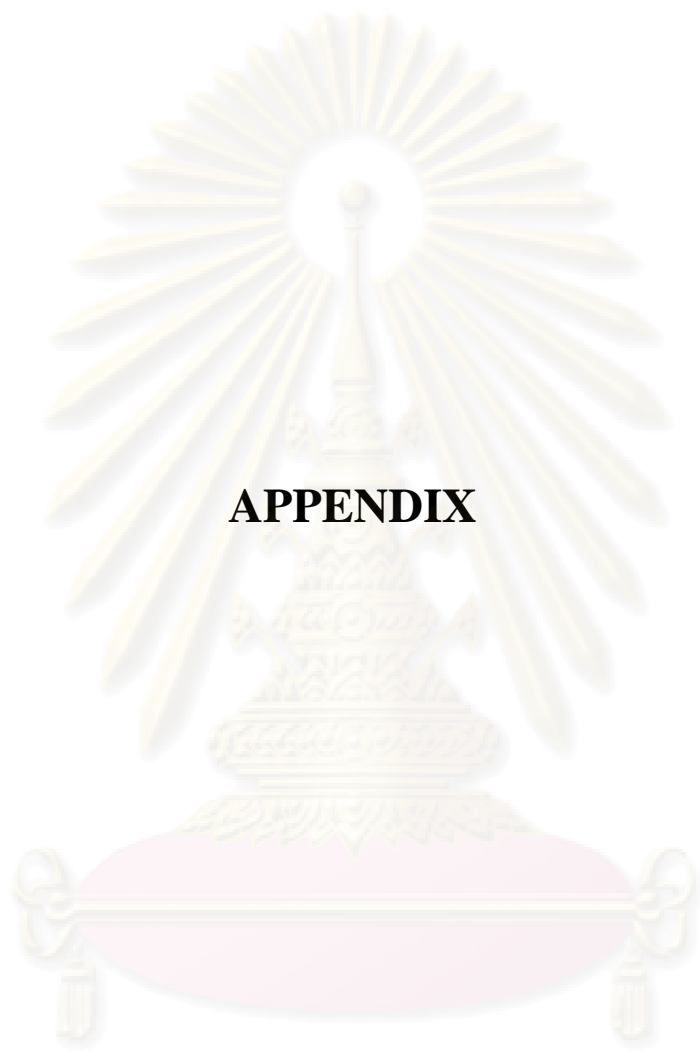
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ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย



**APPENDIX**

ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย

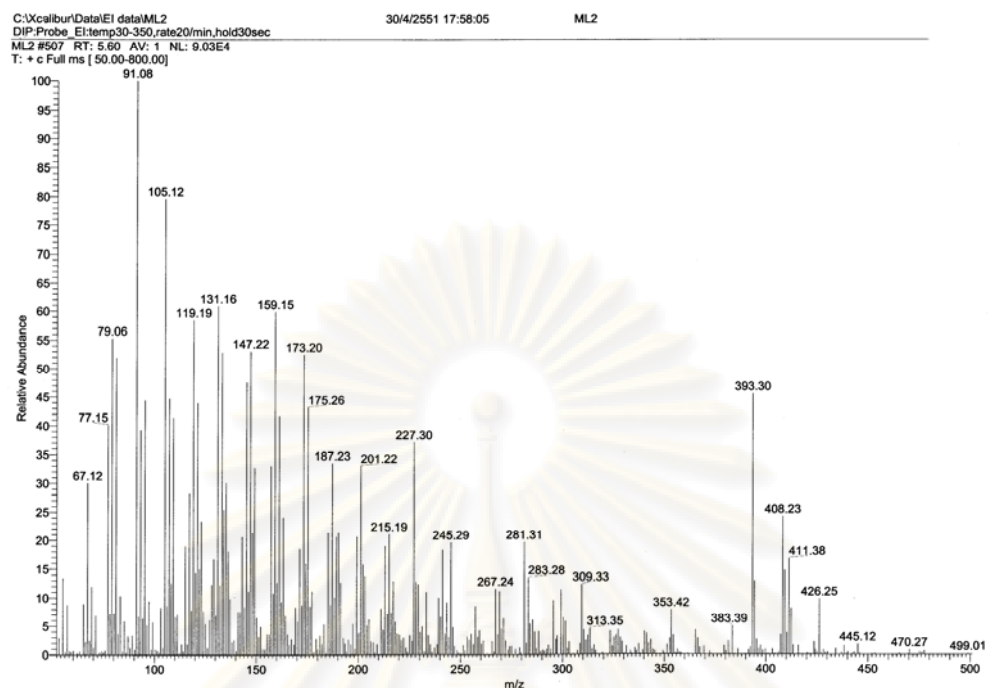


Figure 2 Mass spectrum of compound ML1

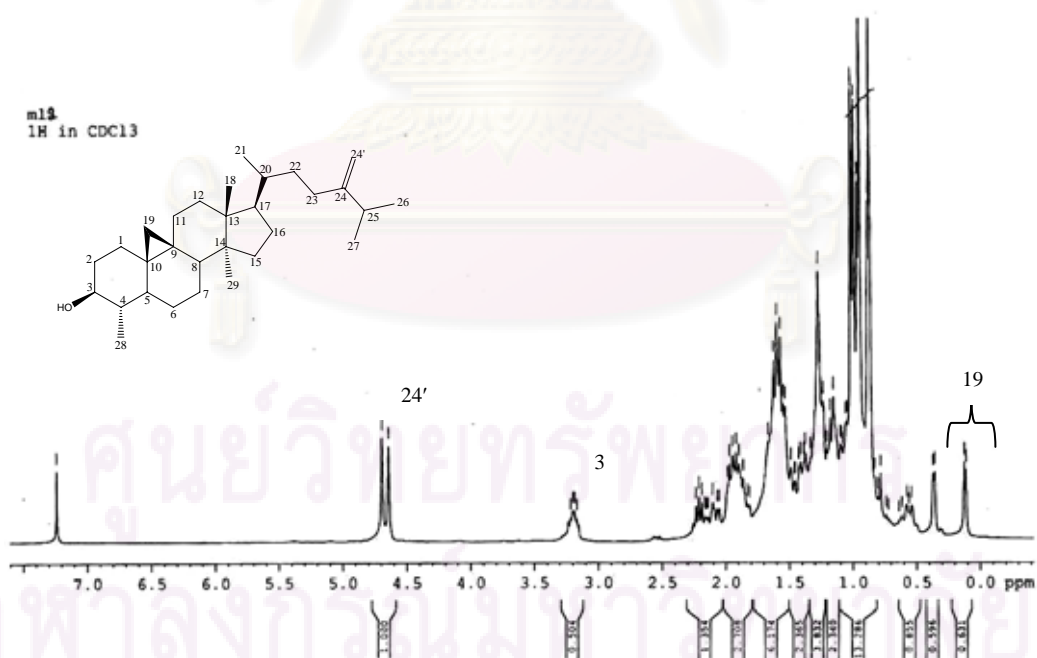
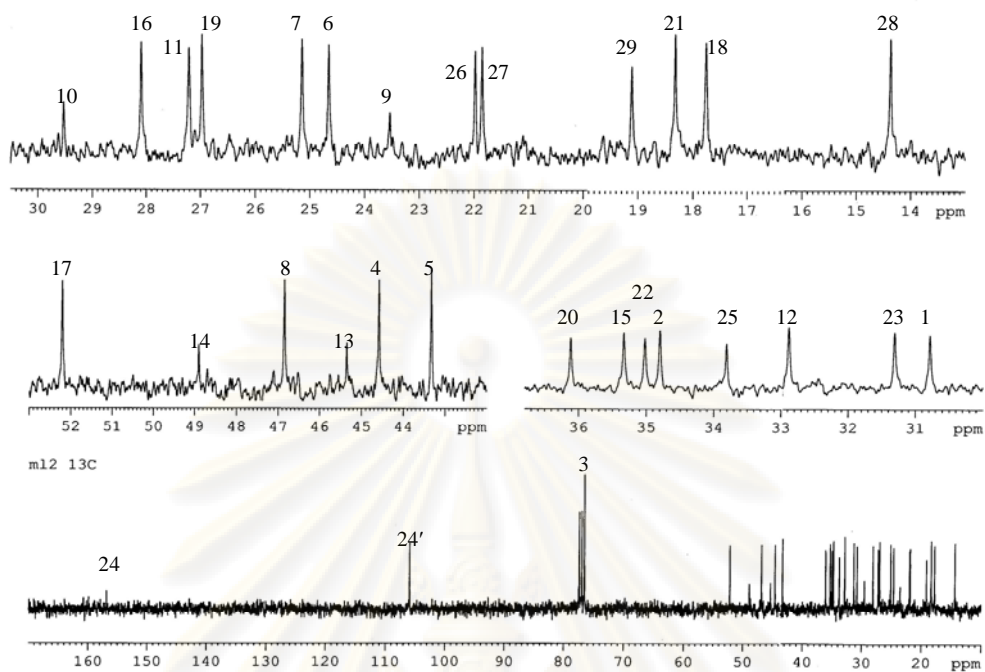
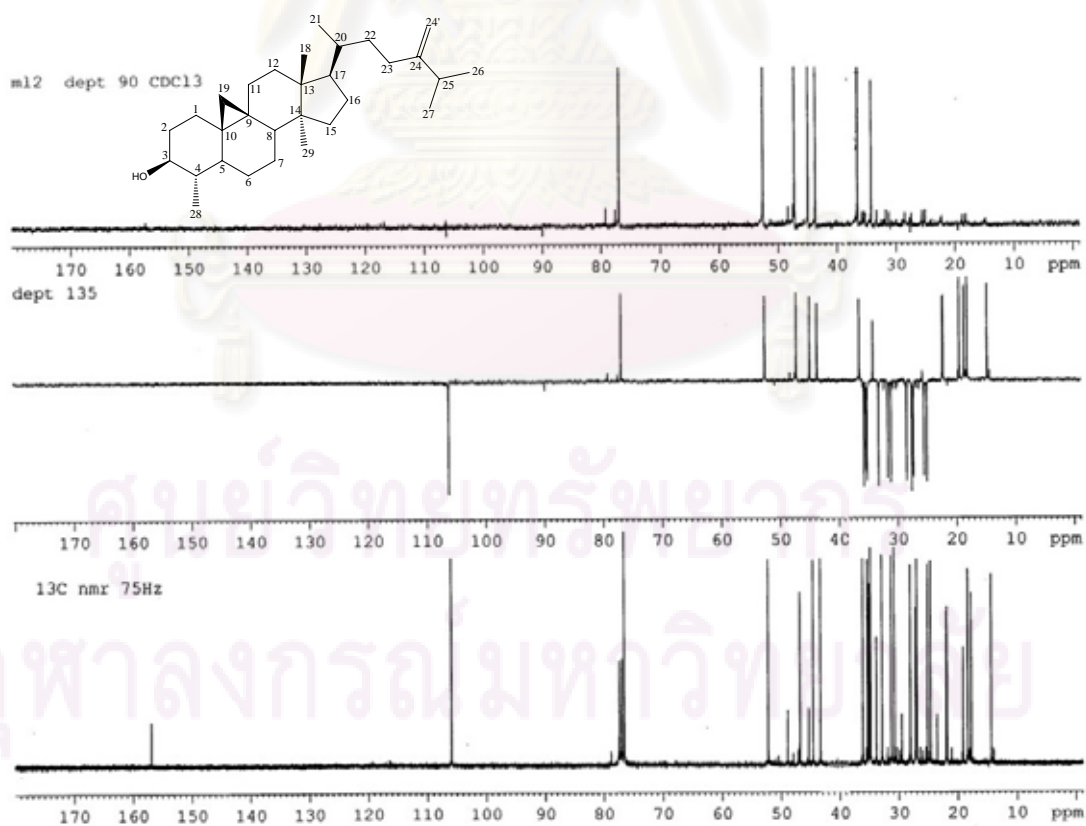


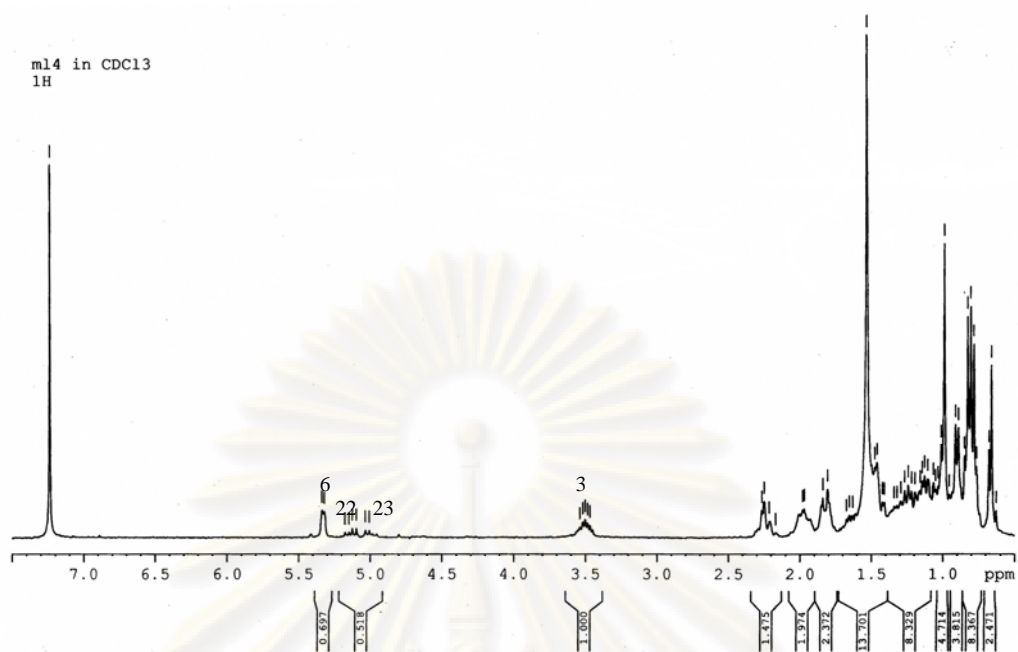
Figure 3 <sup>1</sup>H-NMR (300 MHz) Spectrum of compound ML1 (CDCl<sub>3</sub>)



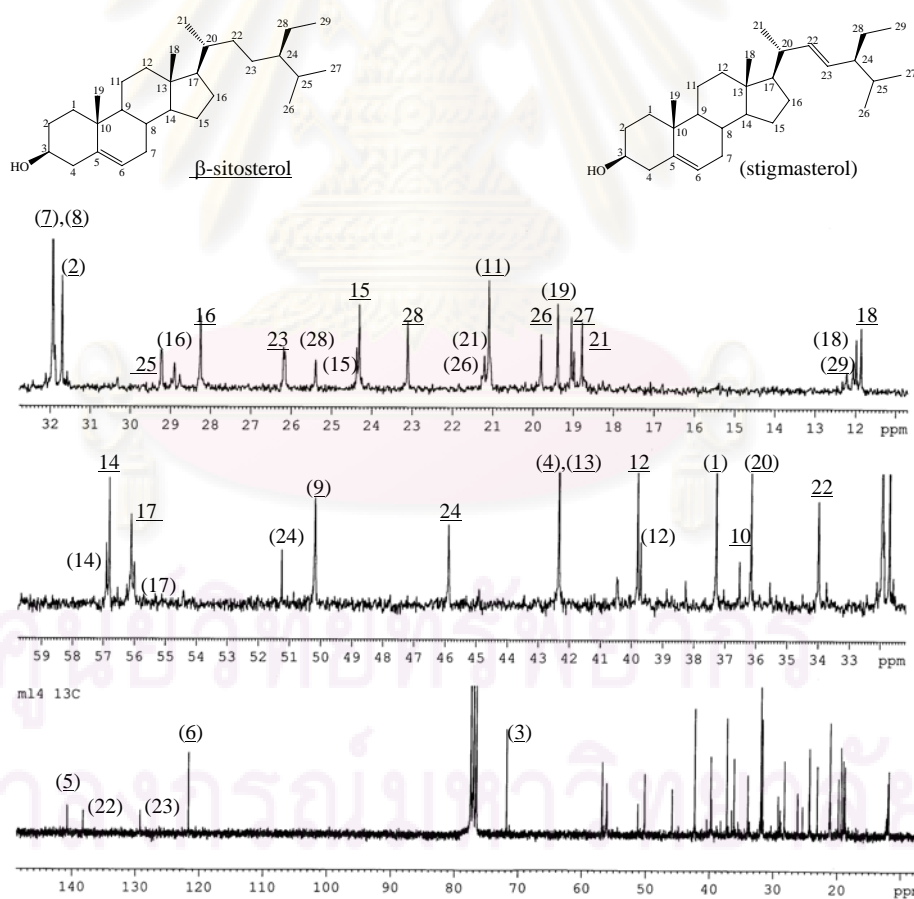
**Figure 4**  $^{13}\text{C}$ -NMR (75 MHz) Spectrum of compound ML1 ( $\text{CDCl}_3$ )



**Figure 5**  $^{13}\text{C}$ -NMR and DEPT Spectra of compound ML1 ( $\text{CDCl}_3$ )

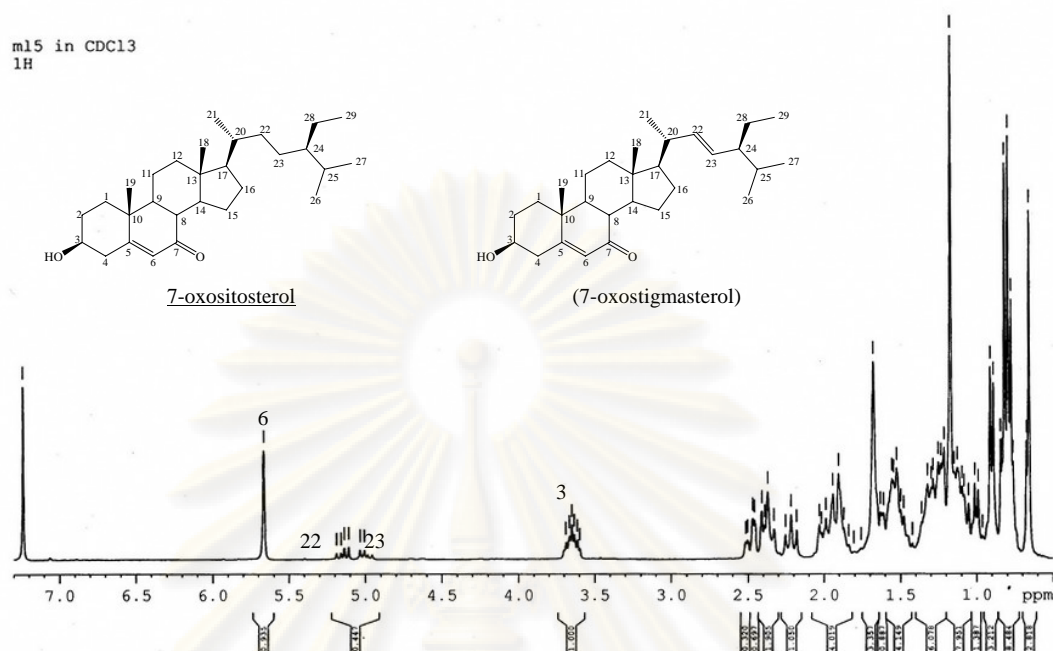


**Figure 6** <sup>1</sup>H-NMR (300 MHz) Spectrum of compound ML2 (CDCl<sub>3</sub>)

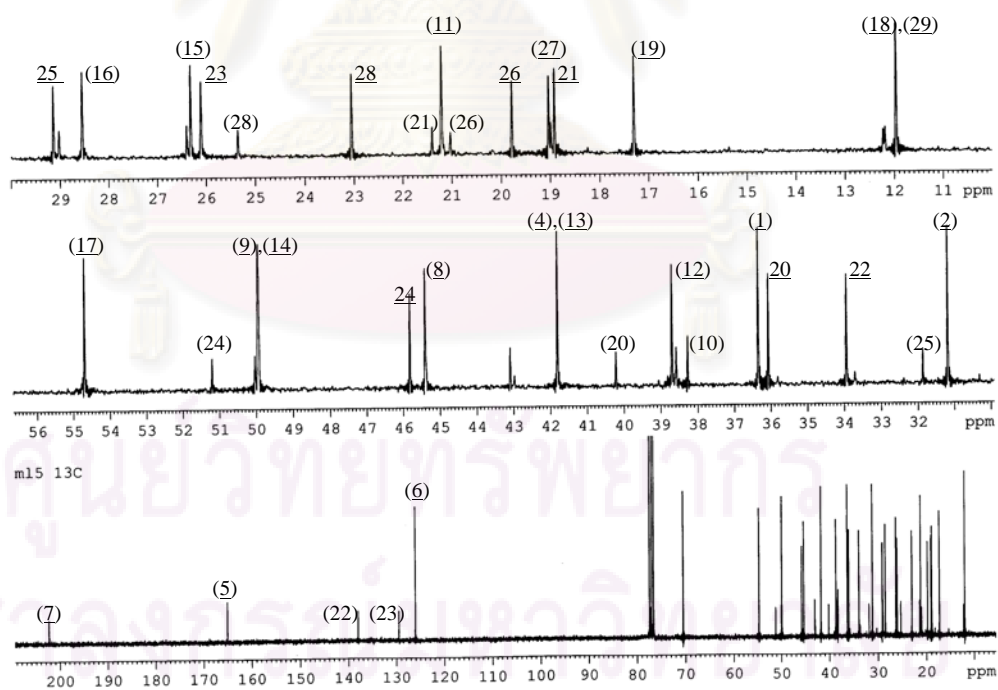


**Figure 7** <sup>13</sup>C-NMR (75 MHz) Spectrum of compound ML2 (CDCl<sub>3</sub>)





**Figure 8** <sup>1</sup>H-NMR (300 MHz) Spectrum of compound ML3 (CDCl<sub>3</sub>)



**Figure 9** <sup>13</sup>C-NMR (75 MHz) Spectrum of compound ML3 (CDCl<sub>3</sub>)

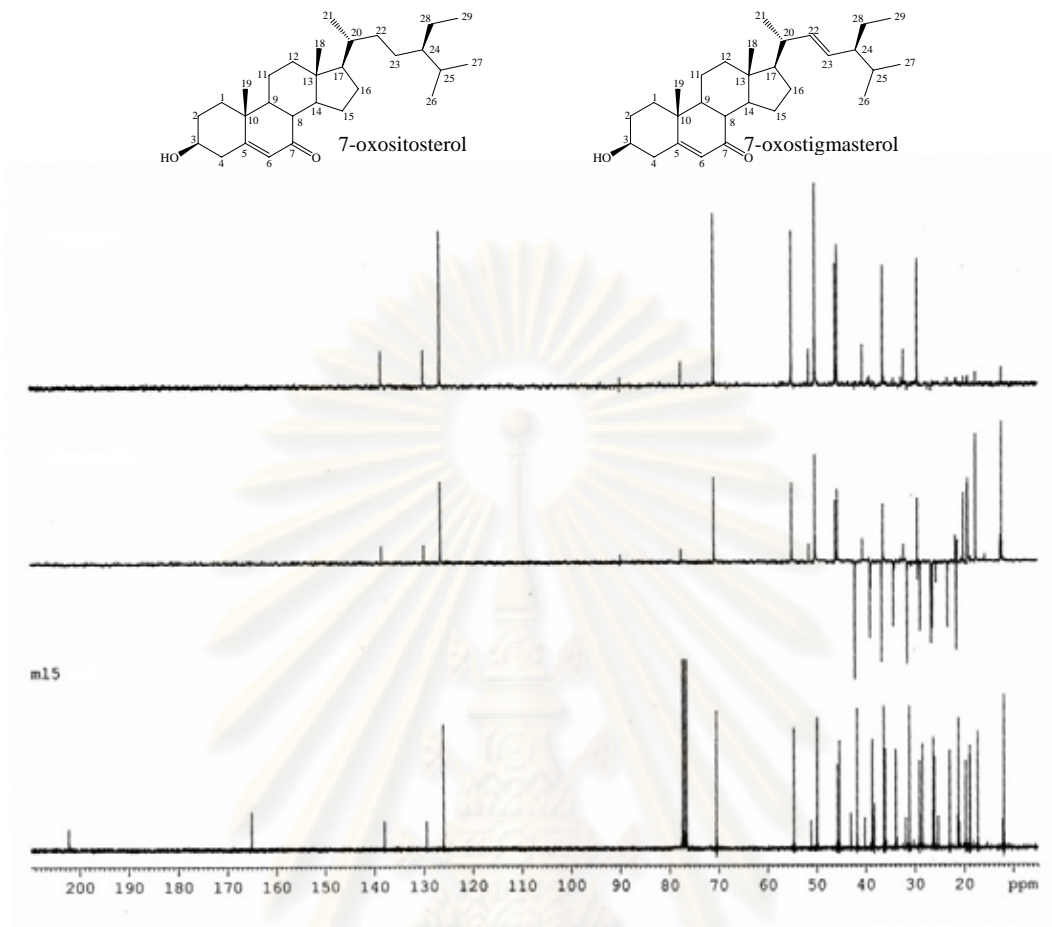


Figure 10  $^{13}\text{C}$ -NMR and DEPT Spectra of compound ML3 ( $\text{CDCl}_3$ )

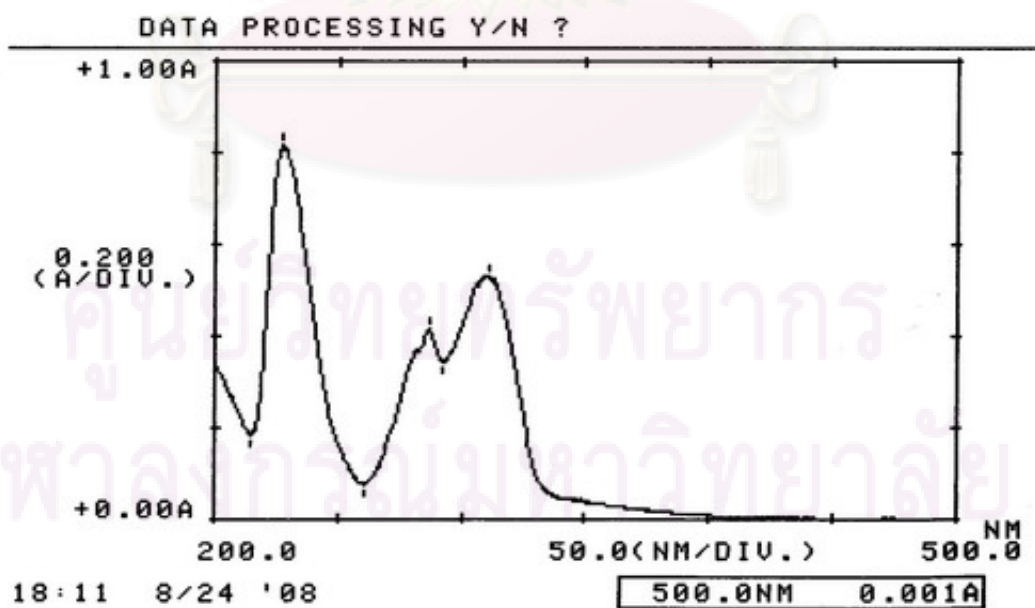


Figure 11 UV spectrum of compound ML4

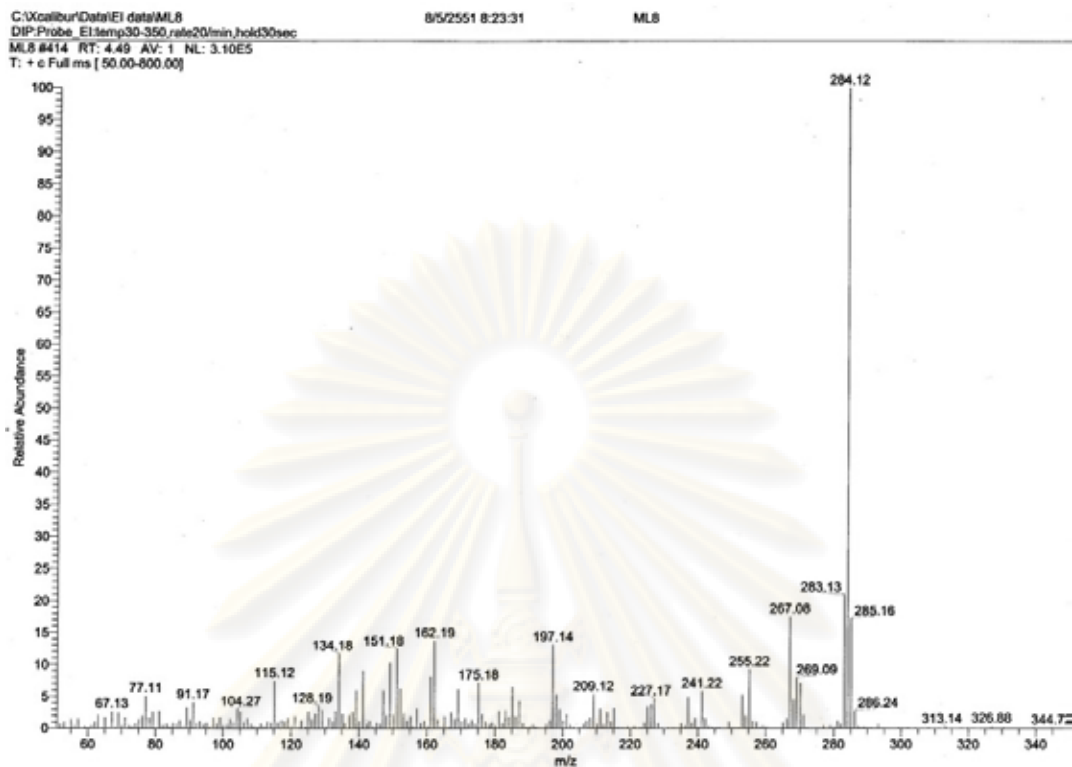


Figure 12 Mass spectrum of compound ML4

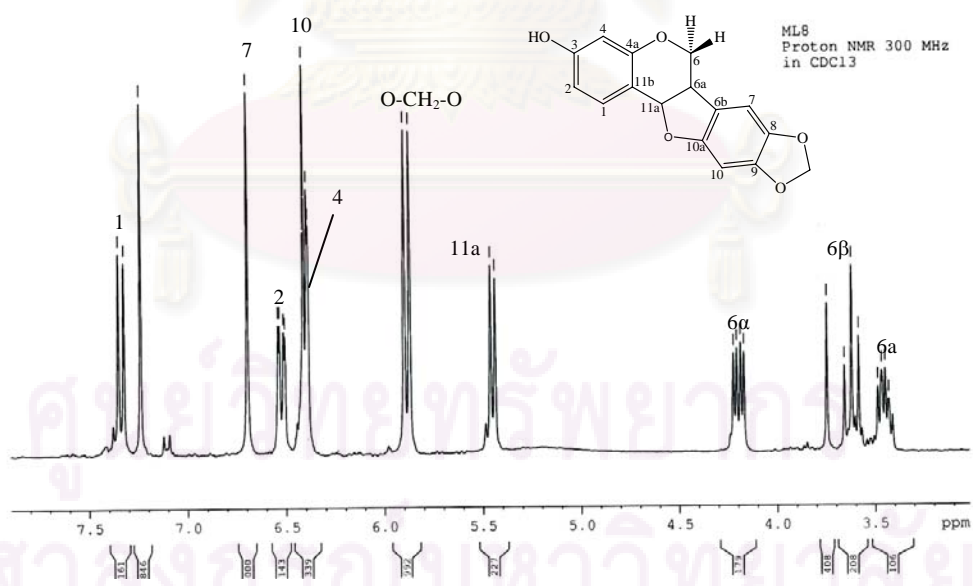
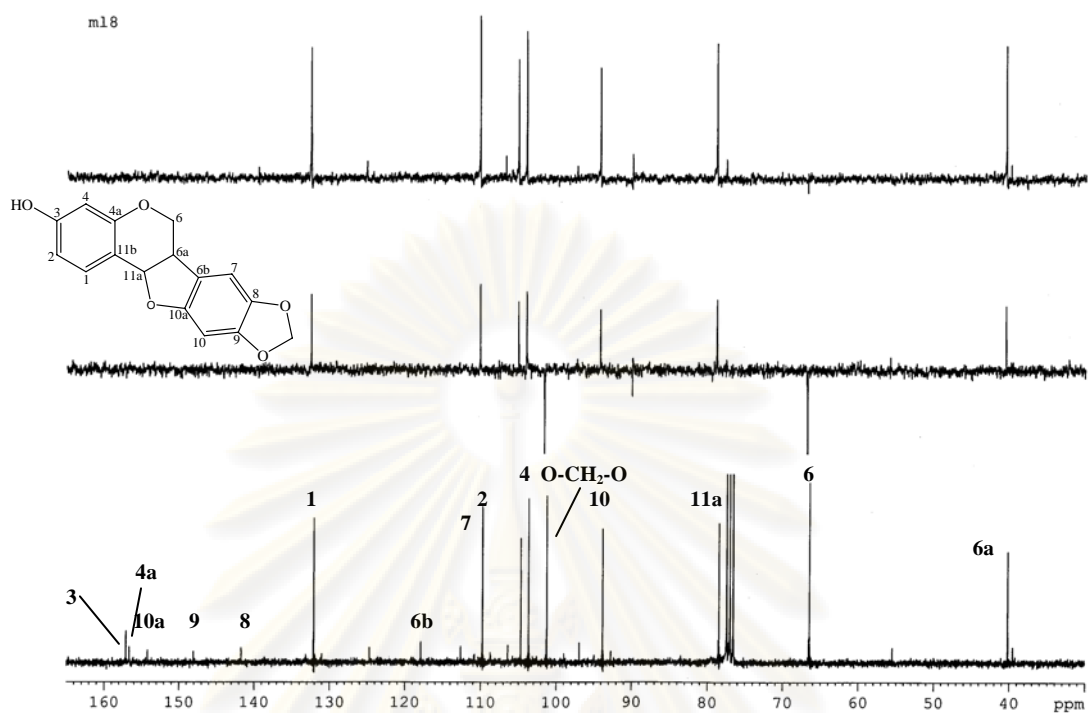
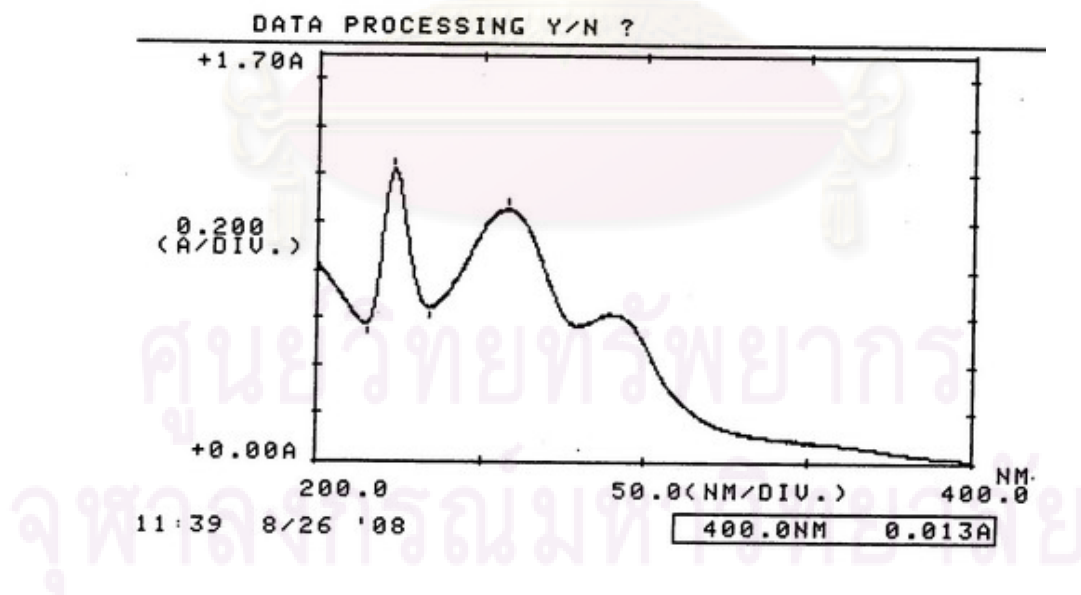


Figure 13 <sup>1</sup>H-NMR (300 MHz) Spectrum of compound ML4 (CDCl<sub>3</sub>)



**Figure 14**  $^{13}\text{C}$ -NMR and DEPT Spectra of compound ML4 ( $\text{CDCl}_3$ )



**Figure 15** UV spectrum of compound ML5

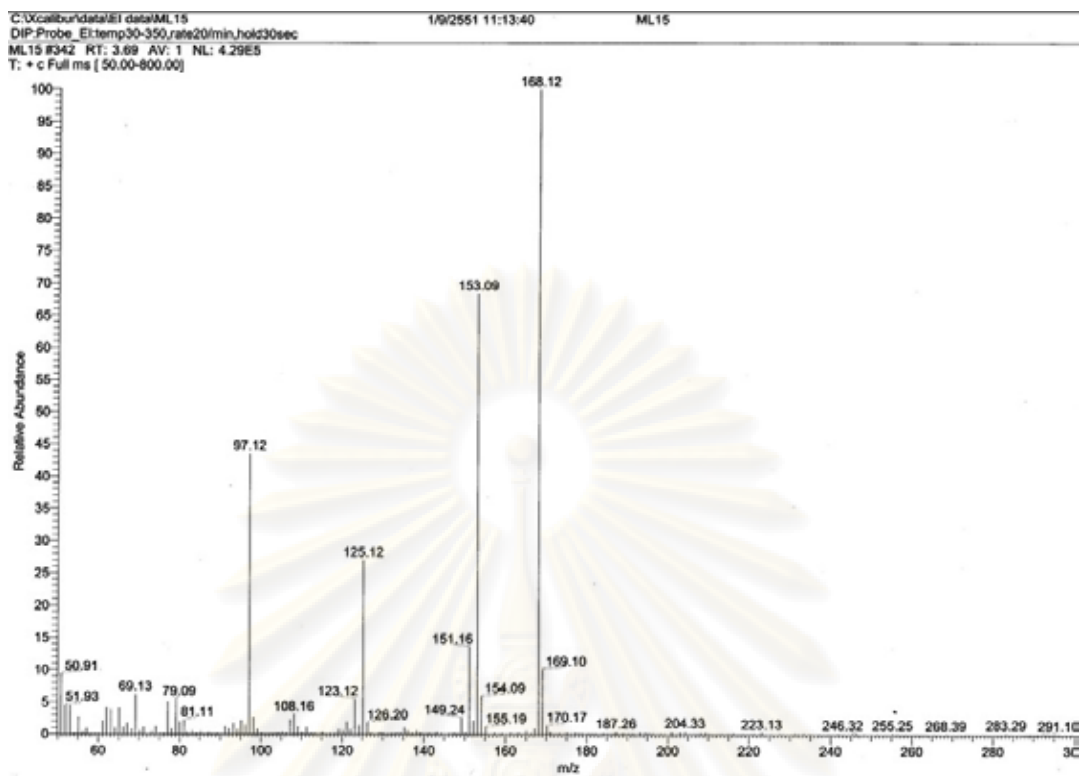


Figure 16 Mass spectrum of compound ML5

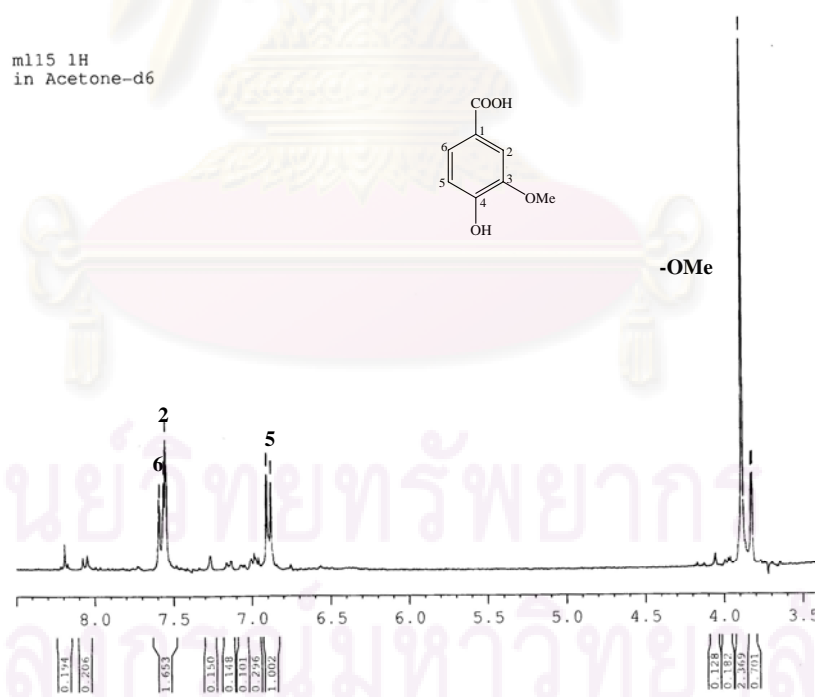
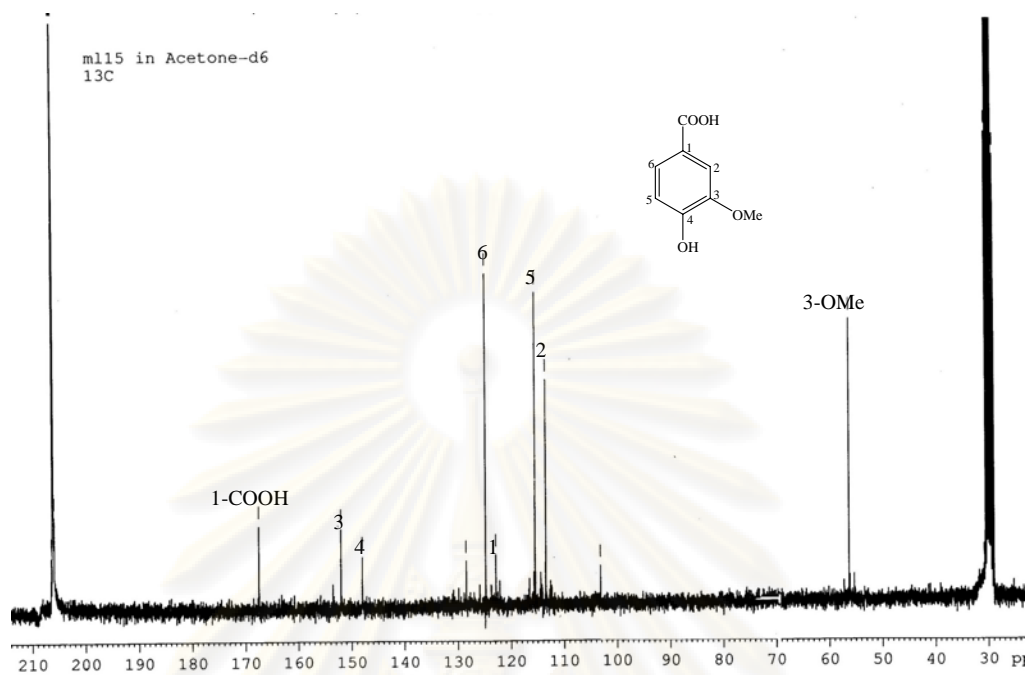
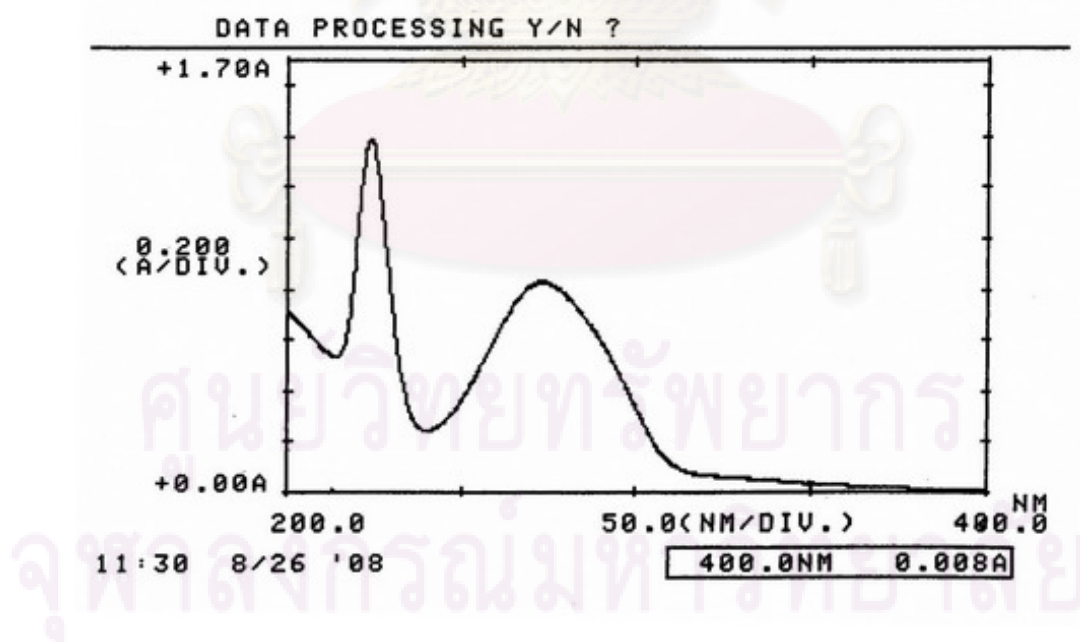


Figure 17 <sup>1</sup>H-NMR (300 MHz) Spectrum of compound ML5 (acetone-d<sub>6</sub>)



**Figure 18**  $^{13}\text{C}$ -NMR (75 MHz) Spectrum of compound ML5 (acetone- $d_6$ )



**Figure 19** UV spectrum of compound ML6

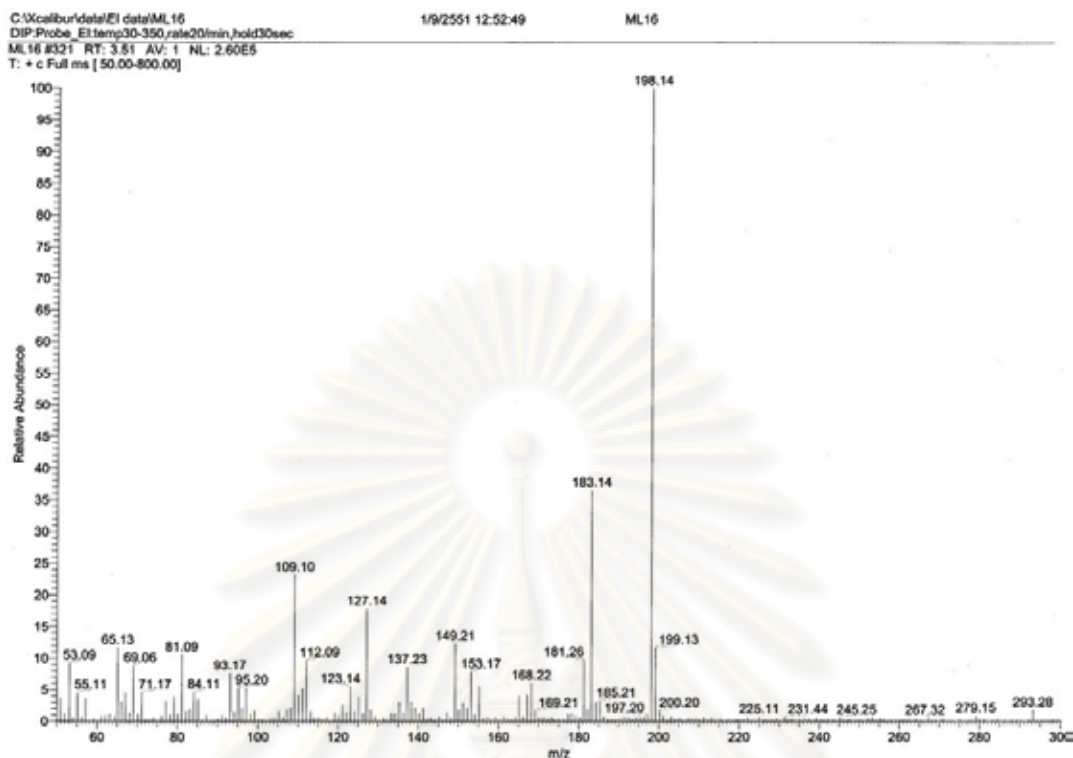


Figure 20 Mass spectrum of compound ML6

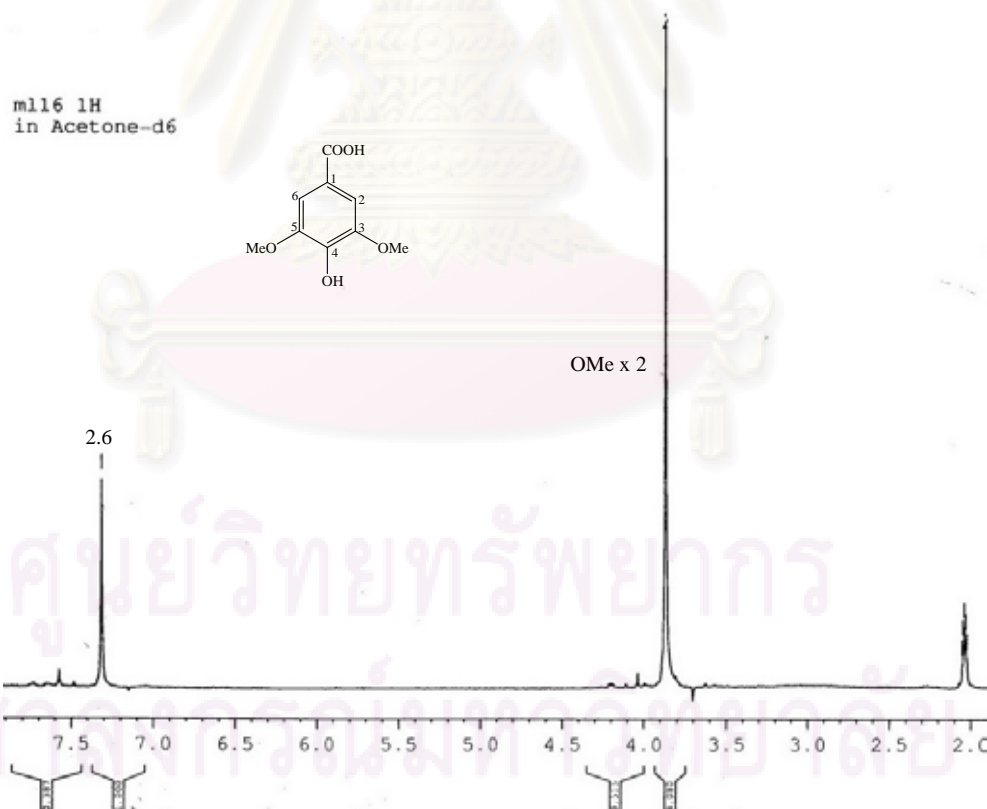


Figure 21 <sup>1</sup>H-NMR (300 MHz) Spectrum of compound ML6 (acetone-d<sub>6</sub>)



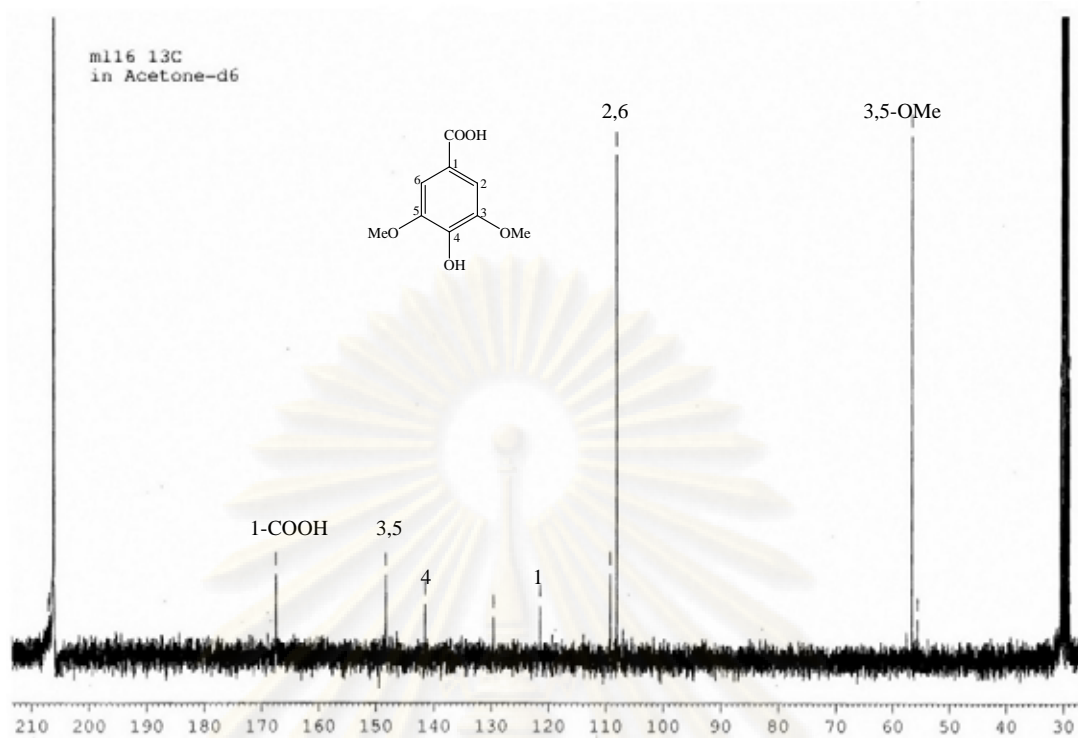


Figure 22  $^{13}\text{C}$ -NMR (75 MHz) Spectrum of compound ML6 (acetone- $d_6$ )

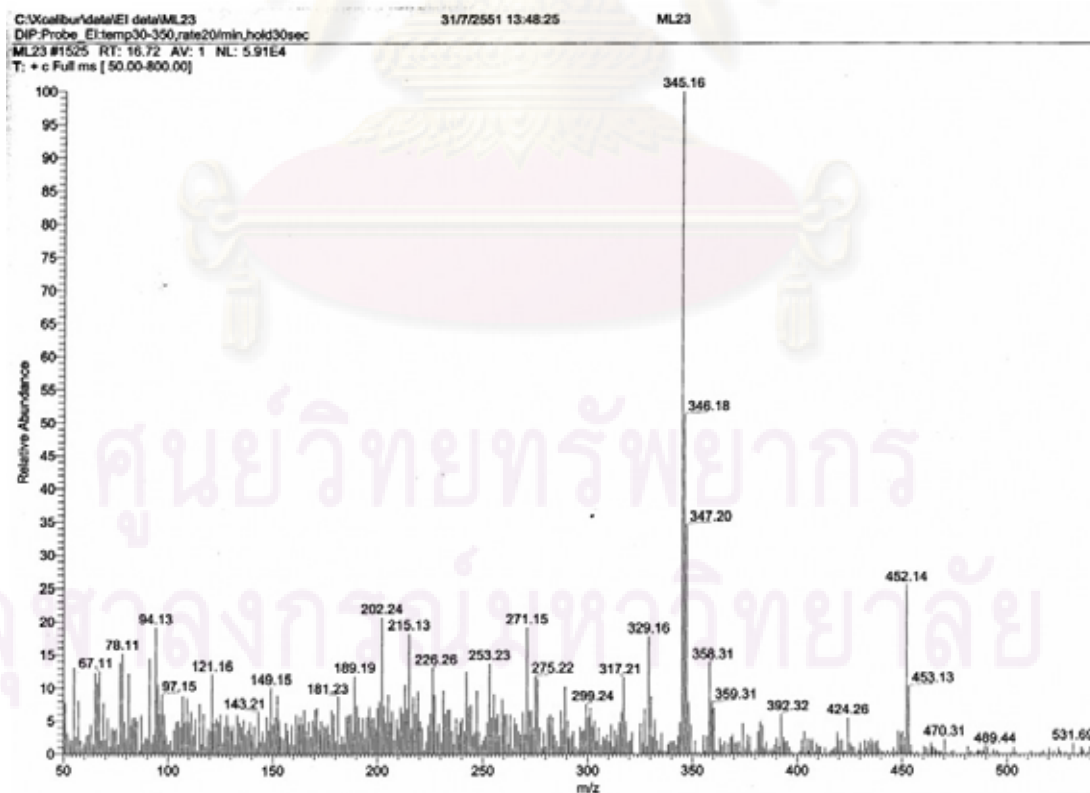


Figure 23 Mass spectrum of compound ML7



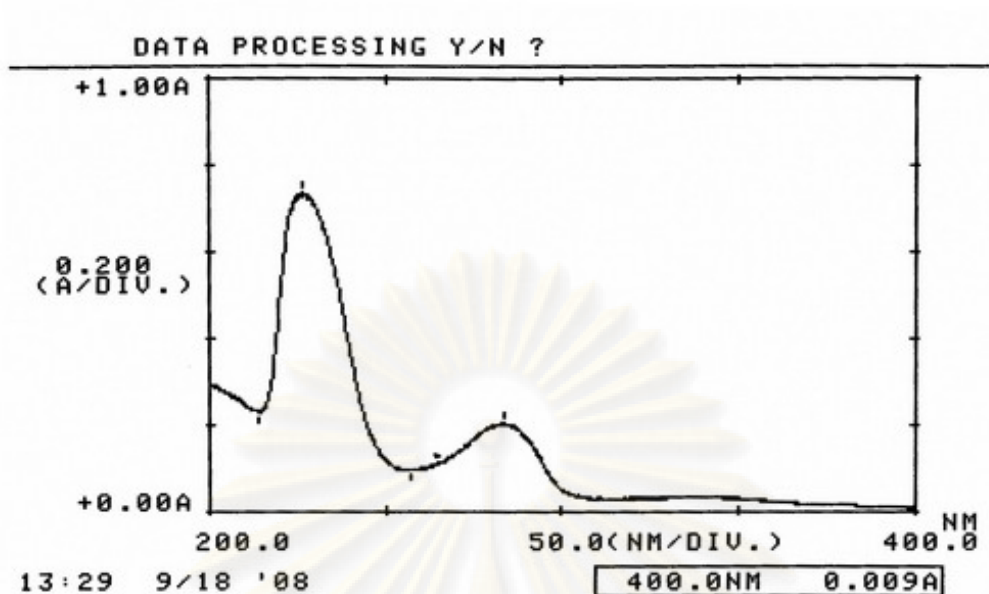


Figure 24 UV spectrum of compound ML7

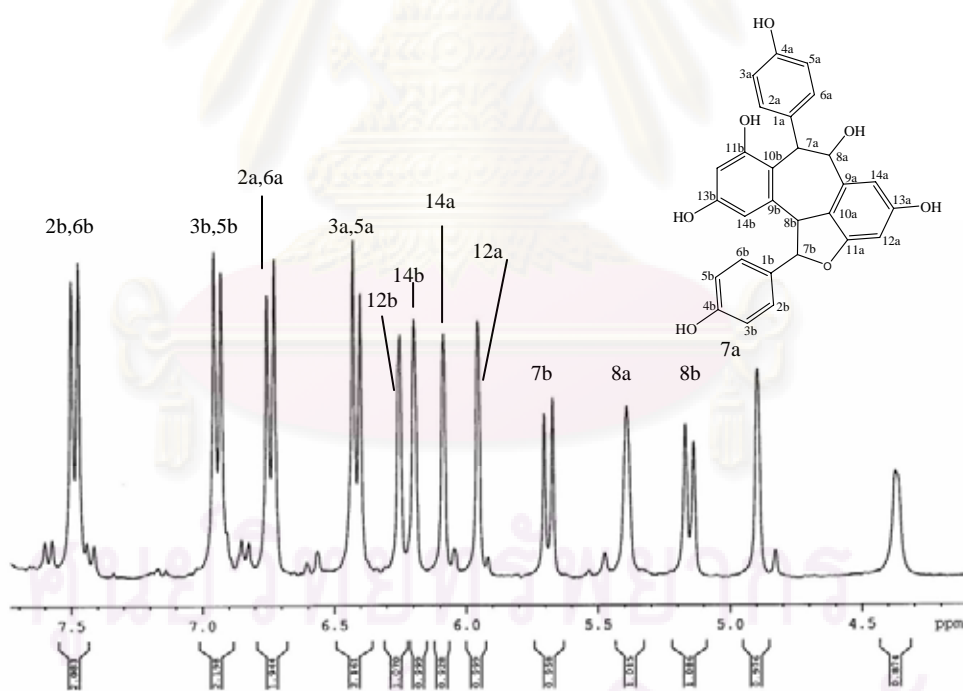
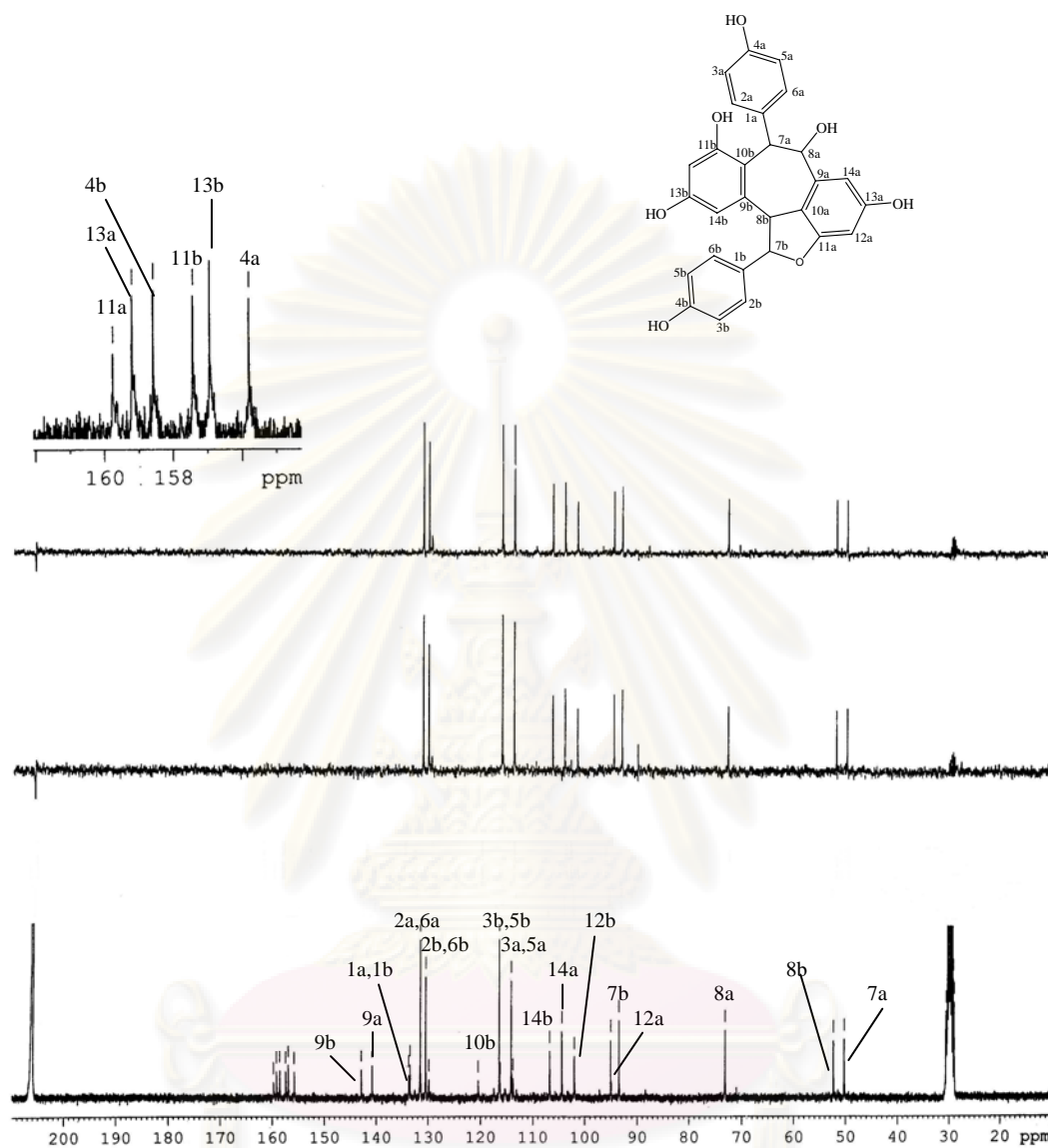


Figure 25  $^1\text{H-NMR}$  (300 MHz) Spectrum of compound ML7 (acetone- $d_6$ )



**Figure 26**  $^{13}\text{C}$ -NMR (75 MHz) and DEPT Spectra of compound ML7 (acetone- $d_6$ )

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จุฬาลงกรณ์มหาวิทยาลัย

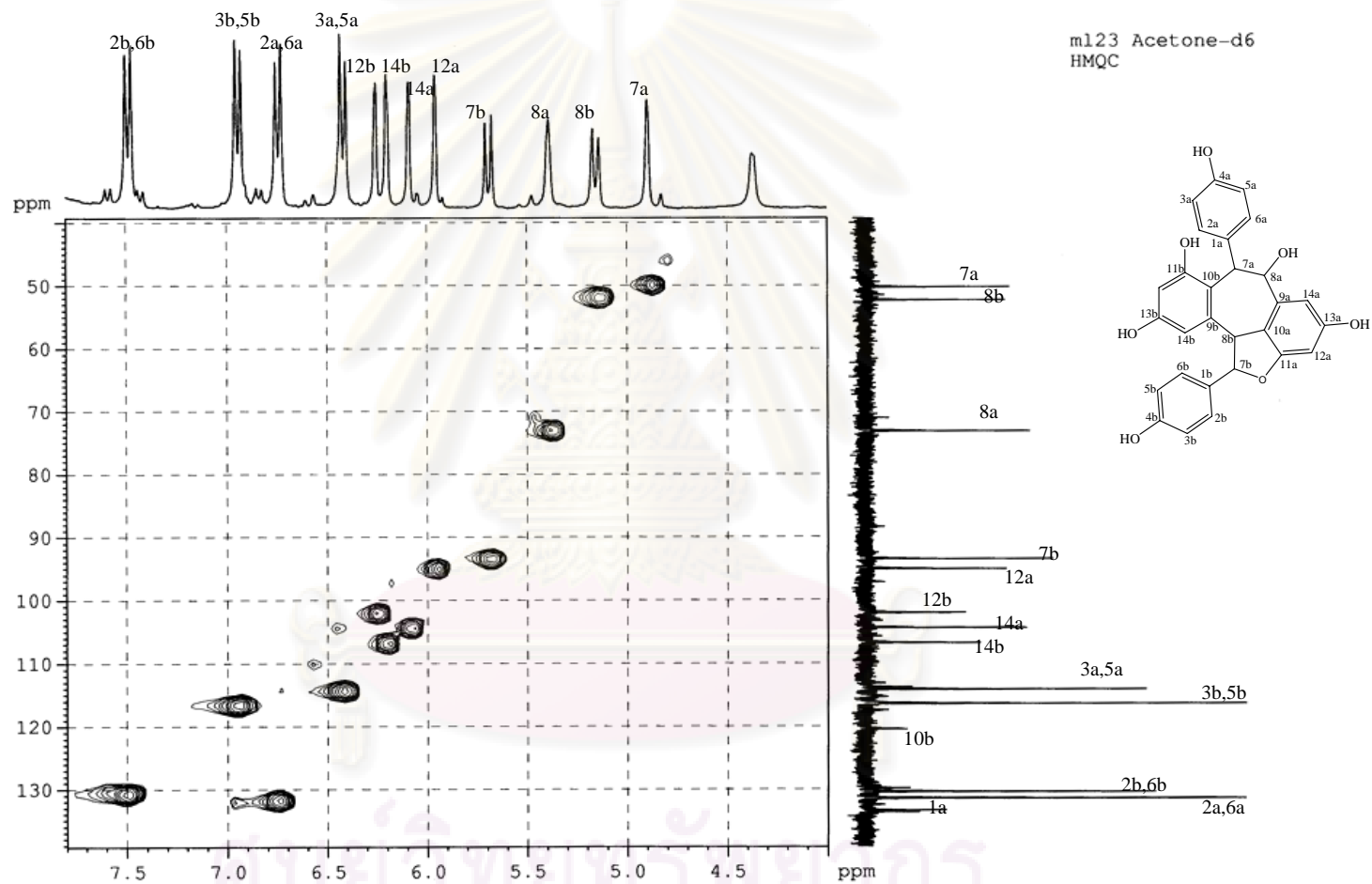


Figure 27 HMQC Spectrum of compound ML7 (acetone-d<sub>6</sub>)

จุฬาลงกรณ์มหาวิทยาลัย

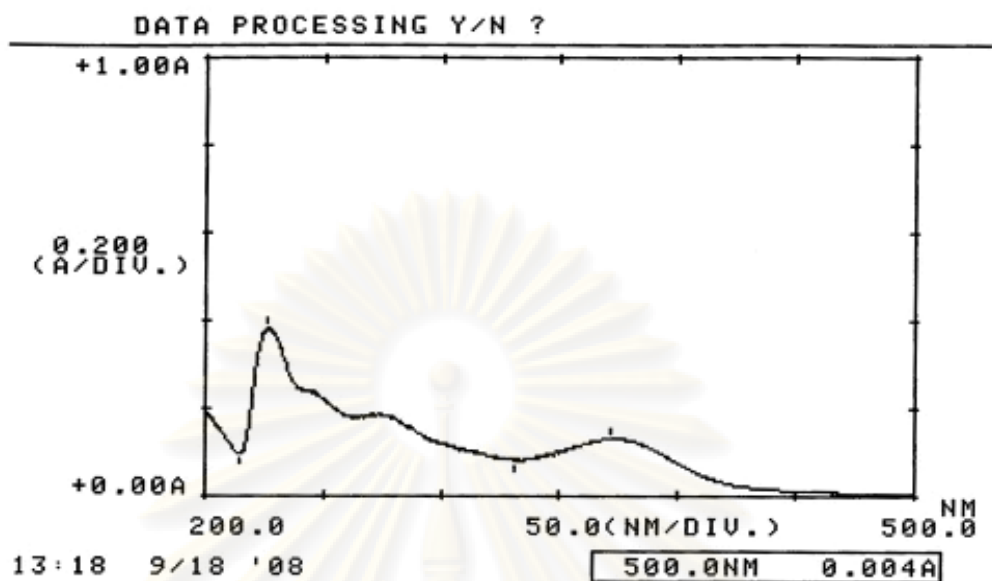


Figure 28 UV spectrum of compound ML8

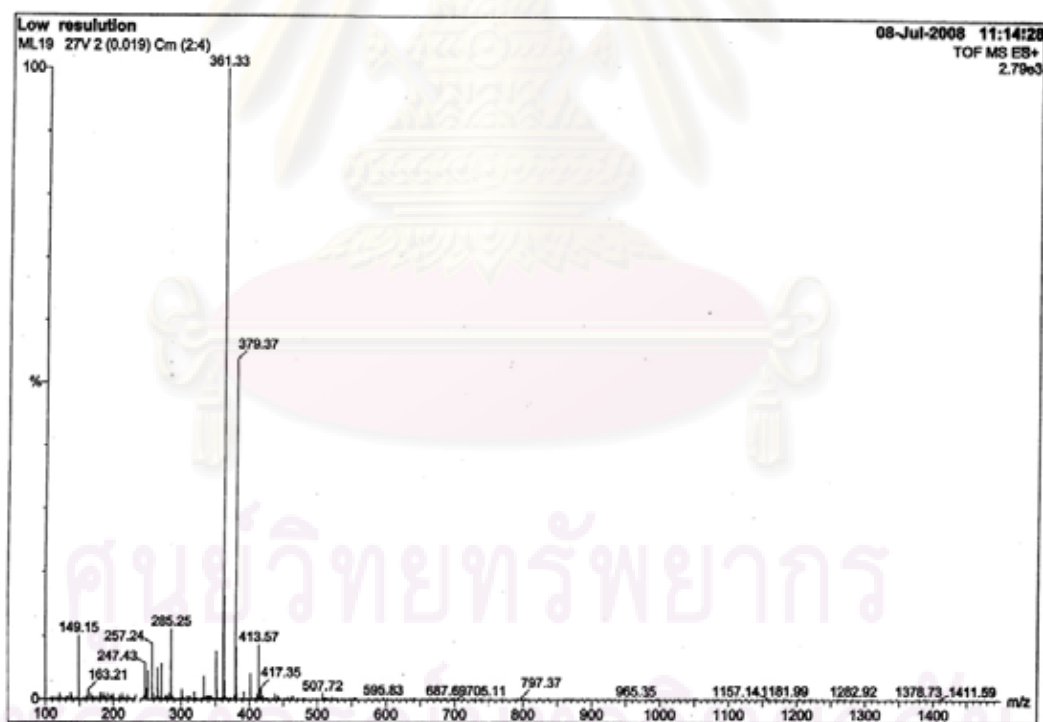
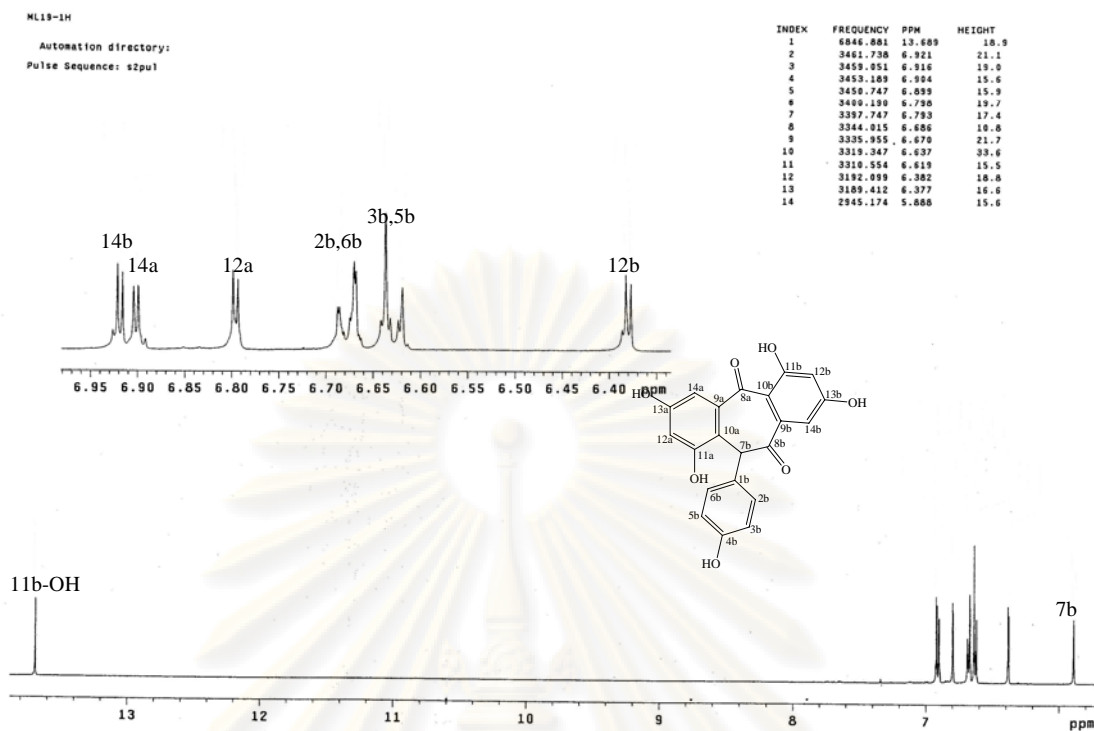
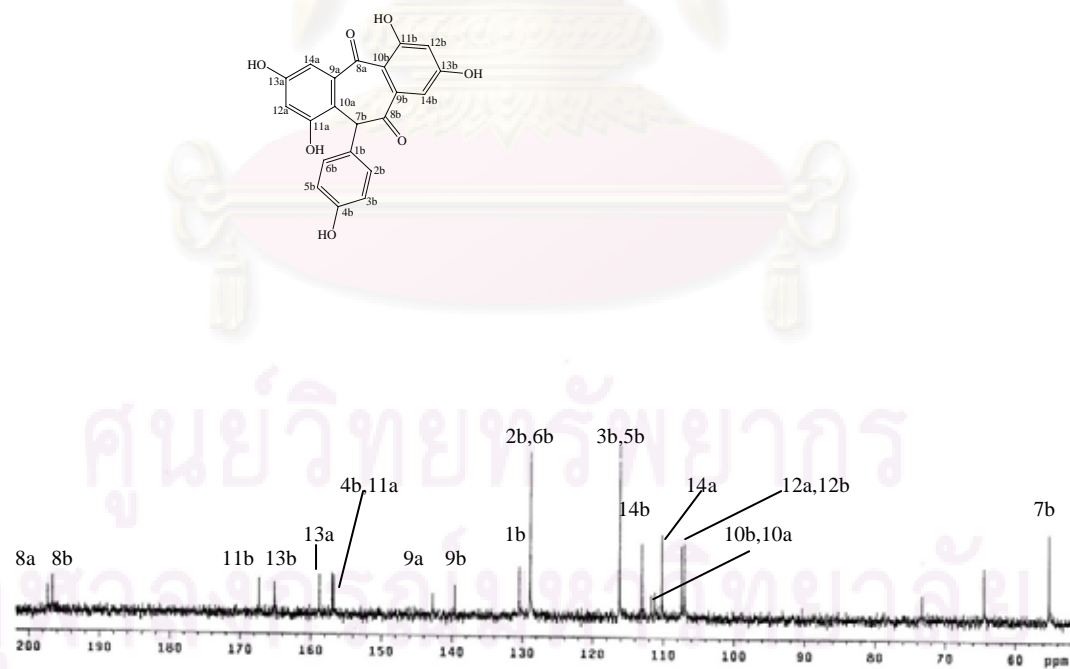


Figure 29 Mass spectrum of compound ML8



**Figure 30**  $^1\text{H-NMR}$  (500 MHz) Spectrum of compound ML8 (acetone- $d_6$ )



**Figure 31**  $^{13}\text{C-NMR}$  (125 MHz) Spectrum of compound ML8 (acetone- $d_6$ )

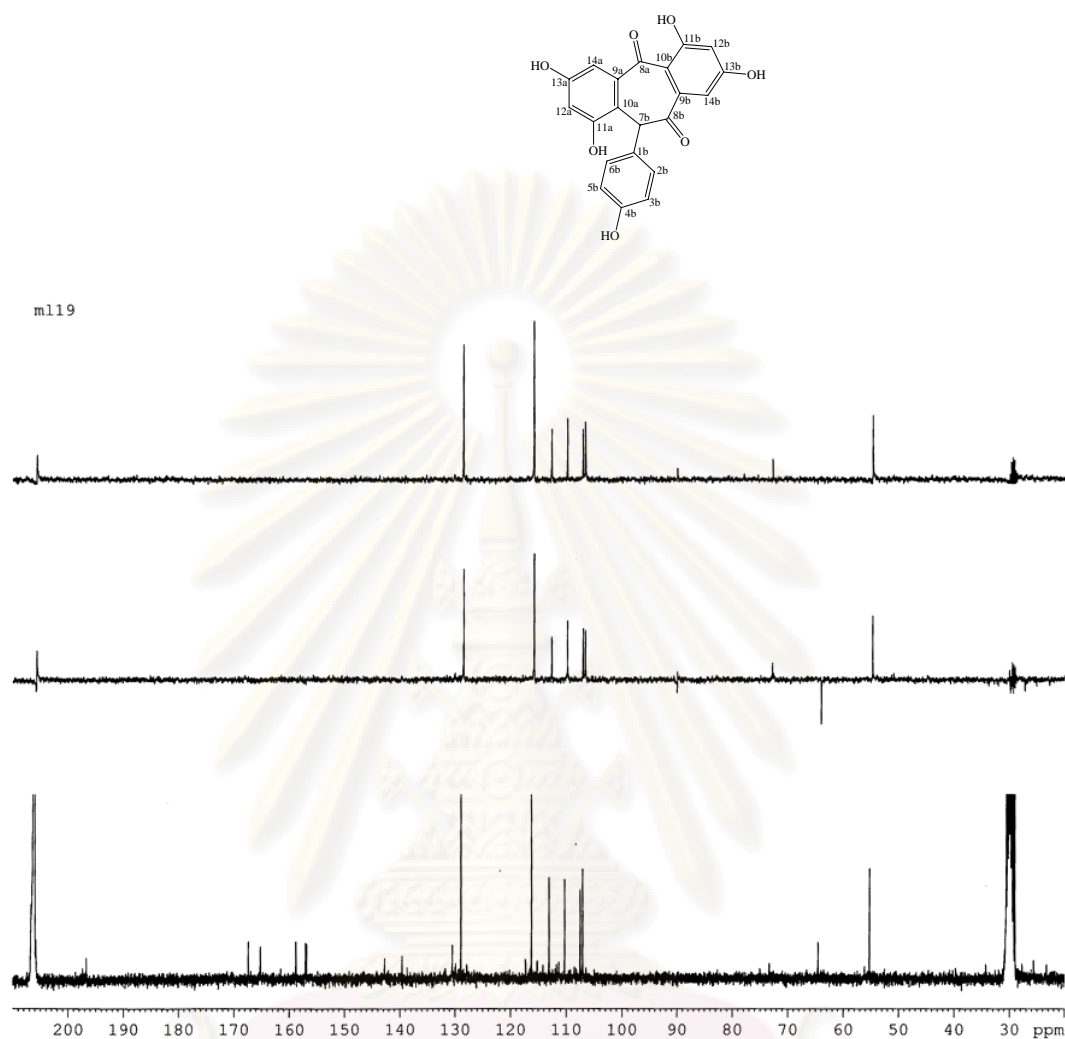


Figure 32 DEPT Spectra of compound ML8 (acetone- $d_6$ )

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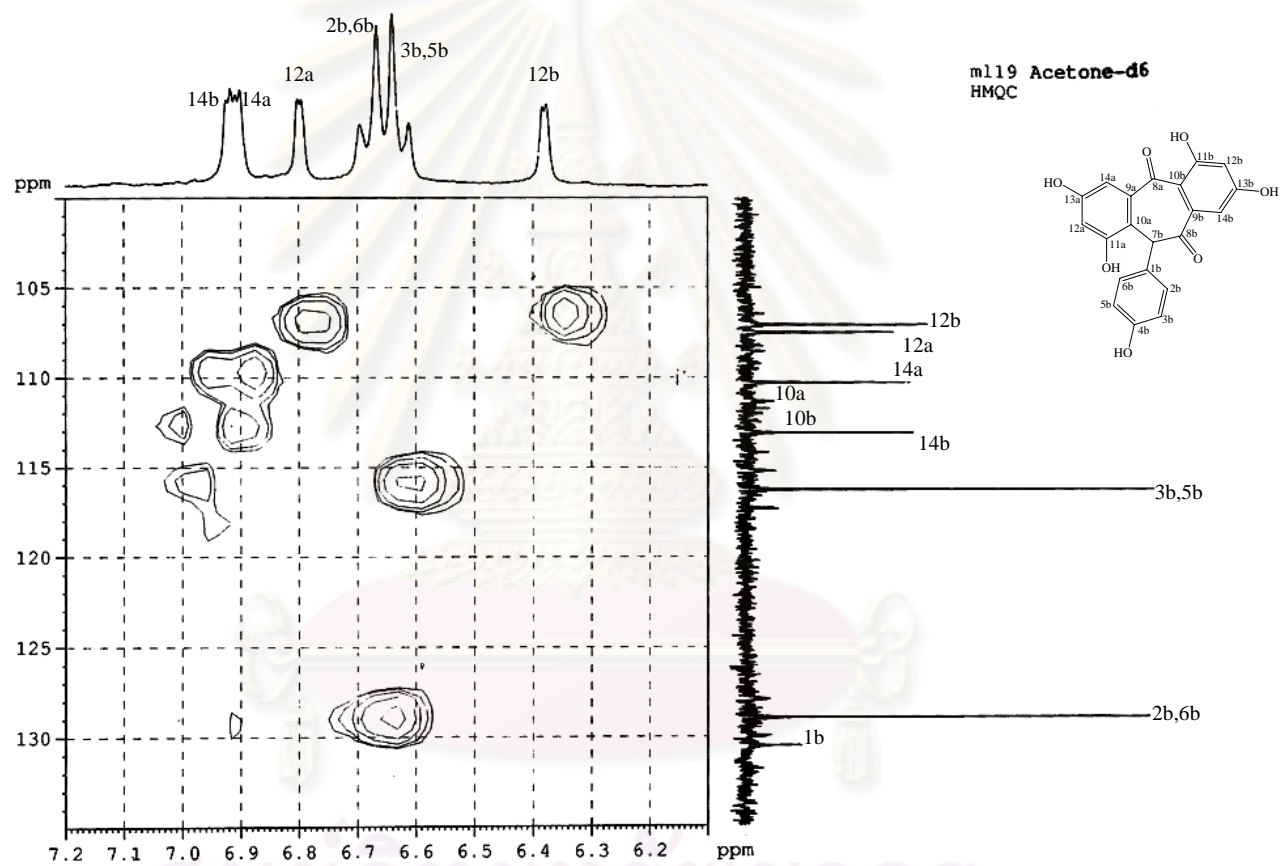
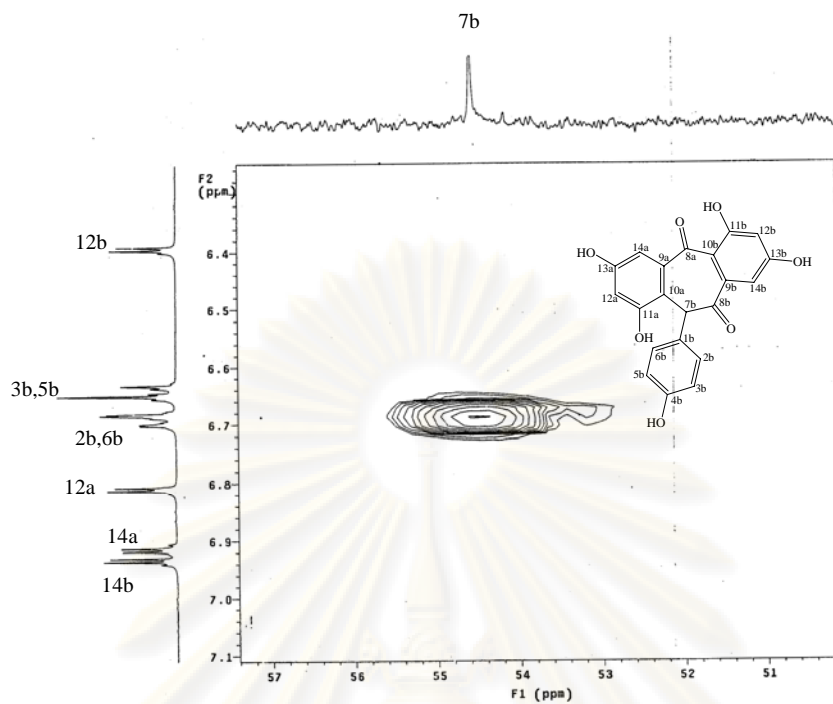
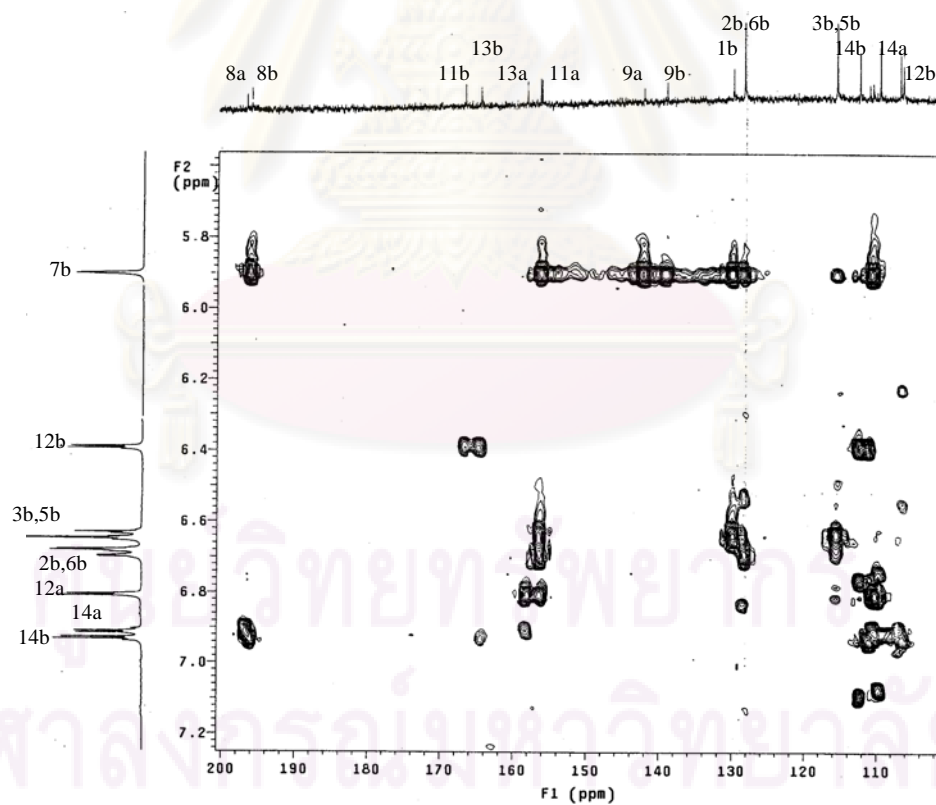


Figure 33 HMQC Spectrum of compound ML8 (acetone-d<sub>6</sub>)

ศูนย์วิทยาศาสตร์การ  
จุฬาลงกรณ์มหาวิทยาลัย

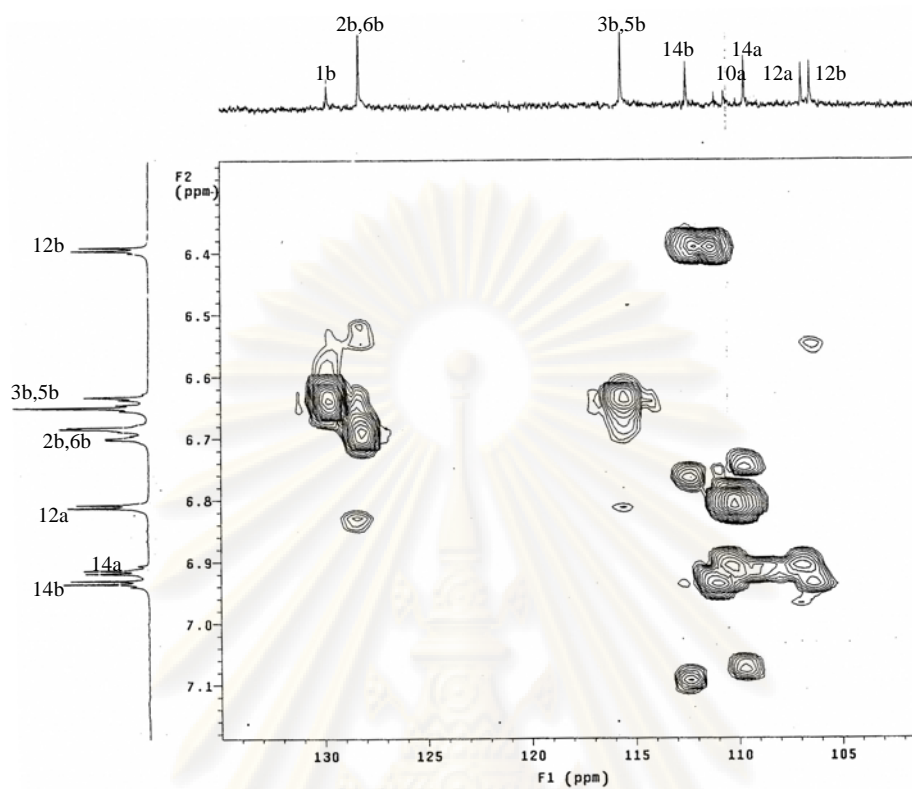


**Figure 34** HMBC Spectrum of compound ML8 (acetone- $d_6$ )

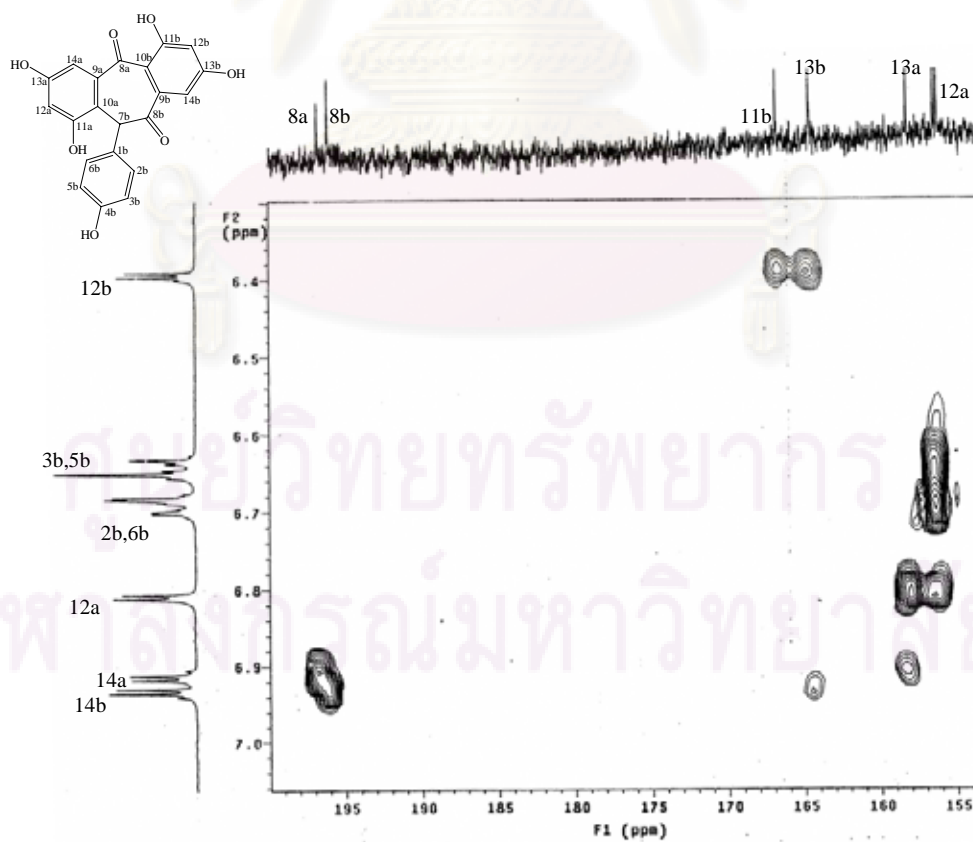


**Figure 35** HMBC Spectrum of compound ML8 (acetone- $d_6$ )

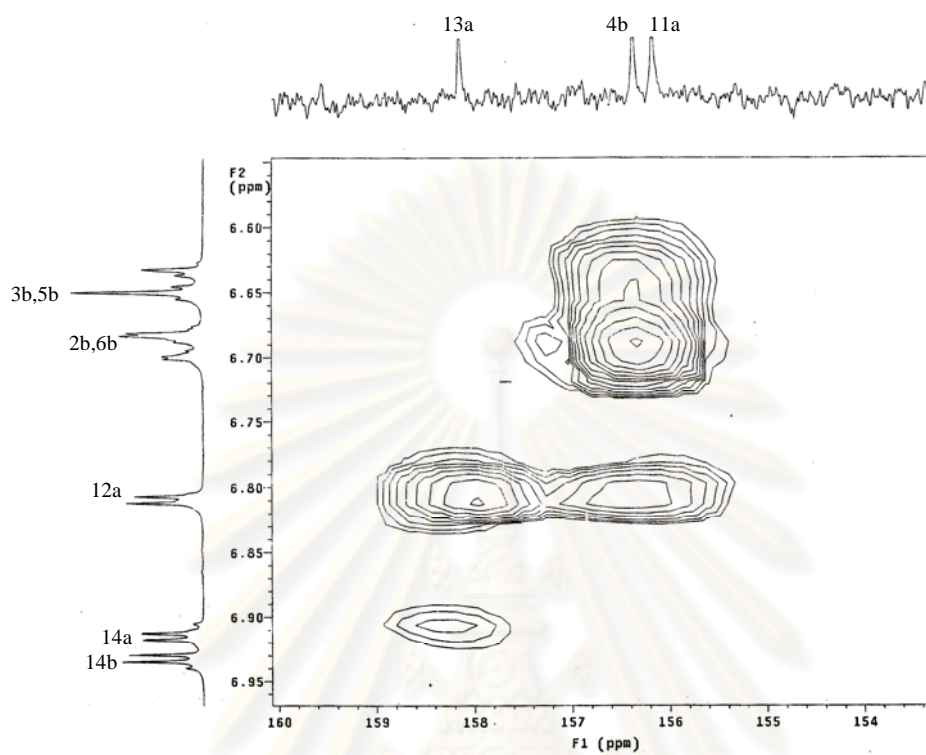




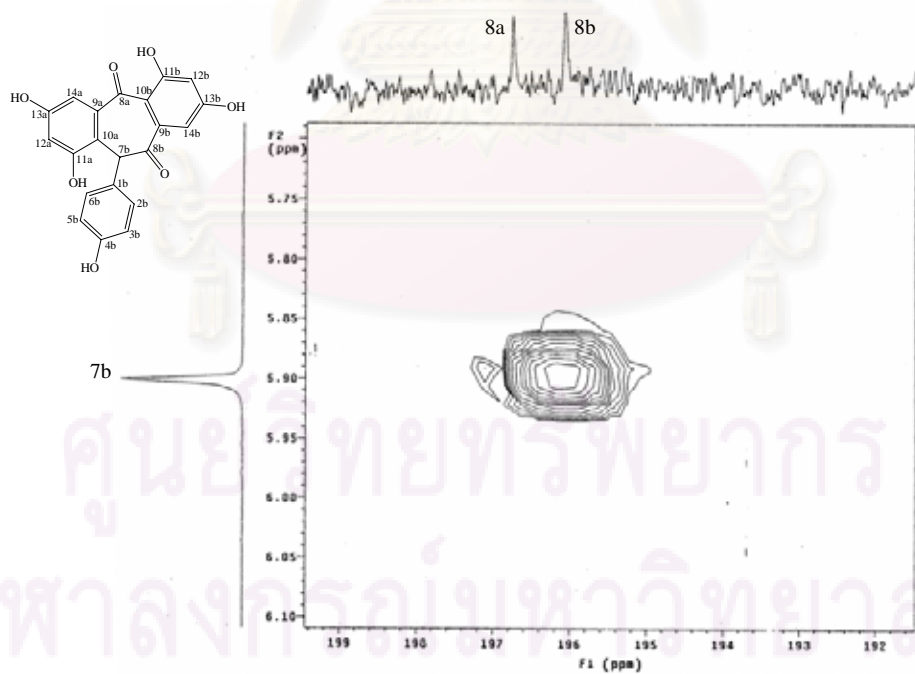
**Figure 36** HMBC Spectrum of compound ML8 (acetone- $d_6$ )



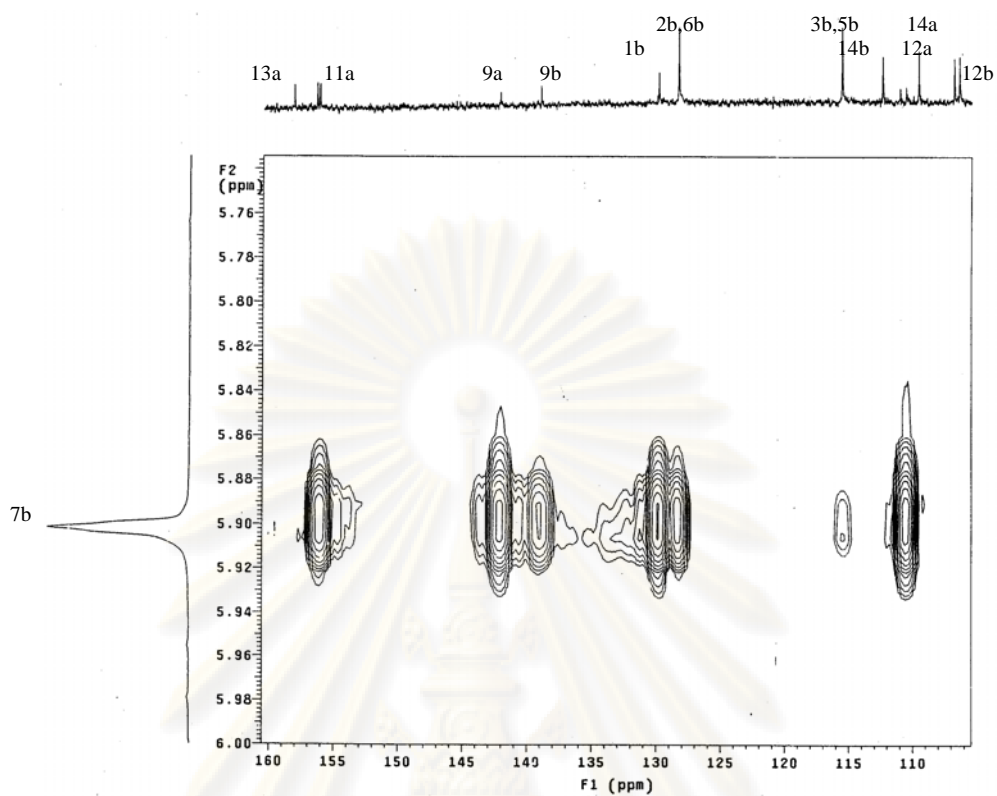
**Figure 37** HMBC Spectrum of compound ML8 (acetone- $d_6$ )



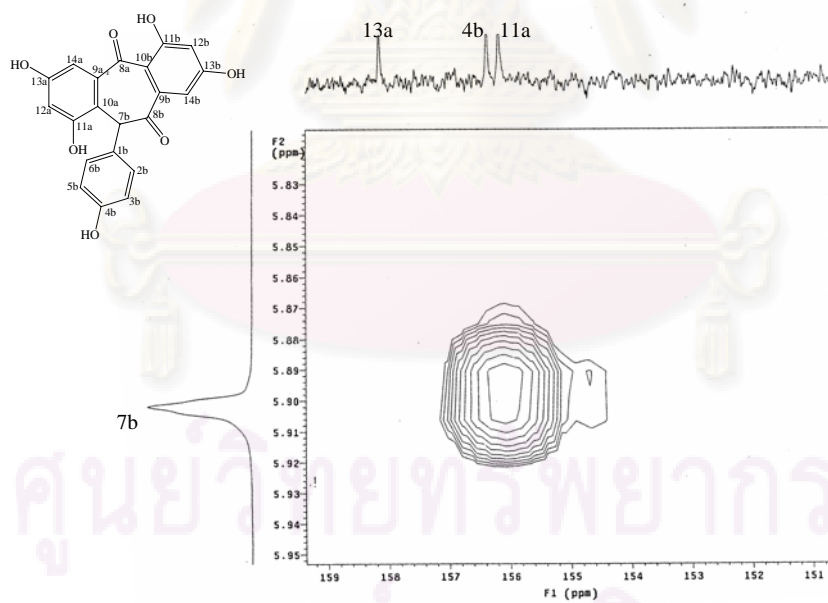
**Figure 38** HMBC Spectrum of compound ML8 (acetone- $d_6$ )



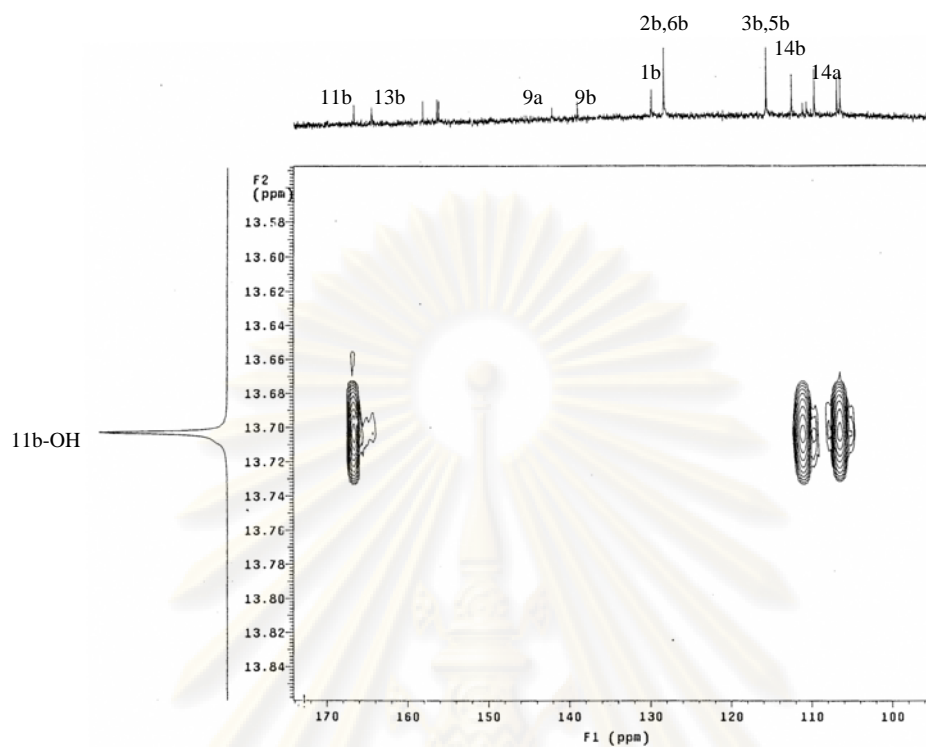
**Figure 39** HMBC Spectrum of compound ML8 (acetone- $d_6$ )



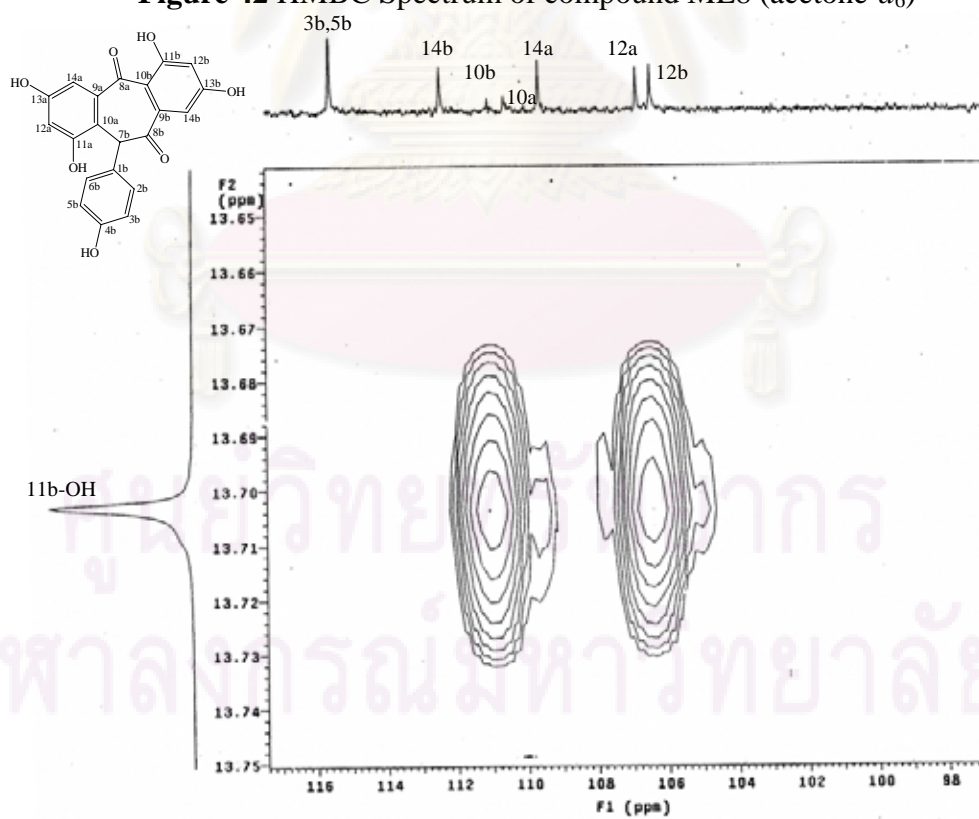
**Figure 40** HMBC Spectrum of compound ML8 (acetone- $d_6$ )



**Figure 41** HMBC Spectrum of compound ML8 (acetone- $d_6$ )



**Figure 42** HMBC Spectrum of compound ML8 (acetone- $d_6$ )



**Figure 43** HMBC Spectrum of compound ML8 (acetone- $d_6$ )

## VITA

Miss Manussanunt Chatsumpun was born on December 29, 1980 in Bangkok, Thailand. She received her Bachelor's degree of Science in Pharmacy in 2003 from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

### Poster Presentation

Chatsumpun, M., Sritularak, B., and Likhitwitayawuid, K. Chemical constituents of *Millettia leucantha* stemwood. Abstract of The 10<sup>th</sup> National Graduate Research Conference, September 11-12, 2008. Sukhothai Thammathirat Open University, Nonthaburi, p. 321.



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