การสร้างสารทุติยภูมิในเซลล์เพาะเลี้ยงของมะหาด

นางสุธิรา มณีฉาย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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SECONDARY METABOLITE PRODUCTION IN CELL CULTURES OF *ARTOCARPUS LAKOOCHA*

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Pharmacognosy
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สุธิรา มณีฉาย : การสร้างสารทุติยภูมิในเซลล์เพาะเลี้ยงของมะหาด.
(SECONDARY METABOLITE PRODUCTION IN CELL CULTURES OF *ARTOCARPUS LAKOOCHA*) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ศ.ภก.ดร. กิตติศักดิ์ ลิขิตวิทยาวุฒิ, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ.ดร. วันชัย ดีเอกนามกูล, 173 หน้า.

การศึกษาการสร้างสารทุติยภูมิในเซลล์เพาะเลี้ยงของมะหาด โดยทำการศึกษาในแคลลัสที่มาจากการชักนำให้เกิดเนื้อเยื่อเพาะเลี้ยงโดยใช้ส่วนลำต้น ใบเลี้ยง และใบอ่อนของพืชที่มาจากการงอกโดยการเพาะเมล็ดในสภาพปราศจากเชื้อ ภายใต้สภาวะของอาหารสูตร Woody Plant Medium ที่ประกอบด้วย 2,4-D 1 มิลลิกรัมต่อลิตร BA 1 มิลลิกรัมต่อลิตร และน้ำตาล 20 กรัมต่อลิตรสามารถแยกสารบริสุทธิ์ได้ 13 ชนิด โดยเป็นสารกลุ่ม flavonoids and stilbenoids ชนิดใหม่ 4 ชนิด คือ 5,7,4′-trihydroxy-2′-methoxy-3-(γ,γ-dimethylallyl)-flavone, 7,2′,4′-trihydroxy-3″,4″-dehydropyrano [1″,4″:5,6] flavone, *trans*-2,4,5′-trihydroxy-3′-methoxy-4′-(3-methyl-*E*-but-1-enyl)-stilbene และ 3-geranyl-2,4,3′,5′-tetrahydroxystilbene และอีก 9 ชนิดเป็นสารที่มีการรายงานมาแล้วคือ aesculin, (−)-catechin, albanin A, isoartocarpesin, norartocarpin, cudraflavone B, cudraflavone C, artotonkin และ chlorophorin การพิสูจน์โครงสร้างทางเคมีของสารที่แยกได้นี้ อาศัยการวิเคราะห์สเปกตรัม ร่วมกับการเปรียบเทียบข้อมูลของสารที่รายงานมาแล้ว เมื่อนำสารบริสุทธิ์ที่แยกได้ไปทำการทดสอบฤทธิ์ในการจับสารอนุมูลอิสระและฤทธิ์ยับยั้งเอนไซม์ tyrosinase พบว่าสารในกลุ่ม stilbenoids มีฤทธิ์แรงในการจับสารอนุมูลอิสระและฤทธิ์ยับยั้งเอนไซม์ tyrosinase ส่วนสารชนิดอื่นมีฤทธิ์ค่อนข้างต่ำ นอกจากนี้ได้พัฒนาวิธีวิเคราะห์สารด้วย TLC densitometry เพื่อวิเคราะห์ปริมาณสาร oxyresveratrol ในแก่นมะหาดและปวกหาด และเพื่อนำไปใช้ในการวิเคราะห์สารทุติยภูมิในเซลล์เพาะเลี้ยงของมะหาด เมื่อทำการศึกษาความสัมพันธ์ของการเจริญของแคลลัสและการสร้างสารทุติยภูมิพบว่า แคลลัสมีการสร้างสารทั้ง 13 ชนิดในทุกช่วงของการเจริญ มีรูปแบบการสร้างสารเหมือนกันแตกต่างเพียงปริมาณสารสำคัญที่สร้าง โดยมีการสร้างสารทุติยภูมิที่คู่ขนานกับการเจริญเติบโตของแคลลัส ซึ่งมีปริมาณสูงสุดในระยะก่อนเข้า stationary phase (ระยะ 15 - 17 วัน) และลดลงอย่างรวดเร็วเมื่อเนื้อเยื่อเข้าสู่ระยะ stationary phase (ระยะ 20 - 25 วัน)

ภาควิชา..เภสัชเวทและเภสัชพฤกษศาสตร์... ลายมือชื่อนิสิต……..
สาขาวิชา…….เภสัชเวท…… ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....
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KEYWORDS : *ARTOCARPUS LAKOOCHA*/ CALLUS CULTURES / SECONDARY METABOLITE PRODUCTION/ TYROSINASE INHIBITORS / QUANTITATIVE ANALYSIS/ FREE RADICAL SCAVENGING ACTIVITY

SUTHIRA MANEECHAI : SECONDARY METABOLITE PRODUCTION IN CELL CULTURES OF *ARTOCARPUS LAKOOCHA*

ADVISOR : PROF. KITTISAK LIKHITWITAYAWUID, Ph.D.,

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173 pp.

A study on the secondary metabolite production in cell cultures of *Artocarpus lakoocha* Roxb. was initiated by inducing callus formation from aerial part explants on Woody Plant Medium (WPM), which contained 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 1 mg/l benzyladenine (BA) and 20 g/l sucrose. A chemical study of the callus cultures of *A. lakoocha* led to the isolation of thirteen pure compounds, including four new compounds, namely, 5,7,4′-trihydroxy-2′-methoxy-3-(γ,γ-dimethylallyl)-flavone, 7,2′,4′-trihydroxy-3″,4″-dehydropyrano [1″,4″:5,6] flavone, *trans*-2,4,5′-trihydroxy-3′-methoxy-4′-(3-methyl-*E*-but-1-enyl)-stilbene and 3-geranyl-2,4,3′,5′-tetrahydroxystilbene together with nine known compounds including aesculin, (–)-catechin, albanin A, isoartocarpesin, norartocarpin, cudraflavone B, cudraflavone C, artotonkin and chlorophorin. Their structures were elucidated through analysis of their spectroscopic data and by comparison with previously reported data. The isolated compounds were evaluated for free radical scavenging and antityrosinase activities. Stilbenoids showed potent free radical scavenging activity and tyrosinase inhibitory activity. A thin-layer chromatography (TLC) densitometric method was also developed for the determination of oxyresveratrol content in *A. lakoocha* heartwood and in the traditional drug ‘Puag-Haad’, and the secondary metabolites in plant cell cultures of *A. lakoocha*. A study on the growth-product relationship during the 25-day growth of *A. lakoocha* callus cultures showed that all the isolated compounds were formed over the peroid of the culture growth. The obtained chemical profiles appeared to proceed parallelly to the growth curve of the callus, with the maximum content in the period of progressive decleration phase (day 15 - day 17) followed by a rapid decline in the stationary phase (day 20 - day 25).

Department : Pharmacognosy and Pharmaceutical Botany Student’s Signature .....

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CONTENTS

Page

CHAPTER

Page

CHAPTER

LIST OF TABLES

LIST OF FIGURES

FIGURE Page

LIST OF SCHEMES

LIST OF ABBREVIATIONS AND SYMBOLS

$[\alpha]^{20}_{D}$	=	Specific rotation at 20ºC and Sodium D line (589 nm)
α	=	Alpha
Acetone-d_6	=	Deuterated acetone
ax	=	Axial
β	=	Beta
br	=	Broad (for NMR spectra)
C	=	Concentration
ºC	=	Degree Celsius
calcd	=	Calculated
CD	=	Circular Dichroism
$CDCl_3$	=	Deuterated chloroform
CH_2Cl_2	=	Dichloromethane
cm	=	Centimeter
^{13}C NMR	=	Carbon-13 Nuclear Magnetic Resonance
^{1}H-^{1}H COSY	=	Homonuclear (Proton-Proton) Correlation Spectroscopy
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)
DPPH	=	1,1-Diphenyl-2-picrylhydrazyl
δ	=	Chemical shift
ED_{50}	=	50% Effective Dose
eq	=	Equatorial
ESI-MS	=	Electrospray Ionization Mass Spectrometry
EtOAc	=	Ethyl acetate
FCC	=	Flash Column Chromatography
g	=	Gram
GF	=	Gel Filtration Chromatography
hr	=	Hour
^{1}H-NMR	=	Proton Nuclear Magnetic Resonance

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

HMBC	=	^{1}H-detected Heteronuclear Multiple Bond Correlation
HMQC	=	^{1}H-detected Heteronuclear Multiple Quantum Coherence
HR- ESI-MS	=	High Resolution Electrospray Ionization Mass Spectrometry
HSQC	=	Heteronuclear Single Quantum Coherence
HSV-1	=	Herpes Simplex Virus type 1
HSV-2	=	Herpes Simplex Virus type 2
Hz	=	Hertz
IC_{50}	=	Concentration showing 50% inhibition
IR	=	Infrared
J	=	Coupling constant
KBr	=	Potassium bromide
Kg	=	Kilogram
L	=	Liter
L-DOPA	=	L-3,4-dihydroxyphenylalanine
μg	=	Microgram
μl	=	Microliter
μM	=	Micromolar
λ_{max}	=	Wavelength at maximal absorption
ε	=	Molar absorptivity
M^{+}	=	Molecular ion
m	=	Meta
m	=	Multiplet (for NMR spectra)
MeOH	=	Methanol
mg	=	Milligram
$[M+H]^{+}$	=	Protonated molecular ion
MHz	=	Megahertz
min	=	Minute
ml	=	Milliliter
mm	=	Millimeter

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

mM	=	Millimolar
m/z	=	Mass to charge ratio
MS	=	Mass spectrum
mult.	=	Multiplicity
MW	=	Molecular weight
NaH_2PO_4	=	Sodium dihydrogen phosphate
Na_2HPO_4	=	Disodium hydrogen phosphate
nm	=	Nanometer
NMR	=	Nuclear Magnetic Resonance
NOESY	=	Nuclear Overhauser Effect Spectroscopy
o	=	Ortho
p	=	Para
ppm	=	Part per million
q	=	Quartet (for NMR spectra)
s	=	Singlet (for NMR spectra)
spp.	=	Species
t	=	Triplet (for NMR spectra)
TLC	=	Thin Layer Chromatography
UV	=	Ultraviolet
UV-VIS	=	Ultraviolet and Visible spectrophotometry
VLC	=	Vacuum Liquid Column Chromatography
υ_{max}	=	Wave number at maximal absorption
2,4-D	=	2,4-Dichlorophenoxy acetic acid
BA or BAP	=	N_6-benzyladenine or N_6-benzylaminopurine
DW	=	Dry weight
FW	=	Fresh weight

CHAPTER I

INTRODUCTION

The genus *Artocarpus* belongs to the family Moraceae of the order Urticarles. This genus consists of 50 species, widely distributed in Sri Lanka, India, Pakistan, Myanmar, Thailand, Indo-china, South-China, Malaysia and Solomon Islands (Kochummen, 1978; Gardner, Sidisunthorn and Anusarnsunthorn, 2000).

Plants in the genus *Artocarpus* are often large trees. They are mostly evergreen trees with abundant white latex in all parts. The leaves are alternate, coriaceous, entire or pinnately lobe rarely pinnate. They have only one main vein from the base. The flowers are minute, tightly packed in oblong or globose heads. Male and female flowers are packed in separate heads. The male flowers have 2-4 sepals and 1 stamen. On the other hand, the female flowers have narrow tubular calyx fused with neighbouring flowers at the base. They consist of 1 slender style and 1 or 2 stigmas that are equal or unequal. The bracts are fused with the flowers to form a syncarp (fruit). The syncarps are irregulary globose or fistshaped with many oblong seeds (J.D. Hooker, 1890; Gardner *et al*., 2000; Wu *et al*., 2003).

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

Artocarpus altilis (Parkinson) Fosberg (*A. communis* J.R.& G. Forst., *A. incisa* Linn. f.)	ขนุนสำปะลอ Khanun sampalo (Central); สาเก Sake (Central); Bread fruit tree; Bread nut tree.
A. altissimus J.J. Smith	ไสน Sanai (Surat Thani).
A. chaplasha Roxb.	หาดส้าน Haat san (Chiang Rai).
A. dadah Miq.	ทังคัน Thang khan; ม่วงกวาง Muang kwang, (Yala); หาดรุม Hat rum, หาดลูกใหญ่, Hat luk yai (Trang); หาดขน Hat khon (Narathiwat).
A. elasticus Reinw. ex Blume	กะออก Ka ok, กะเอาะ Ka-o (Peninsular); ตือกะ Tue-ka (Malay-Yala); เอาะ O (Trang, Ranong).
A. gomezianus Wall. Ex Trécul	ตะปัง Ta pang, ตำปัง Tam-pang (Malay-Peninsular); หาดหนุน Hat nun (Northern); อีโป้ I po (Trang).
A. heterophyllus Lamk.	ขนุน Khanun (General); ขะนู Kha-nu (Chong-

(*A. integrifolia* Linn. f.)	Chanthaburi); ขะเนอ Kha-noe (Khmer); ซีคีย Si-khue, ปะหน่อย Pa-noi (Karen-Maehongson); นะยวยซะ Na-yuai-sa (Karen-Kanchanaburi); นากอ Na-ko (Malay-Pattani); เนน Nen (Chaobon-Nakhonratchasima); มะหนุน Manun (Northern, Peninsular); ล้าง, ลาง Lang (Shan-Northern); หมักหมี้ Makmi (Northeastern); หมากลาง Mak lang (Shan-Maehongson); Jack fruit tree.
A. kemando Miq.	ขนุนป่า Khanun pa (Narathiwat); ยาตู Yatu (Malay-Narathiwat)
A. integer (Thunb.) Merr.	จำปาดะ Champada (General); จำปาเดาะ Champado (Peninsular); Champedak
A. lacucha Roxb. (*A. lakoocha* Roxb.)	กาแย Kaa-yae, ตาแป Ta-pae, ตาแปง Ta-pang (Malay-Narathiwat); มะหาด Mahat (Peninsular); มะหาดใบใหญ่ Mahat bai yai (Trang); หาด
A. lanceifolius Roxb.	ขนุนป่า Khanun pa (Peninsular); หนังกาปิโต Nang-ka-pi-to, หนังกาปีปิ๊ต Nang-ka-pi-pit (Malay-Peninsular); นั่งกาปีแป๊ะ Nang-ka-pi-pae (Malay-Narathiwat).
A. nitidus Tréc subsp. *lingnanensis* Jarrett (*A. parva* Gagnep.)	มะหาดข่อย Mahat khoi (Surat Thani).
A. rigidus Blume subsp. *rigidus*	ขนุนป่า Khanun pa (Peninsular)
A. rigidus Bl. subsp. *asperulus* Jarrett. (*A. calophyllus* Kurz)	ขนุนปาน Khanun pan (Surat Thani)

Artocarpus lakoocha Roxb. is an indigenous plant known in Thai as Mahaad. It is a large deciduous tree reaching 15-18 m. The bark is red brown to dark brown, which becomes rough and scaly with age. The branchets are 3-6 mm. thick. The stipules are ovate-lanceolate, 4-5 cm. in length. The leaves (5-15 cm. x 10-15 cm.) are alternate. Their shape is oval or obovate. The margin of leaves are untoothed or with minute teeth. They have 10-18 secondary veins on each side of the midvein. The leaf texture is leathery. The young red brown shoots are hairy. The stalks are finely hairy, 1.4-3.3 cm. long. The twigs are rather stout without ring scars. The flower heads, which grow from leaf axils, are dirty yellow to pale pink or orange. The male heads (0.8-2 cm.) with globular stalks, while the female heads (1.2-2.3 cm.) are oval or oblong, with 2.5-3 cm. stalks. The fruits are 2.5-8 cm. with smooth velutin surface. Their fresh are pale yellow or orange that change to reddish

brown when dried. The seeds are ellipsoid with bent bristles (Hooker, 1890; Gardner *et al*., 2000; Wu *et al*., 2003).

Cell culture technology has been developed as a possible tool to both study and produce plant secondary metabolites (Zhong, J.J., 2001). Previous reports of *Artocarpus* plants have been focused mainly on micropropagation, for example *A. lakoocha* (Janick., J and Whipkey, A., eds. 2002), *A. altilis* (Murch, S.J. et al., 2008) and *A. heterophyllus* (Lee, C.L. and Keng, C.L., 2005). No reports of secondary metabolite production from cell cultures of *A. lakoocha* have been discussioned. Previous phytochemical studies of stem heartwood and root of this plant have revealed the presence of triterpenoids, flavonoids and stilbenes, some of which possessed biological activities such as antiherpetic, antioxidant and tyrosinase inhibitory activities (Jagtap and Bapat, 2010).

The purpose of this research is to study the secondary metabolite production from *A. lakoocha* cell cultures. The objectives of the research are:

1. To develop a simple, reliable and efficient method for the quantitative analysis of oxyresveratrol in plant extracts and for quantitative analysis in plant cell cultures.

2. To study methods for producing cell cultures of *A. lakoocha*.

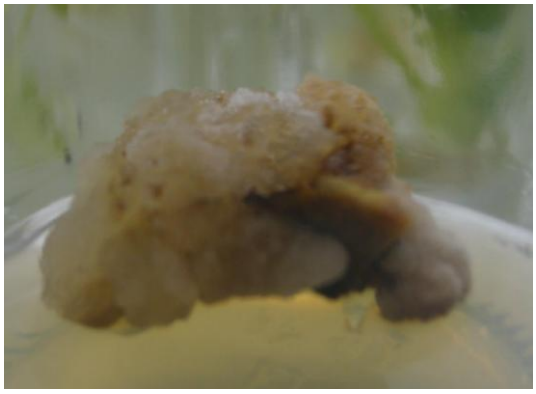
3. To isolate and purify compounds from cell cultures of *A. lakoocha*.

4. To determine the chemical structure of each isolated compound.

5. To evaluate each isolated compound for its free radical scavenging activity, and tyrosinase inhibition potential.

A

B



C

Figure 1 *Artocarpus lakoocha* Roxb.

A) Tree B) Fruit C) Callus culture

CHAPTER II

HISTORICAL

1. Chemical Constituents of *Artocarpus* spp.

A number of compounds have been isolated from the genus *Artocarpus*. They can be classified as flavonoids, triterpenoids, steroids, stilbenoids and miscellaneous substances (Tables 1-4).

Table 1 Distribution of flavonoids in the genus *Artocarpus*

Plant and chemical compound	Plant part	Reference
Artocarpus altilis		
Apigenin [**1**]	Heartwood	Shimizu *et al*., 1998
Artobiloxanthone [**2**] (artocarpus flavone KB-1)	Bark	Aida *et al*., 1997
Artocarpesin [**3**]	Heartwood	Shimizu *et al*., 1998
Artocarpin [**4**]	Heartwood	Venkataraman, 1972
Artocarpus chalcone AC-3-1 [**5**]	Flower Leaves	Fujimoto *et al*., 1987a Wang *et al*., 2007b
Artocarpus chalcone AC-3-3 [**6**]	Flower	Fujimoto *et al*., 1987a
Artocarpus chalcone AC-5-1 [**7**]	Flower Leaves	Fujimoto *et al*., 1987a Wang *et al*., 2007b
Artocarpus chalcone I [**8**]	Flower	Fujimoto, Agusutein, and Made, 1987b
Artocarpus flavanone AC-3-2 [**9**]	Flower	Fujimoto *et al*., 1987a
Artocarpus flavanone AC-5-2 [**10**]	Flower Leaves	Fujimoto *et al*., 1987a Wang *et al*., 2007b
Artocarpus flavone KB-1 [**2**] (Artobiloxanthone)	Bark	Fujimoto *et al*., 1990
Artocarpus flavone KB-2 [**11**]	Bark	Fujimoto *et al*., 1990
Artocarpus flavone KB-3 [**12**] (Artonin E)	Bark	Fujimoto *et al*., 1990
Artochamin B [**13**]	Root cortex	Lin *et al*., 2006
Artochamin D [**14**]	Root cortex	Lin *et al*., 2006

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
Artocommunol CB [**15**]	Root cortex	Chan, Ko, and Lin, 2003
Artocommunol CC [**16**]	Root cortex	Lin *et al*., 2006
Artocommunol CD [**17**]	Root cortex	Chan *et al*., 2003
Artocommunol CE [**18**]	Root cortex	Chan *et al*., 2003
Artoflavone A [**19**]	Root cortex	Lin *et al*., 2009
Artomunoflavanone [**20**]	Root cortex	Lin *et al*., 2006
Artomunoisoxanthone [**21**]	Root cortex	Lin *et al*., 2006
Artomunoxanthentrione [**22**]	Root bark	Shieh and Lin, 1992
Artomunoxanthone [**23**]	Root bark	Shieh and Lin, 1992
Artomunoxanthotrione epoxide [**24**]	Root bark	Lin *et al*., 1992
Artonin E [**12**] (Artocarpus flavone KB-3)	Bark	Hano *et al*., 1990d
Artonin F [**25**]	Bark	Hano *et al*., 1990d
Artonin K [**26**]	Bark	Aida *et al*., 1997
Artonin V [**27**]	Root bark	Hano, Inami and Nomura, 1994
Artonol A [**28**]	Bark	Aida *et al*., 1997
Artonol B [**29**]	Bark	Aida *et al*., 1997
Artonol C [**30**]	Bark	Aida *et al*., 1997
Artonol D [**31**]	Bark	Aida *et al*., 1997
Artonol E [**32**]	Bark	Aida *et al*., 1997
Cudraflavone A [**33**] (Isocyclomorusin)	Root bark Heartwood	Shieh and Lin, 1992 Han *et al*., 2006
Cudraflavone C [**34**]	Heartwood	Han *et al*., 2006
Cycloaltilisin [**35**]	Stem Leaves	Chen *et al*., 1993 Wang *et al*., 2007b

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
Cycloaltilisin 6 [**36**]	Bud cover	Patil *et al*., 2002
Cycloaltilisin 7 [**37**]	Bud cover	Patil *et al*., 2002
Cycloartobiloxanthone [**38**]	Bark	Hano *et al*., 1990d
Cycloartocarpin [**39**]	Heartwood	Venkataraman, 1972
Cycloartomunin [**40**]	Root bark	Lin and Shieh, 1991
Cycloartomunoxanthone [**41**]	Root bark	Lin and Shieh, 1991
Cyclocommunin [**42**]	Root bark	Lin and Shieh, 1991
Cyclocommunol [**43**]	Root bark	Lin and Shieh, 1991
Cyclocommunomethonol [**44**]	Root cortex	Lin *et al*., 2006
Cyclogeracommunin [**45**]	Root cortex	Lin *et al*., 2009
Cyclomorusin [**46**]	Stem Root	Chen *et al*., 1993 Lin and Shieh, 1991
Cyclomulberrin [**47**]	Stem Root bark	Chen *et al*., 1993 Lin and Shieh, 1992
Dihydroartomunoxanthone [**48**]	Root cortex	Lin *et al*., 2006
Dihydrocycloartomunin [**49**]	Root bark	Lin and Shieh, 1991
Dihydroisocycloartomunin [**50**]	Root bark	Lin and Shieh, 1992
Dihydromorin [**51**]	Heartwood	Shimizu *et al*., 1998
Engeletin [**52**]	Stem	Chen *et al*., 1993
(2*S*)-Euchrenone a7 [**53**]	Heartwood	Han *et al*., 2006
Gemichalcone B [**54**]	Heartwood	Han *et al*., 2006
Gemichalcone C [**55**]	Heartwood	Han *et al*., 2006
3′-Geranyl-2′,3,4,4′- tetrahydroxy-chalcone [**56**]	Leaves	Shimizu *et al*., 2000
Hydroxyartocarpin [**57**]	Stem bark	Shamaun *et al*., 2010

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
Isoartocarpesin [**58**]	Heartwood	Shimizu *et al*., 1998
Isobacachalcone [**59**]	Heartwood	Han *et al*., 2006
Isocyclomorusin [**33**] (Cudraflavone A)	Stem	Chen *et al*., 1993
Isocyclomulberrin [**42**] (Cyclocommunin)	Stem	Chen *et al*., 1993
Isolespeol [**60**]	Leaves	Fang *et al*., 2008b
Morin [**61**]	Heartwood	Venkataraman, 1972
Morachalcone A [**62**]	Heartwood	Han *et al*., 2006
Morusin [**63**]	Stem bark	Fujimoto *et al*., 1990
(+)-Norartocarpanone [**64**]	Heartwood	Shimizu *et al*., 1998
Norartocarpetin [**65**]	Heartwood	Venkataraman, 1972
1-(2,4-Dihydroxyphenyl)-3-[3,4-dihydro-3,8-dihydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone [**66**]	Leaves	Wang *et al*., 2007b
1-(2,4-Dihydroxyphenyl)-3-[8-hydroxy- 2-methyl-2-(3,4-epoxy-4-methyl-1-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone [**67**]	Leaves	Wang *et al*., 2007b
1-(2,4-Dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-hydroxy-4-methyl-2-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone [**68**]	Leaves	Wang *et al*., 2007b
1-(2,4-Dihydroxyphenyl)-3-{4-hydroxy-6,6,9-trimethyl-6a,7,8,10a tetrahydro-6H-dibenzo [b,d]pyran-5-yl}-1-propanone [**69**]	Leaves	Wang *et al*., 2007b
3″,3″-Dimethylpyrano[3′,4′]2,4,2′-trihydroxy chalcone [**70**]	Heartwood	Han *et al*., 2006

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
5′-Geranyl-2′,4′,4-trihydroxychalcone [**71**]	Leaves	Fang *et al*., 2008
2-[6-Hydroxy-3,7-dimethylocta-2(*E*),7-dienyl]-2′,3,4,4′-tetrahydroxy dihydrochalcone [**72**]	Leaves	Wang *et al*., 2007b
3,4,2′,4′-Tetrahydroxy-3′-geranyldihydrochalcone [**73**]	Leaves	Fang *et al*., 2008
Artocarpus bracteata		
Artoindonesianin J [**74**]	Root and stem bark	Ersam *et al*., 2002
Carpachromene [**75**]	Root and stem bark	Ersam *et al*., 2002
Kozonol C [**76**]	Root and stem bark	Ersam *et al*., 2002
6-Prenylalpigenin [**77**]	Root and stem bark	Ersam *et al*., 2002
Artocarpus champeden		
Artocarpone A [**78**]	Stem bark	Widyawaruyanti *et al*., 2007
Artocarpone B [**79**]	Stem bark	Widyawaruyanti *et al*., 2007
Artoindonesianin A [**80**]	Root	Hakim *et al*., 1999
Artoindonesianin A-2 [**81**]	Stem bark Heartwood	Syah *et al*., 2006b Widyawaruyanti *et al*., 2007
Artoindonesianin A-3 [**82**]	Heartwood	Syah *et al*., 2006b
Artoindonesianin B [**83**]	Root	Hakim *et al*., 1999
Artoindonesianin E [**84**]	Stem bark	Widyawaruyanti *et al*., 2007
Artoindonesianin M [**85**]	Heartwood	Syah *et al*., 2002a
Artoindonesianin Q [**86**]	Heartwood	Syah *et al*., 2002b

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
Artoindonesianin R [**87**]	Heartwood	Syah *et al*., 2002b
Artoindonesianin S [**88**]	Heartwood	Syah *et al*., 2002b
Artoindonesianin T [**89**]	Heartwood	Syah *et al*., 2002b
Artoindonesianin U [**90**]	Heartwood	Syah *et al*., 2004
Artoindonesianin V [**91**]	Heartwood	Syah *et al*., 2004
Artonin A [**92**]	Root Stem bark	Hakim *et al*., 1999 Widyawaruyanti *et al*., 2007
Artonin B [**93**]	Heartwood	Syah *et al*., 2004
Artopeden A [**94**]	Bark	Wahyuni *et al*., 2009
Cyclochampedol [**95**]	Stem bark	Achmad *et al*., 1996 Parenti *et al*., 1998
Cyclocommunin [**42**]	Heartwood	Syah *et al*., 2004
Cycloheterophyllin [**96**]	Stem bark	Widyawaruyanti *et al*., 2007
Heteroflavanone C [**97**]	Stem bark	Widyawaruyanti *et al*., 2007
Heterophyllin [**98**]	Stem bark	Widyawaruyanti *et al*., 2007
5′-Hydroxycudraflavone A [**99**]	Heartwood	Syah *et al*., 2004
Artocarpus chaplasha (***A. chama***)		
Artocarpesin [**3**]	Heartwood	Rao, Rathi and Venkataraman, 1972
Artocarpin [**4**]	Heartwood Root	Rao *et al*., 1972 Wang *et al*., 2004
Artochamin A [**100**]	Root	Wang *et al*., 2004
Artochamin B [**13**]	Root	Wang *et al*., 2004
Artochamin C [**101**]	Root	Wang *et al*., 2004

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
Artochamin D [**14**]	Root	Wang *et al*., 2004
Artochamin E [**102**]	Root	Wang *et al*., 2004
Artonin A [**92**]	Root	Wang *et al*., 2004
Artonin E [**12**] (Artocarpus flavone KB-3)	Root	Wang *et al*., 2004
Artonin U [**103**]	Root	Wang *et al*., 2004
Chaplashin [**104**]	Heartwood	Rao *et al*., 1972
Cudraflavone A [**33**] (Isocyclomorusin)	Root	Wang *et al*., 2004
Cycloartobiloxanthone [**38**]	Root	Wang *et al*., 2004
Cycloartocarpesin [**105**]	Heartwood	Rao *et al*., 1972
Cycloartocarpin [**39**]	Heartwood	Rao *et al*., 1972
Cycloartocarpin A [**106**]	Root	Wang *et al*., 2004
5′-Hydroxycudraflavone A [**99**]	Root	Wang *et al*., 2004
Artocarpus dadah		
Afzelechin-3-*O*-*α*-L-rhamnoside [**107**]	Stem bark Twig	Su *et al*., 2002
(+)-Catechin [**108**]	Stem bark Twig	Su *et al*., 2002
Dihydromorin [**51**]	Stem bark	Su *et al*., 2002
Engeletin [**52**]	Twig	Su *et al*., 2002
(+)-Epiafzelechin [**109**]	Stem bark	Su *et al*., 2002
(–)-Epiafzelechin-(4*β*→8)-epicatechin [**110**]	Stem bark	Su *et al*., 2002
Gemichalcone B [**54**]	Twig	Su *et al*., 2002
Isogemichalcone B [**111**]	Twig	Su *et al*., 2002

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
Norartocarpetin [**65**]	Twig	Su *et al*., 2002
Steppogenin [**112**]	Twig	Su *et al*., 2002
Artocarpus elasticus		
Artelasticin [**113**]	Heartwood	Kijjoa *et al*., 1996
Artelasticinol [**114**]	Root bark	Ko *et al*., 2005
Artelastin [**115**]	Heartwood	Kijjoa *et al*., 1996
Artelastinin [**116**]	Heartwood	Kijjoa *et al*., 1998
Artelastocarpin [**117**]	Heartwood	Cidade *et al*., 2001
Artelastochromene [**118**]	Heartwood	Kijjoa *et al*., 1996
Artelastofuran [**119**]	Heartwood	Kijjoa *et al*., 1998
Artelastoheterol [**120**]	Root bark	Ko *et al*., 2005
Artelastoxanthone [**121**]	Root bark	Ko *et al*., 2005
Artocarpesin [**3**]	Heartwood	Kijjoa *et al*., 1996
Artocarpin [**4**]	Heartwood	Kijjoa *et al*., 1996
Artoindonesianin E1 [**122**]	Wood	Musthapa *et al*., 2009
Artonin F [**25**]	Root bark	Ko *et al*., 2005
Artonin S [**123**]	Root bark	Ko *et al*., 2005
Artonol A [**28**]	Root bark	Ko *et al*., 2005
Artonol B [**29**]	Root bark	Ko *et al*., 2005
Carpelastofuran [**124**]	Heartwood	Cidade *et al*., 2001
Cycloartelastoxanthendiol [**125**]	Root bark	Ko *et al*., 2005
Cycloartelastoxanthone [**126**]	Root bark	Ko *et al*., 2005
Cycloartobiloxanthone [**38**]	Root bark	Ko *et al*., 2005
Cycloartocarpesin [**105**]	Heartwood	Pendse *et al*., 1976

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
Cycloartocarpin [**39**]	Heartwood	Pendse *et al*., 1976
Cyclomorusin [**46**]	Root bark	Ko *et al*., 2005
Integrin [**127**]	Heartwood	Pendse *et al*., 1976
Norartocarpin [**128**]	Heartwood	Pendse *et al*., 1976
Artocarpus fretessi		
Afzelechin [**129**]	Stem bark	Soekamto *et al*., 2003
Afzelechin-3-*O*-*α*-L-rhamnoside [**107**]	Stem bark	Soekamto *et al*., 2003
Artonin A [**92**]	Stem bark	Soekamto *et al*., 2003
Mulberrin [**130**]	Stem bark	Soekamto *et al*., 2003
Mulberrochromene [**131**]	Stem bark	Soekamto *et al*., 2003
Norartocarpetin [**65**]	Stem bark	Soekamto *et al*., 2003
Artocarpus glaucus		
Cudraflavone C [**34**]	Root bark	Hakim *et al*., 2006
Catechin [**108**]	Root bark	Hakim *et al*., 2006
Artocarpus gomezianus		
Albanin A [**132**]	Root	Likhitwitayawuid, Sritularak and De-Eknamkul, 2000
Artocarpesin [**3**]	Heartwood	Venkataraman, 1972
Artocarpin [**4**]	Heartwood Root	Venkataraman, 1972 Likhitwitayawuid *et al*., 2000
Cudraflavone C [**34**]	Root	Likhitwitayawuid *et al*., 2000
Cycloartocarpin [**39**]	Heartwood Root	Venkataraman, 1972 Likhitwitayawuid *et al*., 2000

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
Isocyclomorusin [**33**]	Root	Likhitwitayawuid *et al*., 2000
Morin [**61**]	Heartwood	Venkataraman, 1972
Norartocarpetin [**65**]	Heartwood Root	Venkataraman, 1972 Likhitwitayawuid *et al*., 2000
Norcycloartocarpin [**133**]	Heartwood	Likhitwitayawuid *et al*., 2006
Artocarpus heterophyllus		
Afzelechin-(4α→8)-catechin [**134**]	Leaves	An *et al*., 1992
Albanin A [**132**]	Wood	Arung, Shimizu and Kondo, 2006b
Artocarpanone [**135**]	Heartwood	Radhakrishnan, Rao and Venkataraman, 1965
Artocarpanone A [**136**]	Root bark	Lin, Lu and Huang, 1995
Artocarpesin [**3**]	Heartwood	Radhakrishnan *et al*., 1965
Artocarpetin [**137**]	Heartwood	Venkataraman, 1972
Artocarpetin A [**138**]	Root bark	Lin *et al*., 1995
Artocarpetin B [**139**]	Root	Chung *et al*., 1995
Artocarpfuranol [**140**]	Wood	Zheng *et al*., 2008
Artocarpin [**4**]	Heartwood	Radhakrishnan *et al*., 1965
Artoflavanone [**141**]	Root	Dayal and Seshadri, 1974
Artoheterophyllin B [**142**]	Twig	Zheng *et al*., 2009
Artoheterophyllin C [**143**]	Twig	Zheng *et al*., 2009

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
Artoheterophyllin D [**144**]	Twig	Zheng *et al*., 2009
Artonin A [**92**]	Root bark	Hano *et al*., 1989
Artonin B [**93**]	Root bark	Hano *et al*., 1989
Artonin C [**145**]	Root bark	Hano, Aida and Nomura, 1990a
Artonin D [**146**]	Root bark	Hano *et al*., 1990a
Artonin I [**147**]	Root bark	Hano *et al*., 1989
Artonin J [**148**]	Root bark	Aida *et al*., 1993
Artonin K [**26**]	Root bark	Aida *et al*., 1993
Artonin L [**149**]	Root bark	Aida *et al*., 1993
Artonin Q [**150**]	Bark	Aida *et al*., 1994
Artonin R [**151**]	Bark	Aida *et al*., 1994
Artonin S [**123**]	Bark	Aida *et al*., 1994
Artonin T [**152**]	Bark	Aida *et al*., 1994
Artonin U [**103**]	Bark	Aida *et al*., 1994
Artonin X [**153**]	Bark	Shinomiya *et al*., 1995
Catechin [**108**]	Leaves	Yamazaki *et al*., 1987
Cudraflavone A [**33**]	Root bark	Lin *et al*., 1995
Cudraflavone C [**34**]	Wood	Arung *et al*., 2006b
Cyanomaclurin [**154**]	Heartwood	Radhakrishnan *et al*., 1965
Cycloartocarpesin [**105**]	Heartwood	Pathasarathy *et al*., 1969
Cycloartocarpin [**39**]	Heartwood	Venkataraman, 1972
Cycloartocarpin A [**106**]	Root bark	Lu and Lin, 1994

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
Cycloheterophyllin [**96**]	Bark	Rao, Varadan, and Venkataraman, 1971
	Root bark	Hano *et al*., 1989
Dihydromorin [**51**]	Heartwood	Venkataraman, 1972
Heteroartonin A [**155**]	Root bark	Chung *et al*., 1995
Heteroflavanone A [**156**]	Root bark	Lu and Lin, 1993
Heteroflavanone B [**157**]	Root bark	Lu and Lin, 1993
Heteroflavanone C [**97**]	Root bark	Lu and Lin, 1994
Heterophyllin [**98**]	Root bark	Hano *et al*., 1989
Heterophyllol [**158**]	Root bark	Lu and Lin, 1993
Isocycloheterophyllin [**159**]	Bark	Rao, Varadan and Venkataraman, 1973
Kuwanon C [**160**]	Wood	Arung *et al*., 2006b
Kuwanon R [**161**]	Root bark	Shinomiya *et al*., 1995
Kuwanon T [**162**]	Root bark	Shinomiya *et al*., 1995
Morin [**61**]	Heartwood	Radhakrishnan *et al*., 1965; Pathasarathy *et al*., 1969; Mu and Li, 1982
Norartocarpetin [**65**]	Heartwood	Radhakrishnan *et al*., 1965
Norartocarpin [**128**]	Heartwood	Venkataraman, 1972
	Wood	Arung *et al*., 2006b
Oxydihydroartocarpesin [**163**]	Heartwood	Pathasarathy *et al*., 1969
6-Prenylapigenin [**77**]	Wood	Arung *et al*., 2006b
3-Prenyl luteolin [**164**]	Wood	Arung *et al*., 2010
Procyanidin B-3 [**165**]	Leaves	An *et al*., 1992

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
Procyanidin C-1 [**166**]	Leaves	An *et al*., 1992
Artocarpus hirsuta		
Artocarpanone [**135**]	Heartwood	Venkataraman, 1972
Artocarpesin [**3**]	Heartwood	Venkataraman, 1972
Artocarpetin [**137**]	Heartwood	Venkataraman, 1972
Artocarpin [**4**]	Heartwood	Venkataraman, 1972; Arung *et al*., 2006b
Cyanomaclurin [**154**]	Heartwood	Venkataraman, 1972
Cycloartocarpesin [**105**]	Heartwood	Venkataraman, 1972
Cycloartocarpin [**39**]	Heartwood	Venkataraman, 1972
Dihydromorin [**51**]	Heartwood	Venkataraman, 1972
Morin [**61**]	Heartwood	Venkataraman, 1972
Norartocarpetin [**65**]	Heartwood	Venkataraman, 1972
Oxydihydroartocarpesin [**163**]	Heartwood	Venkataraman, 1972
Artocarpus integer		
Artocarpanone [**135**]	Heartwood	Pendse *et al*., 1976
Artocarpesin [**3**]	Heartwood	Pendse *et al*., 1976
Artocarpetin [**137**]	Heartwood	Pendse *et al*., 1976
Catechin [**108**]	Leaves	Yamazaki *et al*., 1987
Chaplashin [**104**]	Heartwood	Pendse *et al*., 1976
Cycloartocarpesin [**105**]	Heartwood	Pendse *et al*., 1976
Cycloartocarpin [**39**]	Heartwood	Pendse *et al*., 1976
Cyclointegrin [**167**]	Heartwood	Pendse *et al*., 1976
Cyanomaclurin [**154**]	Heartwood	Pendse *et al*., 1976

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
Dihydromorin [**51**]	Heartwood	Pendse *et al*., 1976
Integrin [**127**]	Heartwood	Pendse *et al*., 1976
Morin [**61**]	Heartwood	Pendse *et al*., 1976
Norartocarpetin [**65**]	Heartwood	Pendse *et al*., 1976
Oxydihydroartocarpesin [**163**]	Heartwood	Pendse *et al*., 1976
Oxyisocyclointegrin [**168**]	Heartwood	Pendse *et al*., 1976
Artocarpus kemando		
Artobiloxanthone [**2**] (artocarpus flavone KB-1)	Stem bark	Seo *et al*., 2003
Artocarpin [**4**]	Root	Hakim *et al*., 2006
Artoindonesianin B [**83**]	Root	Hakim *et al*., 2006
Artoindonesianin D [**169**]	Root	Hakim *et al*., 2006
Artonin E [**12**]	Stem bark	Seo *et al*., 2003
Artonin O [**170**]	Stem bark	Seo *et al*., 2003
Cycloartobiloxanthone [**38**]	Stem bark Root	Seo *et al*., 2003 Hakim *et al*., 2006
Cycloartocarpin [**39**]	Root	Hakim *et al*., 2006
Norartocarpetin [**65**]	Root	Hakim *et al*., 2006
Artocarpus lakoocha		
Artocarpin [**4**]	Heartwood	Venkataraman, 1972
Cycloartocarpin [**39**]	Heartwood	Venkataraman, 1972
5,7-Dihydroxyflavone-3-*O*-*α*-L-rhamnoside [**171**]	Root bark	Chauhan and Kumari, 1979
5-Hydroxy-7,2′,4′-trimetroxyflavone [**172**]	Stemwood	Pavaro and Reutrakul, 1976
Galangin-3-*O*-*α*-L-rhamnoside [**173**]	Root bark	Chauhan and Kumari, 1979
Galangin-3-*O*-*β*-D-galactopyranosyl-(1→4)-*α*-L-rhamnoside [**174**]	Root bark	Chauhan, Kumari and Saraswat, 1979

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
Kaempferol-3-*O*- β-D-xyloside [**175**]	Root bark	Chauhan *et al*., 1982
Norartocarpin [**128**]	Heartwood	Venkataraman, 1972
Norcycloartocarpin [**176**]	Heartwood	Venkataraman, 1972
Quercetin-3-*O*-α-L-rhamnoside [**177**]	Root bark	Chauhan *et al*., 1982
5,7,2',4',-tetrahydroxy-3-prenyl-6-geranylflavone [**178**]	Root bark	Tantrakarnsakul, 2010
Artocarpus lanceifolius		
Artelasticin [**113**]	Heartwood	Syah *et al*., 2001
Artelastofuran [**119**]	Heartwood	Syah *et al*., 2001
Artobiloxanthone [**2**]	Bark	Hakim *et al*., 2002a
Artoindonesianin G [**179**]	Heartwood	Syah *et al*., 2001
Artoindonesianin H [**180**]	Heartwood	Syah *et al*., 2001
Artoindonesianin I [**181**]	Heartwood	Syah *et al*., 2001
Artoindonesianin P [**182**]	Bark	Hakim *et al*., 2002a
Artoindonesianin Z-1 [**183**]	Stem bark	Syah *et al*., 2006a
Artoindonesianin Z-2 [**184**]	Stem bark	Syah *et al*., 2006a
Artoindonesianin Z-3 [**185**]	Stem bark	Hakim *et al*., 2006
Artoindonesianin Z-4 [**186**]	Stem bark	Musthapa *et al*., 2009
Artoindonesianin Z-5 [**187**]	Stem bark	Musthapa *et al*., 2009
Artonin E [**12**]	Stem	Cao *et al*.,2003
Artonol B [**29**]	Bark	Hakim *et al*., 2002a
Cycloartobiloxanthone [**38**]	Bark	Hakim *et al*., 2002a
14-Hydroxyartonin E [**188**]	Stem	Cao *et al*., 2003

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
Artocarpus lowii		
2′,4′-Dihydroxy-4-methoxy-3′-prenyldihydrochalcone [**189**]	Leaves	Jamil *et al*., 2008
Artocarpus maingayi		
Artocarpin [**4**]	Stem bark	Hakim *et al*., 2006
Artoindonesianin D [**169**]	Stem bark	Hakim *et al*., 2006
Chaplashin [**104**]	Stem bark	Hakim *et al*., 2006
Cudraflavone A [**33**]	Stem bark	Hakim *et al*., 2006
Cycloartocarpin **A** [**106**]	Stem bark	Hakim *et al*., 2006
Artocarpus nobilis		
Artobilochromen [**190**]	Bark	Pavanasasivam, Sultanbawa and Mageswaran, 1974; Kumar *et al*., 1977; Sultanbawa and Surendrakumar, 1989
Artobiloxanthone [**2**]	Bark	Sultanbawa and Surendrakumar, 1989
Artonin E 2′-methylether [**191**]	Root bark	Jayasinghe *et al*., 2008
Artonin V 2′-methylether [**192**]	Root bark	Jayasinghe *et al*., 2008
Chromanoartobilochromen A [**193**]	Stem bark	Kumar *et al*., 1977
Chromanoartobilochromen B [**194**]	Stem bark	Pavanasasivam *et al*., 1974; Kumar *et al*., 1977
Chromanoartobilochromene [**195**]	Bark	Pavanasasivam *et al*., 1974
Cycloartobiloxanthone [**38**]	Bark	Pavanasasivam *et al*., 1974
1-(3,4-Dihydro-3,5-dihydroxy-2-methyl-2-(3-methyl-2-butenyl)-2H-1-benzopyran-6-yl-3-(4-hydroxy phenyl)-2(*E*)-propen-1-one [**196**]	Fruit	Jayasinghe *et al*., 2006

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
(–)-Dihydrofuranoartobilochromen A [**197**]	Stem bark	Kumar *et al*., 1977
(–)-Dihydrofuranoartobilochromen B-1 [**198**]	Stem bark	Kumar *et al*., 1977
(–)-Dihydrofuranoartobilochromen B-2 [**199**]	Stem bark	Kumar *et al*., 1977
Dihydroisoartonin E 2′-methylether [**200**]	Root bark	Jayasinghe *et al*., 2008
Furanoartobilochromen A [**201**]	Bark	Pavanasasivam *et al*., 1974
Furanoartobilochromen B-1 [**202**]	Bark	Pavanasasivam *et al*., 1974
Furanoartobilochromen B-2 [**203**]	Bark	Pavanasasivam *et al*., 1974
3′-Geranyl-2′,3,4,4′-tetrahydroxychalcone [**204**]	Leaves Fruit	Jayasinghe *et al*., 2004a Jayasinghe *et al*., 2006
3′-Geranyl-4′,5,7-trihydroxyflavanone [**205**]	Fruit	Jayasinghe *et al*., 2006
8-Geranyl-3′,4′,7-trihydroxyflavanone [**206**]	Fruit	Jayasinghe *et al*., 2006
8-Geranyl-4′,7-dihydroxyflavanone [**207**]	Fruit	Jayasinghe *et al*., 2006
Isoartonin E 2′-methylether [**208**]	Root bark	Jayasinghe *et al*., 2008
Isonymphaeol-B [**209**]	Fruit	Jayasinghe *et al*., 2006
Lespeol [**210**]	Fruit	Jayasinghe *et al*., 2006
2,4,4′-Trihydroxy-3-[(2*E*)-5-methoxy-3,7-dimethylocta-2,6-dienyl]chalcone [**211**]	Fruit	Jayasinghe *et al*., 2006
2′,4′,4-Trihydroxy-3′-geranylchalcone [**212**]	Leaves	Fujimoto *et al*., 2004
2′,4′,4-Trihydroxy-3′-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadiethyl] chalcone [**213**]	Leaves	Fujimoto *et al*., 2004

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
2′,4′,4-Trihydroxy-3′-[2-hydroxy-7-methyl-3-methylene-6-octaethyl] chalcone [**214**]	Leaves	Fujimoto *et al*., 2004
2′,3,4,4′-Tetrahydroxy-3′-geranyl chalcone [**215**]	Leaves	Fujimoto *et al*., 2004
2′,3,4,4′-Tetrahydroxy-3′-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadiethyl] chalcone [**216**]	Leaves	Fujimoto *et al*., 2004
Oxydihydromorusin [**217**]	Stem bark	Kumar *et al*., 1977; Fukai and Nomura, 1993
Xanthoangelol [**218**]	Leaves Fruit	Jayasinghe *et al*., 2004a Jayasinghe *et al*., 2006
Xanthoangelol B [**219**]	Leaves Fruit	Jayasinghe *et al*., 2004a Jayasinghe *et al*., 2006
Artocarpus obtusus		
Dihydroartoindonesianin C [**220**]	Stem bark	Hashim *et al*., 2010
Pyranocycloartobiloxanthone A **[221**]	Stem bark	Hashim *et al*., 2010
Artocarpus odoratissimus		
Artosimmin [**222**]	-	Ee *et al*., 2010
Artocarpus pithecogallus		
Morin [**61**]	Heartwood	Mu and Li, 1982
Artocarpus rigida		
Artobiloxanthone [**2**]	Stem bark	Hano *et al*., 1990b
Artocarpol B [**223**]	Root bark	Ko, Lin, and Yang, 2000
Artocarpol H [**224**]	Root bark	Lu *et al*., 2002
Artonin E [**12**]	Stem bark	Hano *et al*., 1990b
Artonin G [**225**]	Stem bark	Hano *et al*., 1990b
Artonin H [**226**]	Stem bark	Hano *et al*., 1990b

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
Artonin M [**227**]	Stem bark	Hano *et al*., 1990b
Artonin N [**228**]	Stem bark	Hano *et al*., 1990b
Artonin O [**170**]	Stem bark	Hano *et al*., 1990b
Artonin P [**229**]	Stem bark	Hano *et al*., 1990b
Cycloartobiloxanthone [**38**]	Stem bark	Hano *et al*., 1990b
Rubraflavone C [**230**]	Root bark	Lu *et al*., 2002
Artocarpus rotundo		
Artoindonesianin L [**231**]	Root bark	Suhartati *et al*., 2001
Artonin E [**12**]	Root bark	Suhartati *et al*., 2001
Artonin M [**226**]	Root bark	Suhartati *et al*., 2001
Artonin O [**170**]	Root bark	Suhartati *et al*., 2001
Cycloartobiloxanthone [**38**]	Root bark	Suhartati *et al*., 2001
Artocarpus scotehinii		
Artobiloxanthone [**2**] (artocarpus flavone KB-1)	Stem bark	Hakim *et al*., 2006
Artonin E [**12**] (artocarpus flavone KB-3)	Stem bark	Hakim *et al*., 2006
Cycloartobiloxanthone [**38**]	Stem bark	Hakim *et al*., 2006
Norartocarpetin [**65**]	Stem bark	Hakim *et al*., 2006
5′-Hydroxycudraflavone A [**99**]	Stem bark	Hakim *et al*., 2006
Artocarpus sepicanus		
Sepicanin A [**232**]	Leaves	Radwan *et al*., 2009
Artocarpus styracifolius		
Styracifolin A [**233**]	Stem bark	Bourjot *et al*., 2010

Table 1 (continued)

Plant and chemical compound	Plant part	Reference
Styracifolin B [**234**]	Stem bark	Bourjot *et al*., 2010
Artocarpus teysmanii		
Artoindonesianin C [**235**]	Root bark	Makmur *et al*., 2000
Artonin J [**148**]	Root bark	Makmur *et al*., 2000
Cycloartobiloxanthone [**38**]	Root bark	Makmur *et al*., 2000
Artocarpus tonkinensis		
Alphitonin-4-*O*-glucoside [**236**]	Leaves	Thuy *et al*., 2004
Artokin-4′-*O*-glucoside [**237**]	Leaves	Dang *et al*., 2009
Artotonin A [**238**]	Root	Ma *et al*., 2010
Artotonin B [**239**]	Root	Ma *et al*., 2010
Maesopsin-4-*O*-glucoside [**240**]	Leaves	Thuy *et al*., 2004
Artocarpus venenosa		
Paratocarpin A [**241**]	Stem bark	Nomura *et al*., 1998
Paratocarpin B [**242**]	Stem bark	Nomura *et al*., 1998
Paratocarpin C [**243**]	Stem bark	Nomura *et al*., 1998
Paratocarpin D [**244**]	Stem bark	Nomura *et al*., 1998
Paratocarpin E [**245**]	Stem bark	Nomura *et al*., 1998
Paratocarpin F [**246**]	Stem bark	Nomura *et al*., 1998
Paratocarpin G [**247**]	Stem bark	Nomura *et al*., 1998
Paratocarpin H [**248**]	Stem bark	Nomura *et al*., 1998
Paratocarpin I [**249**]	Stem bark	Nomura *et al*., 1998
Paratocarpin J [**250**]	Stem bark	Nomura *et al*., 1998
Paratocarpin K [**251**]	Stem bark	Nomura *et al*., 1998
Paratocarpin L [**252**]	Stem bark	Nomura *et al*., 1998

Table 2 Distribution of triterpenoids and steroids in the genus *Artocarpus*

Plant and chemical compound	Category	Plant part	Reference
Artocarpus altilis			
α-Amyrin [**253**]	Triterpene	Latex	Ultee, 1949
α-Amyrin acetate [**254**]	Triterpene	Fruit	Altman and Zito, 1976
β-Amyrin acetate [**255**]	Triterpene	Latex	Ultee, 1949
Cycloart-23-ene-3β,25-diol [**256**]	Triterpene	Fruit	Altman and Zito, 1976
Cycloart-24-ene-3β-ol [**257**] (Cycloartenol)	Triterpene	Fruit Stem bark	Altman and Zito, 1976 Pavanasasivum and Sultanbawa, 1973
Cycloart-25-ene-3β,24-diol [**258**]	Triterpene	Fruit	Altman and Zito, 1976
Cycloartenone [**259**]	Triterpene	Stem bark	Pavanasasivum and Sultanbawa, 1973
Cycloartenyl acetate [**260**]	Triterpene	Stem bark	Pavanasasivum and Sultanbawa, 1973
Lupeol acetate [**261**]	Triterpene	Root bark	Shieh and Lin, 1992
β-Sitosterol [**262**]	Steroid	Root bark	Shieh and Lin., 1992
Artocarpus champeden			
Cycloartenone [**259**]	Triterpene	Stem bark	Achmad *et al*., 1996
Cycloeucalenol [**263**]	Triterpene	Stem bark	Achmad *et al*., 1996
Glutinol [**264**]	Triterpene	Stem bark	Achmad *et al*., 1996
24-Methylenecycloartenone [**265**]	Triterpene	Stem bark	Achmad *et al*., 1996
Artocarpus chaplasha			
Cycloartenyl acetate [**260**]	Triterpene	Stem bark	Mahato, Banerjee and Chakravarti, 1971

Table 2 (continued)

Plant and chemical compound	Category	Plant part	Reference
Isocycloartenol acetate [**266**]	Triterpene	Stem bark	Mahato *et al*., 1971
Lupeol acetate [**261**]	Triterpene	Stem bark	Mahato *et al*., 1971
β-Sitosterol [**262**]	Steroid	Root bark	Shieh and Lin., 1992
Artocarpus elasticus			
β-Amyrin acetate [**254**]	Triterpene	Latex	Ultee, 1949
Lupeol acetate [**261**]	Triterpene	Latex	Ultee, 1949
β-Sitosterol [**262**]	Steroid	Root bark	Shieh and Lin., 1992
Artocarpus gomezianus			
Lupeol acetate [**261**]	Triterpene	Leaves	Kingroungpet, 1994
Simiarenol [**267**]	Triterpene	Leaves	Kingroungpet, 1994
β-Sitosterol [**262**]	Steroid	Root bark	Shieh and Lin., 1992
Stigmasterol [**268**]	Steroid	Leaves	Kingroungpet, 1994
Artocarpus heterophyllus			
Betulin [**269**]	Triterpene	Root bark	Lu and Lin, 1994
Betulinic acid [**270**]	Triterpene	Root Root bark	Dayal and Seshadri, 1974 Lu and Lin, 1994
Butyrospermol [**271**]	Triterpene	Fruit	Barton, 1951
Cycloartenone [**257**]	Triterpene	Stem Bark	Pavanasasivam and Sultanbawa, 1973
Cycloartenyl acetate [**260**]	Triterpene	Stem Bark	Pavanasasivam and Sultanbawa, 1973
9,19-Cyclolanost-23-ene-3*β*,25-diol (Cycloart-23-ene-3*β*,25-diol) [**256**]	Triterpene	Fruit	Kielland and Malterud, 1994
Cycloartenone [**257**]	Triterpene	Stem Bark	Pavanasasivam and Sultanbawa, 1973

Table 2 (continued)

Plant and chemical compound	Category	Plant part	Reference
9,19-Cyclolanost-25-ene-3β,24-diol [**258**]	Triterpene	Fruit	Kielland and Malterud, 1994
9,19-Cyclolanost-3-one-24,25-diol [**272**]	Triterpene	Fruit	Kielland and Malterud, 1994
Ursolic acid [**273**]	Triterpene	Root	Dayal and Seshadri, 1974
		Root bark	Lu and Lin, 1994
β-Sitosterol [**262**]	Steroid	Root bark	Shieh and Lin., 1992
Artocarpus integer			
Cycloartenone [**259**]	Triterpene	Latex	Pant and Chaturvedi, 1989
β-Sitosterol [**262**]	Steroid	Root bark	Shieh and Lin., 1992
Artocarpus lakoocha			
β-Amyrin acetate [**254**]	Triterpene	Stem Bark	Kapil and Joshi, 1960
Cycloartenol [**257**] (Cycloart-24-ene-3β-ol)	Triterpene	Stem Bark	Pavanasasivam and Sultanbawa, 1973
Cycloartenone [**259**]	Triterpene	Stem Bark	Pavanasasivam and Sultanbawa, 1973
Cycloartenyl acetate [**260**]	Triterpene	Stem Bark	Pavanasasivam and Sultanbawa, 1973
Lupeol [**274**]	Triterpene	Root Bark	Chauhan and Kumari, 1979
β-Sitosterol [**262**]	Steroid	Root bark	Shieh and Lin., 1992
Artocarpus nobilis			
Cycloartenol [**257**] (Cycloart-24-ene-3β-ol)	Triterpene	Stem Bark Heartwood	Pavanasasivam and Sultanbawa, 1973
Cycloartenone [**259**]	Triterpene	Stem Bark Heartwood	Pavanasasivam and Sultanbawa, 1973
Cycloartenyl acetate [**260**]	Triterpene	Stem Bark Heartwood	Pavanasasivam and Sultanbawa, 1973

Table 3 Distribution of stilbenoids in the genus *Artocarpus*

Plant and chemical compound	Plant part	Reference
Artocarpus altilis		
Artocarbene [**275**]	Heartwood	Shimizu *et al*., 1997
4-Prenyloxyresveratrol [**276**]	Heartwood	Shimizu *et al*., 1997
Artocarpus chaplasha		
Artochamin H [**277**]	Stem	Wang *et al*., 2006
Artochamin I [**278**]	Stem	Wang *et al*., 2006
Artochamin J [**279**]	Stem	Wang *et al*., 2006
Artochamin K [**280**]	Stem	Wang *et al*., 2006
Artochamin F [**281**]	Stem	Wang *et al*., 2006
Artochamin G [**282**]	Stem	Wang *et al*., 2006
Artostilbene A [**283**]	Stem	Wang *et al*., 2007a
Artostilbene B [**284**]	Stem	Wang *et al*., 2007a
Oxyresveratrol [**285**]	Heartwood	Rao *et al*., 1972
Resveratrol [**286**]	Heartwood	Rao *et al*., 1972
Artocarpus dadah		
3-(2,3-Dihydroxy-3-methylbutyl)-resveratrol [**287**]	Stem bark	Su *et al*., 2002
3-(*γ*,*γ*-Dimethylallyl)- oxyresveratrol [**288**]	Stem bark	Su *et al*., 2002
3-(*γ*,*γ*-Dimethylallyl)-resveratrol [**289**]	Stem bark	Su *et al*., 2002
3-(*γ*,*γ*-Dimethylpropenyl) moracin M [**290**]	Stem bark	Su *et al*., 2002
Moracin M [**291**]	Twig	Su *et al*., 2002
Oxyresveratrol [**285**]	Stem bark Twig	Su *et al*., 2002
Resveratrol [**286**]	Twig	Su *et al*., 2002

Table 3 (continued)

Plant and chemical compound	Plant part	Reference
Artocarpus fretessi		
Artoindonesianin X [**292**]	Root bark	Soekamto *et al*., 2003
Artoindonesianin Y [**293**]	Root bark	Soekamto *et al*., 2003
Artocarpus gomezianus		
Andalasin [**294**]	Root	Likhitwitayawuid *et al*., 2001
Artogomezianol [**295**]	Root	Likhitwitayawuid *et al*., 2001
Artoindonesianin N [**296**]	Bark	Hakim *et al*., 2002b
Artoindonesianin O [**297**]	Bark	Hakim *et al*., 2002b
Oxyresveratrol [**285]**	Heartwood	Likhitwitayawuid *et al*., 2006
Resveratrol [**286**]	Root	Likhitwitayawuid *et al*., 2000
Artocarpus heterophyllus		
Artoheterophyllin A [**298**]	Twig	Zheng *et al*., 2009
Artocarpus integer		
Artocarbene [**275**]	Aerial part	Boonlaksiri *et al*., 2000
4-Methoxy-2,2-dimethyl-6-(2-(2,4-dihydroxy)phenyl-trans-ethenyl) chromene [**299**]	Aerial part	Boonlaksiri *et al*., 2000
4-Prenyloxyresveratrol [**276**] (*trans*-4-Isopentenyl-3,5,2′,4′ tetrahydroxy stilbene)	Aerial part	Boonlaksiri *et al*., 2000
trans-4-(3-Methyl-*E*-but-1-enyl)-3,5,2′,4′-tetrahydroxystilbene [**300**] (Artoindonesianin F)	Aerial part	Boonlaksiri *et al*., 2000
Artocarpus lakoocha		
Artolakoochol [**301**]	Root bark	Sritularak *et al*., 2010
cycloartolakoochol [**302**]	Root bark	Sritularak *et al*, 2010

Table 3 (continued)

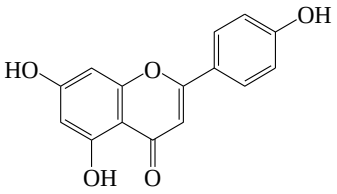
Plant and chemical compound	Plant part	Reference
4-Hydroxyartolakoochol [**303**]	Root bark	Sritularak *et al*, 2010
Lakoochin A [**304**]	Root	Puntumchai *et al*., 2004
Lakoochin B [**305**]	Root	Puntumchai *et al*., 2004
Oxyresveratrol [**285]**	Heartwood	Venkataraman, 1972; Likhitwitayawuid *et al*., 2001
Resveratrol [**286**]	Heartwood	Venkataraman, 1972
Artocarpus nitidus		
Artonitidin A [**306**]	Stem	Zhao *et al*., 2009
Artonitidin B [**307**]	Stem	Zhao *et al*., 2009
Artocarpus nobilis		
trans-4-(3-Methyl-E-but-1-enyl)-3,5,2′,4′-tetrahydroxystilbene [**300**] (Artoindonesianin F)	Stem bark	Jayasinghe *et al*., 2004b
trans-4-Isopentenyl-3,5,2′,4′-tetra hydroxy stilbene [**276**] (4-prenyloxyresveratrol)	Stem bark	Jayasinghe *et al*., 2004b
Artocarpus petelotii		
Artopetelin L [**308**]	Root bark	Shen and Hou, 2008
Artopetelin M [**309**]	Root bark	Shen and Hou, 2008
Artocarpus rigida		
Artocarpol A [**310**]	Root bark	Ko, Lin and Yang, 2001
Artocarpol C [**311**]	Root bark	Ko *et al*., 2001
Artocarpol D [**312**]	Root bark	Ko *et al*., 2001
Artocarpol E [**313**]	Root bark	Ko *et al*., 2001
Artocarpol F [**314**]	Root bark	Ko *et al*., 2001
Artocarpus tonkinensis		
Artotonkin [**315**]	Stem bark	Lien *et al*., 1998

Table 4 Distribution of miscellaneous constituents in the genus *Artocarpus*

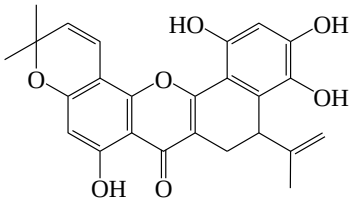
Plant and chemical compound	Category	Plant part	Reference
Artocarpus altilis			
γ-Aminobutyric acid [**316**]	Amino acid	Leaves	Durand *et al*., 1962
Artocarpus chaplasha			
Resorcinol [**317**]	Benzenoid	Heartwood	Rao *et al*., 1972
β-Resorcylaldehyde [**318**]	Benzenoid	Heartwood	Rao *et al*., 1972
Artocarpus dadah			
Dadahol A [**319**]	Neolignan	Twig	Su *et al*., 2002
Dadahol B [**320**]	Neolignan	Twig	Su *et al*., 2002
Artocarpus gomezianus			
Arbutin [**321**]	Phenolic glycoside	Leaves	Kingroungpet, 1994
1-Dotriacontanol [**322**]	Alcohol	Leaves	Kingroungpet, 1994
Phenyl-*β*-naphthylamine [**323**]	Naphthalene	Root	Likhitwitayawuid and Sritularak, 2001
Resorcinol [**317**]	Benzenoid	Root	Sritularak, 1998
Artocarpus heterophyllus			
Acetylcholine [**324**]	Amine	Seed	Pereira *et al*., 1962
Artocarpus integra *α* -D-Galactose specific lectin [**325**]	Lectin	Seed	Suresh, Appukuttan and Basu, 1982
Artocarpus integrifolia lectin [**326**]	Lectin	Seed	Chatterjee *et al*., 1982; Namjuntra and Culavatnatol, 1984
Artocarpus lectin CE-A-I [**327**]	Lectin	Seed	Ferreira *et al*., 1992
Aurantiamide acetate [**328**]	Protein	Seed	Chakraborty and Mandal, 1981
9-Hydroxytridecyl docosanoate [**329**]	Fatty acid	Latex	Pant and Chaturvedi, 1989

Table 4 (continued)

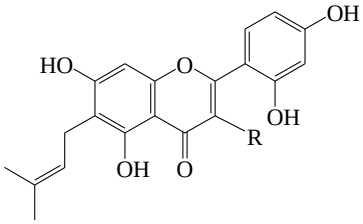
Plant and chemical compound	Category	Plant part	Reference
4-Hydroxyundecyl docosanoate [**330**]	Fatty acid	Latex	Pant and Chaturvedi, 1989
Jacalin [**331**]	Lectin	Seed	Hagiwara *etal*., 1988 Ferreira *et al*., 1992
Lymphoagglutinin [**332**]	Lectin	Seed	Arora *et al*., 1987
Recinoleic acid [**333**]	Fatty acid	Seed oil	Daulatabad and Mirajkar, 1989
Artocarpus hirsuta			
Lymphoagglutinin [**332**]	Lectin	Seed	Arora *et al*., 1987
Artocarpus integer			
Artocarpus lectin C [**334**]	Lectin	Seed	Hashim, Gendeh and Jaafar, 1992
4-Hydroxyundecyl docosanoate [**330**]	Fatty acid	Latex	Pant and Chaturvedi, 1989
Artocarpus lakoocha			
ALA-I [**335**]	Isolectin	Seed	Wongkham *et al*., 1995
ALA-II [**336**]	Isolectin	Seed	Wongkham *et al*., 1995
ALA-III [**337**]	Isolectin	Seed	Promdee, 1996
Artocarpus lakoocha lectin [**338**]	Lectin	Seed	Chatterjee *et al*., 1982
Lymphoagglutinin [**332**]	Lectin	Seed	Arora *et al*., 1987
Resorcinol [**317**]	Benzenoid	Heartwood	Venkataraman, 1972
Artocarpus lignanensis			
Artocarpus lectin [**339**]	Lectin	Seed	Zhang *et al*., 1999
Artocarpus masticatus			
Artocarpus lectin AM [**340**]	Lectin	Seed	Blasco *et al*., 1996
Artocarpus melinoxylus			
Artocarpus lectin AME [**341**]	Lectin	Seed	Blasco *et al*., 1996



[**1**] Apigenin

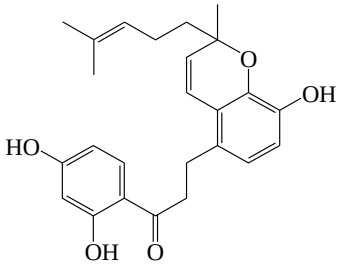


[**2**] Artobiloxanthone
(artocarpus flavone KB-1)

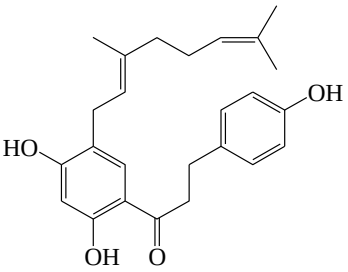


[**3**] Artocarpesin R=H

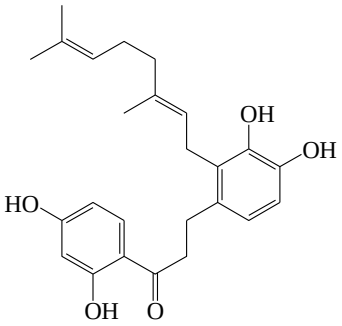
[**4**] Artocarpin R=



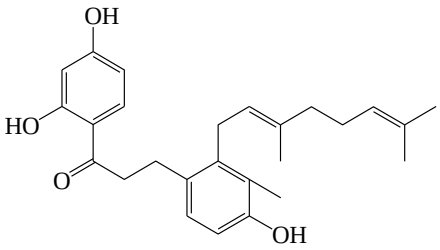
[**5**] Artocarpus chalcone AC-3-1



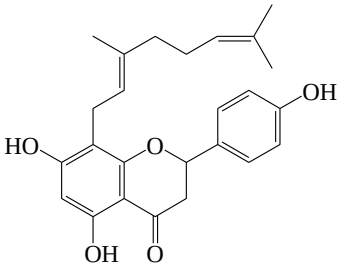
[**6**] Artocarpus chalcone AC-3-3



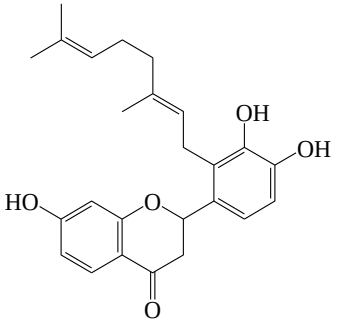
[**7**] Artocarpus chalcone AC-5-1



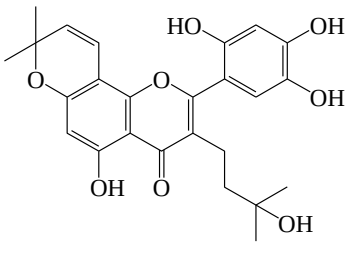
[**8**] Artocarpus chalcone I



[**9**] Artocarpus flavanone AC-3-2

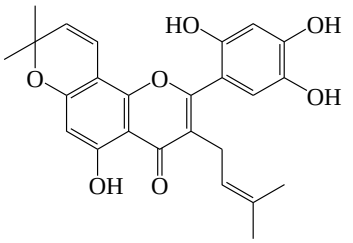


[**10**] Artocarpus flavanone AC-5-2

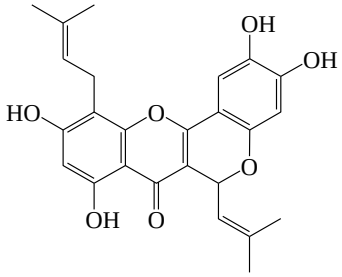


[**11**] Artocarpus flavone KB-2

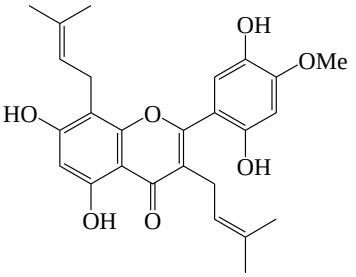
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp.



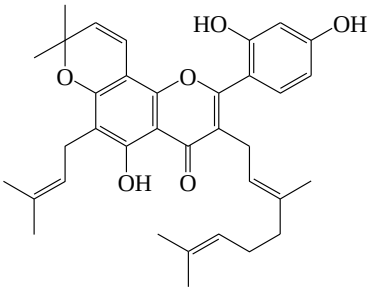
[**12**] Artocarpus flavone KB-3 (Artonin E)



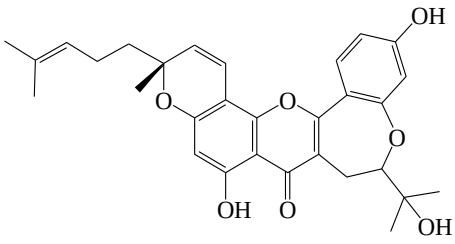
[**13**] Artochamin B



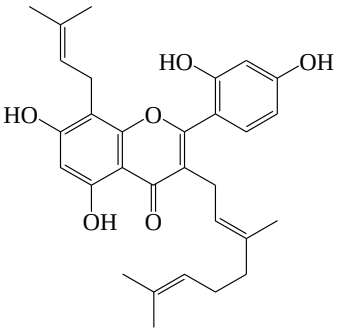
[**14**] Artochamin D



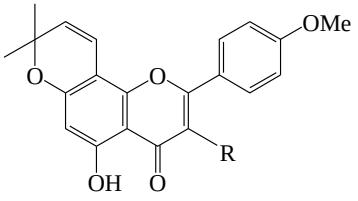
[**15**] Artocommunol CB

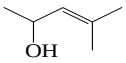


[**16**] Artocommunol CC

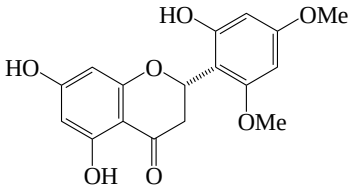


[**17**] Artocommunol CD

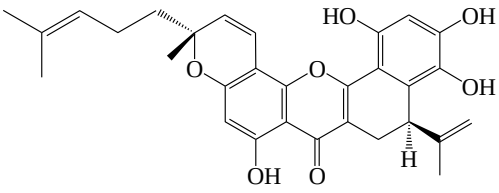


[**18**] Artocommunol CE R= 

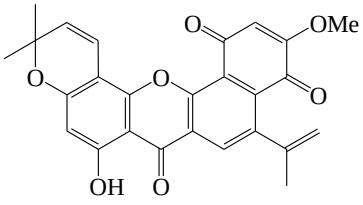
[**19**] Artoflavone A R= 



[**20**] Artomunoflavanone

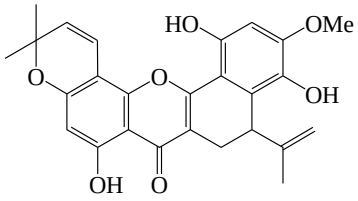


[**21**] Artomunoisoxanthone

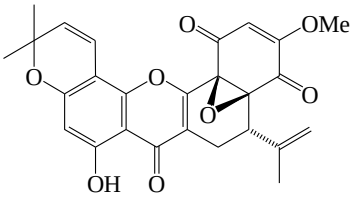


[**22**] Artomunoxanthentrione

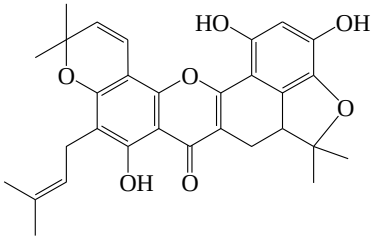
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



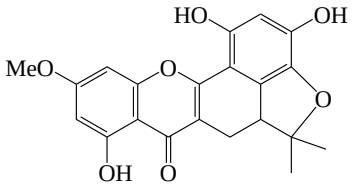
[**23**] Artomunoxanthone



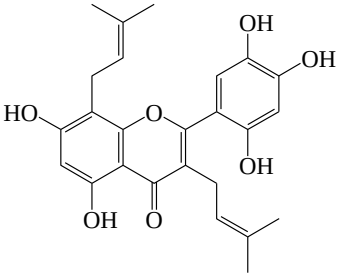
[**24**] Artomunoxanthotrione epoxide



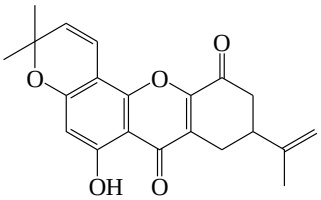
[**25**] Artonin F



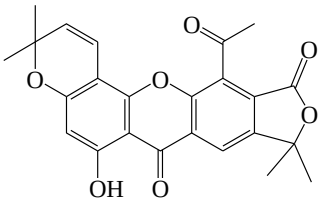
[**26**] Artonin K



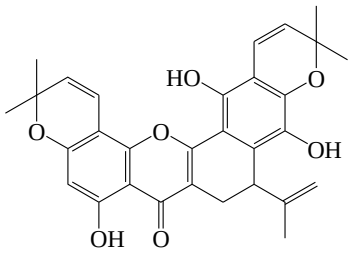
[**27**] Artonin V



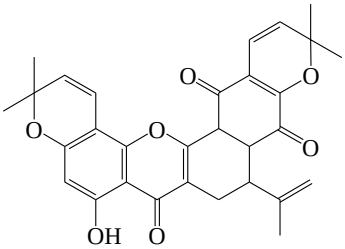
[**28**] Artonol A



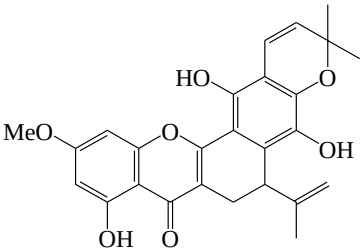
[**29**] Artonol B



[**30**] Artonol C

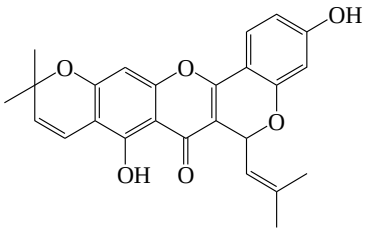


[**31**] Artonol D

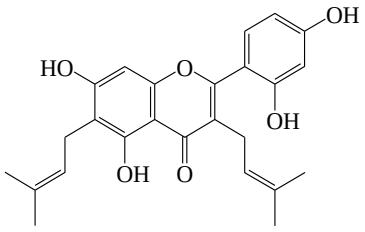


[**32**] Artonol E

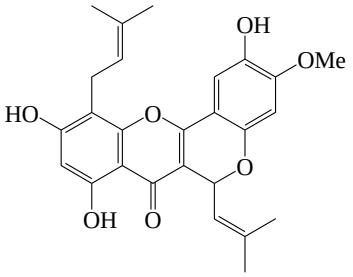
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



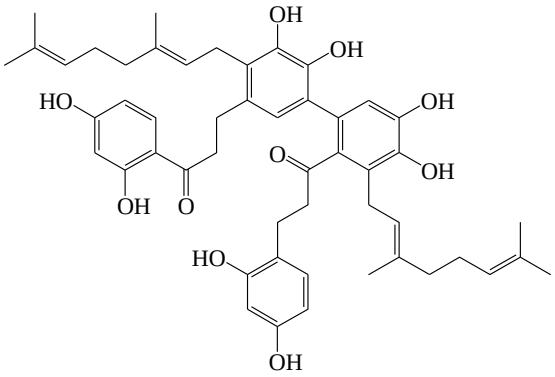
[**33**] Cudraflavone A
(Isocyclomorusin)



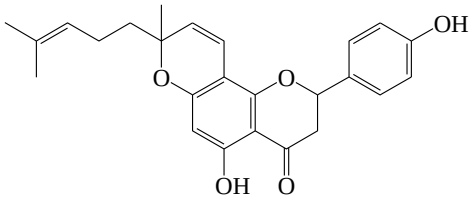
[**34**] Cudraflavone C



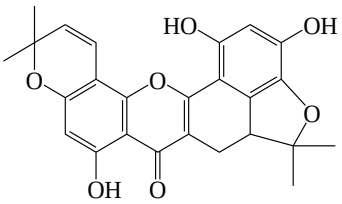
[**35**] Cycloaltilisin



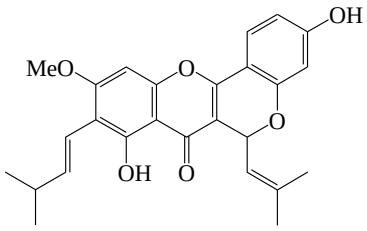
[**36**] Cycloaltilisin 6



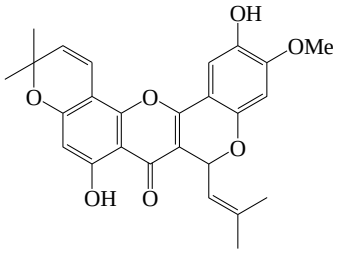
[**37**] Cycloaltilisin 7



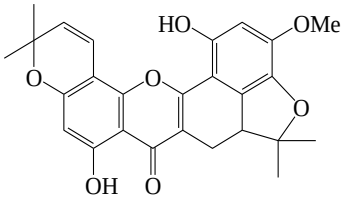
[**38**] Cycloartobiloxanthone



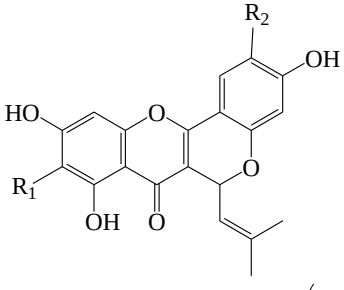
[**39**] Cycloartocarpin



[**40**] Cycloartomunin

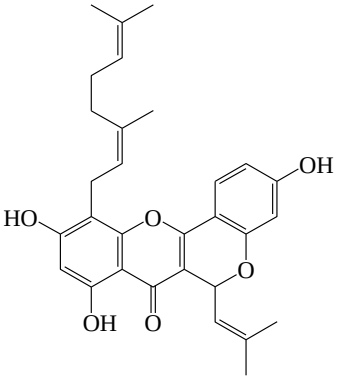


[**41**] Cycloartomunoxanthone

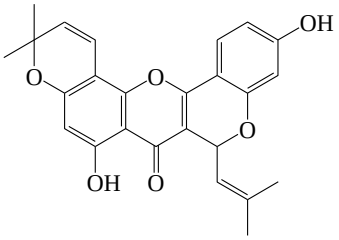


[**42**] Cyclocommunin R_1 =  R_2= H
[**43**] Cyclocommunol $R_1 = R_2 = H$
[**44**] Cyclocommunomethonol R_1=H R_2=OMe

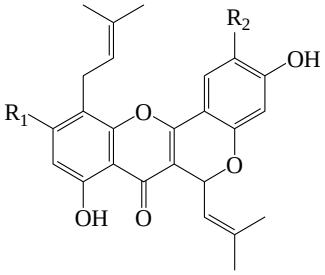
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



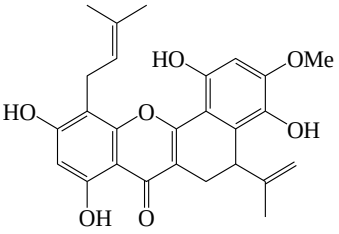
[**45**] Cyclogeracommunin



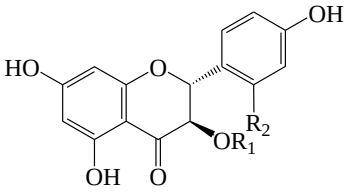
[**46**] Cyclomorusin



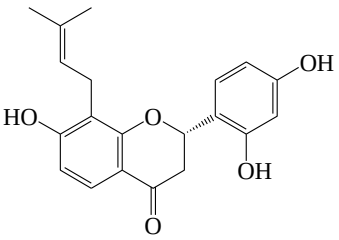
[**47**] Cyclomulberrin R_1=OH R_2=H
[**49**] Dihydrocycloartomunin
R_1=OMe R_2=OH
[**50**] Dihydroisocycloartomunin
R_1=OH R_2=OMe



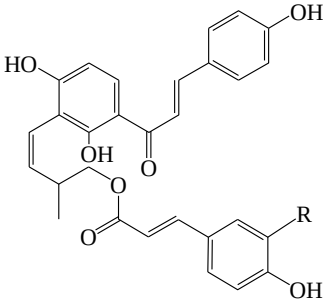
[**48**] Dihydroartomunoxanthone



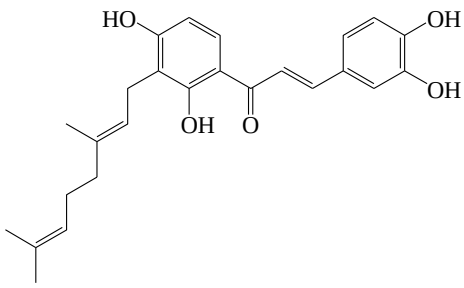
[**51**] Dihydromorin R_1=H R_2=OH
[**52**] Engeletin R_1=rhamnose R_2=H



[**53**] (2*S*)-Euchrenone a7

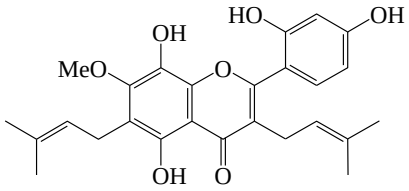


[**54**] Gemichalcone B R=H
[**55**] Gemichalcone C R=OMe

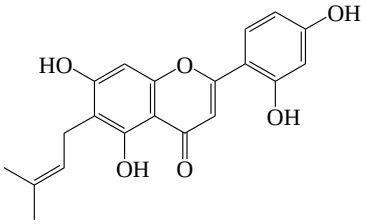


[**56**] 3′-Geranyl-2′,3,4,4′- tetrahydroxy-chalcone

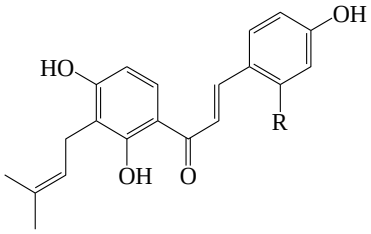
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



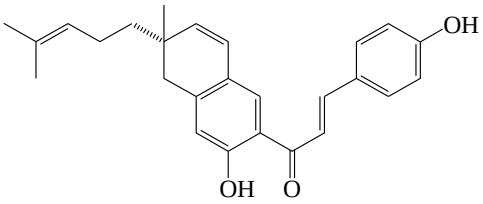
[**57**] Hydroxyartocarpin



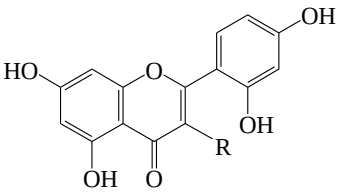
[**58**] Isoartocarpesin



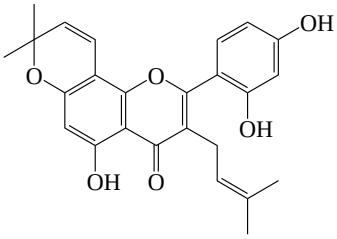
[**59**] Isobacachalcone R=H
[**62**] Morachalcone A R=OH



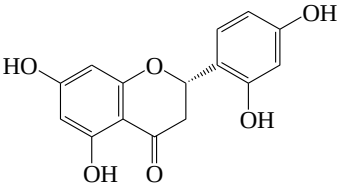
[**60**] Isolespeol



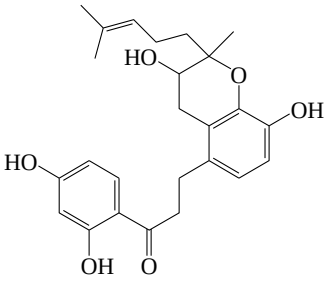
[**61**] Morin R=OH
[**65**] Norartocarpetin R=H



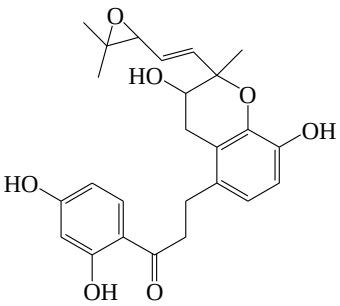
[**63**] Morusin



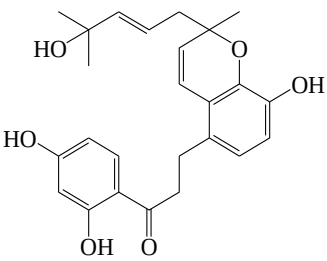
[**64**] (+)-Norartocarpanone



[**66**]1-(2,4-Dihydroxyphenyl)-3-[3,4-dihydro -3,8-dihydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone

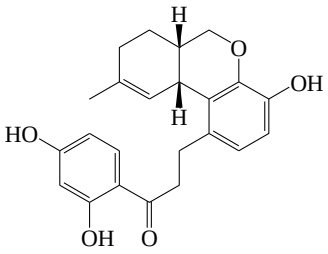


[**67**] 1-(2,4-Dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(3,4-epoxy-4-methyl-1-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone

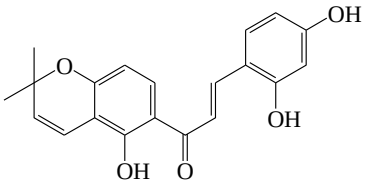


[**68**] 1-(2,4-Dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-hydroxy-4-methyl-2-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone

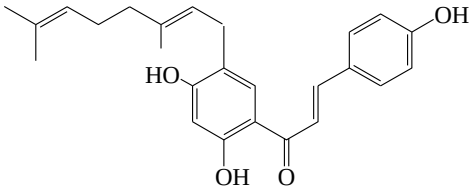
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



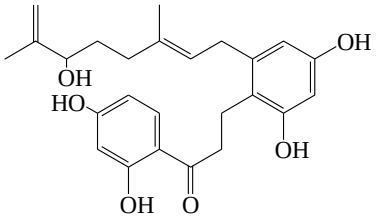
[**69**] 1-(2,4-Dihydroxyphenyl)-3-{4-hydroxy-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6H-dibenzo [b,d]pyran-5-yl}-1-propanone



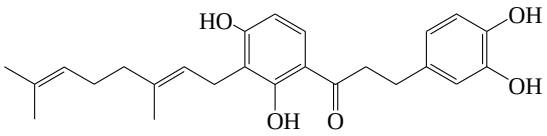
[**70**] 3″,3″-Dimethylpyrano[3′,4′]2,4,2′-trihydroxychalcone



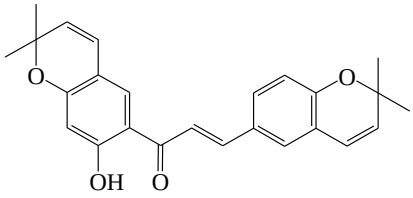
[**71**] 5′-Geranyl-2′,4′,4-trihydroxychalcone



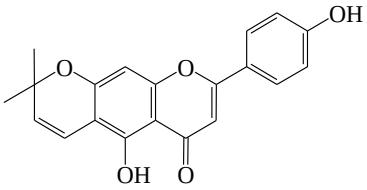
[**72**] 2-[6-Hydroxy-3,7-dimethylocta-2(E),7-dienyl]-2′,3,4,4′ tetrahydroxy dihydrochalcone



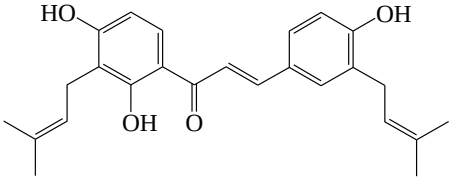
[**73**] 3,4,2′,4′-Tetrahydroxy-3′-geranyl dihydrochalcone



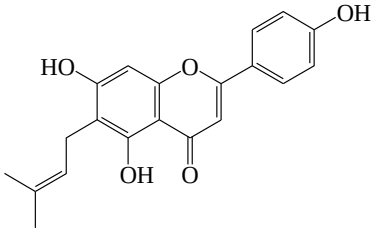
[**74**] Artoindonesianin J



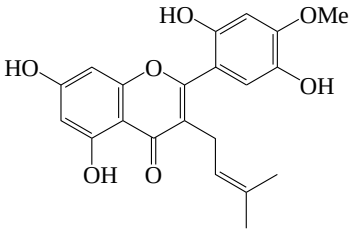
[**75**] Carpachromene



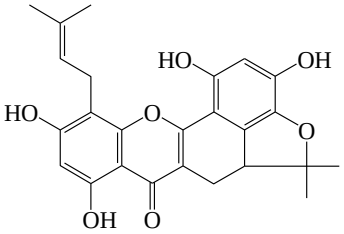
[**76**] Kozonol C



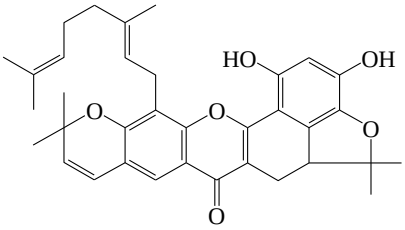
[**77**] 6-Prenylalpigenin



[**78**] Artocarpone A

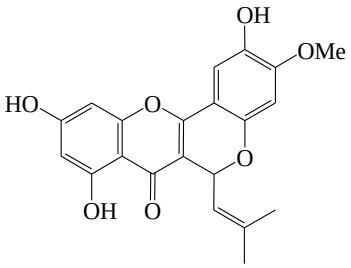


[**79**] Artocarpone B

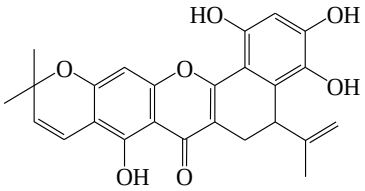


[**80**] Artoindonesianin A

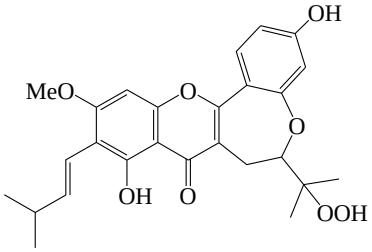
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



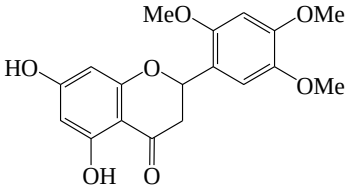
[**81**] Artoindonesianin A-2



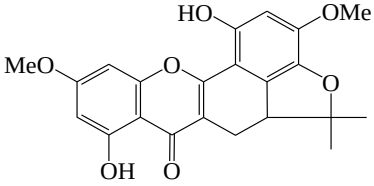
[**82**] Artoindonesianin A-3



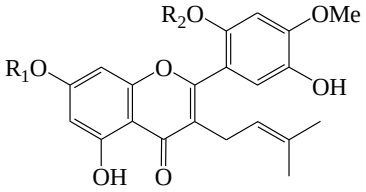
[**83**] Artoindonesianin B



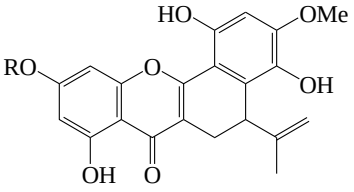
[**84**] Artoindonesianin E



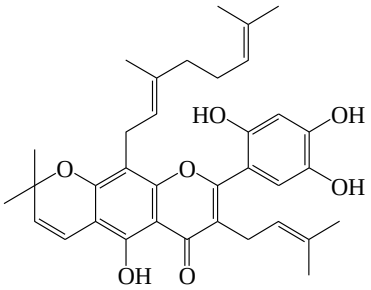
[**85**] Artoindonesianin M



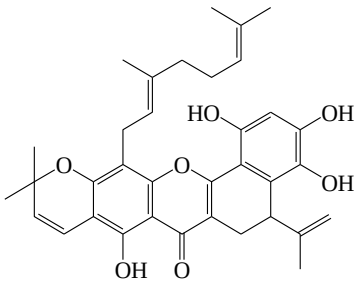
[**86**] Artoindonesianin Q R_1=Me R_2=H
[**87**] Artoindonesianin R R_1=H R_2=Me



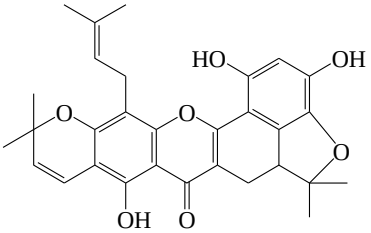
[**88**] Artoindonesianin S R=Me
[**89**] Artoindonesianin T R=H



[**90**] Artoindonesianin U

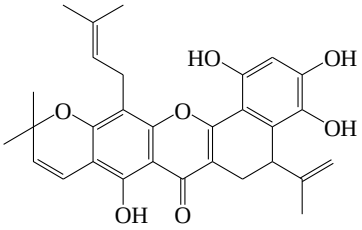


[**91**] Artoindonesianin V

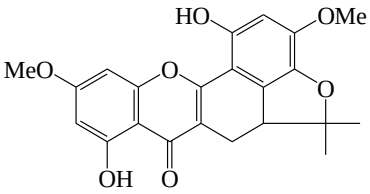


[**92**] Artonin A

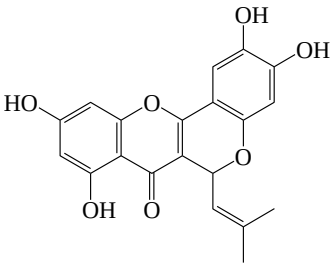
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



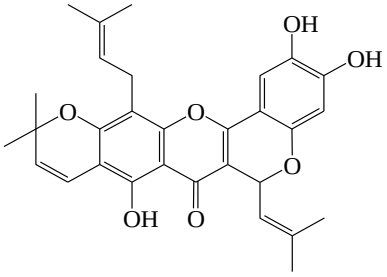
[**93**] Artonin B



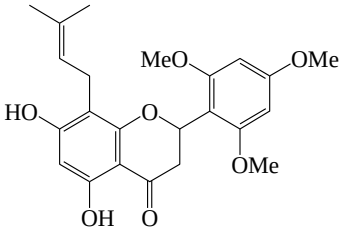
[**94**] Artopeden A



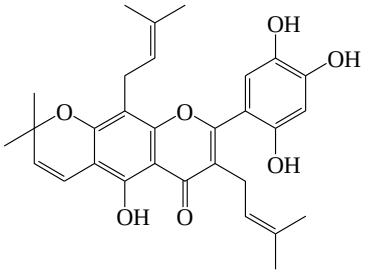
[**95**] Cyclochampedol



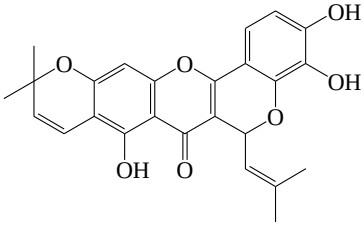
[**96**] Cycloheterophyllin



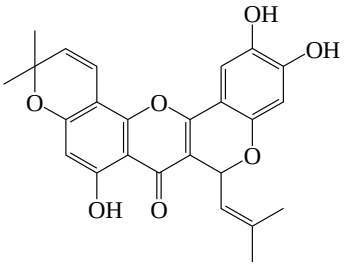
[**97**] Heteroflavanone C



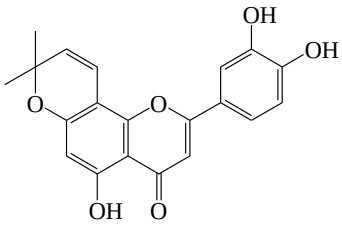
[**98**] Heterophyllin



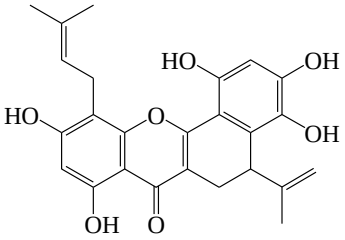
[**99**] 5′-Hydroxycudraflavone A



[**100**] Artochamin A

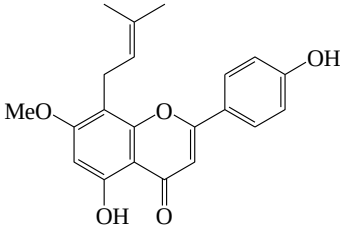


[**101**] Artochamin C

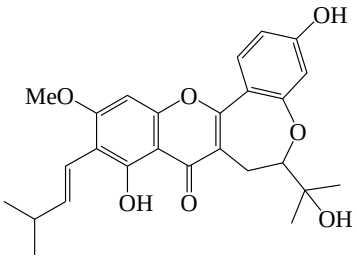


[**102**] Artochamin E

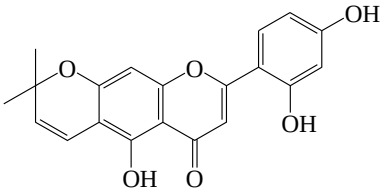
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



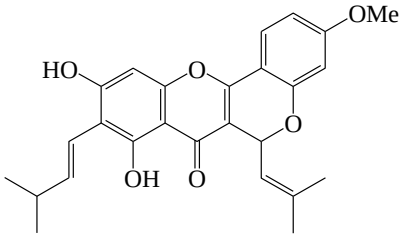
[**103**] Artonin U



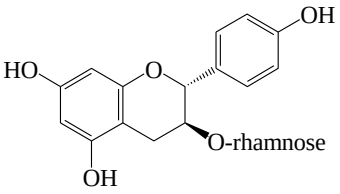
[**104**] Chaplashin



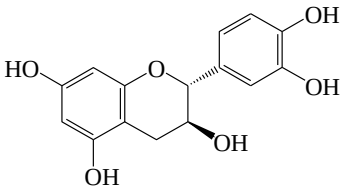
[**105**] Cycloartocarpesin



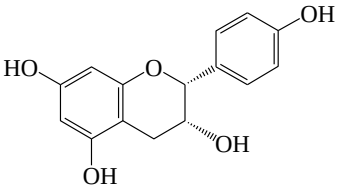
[**106**] Cycloartocarpin A



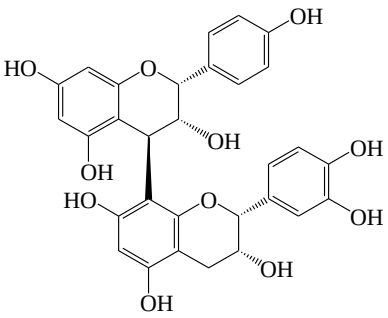
[**107**] Afzelechin-3-O-α-L-rhamnoside



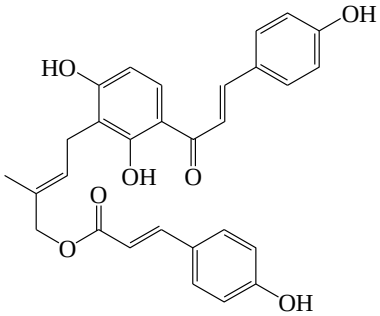
[**108**] (+)-Catechin



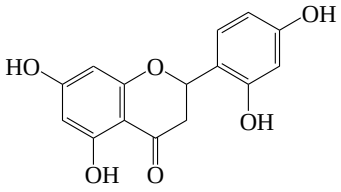
[**109**] (+)-Epiafzelechin



[**110**] (–)-Epiafzelechin-(4β→8)-epicatechin

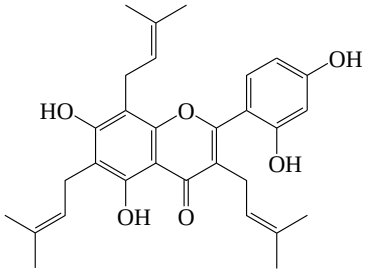


[**111**] Isogemichalcone B

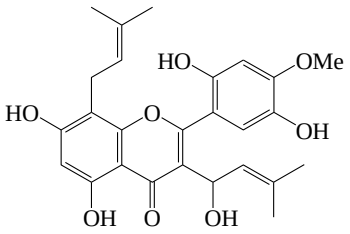


[**112**] Steppogenin

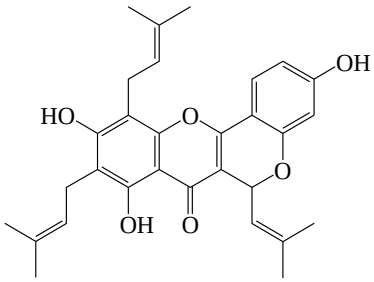
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



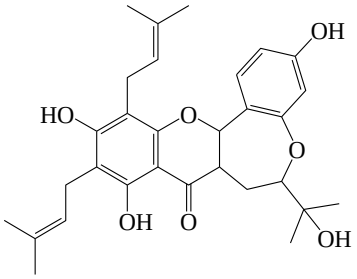
[**113**] Artelasticin



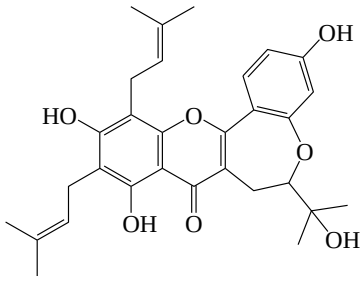
[**114**] Artelasticinol



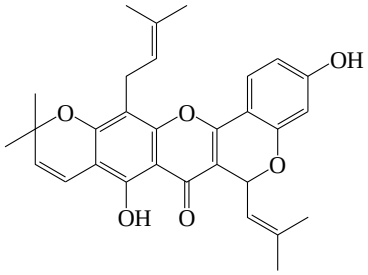
[**115**] Artelastin



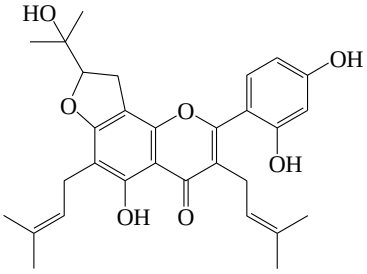
[**116**] Artelastinin



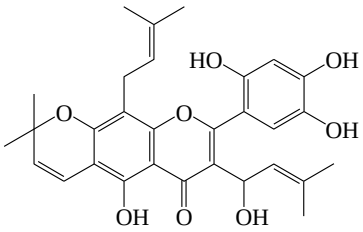
[**117**] Artelastocarpin



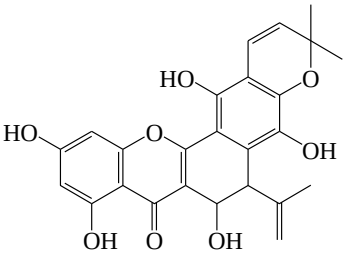
[**118**] Artelastochromene



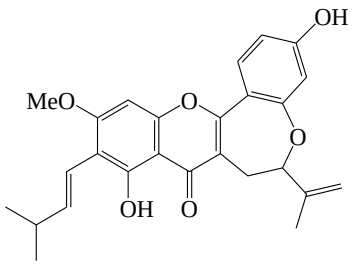
[**119**] Artelastofuran



[**120**] Artelastoheterol

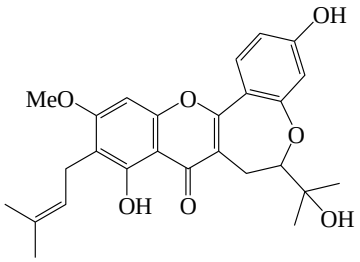


[**121**] Artelastoxanthone

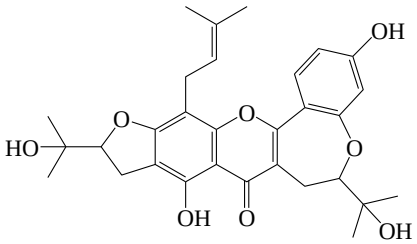


[**122**] Artoindonesianin E1

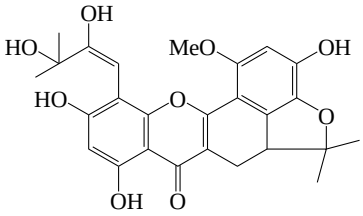
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



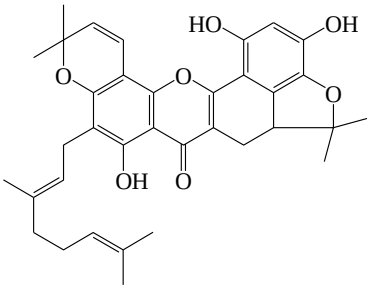
[**123**] Artonin S



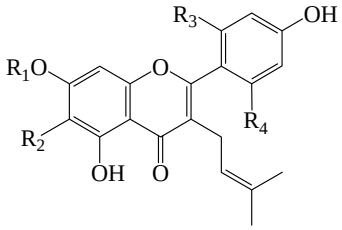
[**124**] Carpelastofuran



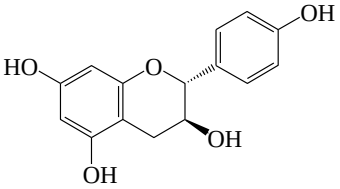
[**125**] Cycloartelastoxanthendiol



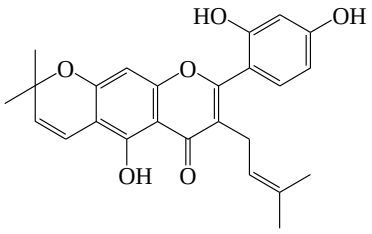
[**126**] Cycloartelastoxanthone



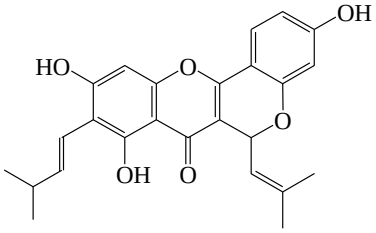
	R_1	R_2	R_3	R_4
[**127**] Integrin	Me	H	H	OH
[**128**] Norartocarpin	H		H	OH
[**130**] Mulberrin	H		OH	H
[**132**] Albanin A	H	H	H	OH



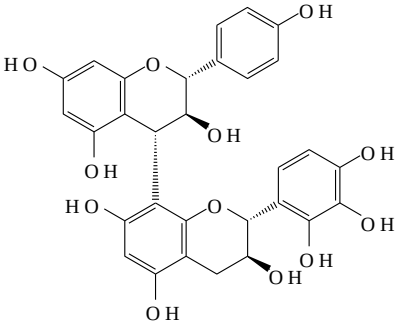
[**129**] Afzelechin



[**131**] Mulberrochromene

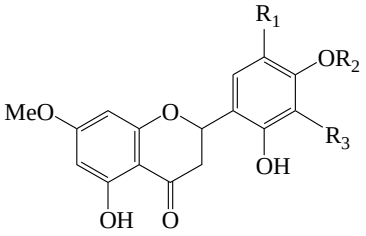


[**133**] Norcycloartocarpin

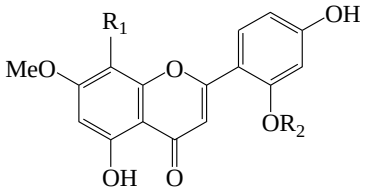


[**134**] Afzelechin-(4α→8)-catechin

Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



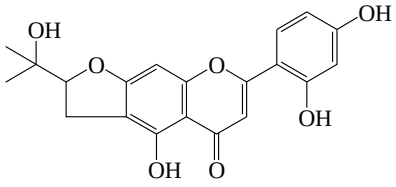
[**135**] Artocarpanone $R_1=R_2=R_3=H$
[**136**] Artocarpanone A $R_1=R_3=H$ $R_2=Me$
[**141**] Artoflavanone $R_1=R_3=OMe$ $R_2=Me$



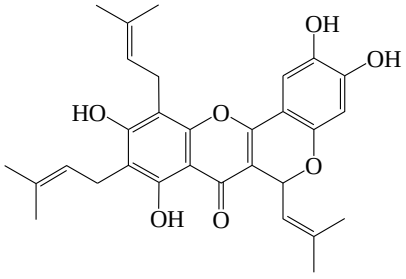
[**137**] Artocarpetin $R_1=H$ $R_2=H$

[**138**] Artocarpetin A $R_1=$  $R_2=H$

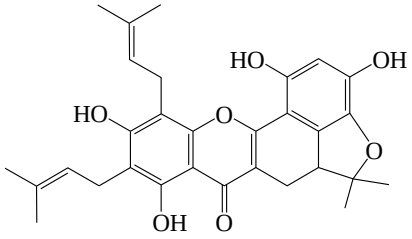
[**139**] Artocarpetin B $R_1=$  $R_2=Me$



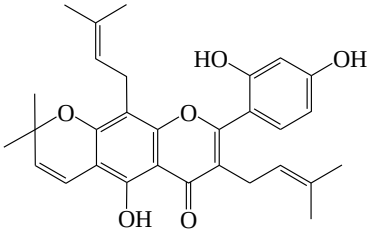
[**140**] Artocarpfuranol



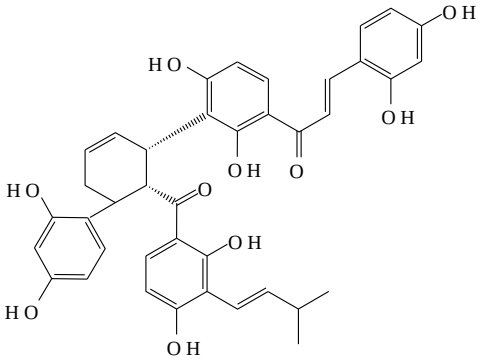
[**142**] Artoheterophyllin B



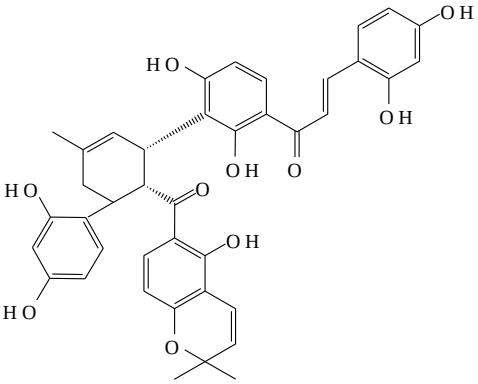
[**143**] Artoheterophyllin C



[**144**] Artoheterophyllin D

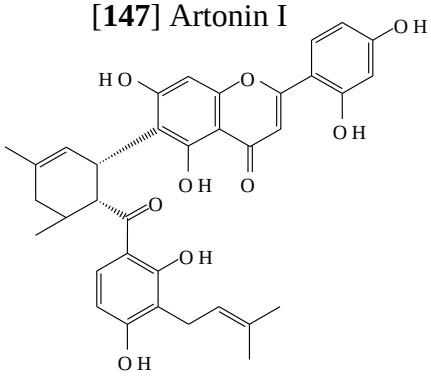


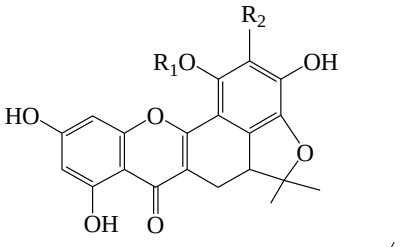
[**145**] Artonin C



[**146**] Artonin D

[**147**] Artonin I

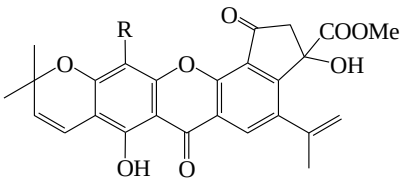




[**148**] Artonin J $R_1=H$ $R_2=$ 

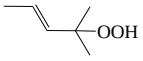
[**149**] Artonin L $R_1=Me$ $R_2=H$

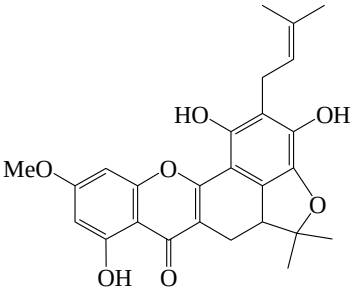
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



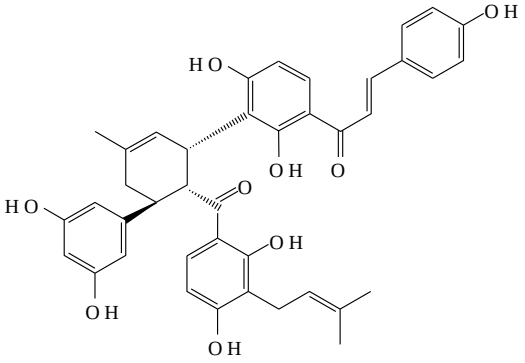
[**150**] Artonin Q R =

[**151**] Artonin R R=

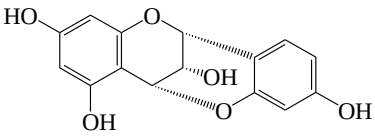




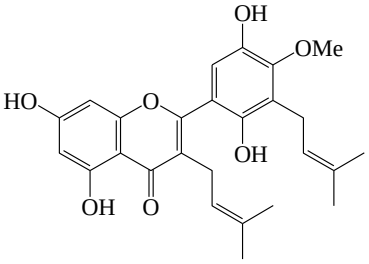
[**152**] Artonin T



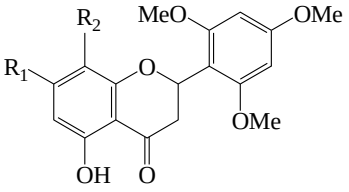
[**153**] Artonin X



[**154**] Cyanomaclurin



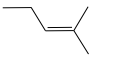
[**155**] Heteroartonin A

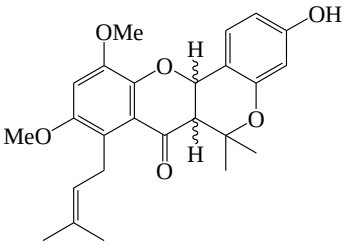


[**156**] Heteroflavanone A R_1=OH R_2=H

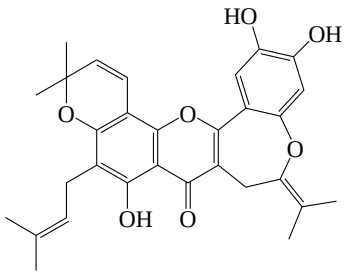
[**157**] Heteroflavanone B R_1=OMe

R_2=



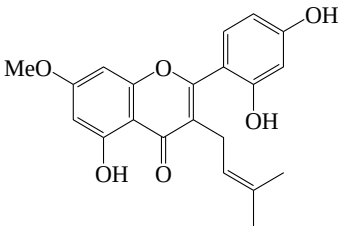


[**158**] Heterophyllol

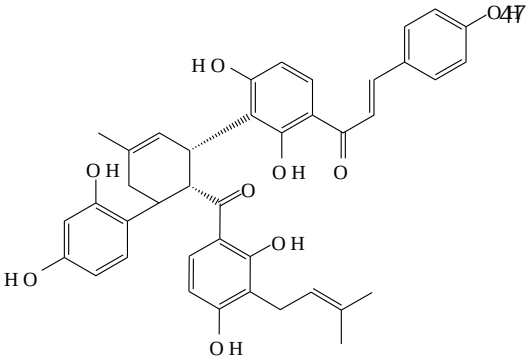


[**159**] Isocycloheterophyllin

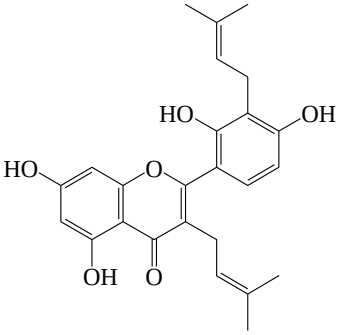
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



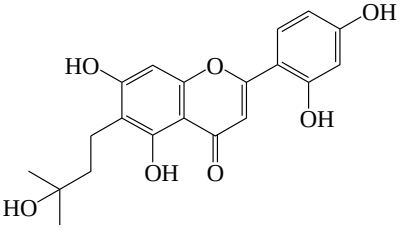
[**160**] Kuwanon C



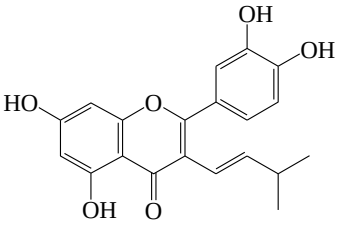
[**161**] Kuwanon R



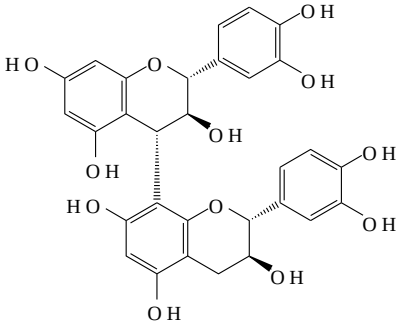
[**162**] Kuwanon T



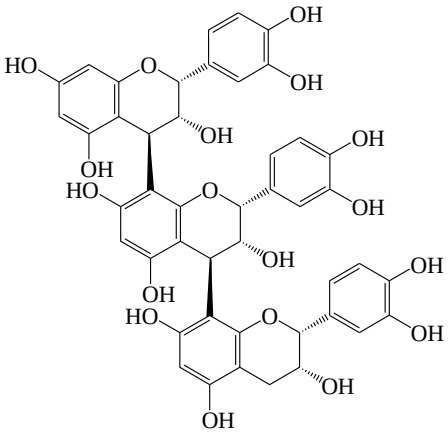
[**163**] Oxydihydroartocarpesin



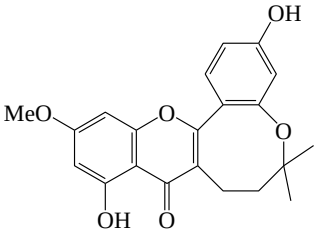
[**164**] 3-Prenyl luteolin



[**165**] Procyanidin B-3

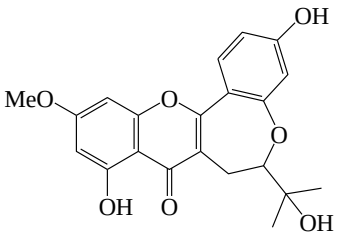


[**166**] Procyanidin C-1

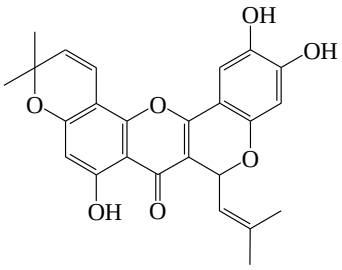


[**167**] Cyclointegrin

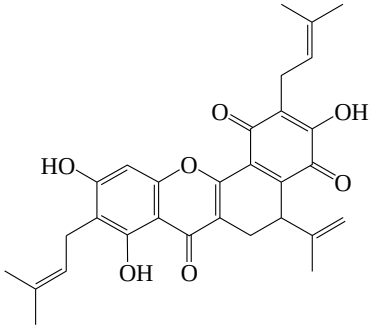
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



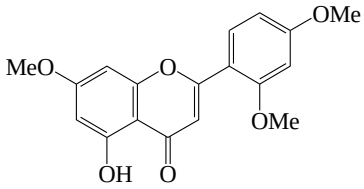
[**168**] Oxyisocyclointegrin



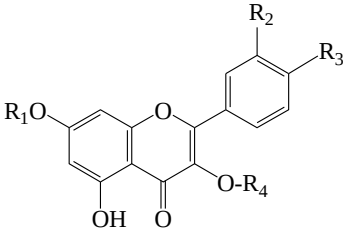
[**169**] Artoindonesianin D



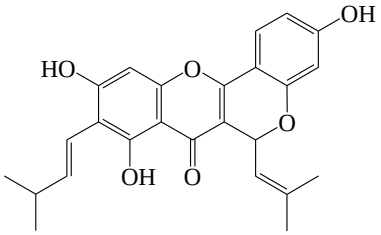
[**170**] Artonin O



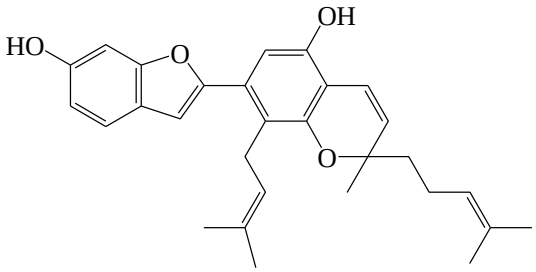
[**172**] 5-Hydroxy-7,2′,4′-trimethoxyflavone



	R_1	R_2	R_3	R_4
[**171**] 5,7-Dihydroxyflavone-3-*O*-*α*-L-rhamnoside	H	H	H	rhamnose
[**173**] Galangin-3-*O*-*α*-L-rhamnoside	Me	H	H	rhamnose
[**174**] Galangin-3-*O*-*β*-D-galactopyranosyl-(1→4)-*α*-L-rhamnoside	Me	H	H	galactose-rhamnose
[**175**] Kaempferol-3-*O*-*β*-D-xyloside	H	H	OH	xylose
[**177**] Quercetin-3-*O*-*α*-L-rhamnoside	H	OH	OH	rhamnose

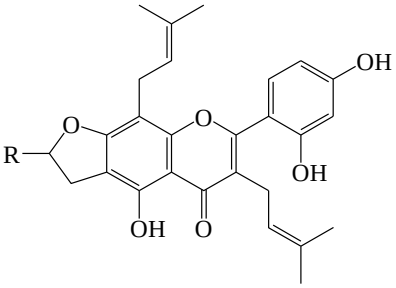


[**176**] Norcycloartocarpin

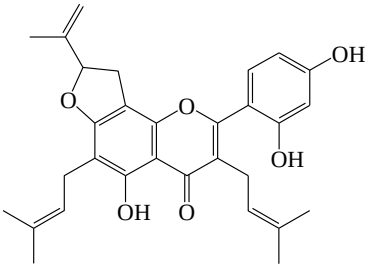


[178] Artolakoochol

Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)

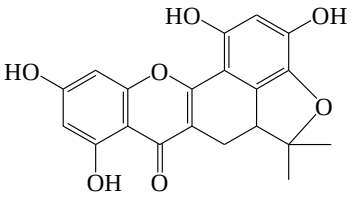


[**179**] Artoindonesianin G R=

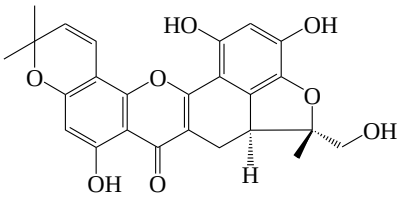


[**180**] Artoindonesianin H

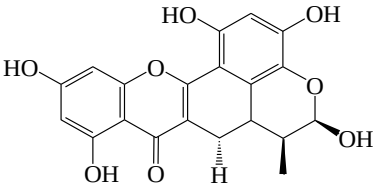
[**181**] Artoindonesianin I R=



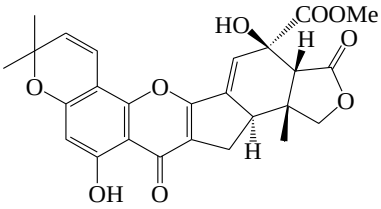
[**182**] Artoindonesianin P



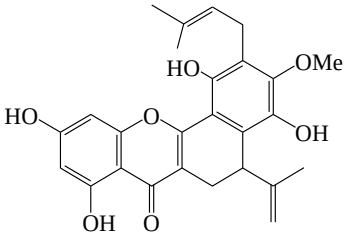
[**183**] Artoindonesianin Z-1



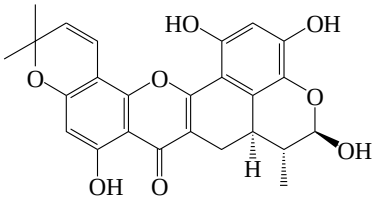
[**184**] Artoindonesianin Z-2



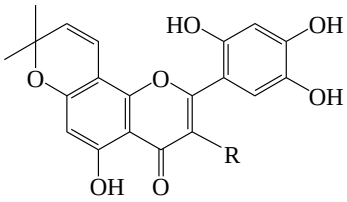
[**185**] Artoindonesianin Z-3



[**186**] Artoindonesianin Z-4

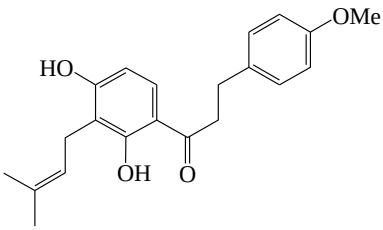


[**187**] Artoindonesianin Z-5



[**188**] 14-Hydroxyartonin E

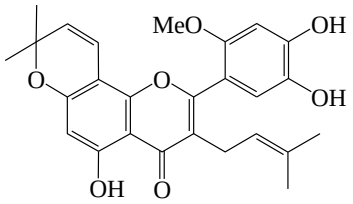
R=



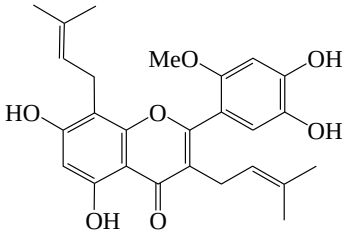
[**189**] 2′,4′-dihydroxy-4-methoxy-3′-prenyl dihydrochalcone

[**190**] Artobilochromen R=

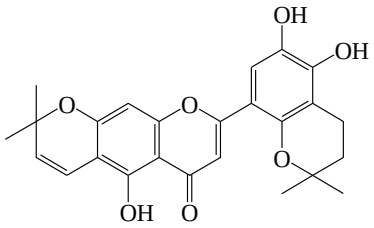
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



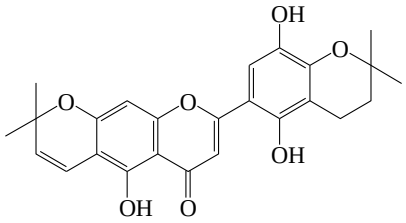
[**191**] Artonin E 2′-methylether



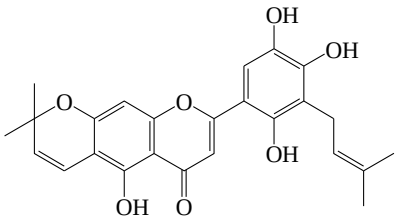
[**192**] Artonin V 2′-methylether



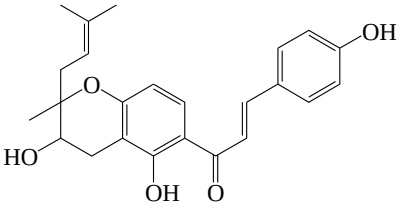
[**193**] Chromanoartobilochromen A



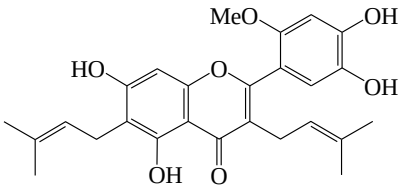
[**194**] Chromanoartobilochromen B



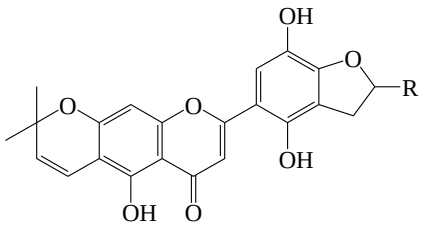
[**195**] Chromanoartobilochromene



[**196**]1-(3,4-Dihydro-3,5-dihydroxy-2-methyl-2-(3-methyl-2-butenyl)-2H-1-benzopyran-6-yl-3-(4-hydroxy phenyl)-2(*E*)-propen-1-one



[**200**] Dihydroisoartonin E 2′-methylether



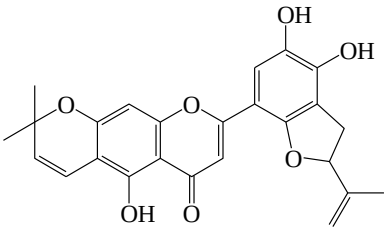
[**198**] (–)-Dihydrofuranoartobilochromen B-1

R=

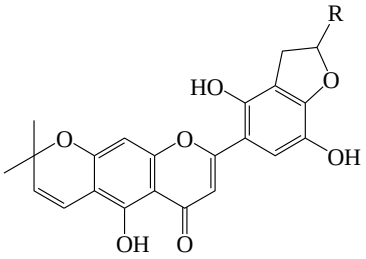
[**199**](–)-Dihydrofuranoartobilochromen B-2

R=

Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)

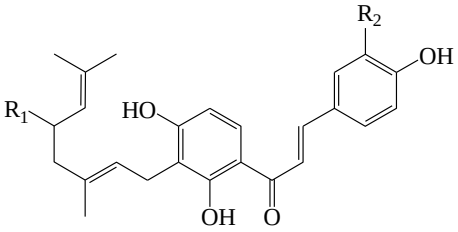


[**201**] Furanoartobilochromen A



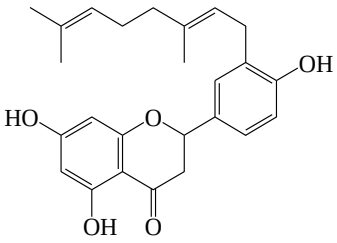
[**202**] Furanoartobilochromen B-1 R= 

[**203**] Furanoartobilochromen B-2 R= 

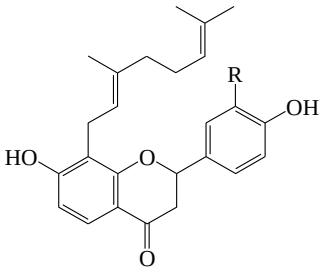


[**204**] 3′-Geranyl-2′,3,4,4′-tetrahydroxy chalcone R_1=H R_2=OH

[**211**] 2,4,4′-Trihydroxy-3-[(2*E*)-5-methoxy-3,7-dimethylocta-2,6-dienyl]chalcone R_1=OMe R_2=H

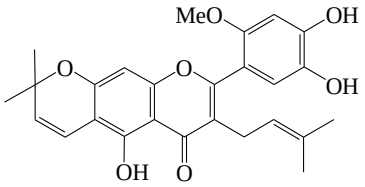


[**205**] 3′-Geranyl-4′,5,7-trihydroxyflavanone

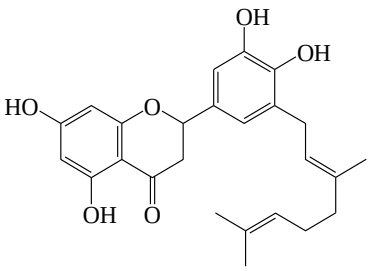


[**206**] 8-Geranyl-3′,4′,7-trihydroxy flavanone R=OH

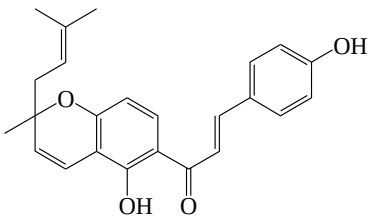
[**207**] 8-Geranyl-4′,7-dihydroxyflavanone R=H



[**208**] Isoartonin E 2′-methylether

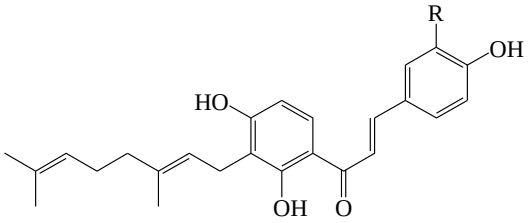


[**209**] Isonymphaeol-B



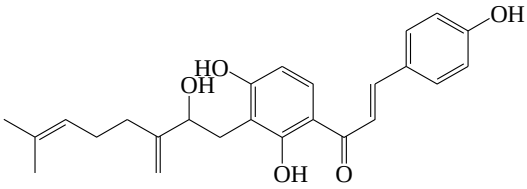
[**210**] Lespeol

Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)

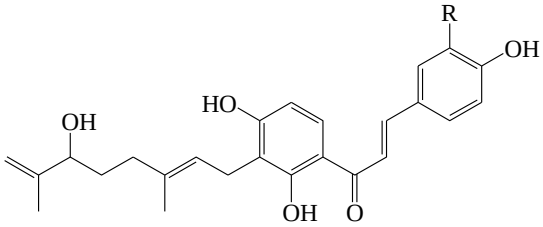


[**212**] 2′,4′,4-Trihydroxy-3′-geranylchalcone R= H

[**215**] 2′,3,4,4′-Tetrahydroxy-3′-geranyl chalcone R= OH

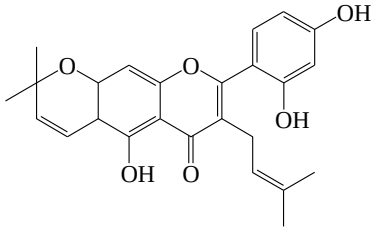


[**213**] 2′,4′,4-Trihydroxy-3′-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadiethyl]chalcone

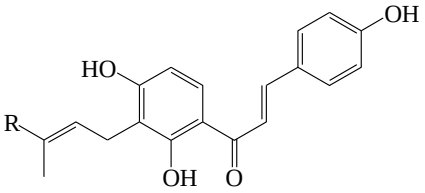


[**214**] 2′,4′,4-Trihydroxy-3′-[2-hydroxy-7-methyl-3-methylene-6-octaethyl] chalcone R=H

[**216**] 2′,3,4,4′-Tetrahydroxy-3′-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadiethyl] chalcone R= OH

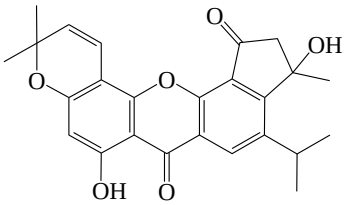


[**217**] Oxydihydromorusin

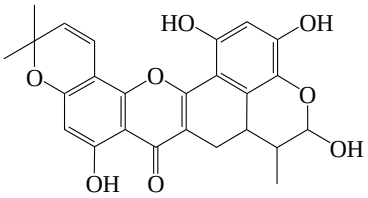


[**218**] Xanthoangelol R= 

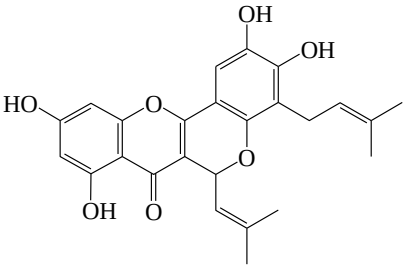
[**219**] Xanthoangelol B R= 



[**220**] Dihydroartoindonesianin C

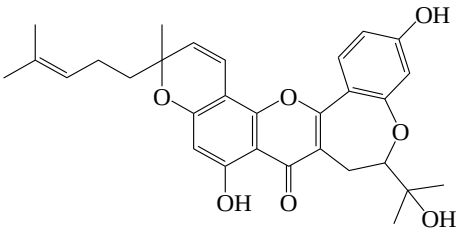


[**221**] Pyranocycloartobiloxanthone A

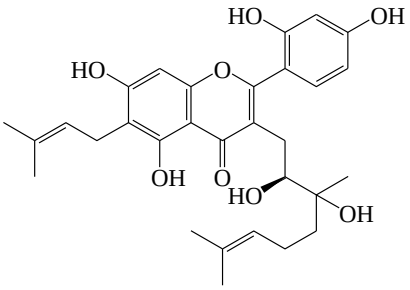


[**222**] Artosimmin

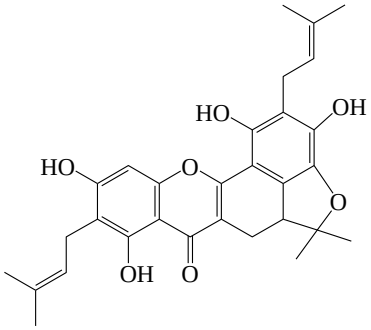
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



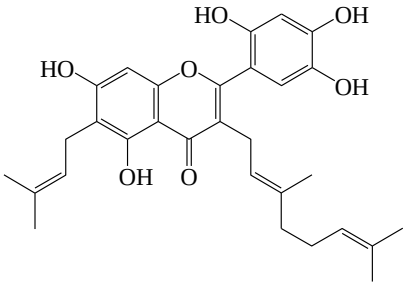
[**223**] Artocarpol B



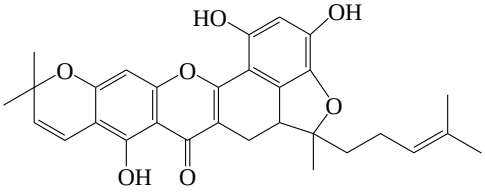
[**224**] Artocarpol H



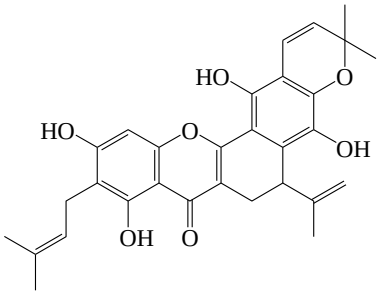
[**225**] Artonin G



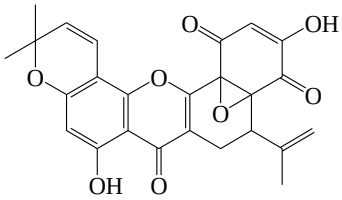
[**226**] Artonin H



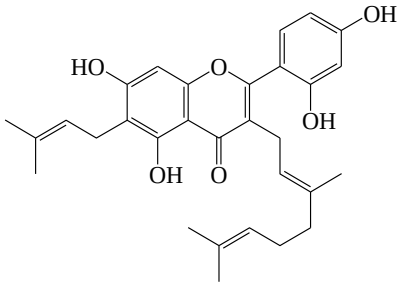
[**227**] Artonin M



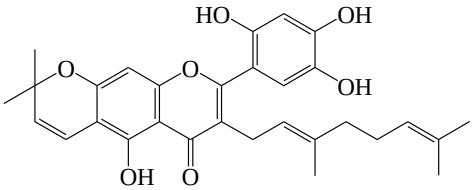
[**228**] Artonin N



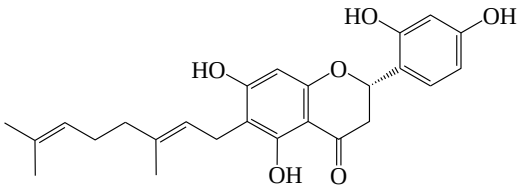
[**229**] Artonin P



[**230**] Rubraflavone C

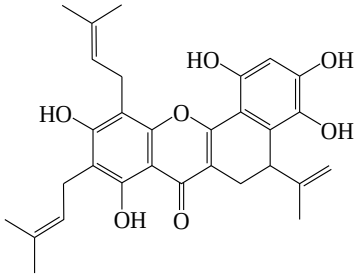


[**231**] Artoindonesianin L

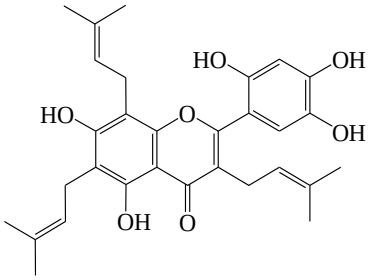


[**232**] Sepicanin A

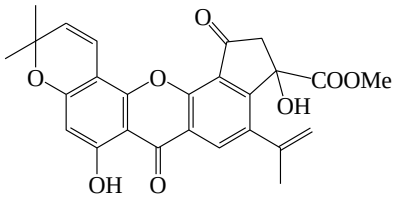
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



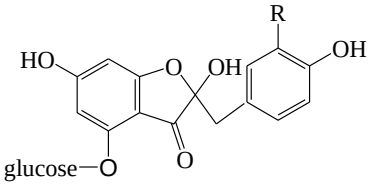
[**233**] Styracifolin A



[**234**] Styracifolin B

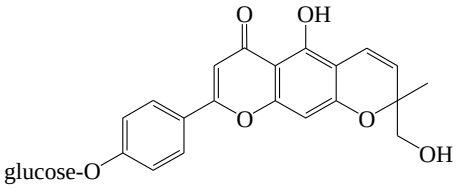


[**235**] Artoindonesianin C

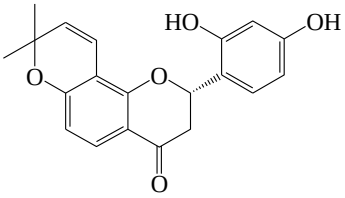


[**236**] Alphitonin-4-*O*-glucoside R=H

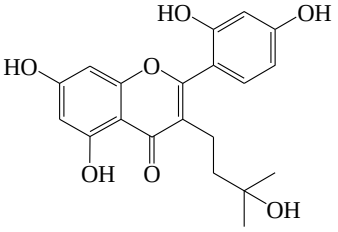
[**240**] Maesopsin-4-*O*-glucoside R=OH



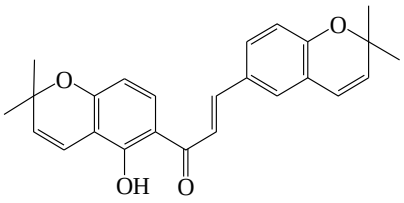
[**237**] Artokin-4′-*O*-glucoside



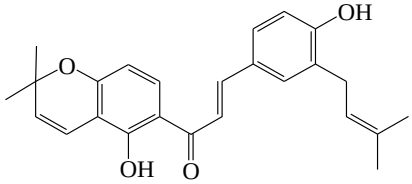
[**238**] Artotonin A



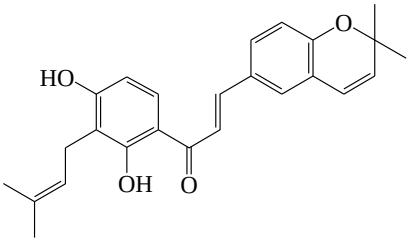
[**239**] Artotonin B



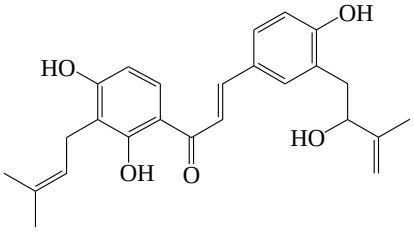
[**241**] Paratocarpin A



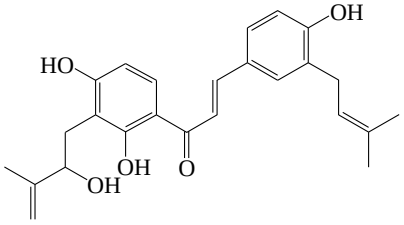
[**242**] Paratocarpin B



[**243**] Paratocarpin C

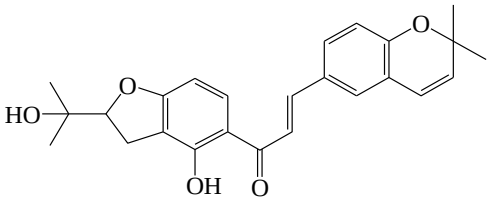


[**244**] Paratocarpin D

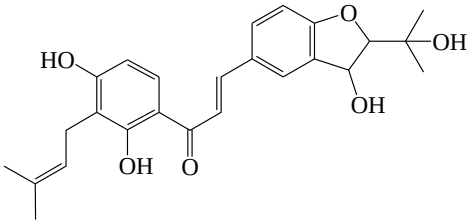


[**245**] Paratocarpin E

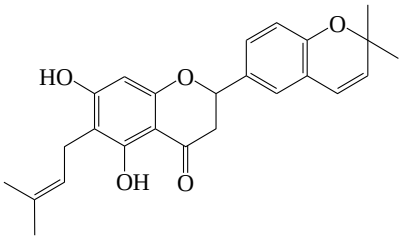
Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)



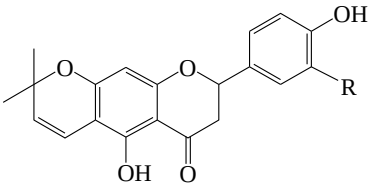
[**246**] Paratocarpin F



[**247**] Paratocarpin G

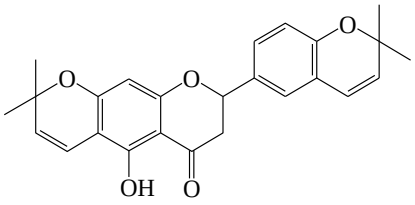


[**248**] Paratocarpin H

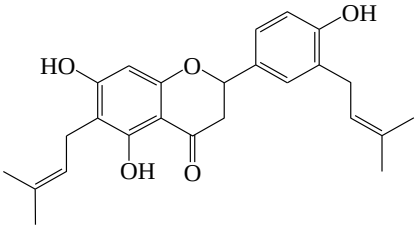


[**249**] Paratocarpin I R=

[**251**] Paratocarpin K R=H

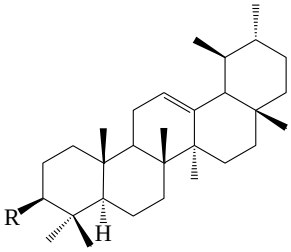


[**250**] Paratocarpin J

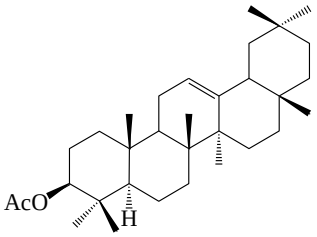


[**252**] Paratocarpin L

Figure 2 Structures of flavonoids previously isolated from *Artocarpus* spp. (continued)

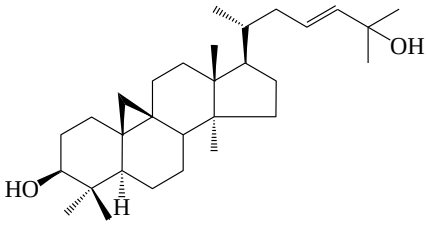


[**253**] α-Amyrin R=OH

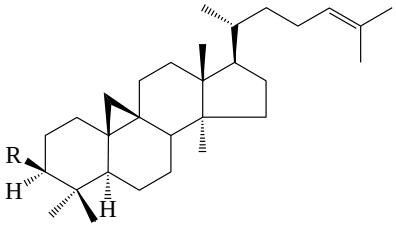


[**254**] α-Amyrin acetate R=OAc

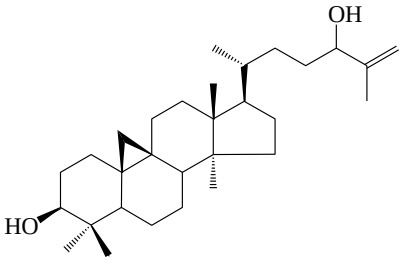
[**255**] β-Amyrin acetate



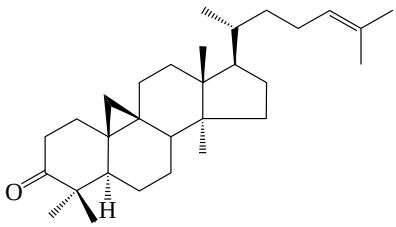
[**256**] Cycloart-23-ene-3β,25-diol
(9,19-Cyclolanost-3-one-24,25-diol)



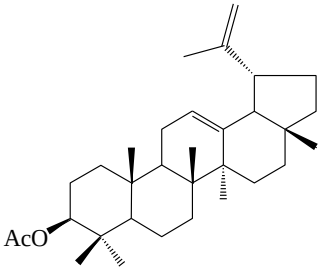
[**257**] Cycloartenol
(Cycloart-24-ene-3β-ol) R=OH
[**260**] Cycloartenyl acetate R=OAc



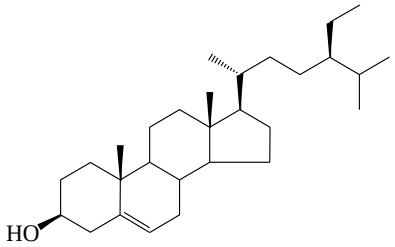
[**258**] Cycloart-25-ene-3β,24-diol
(9,19-Cyclolanost-23-ene-3β,25-diol)



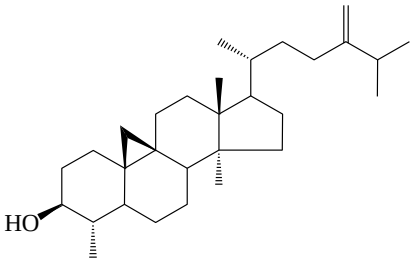
[**259**] Cycloartenone



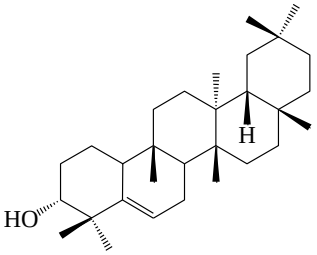
[**261**] Lupeol acetate



[**262**] β-Sitosterol

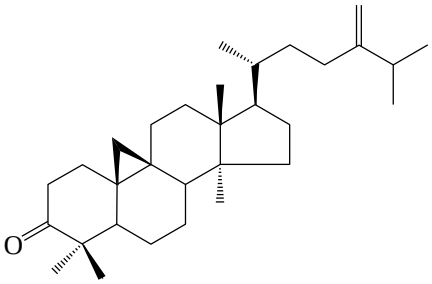


[**263**] Cycloeucalenol

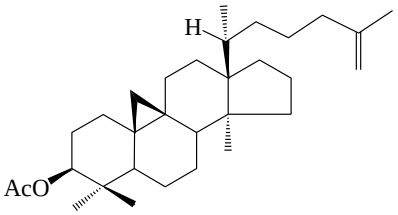


[**264**] Glutinol

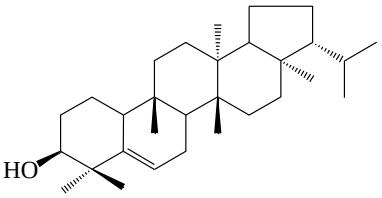
Figure 3 Structures of triterpenoids and steroids previously isolated from *Artocarpus* spp.



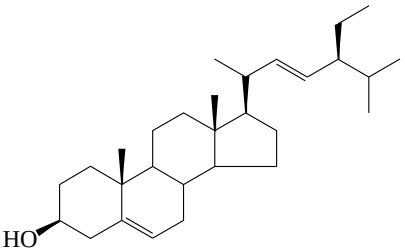
[**265**] 24-Methylenecycloartenone



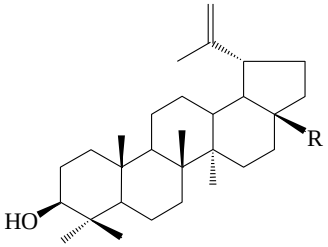
[**266**] Isocycloartenol acetate



[**267**] Simiarenol



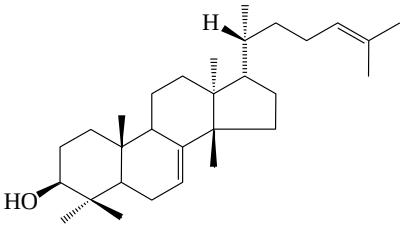
[**268**] Stigmasterol



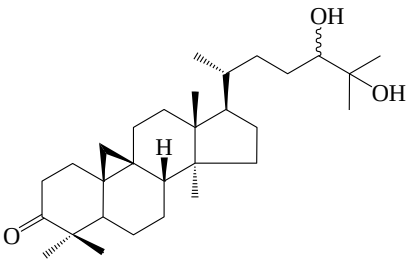
[**269**] Betulin R=CH_2OH

[**270**] Betulinic acid R=COOH

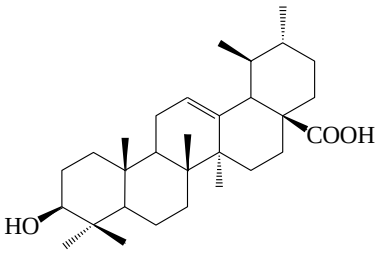
[**274**] Lupeol R=CH_3



[**271**] Butyrospermol

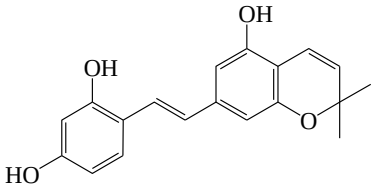


[**272**] (9,19-Cyclolanost-3-one-24,25-diol)

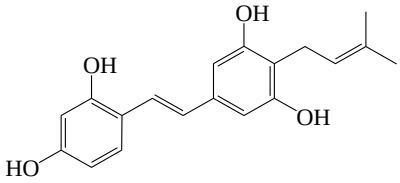


[**273**] Ursolic acid

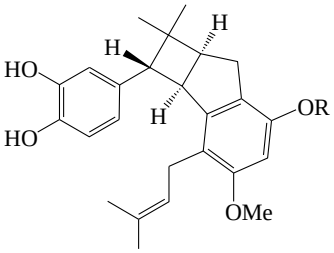
Figure 3 Structures of triterpenoids previously isolated from *Artocarpus* spp. (continued)



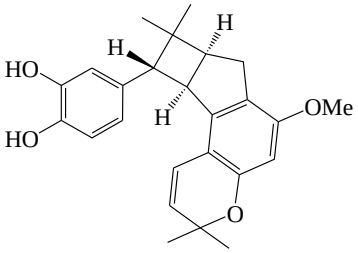
[**275**] Artocarbene



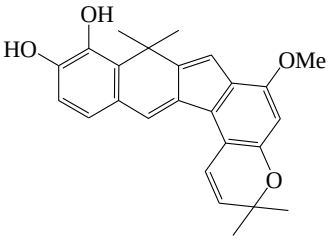
[**276**] 4-Prenyloxyresveratrol (*trans*-4-Isopentenyl-3,5,2′,4′tetrahydroxystilbene)



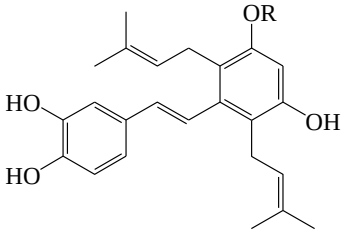
[**277**] Artochamin H R=H
[**278**] Artochamin I R=Me



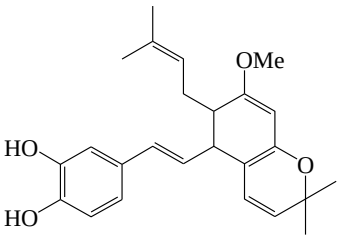
[**279**] Artochamin J



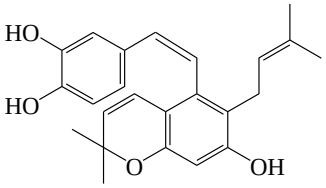
[**280**] Artochamin K



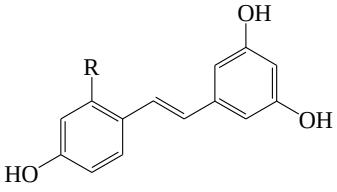
[**281**] Artochamin F R=H
[**282**] Artochamin G R=Me



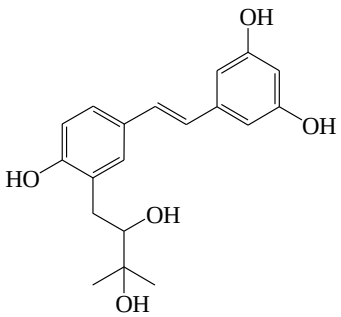
[**283**] Artostilbene A



[**284**] Artostilbene B

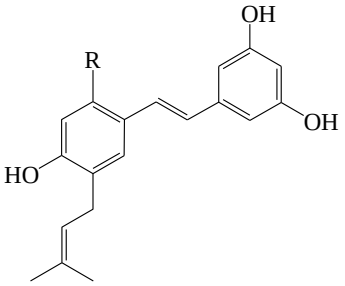


[**285**] Oxyresveratrol R=OH
[**286**] Resveratrol R=H

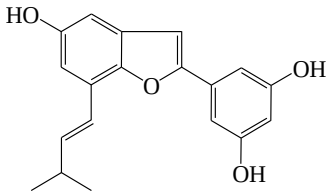


[**287**] 3-(2,3-Dihydroxy-3-methylbutyl)-resveratrol

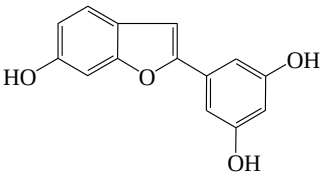
Figure 4 Structures of stilbenoids previously Isolated from *Artocarpus* spp.



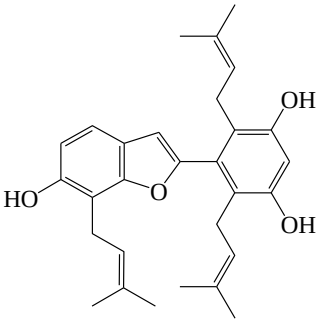
[**288**] 3-(*γ*,*γ*-Dimethylallyl) oxyresveratrol
R=OH
[**289**] 3-(*γ*,*γ*-Dimethylallyl) resveratrol
R=H



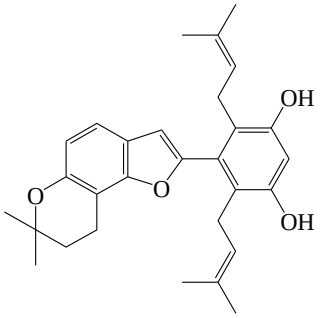
[**290**] 3-(*γ*,*γ*-Dimethylpropenyl)
Moracin M



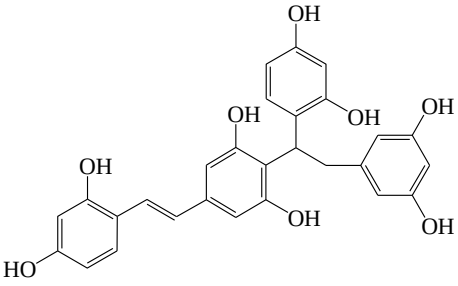
[**291**] Moracin M



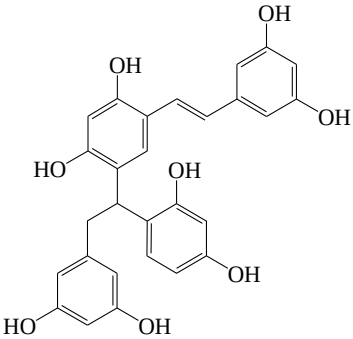
[**292**] Artoindonesianin X



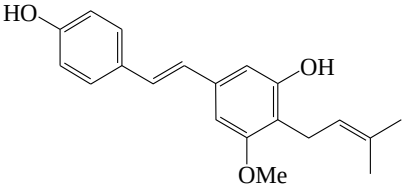
[**293**] Artoindonesianin Y



[**294**] Andalasin

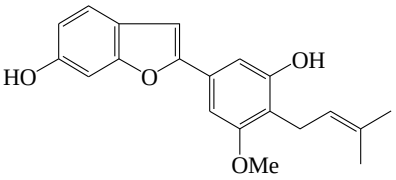


[**295**] Artogomezianol

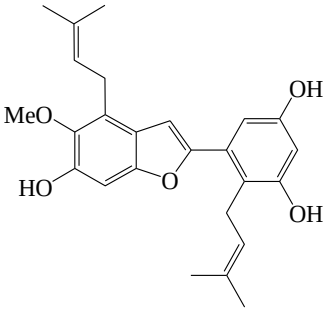


[**296**] Artoindonesianin N

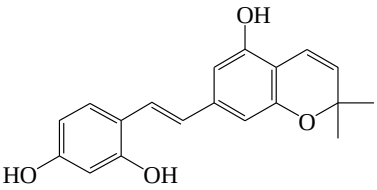
Figure 4 Structures of stilbenoids previously Isolated from *Artocarpus* spp.



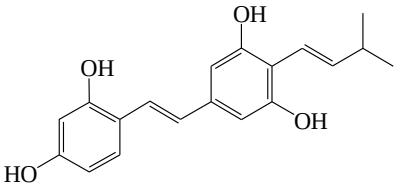
[**297**] Artoindonesianin O



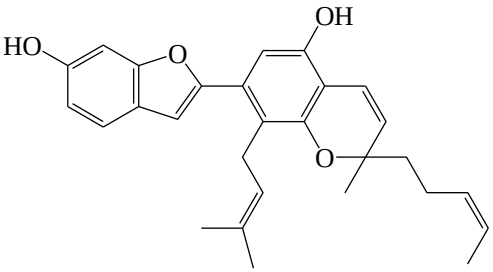
[**298**] Artoheterophyllin A



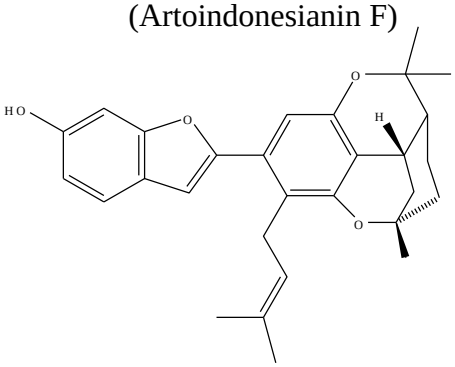
[**299**] 4-Methoxy-2,2-dimethyl-6-(2-(2,4-dihydroxy) phenyl-*trans*-ethenyl) chromene



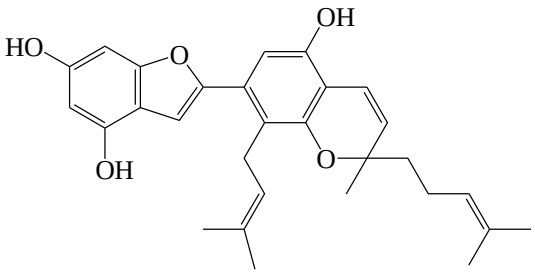
[**300**] *trans*-4-(3-Methyl-*E*-but-1-enyl)-3,5,2′,4′-tetrahydroxystilbene (Artoindonesianin F)



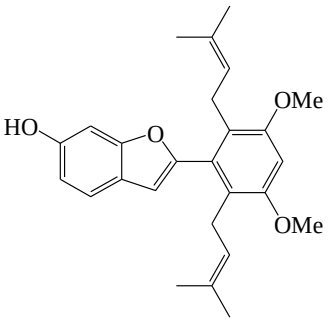
[**301**] Artolakoochol



[**302**] Cycloartolakoochol

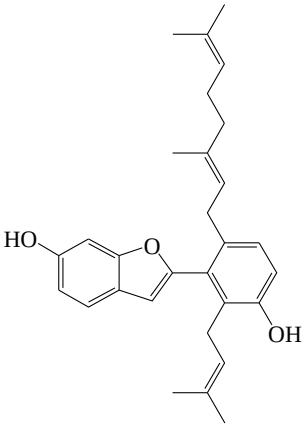


[**303**] 4-Hydroxyartolakoochol

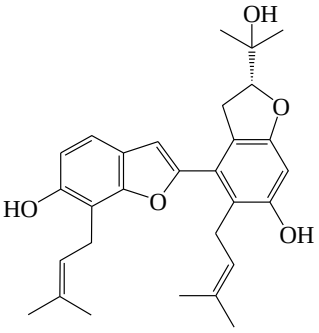


[**304**] Lakoochin A

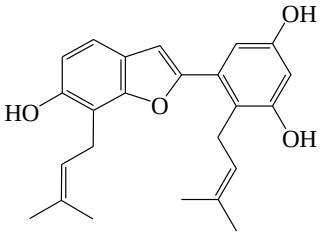
Figure 4 Structures of stilbenoids previously isolated from *Artocarpus* spp. (continued)



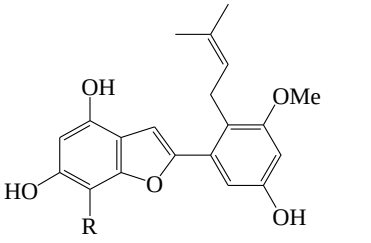
[**305**] Lakoochin B



[**306**] Artonitidin A

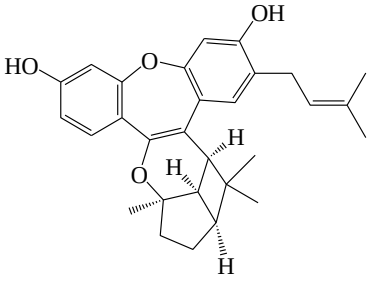


[**307**] Artonitidin B

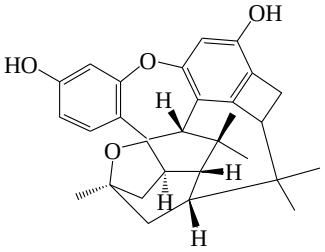


[**308**] Artopetelin L R=

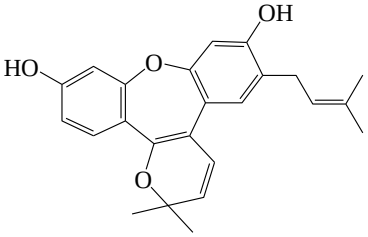
[**309**] Artopetelin M R=



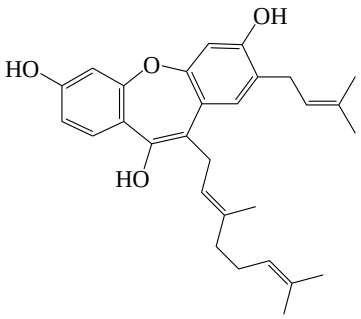
[**310**] Artocarpol A



[**311**] Artocarpol C

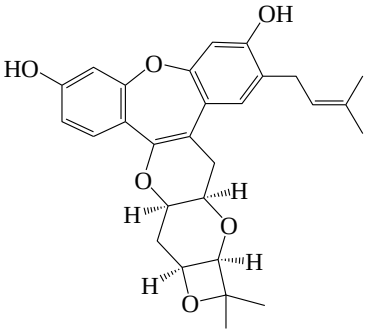


[**312**] Artocarpol D

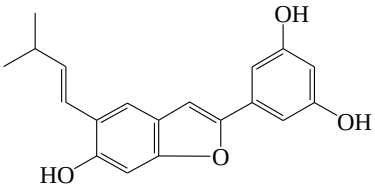


[**313**] Artocarpol E

Figure 4 Structures of stilbenoids previously isolated from *Artocarpus* spp. (continued)

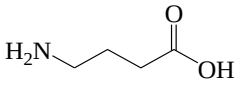


[**314**] Artocarpol F

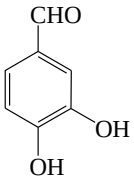


[**315**] Artotonkin

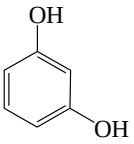
Figure 4 Structures of stilbenoids previously isolated from *Artocarpus* spp. (continued)



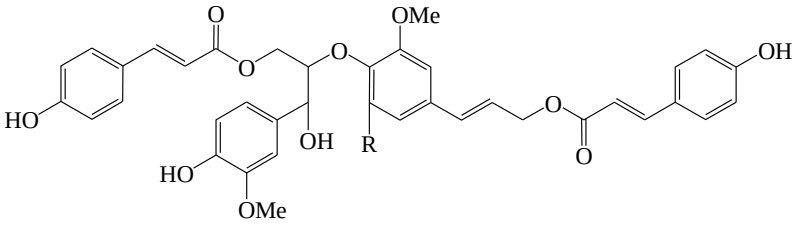
[**316**] γ-Aminobutyric acid



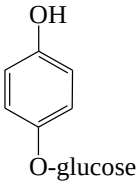
[**317**] Resorcinol



[**318**] *β* Resorcylaldehyde



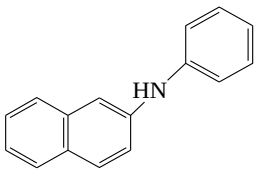
[**319**] Dadahol A R=OMe
[**320**] Dadahol B R=H



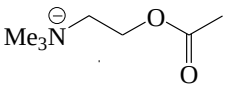
[**321**] Arbutin

$HOCH_2CH_2(CH_2)_{29}CH_3$

[**322**] 1-Dotriacontanol



[**323**] Phenyl- *β*-naphthylamine



[**324**] Acetylcholine

$CH_3(CH_2)_{20}COO(CH_2)_8CH(OH)(CH_2)_3CH_3$

[**329**] 9-Hydroxytridecyl docosanoate

$CH_3(CH_2)_{20}COO(CH_2)_3CH(OH)(CH_2)_6CH_3$

[**330**] 4-Hydroxyundecyl docosanoate

$CH_3(CH_2)_5CH(OH)CH_2CH=CH(CH_2)_7COOH$

[**333**] Ricinoleic acid

Figure 5 Structures of miscellaneous compounds previously isolated from *Artocarpus* spp.

2. Traditional Uses and Biological Activities of *Artocarpus* spp.

2.1 Traditional uses of *Artocarpus* spp.

Many members of the genus *Artocarpus* have been used in traditional medicine in South-East Asia for the treatment of several disceases and disorders, such as inflammation, malarial fever, ulcer, abscess and diarrhea. *A. heterophyllus* is used in the treatment of various symptoms. For example, the pulp and seeds are used as a cooling tonic and pectoral. The roots are used to treat diarrhea and skin disease, whereas its leaves are used to promote lactation in women. Its latex is mixed with vinegar as a treatment for abscess, and the wood, which is used by monks to dye their robes, has sedative effect in convulsions. (Jagtap and Bapat, 2010). The leaves of *A. altilis* have been used for the treatment of liver cirrhosis, hypertention and diabetes (Wang *et al*., 2006). In Indonesia, the seeds of *A. champeden* have been used against diarrhea while its roots are used against malaria fever. In western Java, *A. elasticus* has been used to treat inflammation. The bark of this plant is used as a contraceptive, while the young leaves are used to treat tuberculosis (Jagtap and Bapat, 2010). The sap from *A. lowii* is traditionally used as an ointment and as cooking oil (Jamil *et al*., 2008). Wood is used in making furniture, oars and musical instruments, in building construction and boat, and as a yellow dye for dyeing cotton and silk. (Rujinirum *et al*., 2005)

Artocarpus lakoocha Roxb. is a valuable tropical tree species native to India and used for fruit, furniture, timber, and feed. The lakoocha fruits are generally eaten fresh. The edible fruit pulp is believed to act as a tonic for the liver. (Joshee *et al*., 2002). The brown powder from aqueous extraction of its wood, called Puag-Haad in Thai, has been used as a traditional anthelmintic drug to treat tapeworm infection. In addition, *A. lakoocha* roots yield a color dye (Charoenlarp *et al*., 1981; Jagtap and Bapat, 2010).

2.2 Biological activities of *Artocarpus* spp.

Compounds from *Artocarpus* plants showed various activities such as antimicrobial, cytotoxic, anti-inflammatory, anti-platelet aggregration and antioxidant properties.

Artocarpin [**4**] and artocarpesin [**3**], isolated from *A. heterophyllus*, inhibited the growth of cariogenic bacteria such as *Streptococci mutans***,** *Actinomyces viscosus***,** *Lactobacillus casei* (Sato *et al*., 1996). Artonin E [**12**], isolated from the bark of *A. rigida*,

showed antimicrobial activity against the gram-negative bacteria *Escherichia coli* and *Bacillus subtilis* (Suhartati and Yandri, 2008). Lakoochins A [**327**] and B [**328**], from the root of *A. lakoocha*, displayed anti-tuberculosis activity by inhibiting the growth of *Mycobacterium tuberculosis* (Puntumchai *et al*., 2004). Oxyresveratrol [**28**5] from *A. lakoocha* possessed moderate activity against herpes simplex virus (HSV) type I and type II and against a wild-type human immunodeficiency virus type I (HIV-1/LAI) (Likhitwitayawuid *et al*., 2005). Morusin [**63**], cycloartobiloxanthone [**38**] and artonin E [**12**] from the root bark of *A. altilis* exhibited moderate antiplasmodial activity against *Plasmodium falciparum* (Boonphong *et al*., 2007). Cycloartocarpin [**39**], isocyclomorusin [**33**] and norartocarpetin [**65**], compounds isolated from *A. gomezianus*, showed moderate activity against both types of HSV (Likhitwitayawuid *et al*., 2006).

Prenylated flavones such as artoindonesianins U [**90**] and V [**91**] (Syah *et al*., 2004), artoindonesianins A-2 [**81**] artoindonesianins A-3 [**82**] and artoindonesianins T [**89**], heterophyllin [**98**] and cudraflavone C [**34**] from the heartwood of *A. champeden* (Syah *et al*., 2006b) and cycloartobiloxanthone [**38**] and artonin E [**12**] from *A. rigida* (Suhartati and Yandri, 2008) showed strong cytotoxicity against the murine leukemia P-388 cell line. In another report , artosimmin [**221**], isolated from *A. odoratissimus*, exhibited significant cytotoxicity against HL-60 and MCF-7 cancer cell lines (Ee *et al*., 2010).

Dihydroartomunoxanthone [**48**], artochamin B [**13**] and artocommunol CC [**16**], isolated from the roots of *A. communis*, significanly inhibited secondary platelet aggregation induced by adrenaline, resulting in the inhibitory effect on thromboxane formation (Weng *et al*., 2006).

The anti-inflammatory activity of artocarpesin [**3**], norartocarpetin [**65**] and oxyresveratrol [**284**] from *A. heterophyllus* has been demonstrated against production of proinflamatory mediators in lipopolysaccharide (LPS)-activated RAW 264.7 murine macrophage cells (Fang, Hsu, and Yen, 2008a).

Artocarpin [**4**]**,** isolated from a diethyl ether extract of heartwood of *A. incisus*, possessed potent 5α- reductase inhibitory effect resulting in the inhibiton of the conversion of testosterone into 5α-dihydrotestosterone. The 5α-reductase inhibitor acts on androgen receptors which are found in both preputial skin and nongenital skin. Therefore artocarpin might be useful in selective treatment of androgen-dependent disorders such as male pattern alopecia and achne (Shimizu *et al*., 2000).

Norartocarpin [**128**], cudraflavone C [**34**], artotonkin [**337**], albanin A [**132**] and artopetelin M [**331**], isolated from *A. nitidus*, showed inhibitory effect on pancreatic lipase with IC_{50} values ranging from 1.8 ± 0.1 to 63.8 ± 3.6 µM (Zhao *et al*., 2009).

Several phenolic compounds found in plants of this genus were shown to be inhibitors of tyrosinase activity. For example, norartocarpetin [**65**] and resveratrol [**287**] from *A. gomezianus* exhibited potent tyrosinase inhibitory activity (Likhitwitayawuid *et al*., 2000). Artocarpanone [**135**] inhibited both mushroom tyrosinase enzyme activity and melanin production in B16 melanoma cells (Arung *et al*., 2006a). Artocarpin [**4**], cudraflavone C [**34**], 6-prenylapigenin [**77**], kuwanon C [**160**], norartocarpin [**128**] and albanin A [**132**] inhibited melanin biosynthesis in B16 melanoma cells without inhibiting the tyrosinase enzyme (Arung *et al*., 2006b). The heartwood extract of *A. lakoocha* showed both tyrosinase-inhibitory activity *in vitro* and melanin-reducing efficacy in humans (Tengamnuay *et al*., 2006).

The prenylated flavones cycloheterophyllin [**96**] and artonins A [**92**] and B [**93**] inhibited lipid peroxidation and were antioxidants against DPPH, peroxyl and hydroxyl radicals and H_2O_2 (Ko *et al*., 1998). Artelastin [**115**], another prenylated flavone isolated from *A. elasticus*, showed strong $O_2^{\bullet-}$ scavenging activity (Cerqueira *et al*., 2008). In another study, cyclogeracommunin [**45**], artoflavone A [**19**], artomunoisoxanthone [**21**], artocommunol CC [**16**], artochamin D [**14**], artochamin B [**13**] and dihydroartomunoxanthone [**48**] isolated from the root cortex of *A. communis*, and cycloartelastoxanthone [**126**], artelastoheterol [**120**], cycloartobiloxanthone [**38**] and artonol A [**28**] isolated from *A. elasticus* all showed inhibition of oxidative DNA damage. Compounds [**19**], [**126**], [**120**] and [**38**] showed significant DPPH-scavenging activity, while compounds [**45**] and [**28**] significantly inhibited xanthine oxidase (XO) activity (Lin *et al*., 2009). The xanthone pyranocycloartobiloxanthone A [**220**], isolated from the stem bark of *A. obtusus*, showed strong DPPH-scavenging activity (Hashim *et al*., 2010).

3. Tissue Cultures of *Artocarpus* spp.

With regard to cell cultures of plants of the genus *Artocarpus*, there have been a few reports which focused on micropropagation. For example, a micropropagation protocol for *Artocarpus lakoocha* using leaf disc, nodal segment, and shoot tip explants was described. Explants taken from seedling by air dried seeds of this plant were germinated on sterile MS medium containing 0.8% agar and 0.5% sucrose. Optimum results for

multiple shoot induction were obtained with MS medium supplemented with 1.0 to 2.0 mg/l BA.(Joshee *et al.,* 2002). For *A. altilis*, shoot tip explants taken from mature trees were used. Cultures were initiated from shoot tip explants maintained on MS medium supplemented with 6- benzyladenine (BA, 4.4 mM) (Rouse-Miller and Duncan, 2000). A micropropagation protocol of *A. heterophyllus* using shoot explants cultured on MS medium supplement with BA 4.5 mg/l was reported.

4. Quantitative Analysis of Chemical Constitutents of *Artocarpus* spp.

With regard to analysis of oxyresveratrol in *A. lakoocha*, a few methods for the quantitation of oxyresveratrol in *A. lakoocha* heartwood have been described, and no quantitative analyses of the traditional drug Puag-Haad have been reported. The previously reported analytical methods include gravimetric, high performance liquid chromatographic (HPLC) and capillary zone electrophoretic techniques. These procedures, however, have some disadvantages. The gravimetric method is a laborious process and has a wide margin of errors. The high-performance liquid chromatography (HPLC) and the electrophoresis methods require careful optimization of chromatographic conditions, such as well chosen stationary and mobile phases and proper treatments of the sample and solvent.

The determination of other compounds such as β-sitosterol and Lupeol in *A. lakoocha* has been reported. Quantitative analysis of β-sitosterol and Lupeol in leaf powder was performed on silica gel 60 F_{254} HPTLC (High-performance thin layer chromatographic) plate, with toluene: methanol: formic acid, 7.0:2.0:0.3 (v/v/v), as mobile phase and treated with methanolic sulphuric acid reagent. The β- sitosterol and Lupeol contents in leaf powder were found to be 0.176 and Lupeol 0.217 mg/ 500 mg plant power (Vaidya *et al.*, 2011).

CHAPTER III

EXPERIMENTAL

1. Plant Materials

1.1 Sample source

Natural samples of *Artocarpus lakoocha* heartwood (ALH1-ALH3) were collected from natural habitats: Chiang Rai (Northern province); Kanchanaburi (Western) and Bangkok (Central). Commercial samples (ALH-4 to ALH-14) were purchased from drugstores as indicated in Table 11. Two samples of the traditional drug Puag-Haad (PH1 and PH2) were obtained from drugstores (Table 11).

1.2 Plant cell cultures

The seeds of *A. lakoocha* Roxb. were collected from the Eastern Botanical Garden (Khao Hin Son) Chachoengsao Province, Thailand in February 2007. The plant was identified by officials of the Eastern Botanical Garden. Authentication was performed by comparison with herbarium specimens (BKF 29303) at the Royal Forest Department, Ministry of Natural Resources and Environment of Thailand. A voucher specimen (SS00101) has been deposited at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

2. Quantitative Analysis of Oxyresveratrol in *A. lakoocha* and 'Puag-Haad'

2.1 Sample Preparation

Each sample of dried heartwood of *A. lakoocha* (5 g) was extracted with 250 ml ethanol in a Soxhlet apparatus for 8 h. The organic layer, after removal of the organic solvent and drying in a desiccator, gave a dried extract. This extract (10 mg) was dissolved in 100 ml methanol to give a sample solution. For Puag-Haad, a sample solution was prepared by dissolving Puag-Haad (1 mg) in 10 ml methanol.

2.2 Preparation of Standard Solutions

Oxyresveratrol was obtained from a previous study (Likhitwittayawut, *et al*. 2005). A standard solution with an accurate concentration of 0.16 mg/ml was prepared by dissolving oxyresveratrol (16 mg) in methanol (100 ml). From this solution, 4 additional standard solutions (0.08, 0.04, 0.02 and 0.01 mg/ml) were prepared by serial dilution.

2.3 Instruments and Chromatographic Conditions

2.3.1 HPLC method

The reversed-phase HPLC system consisted of a pump equipped with a UV detector Shimadzu SPD-10 Aλ) and a Phenomenex Luna Phenyl-Hexyl (150×4.60 mm) column connected to security guard (HPLC Guard Cartridge System). The mobile phase contained MeOH:H_2O (80: 20 v/v) and the flow rate was 0.1 ml/min. The mobile phase was filtered through Millipore filter 0.45 mm, white nylon HNWP 47mm and was degassed before use. The wavelength was set at 254 nm. The samples were manually injected (20 µl) and the concentration of oxyresveratrol in the samples was calculated using the average peak area compared between standard and sample after triplicate injections.

2.3.2 TLC densitometric method

A Camag TLC system (Linomat, Switzerland), which included an automatic TLC sampler, a TLC scanner and a CATS software, was used. Chromatography was performed on a TLC aluminum sheet (silica gel 60 F 254 plate, 20 × 10 cm) using methylene chloride/ methanol (85: 15) as the mobile phase. Each sample (20 µl) was applied as a band (5 × 0.30 mm) in triplicate. Plates were developed in a glass tank preequilibrated with the mobile phase. The solvent was allowed to run up the plate to a height of 8 cm. Chromatograms were evaluated by measuring the peak area with the TLC scanner in the absorbance mode at 254 nm.

2.4 Method Validation

2.4.1. Linearity

For the HPLC method, linearity was determined over the range of 0.2–3.2 µg/injection. Five standard oxyresveratrol solutions with different concentration were prepared by serial dilution. Each of the standard solutions was evaluated by triplicate. For the TLC denditometric method, linearity was determined over the range of 0.2-3.2µg/spot. For both methods, a plot of average area under curve (AUC) versus concentration was obtained. Linearity was expressed as correlation coefficient (r^2).

Table 5 Concentrations of standard solution (HPLC method)

Standard	Concentation		First	Second	Third	Average of AUC
	mg/ml	µg/injection	AUC	AUC	AUC	
S1	0.16	3.2	27863410	27856466	28162246	27960707
S2	0.08	1.6	15101118	15223273	14769249	15031213
S3	0.04	0.8	8859345	8772674	8679064	8770361
S4	0.02	0.4	5402098	5455891	5311082	5389690
S5	0.01	0.2	3562417	3630490	3660043	3617650

Table 6 Concentrations of standard solution (TLC densitometric method).

Standard	Concentation		First	Second	Third	Average of AUC
	mg/ml	µg/spot	AUC	AUC	AUC	
S1	0.16	3.2	24056	21779	22895	22910
S2	0.08	1.6	12029	11825	11027	11627
S3	0.04	0.8	7619	7118	6991	7243
S4	0.02	0.4	4194	4165	3929	4096
S5	0.01	0.2	1777	1746	1699	1741

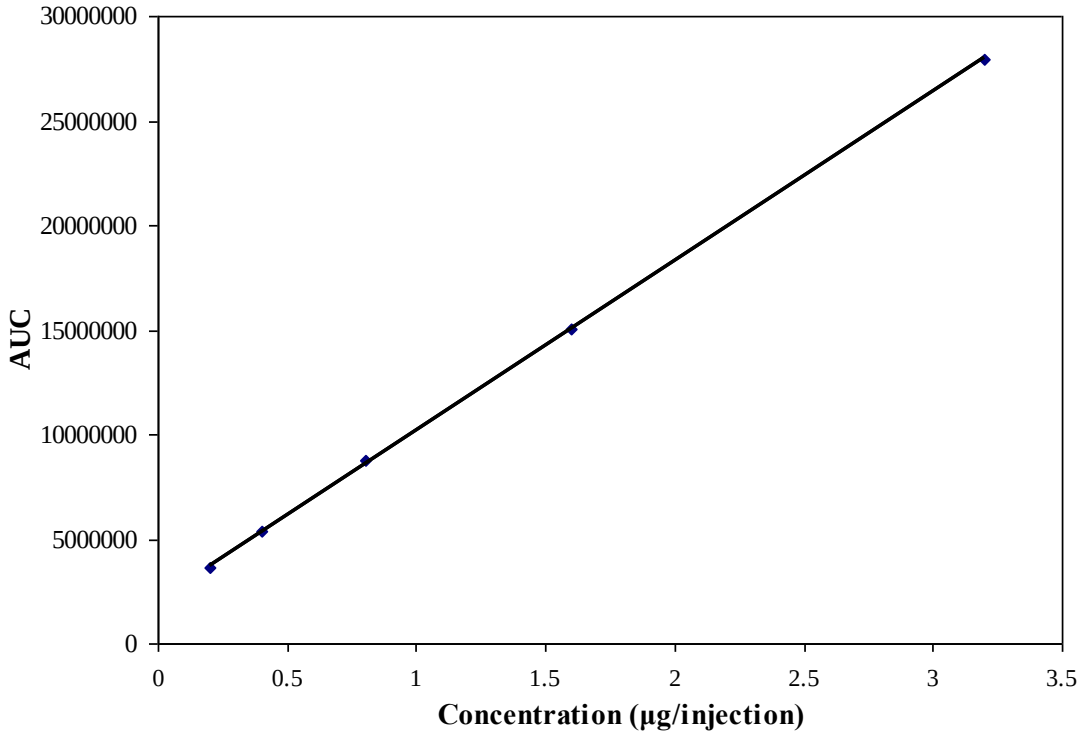


Figure 6 Calibration curve for standard solution of oxyresveratrol (HPLC method)

$y = 8075532.25x + 2140264.21$ ($r^2 = 1.00$)

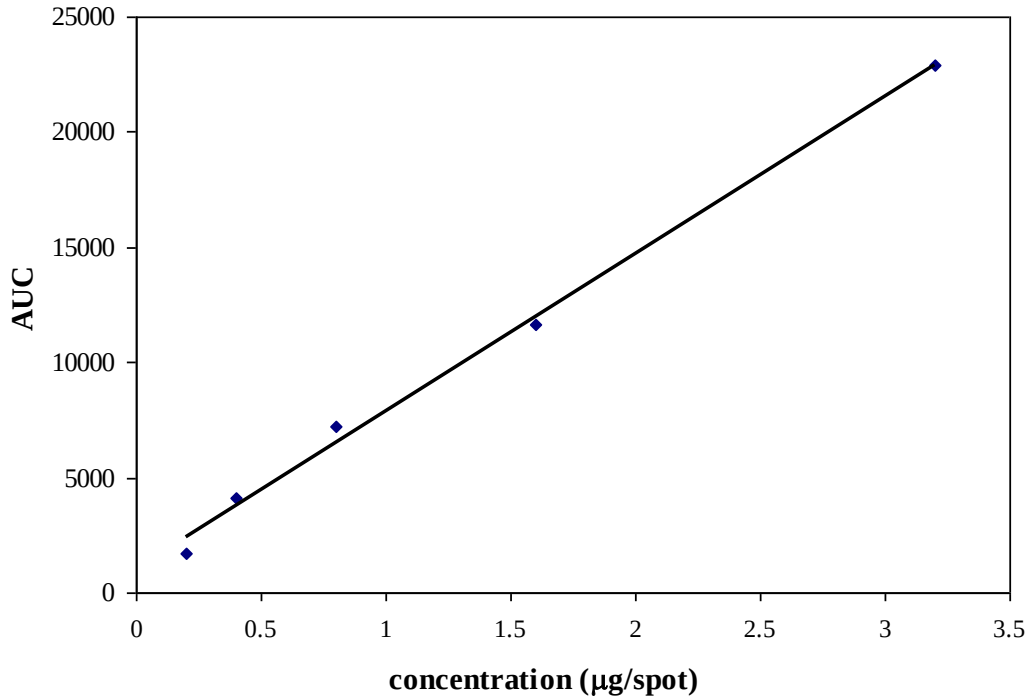


Figure 7 Calibration curve for standard solution of oxyresveratrol (TLC densitometric method) Y= 6829.825x+1054.417 (r^2= 0.996)

2.4.2 Accuracy

Determination of accuracy was done using the recovery method. One milliliter of the *A. lakoocha* heartwood sample solution was spiked with 0.05, 0.25, 0.45, 0.65, 0.85 and 1.05 ml of oxyresveratrol solution in methanol (0.20 mg/ml). Each of these solutions (20 µl/spot for TLC densitometric method; 20 µl/injection for HPLC method) was then analyzed, and the percentage recovery of oxyresveratrol was determined. The theoretical content of oxyresveratrol in each spot/injection was determined from the equation: theoretical content (µg/spot or injection = 20(8.4+0.2a) /(1,000+a), where 8.4 is the initial amount of oxyresveratrol (µg) in 1,000 µl of sample solution (from 18 determinations), and a is the volume (µl) of oxyresveratrol solution added.

Table 7 Accuracy of HPLC method.

Sample number	Oxyresveratrol added (µg)	Theoretical content (µg/injection)	HPLC	
			Experimental content (ug/injection) ± SD	Recovery (%) ± SD
1	10	0.3505	0.3594 ±0.0171	102.54 ±4.75
2	50	0.9344	0.9273 ± 0.0389	99.24 ± 4.19
3	90	1.2682	1.2241 ± 0.0533	96.52 ± 4.36
4	130	1.6776	1.6238 ± 0.0555	97.24 ± 3.40
5	170	1.9287	1.9320 ±0.0782	100.17 ± 4.05
6	210	2.1307	2.0921 ± 0.0847	98.19 ± 4.05

Table 8 Accuracy of TLC densitometric method.

Sample number	Oxyresveratrol added (µg)	Theoretical content(µg/spot)	TLC	
			Experimental content (ug/spot) ± SD	Recovery (%) ± SD
1	10	0.3505	0.3493± 0.0143	99.65 ± 4.07
2	50	0.9344	0.9349± 0.0300	100.05 ± 3.21
3	90	1.3572	1.37458±0.0467	101.288±3.44
4	130	1.6776	1.7204± 0.0540	102.55 ± 3.22
5	170	1.9287	1.9116± 0.0589	99.11 ± 3.05
6	210	2.1307	2.1860± 0.0643	102.60 ± 3.02

2.4.3 Precision

The extract (1 mg) was dissolved in the methanol (10 ml) to give a solution with an accurate concentration of 0.1 mg/ml (SM1). The AUC and concentration of oxyresveratrol was calculated from the standard curve. The mean concentration was then calculated from 8 sucessive injections of the sample (HPLC method). The mean concentration was then calculated from 9 spots of the sample (TLC densitometric method). The precision was expressed as percent coefficient of variation (% CV), which was obtained from the standard deviation (SD).

Table 9 Precision of HPLC method (n=8)

Intra day			Inter day		
Spot number	AUC of SM1 (0.1 mg/ml) 2 µg	Concentation (µg/injection)	Spot number	AUC of SM1 (0.1 mg/ml) 2 µg	Concentation (µg/injection)
1	14580816	1.540524	1	14763915	1.563197
2	14504556	1.531081	2	16168672	1.737150
3	16163526	1.736512	3	16069212	1.724833
4	15143544	1.610207	4	15101928	1.605054
5	15658686	1.673998	5	15658835	1.674016
6	15455945	1.648892	6	16163528	1.736513
7	16037244	1.720875	7	14964828	1.588077
8	15285222	1.627751	8	16469443	1.774394
Mean		1.63623	Mean		1.675404
SD		0.07537	SD		0.080139
% CV		4.61	% CV		4.78
Total Mean (intra+inter day)			1.655817		
SD			0.077832		
% CV			4.70		

Table 10 Precision of TLC densitometric method (n=9)

Intra day			Inter day		
Spot number	AUC of SM1 (0.1 mg/ml) 2 µg	Concentation (µg/spot)	Spot number	AUC of SM1 (0.1 mg/ml) 2 µg	Concentation (µg/ spot)
1	11893.44	1.587013	1	11778.51	1.570186
2	12208.91	1.633203	2	12294.66	1.645759
3	12287.44	1.644701	3	12621.54	1.693619
4	12388.02	1.659428	4	12761.39	1.714096
5	12222.95	1.635259	5	13220.68	1.781343
6	12099.19	1.617139	6	13073.30	1.759764
7	12238.30	1.637507	7	13083.51	1.761259
8	12495.18	1.675118	8	13152.39	1.771345
9	12461.05	1.670121	9	13229.23	1.782595
Mean		1.64	Mean		1.72
SD		0.0273	SD		0.0727
% CV		1.66	% CV		4.23
Total Mean (intra+inter day)			1.68		
SD			0.0673		
% CV			4.01		

2.4.4 Limit of Detection and Limit of Quantitation

A plot of experimental concentration against SD was performed to reflect a linear correlation, and the value of the SD on the Y-axis intercept was obtained. This intercept value was then multiplied by a factor of 3 to give the limit of detection, or by 10 to give the limit of quantitation according to the protocols described by Taylor (Taylor, J.K. 1987).

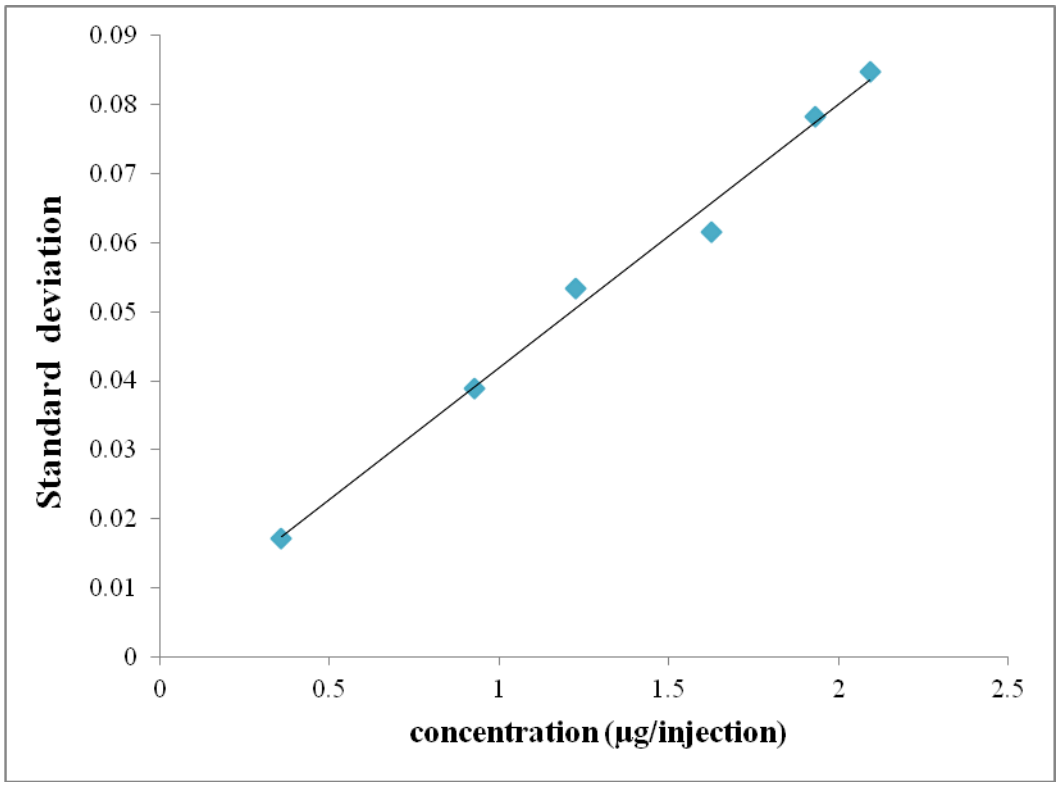


Figure 8 Plot of oxyresveratrol content in spiked samples vs. SD (HPLC method)

Y= 0.0383x+0.0036 (r^2= 0.9913)

Y-intercept = 0.0036 µg/injection

LOD = 3 × Y-intercept

LOD = 3 × 0.0036= 0.0108 µg/injection

LOQ = 10 × Y-intercept

LOQ = 10 × 0.0036 = 0.036 µg/injection

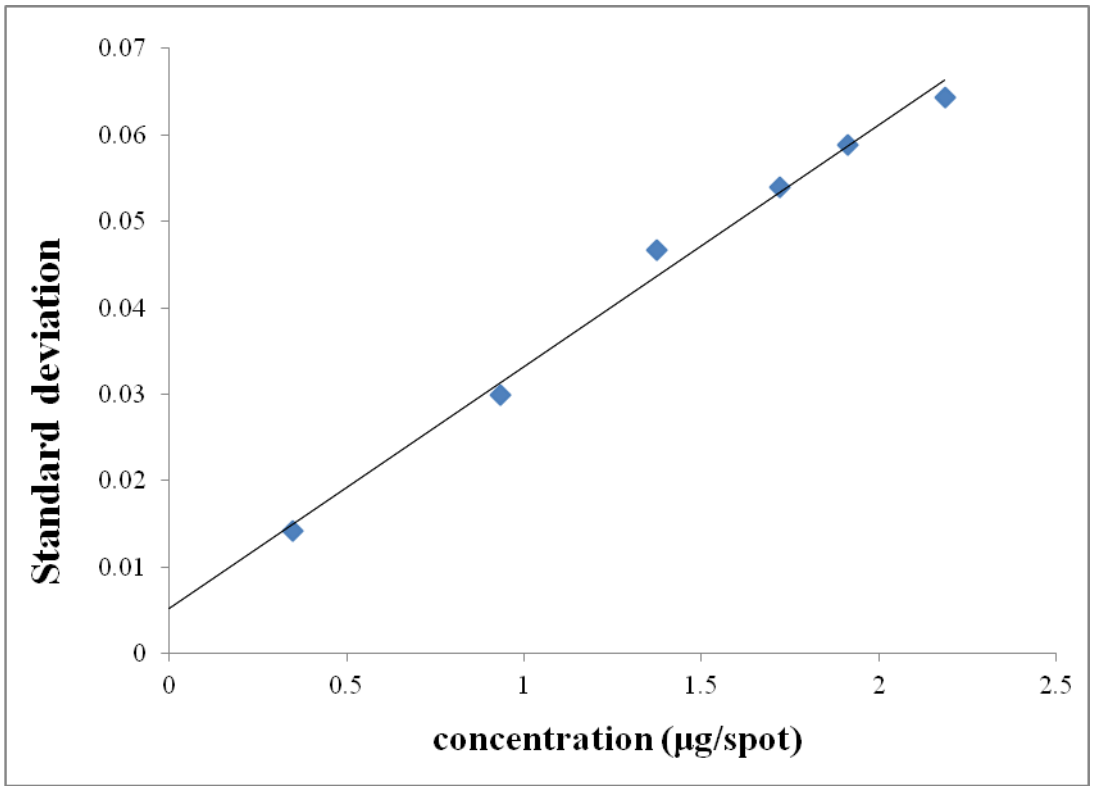


Figure 9 Plot of oxyresveratrol content in spiked samples vs. SD (TLC densitometric method)

Y= 0.0280x+0.0052 (r^2= 0.9909)

Y-intercept = 0.0052 µg/spot

LOD = 3 × Y-intercept

LOD = 3 × 0.0052= 0.0156 µg/spot

LOQ = 10 × Y-intercept

LOQ = 10 × 0.0052 = 0.052 µg/spot

3. *In Vitro* Cultures of *A. lakoocha*

3.1 Callus cultures

The seeds of *A. lakoocha* were surface sterilized and germinated on free hormone MS medium (Murashige and skoogs, 1962), B5 medium (Gamborg, 1968), SH medium (Schenk and Hildebrandt 1939) and Woody plant medium (Lloyd and Mc. Cown, 1980). After seedling 1-2 months, the calli derived on the explants were transferred to the same medium with various combinations of 2,4-dichlorophenoxyacetic acid (2,4-D) and benzyladenine (BA); 1-Naphthaleneacetic acid (NAA) and 6-Furfurylaminopurine (kinetin). Explants from 1–2 months old

sterile seedlings were used for induction of callus cultures. Callus formed after 3 weeks from explants was then subcultured onto fresh medium every 3 weeks. WPM medium was found to be most suitable for callus induction and seed germination. Explants were inoculated on agar solidified (0.8%, w/v) WPM basal medium with 2% sucrose and incorporated with 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 1 mg/l benzyladenine (BA). All the cultures were incubated at 25±2 ◦C under fluorescent light (16/8 photoperiod). Cultures were maintained by subculturing and transferring after every 25 days. The induced calli were subcultured on the same medium in 3 months for mass production and then transferred to the dark for over 6 months for accumulated secondary metabolited.

3.2 Cell Suspension Cultures

Cell suspension cultures were initiated by inoculation of 1 g fresh weight of friable callus into a 125 ml Erlenmeyer flask containing 25 ml of liquid WPM medium supplemented with 1 mg/l 2, 4-dichlorophenoxyacetic acid (2,4-D), 1 mg/l benzyladenine (BA) and 20 g/l sucrose. The flasks were placed on the rotary shaker (100 rpm) at 25 ± 2 ˚C in darkness. Subculturing was done every 3 weeks.

3.3 Study on the Relationship between the Growth of Callus Cultures and Secondary Metabolites Production in Callus Cultures

Calli derived from the aerial part of sterilized explants were maintained on WPM medium supplemented with 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 1 mg/l benzyladenine (BA) and 20 g/l sucrose. Subculturing was done every 3 weeks over 6 months in dark condition.Practically, 1g FW of calli was transferred to the fresh medium. The culltures were were grown under the dark condition at 25 ± 2 ˚C. Each treatment will be done in three replicates.

The dry weight of callus culture were determined at different ages of 5, 10, 12, 15, 17, 20, 22 and 25 day-after-culture. The secondary metabolite contents were also measured using TLC densitometric method as described above.

4. General Techniques for Isolation of Secondary Metabolites in Callus Cultures

4.1 Analytical Thin-layer Chromatography (TLC)

Technique	:	One dimension, ascending
Adsorbent	:	Siliga gel 60 F_{254} (E. Merck) percoated plate
Layer thickness	:	0.25 mm
Distence	:	8 cm

Temperature : Laboratory temperature (30-35 ˚C)

Detection : 1. Ultraviolet light (254 and 365 nm)

2. 50% H_2SO_4 in H_2O and heating at 105 ˚C for 10 min.

4.2 Column Chromatography

4.2.1 Vacuum Liquid Column Chromatography

Adsorbent : Silica gel 60 particle size 0.063-0.200 nm (70-230 mesh ASTM) (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 observing under UV light (254 and 365 nm), and 50% H_2SO_4 spraying reagent in hot condition.

4.2.2 Porus Polymer gel Chromatography

Adsorbent : Diaion HP-20

Packing method : Wet packing

Sample loading : The sample was dissolved in water and then placed on top of the column.

Detection : Fractions were examined by TLC observing under UV light (254 and 365 nm) and spraying with sulfuric acid and then heating at 105 ˚C for 10 min.

4.2.3 High Pressure Liquid Chromatography (HPLC)

Semi-preparative column : 1. TSK-Gel ODS-100V, 5 μm, 2 × 25 cm (Tosoh Chemicals Co., Ltd.)

2. Capcell Pak ODS, 5 μm, 2 × 25 cm (Shiseido Fine Chemical Co., Ltd.)

Analytical column : Capcell Pak ODS, 5 μm, 0.46 × 25 cm

Flow rate : 1. 1 ml/min for analytical column

2. 9 ml/min for semi-preparative column

Mobile phase : methanol or acetonitrile in water

Sample preparation : The sample was dissolved in a small amount of

		eluent and injected into the column.
Injection volume	:	10 µl for analytical column 500 µl for semi-preparative column
Pump	:	1. 880-PU (JASCO) for semi-preparative column and analytical column 2. PU-2089 Plus (JASCO) for semi-preparative column
Detector	:	UV-Vis detector 875-UV (JASCO) variable-wavelength
Recorder	:	1. Hitachi 561 2. Sekonic SS-250 F
Temperature	:	Room temperature

4.3 Spectroscopy

4.3.1 Ultraviolet (UV) Absorption Spectra

UV (in methanol) spectra were obtained on a Milton Roy Spectronic 3000 Array spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Science, Chulalongkorn University).

4.3.2 Mass Spectra

Mass spectra were recorded on a Bruker micro TOF mass spectrometer (National Center for Genetic Engineering and Biotechnology).

4.3.3 Proton and Carbon-13 Nuclear Magnetic Resonance (^{1}H and ^{13}C-NMR) Spectra

^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a JEOL JMN-α 400 instrument. (School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan)

Solvent for NMR spectra were deuterated acetone, deuterated methanol. Chemical shifts were given in δ (ppm) with the solvent as an internal standard.

4.4 Optical Rotations

Optical rotations were measured on a JASCO DIP-360 digital polarimeter. (School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan)

4.5 Solvents

Throughout this work, all organic solvents were of commercial grade and were redistilled prior to use.

5. Isolation and Extraction of Callus Cultures

A study on the isolation and extraction used callus cultures in day 17 of callus growth. The dried callus of *Artocarpus lakoocha* (40 g) was extracted three times with ethanol (3 × 500 ml) by maceration method. The extracts were combined and evaporated under reduced pressure to yield 8 g of viscous mass.

The water soluble extract was subjected to HP-20 column chromatography, using 50% methanol (4 l), 75 % methanol (7 l) and 100% methanol (7 l), respectively as elutents, to yield 50% methanol fraction (0.597 g), 75 % methanol fraction (1.074 g) and 100% methanol fraction (2.016 g) eluate, respectively after removal of the solvent.

The 50 % methanol fraction (0.597 g) was chromatographed on a silica gel column, using chloroform-methanol (95:5) 500 ml, (9:1) 200 ml, (8:2) 300 ml and then fractionated using chloroform-methanol-water (8:2:0.2) 300 ml. Fractions were collected and pooled by TLC analysis to afford 8 combined fractions.

From the combined fractions, fraction 6 (0.133 g) was subjected to semipreparative HPLC [ODS-EP, 20 × 250 mm; solvent, methanol-water (15:85), UV-detector 205 nm, room temperature] to yield aesculin (**CAL13**) (4 mg, t_R 32 min) and (–)-catechin (**CAL12**) (2 mg, t_R 88 min).

The 75 % methanol fraction (1.074 g) was chromatographed on a silica gel column and fractionated using hexane: chloroform-methanol (95:5); 1:3 400 ml, hexane: chloroform-methanol (92:8); 1:3 400 ml, chloroform-methanol (88:22) 500 ml, chloroform-methanol (8:2) 100 ml and then chloroform-methanol (1:1) 40 ml. Fractions were collected and pooled by TLC analysis to afford 8 combined fractions.

From the combined fractions, fraction 75MF2 (0.046 g) was subjected to semipreparative HPLC [ODS-EP, 20 × 250 mm; solvent, methanol-water (65:35), UV-detector 205 nm, room temperature] to yield compound **CAL1** (2.8 mg, t_R 42 min).

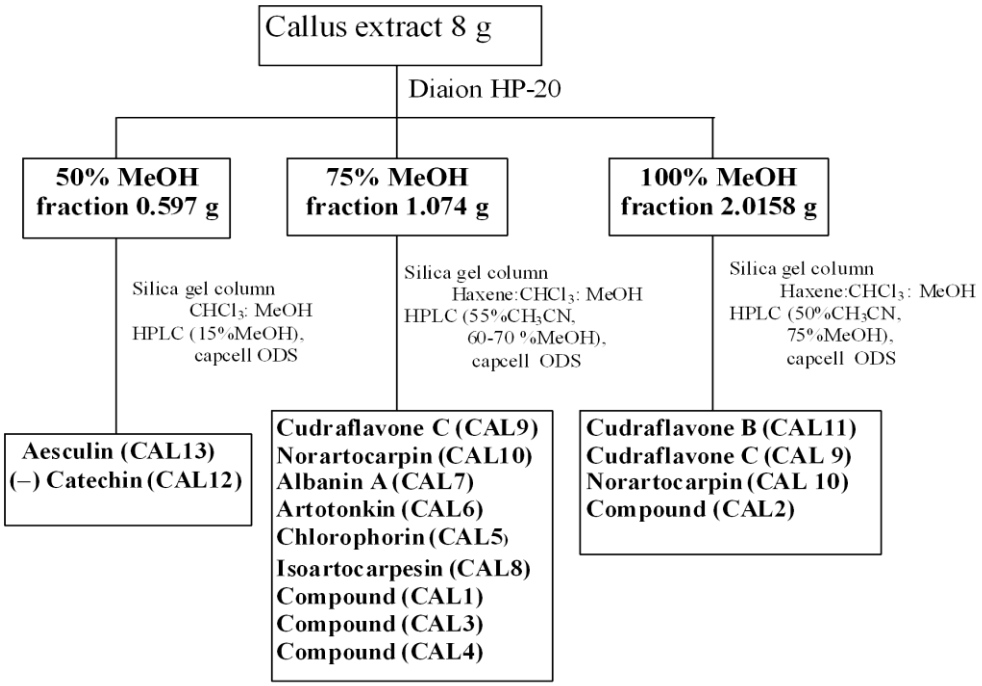
From the combined fractions, fraction 75MF3 (0.267 g) was subjected to semipreparative HPLC [ODS-EP, 20 × 250 mm; solvent, methanol-water (70:30), UV-detector 205 nm, room temperature] to yield compound **CAL3** (5 mg, t_R 25 min), compound **CAL4** (5.8 mg, t_R 28 min) cudraflavone C (**CAL9**) (49 mg, t_R 77 min) and Norartocarpin (**CAL10**) (99 mg, t_R 91 min)

From the combined fractions, fraction 75MF4 (0.102 g) was subjected to semipreparative HPLC [ODS-EP, 20 × 250 mm; solvent, methanol-water (70:30), UV-detector 205 nm, room temperature] to yield albanin A (**CAL7**) (10 mg, t_R 17 min), artotonkin (**CAL6**) (28.7 mg, t_R 23 min), chlorophorin (**CAL5**) (4 mg, t_R 30 min) and isoartocarpesin (**CAL8**) (7 mg, t_R 39 min).

The 100 % methanol fraction (2.016 g) was chromatographed on a silica gel column and eluted with hexane-(chloroform-methanol) [1:3 (95:5)] 600 ml, chloroform-methanol (95:5) 550 ml, (92:8) 300 ml, chloroform-methanol (88:12) 200 ml and (1:1) 200 ml. Fractions were collected and pooled by TLC analysis to afford 9 combined fractions.

From the combined fractions, fraction 100MF3 (0.099 g) was subjected to semipreparative HPLC [ODS-EP, 20 × 250 mm; solvent, methanol-water (75:25), UV-detector 205 nm, room temperature] to yield cudraflavone B (**CAL11**) (52.3 mg, t_R 60 min).

From the combined fractions, fraction 100MF5 (0.149 g) was subjected to semipreparative HPLC [ODS-EP, 20 × 250 mm; solvent, acetonitrile-water (50:50), UV-detector 205 nm, room temperature] to yield compound **CAL2** (1.7 mg, t_R 60 min), cudraflavone C (**CAL9**) (8 mg, t_R 93 min) and norartocarpin (**CAL10**) (19.8 mg, t_R 111 min)



Scheme 1 Separation of the callus cultures of *Artocarpus lakoocha*

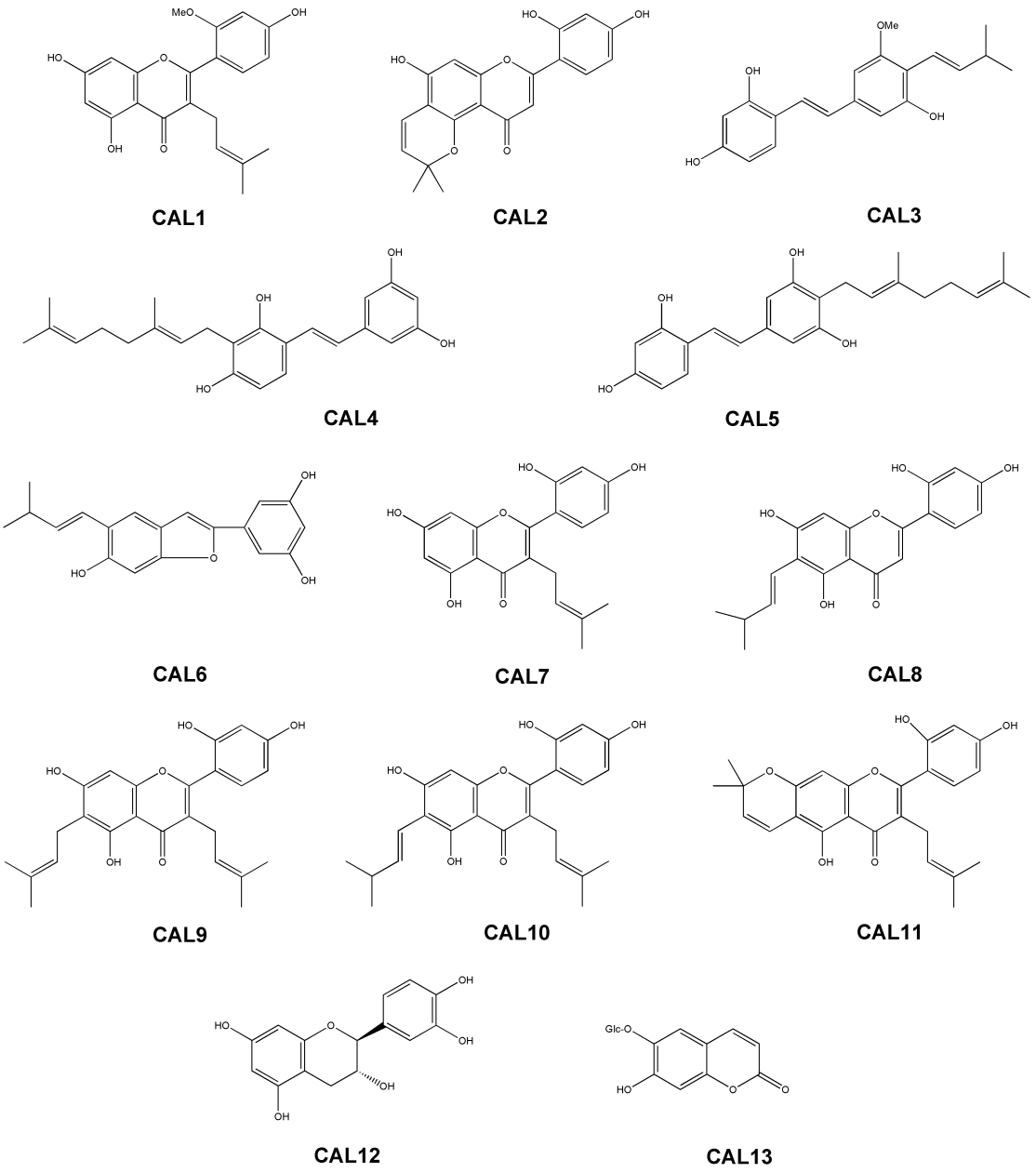


Figure 10 Compounds from callus cultures of *A. lakoocha*

6. Physical and Spectral Data of Isolated Compounds

6.1 Compound CAL1

Compound CAL1 was obtained as a yellow amorphous solid, soluble in methanol (2.8 mg, 7×10^{-3} % based on dried weight of callus cultures).

HRESIMS : $[M+H]^+$ at *m/z* 369.1340 (calcd for $C_{21}H_{21}O_6$ 369.1333), Figure 29

UV : λ_{max} nm (log ε), in methanol
209 (4.50), 242(4.28), 258 (4.32), 303 (3.92), Figure 30

^{1}H NMR : δ ppm, 400 MHz, in methanol-d_4; see Table 12, Figure 31

^{13}C NMR : δ ppm, 100 MHz, in methanol-d_4; see Table 12, Figure 32

6.2 Compound CAL2

Compound CAL2 was obtained as a yellow amorphous solid, soluble in acetone (1.7 mg, 3.75×10^{-3} % based on dried weight of callus cultures).

HRESIMS : $[M+H]^+$ at *m/z* 353.1015 (calcd for $C_{20}H_{16}O_6$, 353.1020), Figure 36

UV : λ_{max} nm (log ε), in methanol
221 (4.39), 287 (4.29), 346 (4.29), Figure 37

^{1}H NMR : δ ppm, 400 MHz, in acetone-d_6; see Table 13, Figure 38

^{13}C NMR : δ ppm, 100 MHz, in acetone-d_6; see Table 13, Figure 39

6.3 Compound CAL3

Compound CAL3 was obtained as a white powder, soluble in acetone (5 mg, 12.5×10^{-3} % based on dried weight of callus cultures).

HRESIMS : $[M+H]^+$ at *m/z* 327.1621 (calcd for $C_{20}H_{22}O_4$ 327.1597), Figure 42

UV : λ_{max} nm (log ε), in methanol
218 (5.38), 346 (5.39), Figure 43

^{1}H NMR : δ ppm, 400 MHz, in acetone-d_6; see Table 14, Figure 44

^{13}C NMR : δ ppm, 100 MHz, in acetone-d_6; see Table 14, Figure 45

6.4 Compound CAL4

Compound CAL4 was obtained as a white amorphous solid, soluble in acetone (5 mg, 12.5×10^{-3} % based on dried weight of callus cultures).

HRESIMS : $[M+H]^+$ at *m/z* 381.2076 (calcd for $C_{24}H_{28}O_4$ 381.2067) Figure 49

UV : λ_{max} nm (log ε), in methanol
211 (4.58), 330 (4.27), Figure 50

^{1}H NMR : δ ppm, 400 MHz, in acetone-d_6; see Table 15, Figure 51

^{13}C NMR : δ ppm, 100 MHz, in acetone-d_6; see Table 15, Figure 52

6.5 Compound CAL5

Compound CAL5 was obtained as a white amorphous solid, soluble in acetone (4 mg, 10×10^{-3} % based on dried weight of callus cultures).

ESIMS **:** $[M+H]^+$ at *m/z* 381.21, Figure 55

UV **:** λ_{max} nm (log ε), in methanol
220 (3.37), 261 (2.73), 314 (3.95), Figure 56

^{1}H NMR **:** δ ppm, 400 MHz, in acetone- d_6; see Table 16, Figure 57

^{13}C NMR **:** δ ppm, 100 MHz, in acetone- d_6; see Table 16, Figure 58

6.6 Compound CAL6

Compound CAL6 was obtained as a white amorphous solid, soluble in acetone (28.7 mg, 71.8×10^{-3} % based on dried weight of callus cultures).

ESIMS **:** $[M+H]^+$ at *m/z* 481.21, Figure 59

UV : λ_{max} nm (log ε), in methanol
228 (4.57), 302 (4.33), 339 (4.49), Figure 60

^{1}H NMR : δ ppm, 400 MHz, in acetone- d_6; see Table 17, Figure 61

^{13}C NMR : δ ppm, 100 MHz, in acetone- d_6; see Table 17, Figure 62

6.7 Compound CAL7

Compound CAL7 was obtained as a yellow amorphous solid, soluble in acetone (10 mg, 20.5×10^{-3} % based on dried weight of callus cultures).

ESIMS **:** $[M+H]^+$ at *m/z* 355.12, Figure 63

UV : λ_{max} nm (log ε), in methanol
210 (4.50), 256 (4.55), Figure 64

^{1}H NMR : δ ppm, 400 MHz, in acetone- d_6; see Table 18, Figure 65

^{13}C NMR : δ ppm, 100 MHz, in acetone- d_6; see Table 18, Figure 66

6.8 Compound CAL8

Compound CAL8 was obtained as a yellow amorphous solid, soluble in methanol (7 mg, 17.5×10^{-3} % based on dried weight of callus cultures).

ESIMS **:** $[M+H]^+$ at *m/z* 355.12, Figure 67

UV : λ_{max} nm (log ε), in methanol
211 (4.75), 287 (4.66), 347 (4.74), Figure 68

^{1}H NMR : δ ppm, 400 MHz, in methanol-d_4; see Table 19, Figure 69

^{13}C NMR : δ ppm, 100 MHz, in methanol-d_4; see Table 19, Figure 70

6.9 Compound CAL9

Compound CAL9 was obtained as a yellow amorphous solid, soluble in acetone (118 mg, 0.295 % based on dried weight of callus cultures).

ESIMS **:** $[M+H]^+$ at *m/z* 423.18, Figure 71

UV : λ_{max} nm (log ε), in methanol
212 (4.50), 262 (4.35), 312 (4.02), Figure 72

^{1}H NMR : δ ppm, 400 MHz, in acetone- d_6; see Table 20, Figure 73

^{13}C NMR : δ ppm, 100 MHz, in acetone- d_6; see Table 20, Figure 74

6.10 Compound CAL10

Compound CAL10 was obtained as a yellow amorphous solid, soluble in acetone (57 mg, 0.143 % based on dried weight of callus cultures).

ESIMS **:** $[M+H]^+$ at *m/z* 423.18, Figure 75

UV : λ_{max} nm (log ε), in methanol
210 (4.07), 279 (4.54), 325 (4.14), Figure 76

^{1}H NMR : δ ppm, 400 MHz, in acetone- d_6; see Table 21, Figure 77

^{13}C NMR : δ ppm, 100 MHz, in acetone- d_6; see Table 21, Figure 78

6.11 Compound CAL11

Compound CAL11 was obtained as a yellow amorphous solid, soluble in acetone (52.3 mg, 0.131 % based on dried weight of callus cultures).

ESIMS **:** $[M+Na]^+$ at *m/z* 423.18, Figure 79

UV : λ_{max} nm (log ε), in methanol
210 (4.23), 278 (4.52), 330 (4.05), Figure 80

^{1}H NMR : δ ppm, 400 MHz, in acetone- d_6; see Table 22, Figure 81

^{13}C NMR : δ ppm, 100 MHz, in acetone- d_6; see Table 22, Figure 82

6.12 Compound CAL12

Compound CAL12 was obtained as a yellow amorphous solid, soluble in methanol (2 mg, 5×10^{-3} % based on dried weight of callus cultures).

ESIMS **:** $[M+H]^+$ 291.09 at *m/z* , Figure 83

$[\alpha]^{28}_D$: – 48.0 ° (*c* 0.1; MeOH)

UV : λ_{max} nm (log ε), in methanol
211 (3.32), 280 (2.50), Figure 84

^{1}H NMR : δ ppm, 400 MHz, in methanol-d_4; see Table 23, Figure 85

^{13}C NMR : δ ppm, 100 MHz, in methanol-d_4; see Table 23 , Figure 86

6.13 Compound CAL13

Compound CAL13 was obtained as a yellow amorphous solid, soluble in methanol (4 mg, 10×10^{-3} % based on dried weight of callus cultures).

ESIMS **:** $[M+H]^+$ at *m/z* 341.08, Figure 87

UV : λ_{max} nm (log ε), in methanol
207 (4.39), 221 (4.35), 331 (3.98), Figure 88

^{1}H NMR : δ ppm, 400 MHz, in methanol-d_4; see Table 24 , Figure 89

^{13}C NMR : δ ppm, 100 MHz, in methanol-d_4; see Table 24, Figure 90

7. Analysis of Secondary Metabolites in the Callus Cultures of *A. lakoocha*.

7.1 Preparation of crude extracts of callus cultures

The dried calli of *A. lakoocha* (100 mg) were extracted with 10 ml ethanol by maceration for 24 h, followed by sonicated at 50˚ C for 20 min and centrifugation at 14,00 rpm for 10 min The supernatant part was concentrated by evaporator to give a dried extract. This extract was dissolved in 0.5 ml methanol to give a sample solution.

In our cell cultures of *A lakoocha*, thirteen compounds were isolated and characterized. Only a trace amount of oxyresveratrol was detected on TLC densitometric plate (Fig 11) in the same condition used for analysis of oxyresveratrol in the heartwood of *A lakoocha* (methylene chloride/ methanol (85: 15)). Comparison of the UV–vis spectra of the callus extract with reference that of standard confirmed the finding. (Fig 12), However, the compound could not be fully identified.

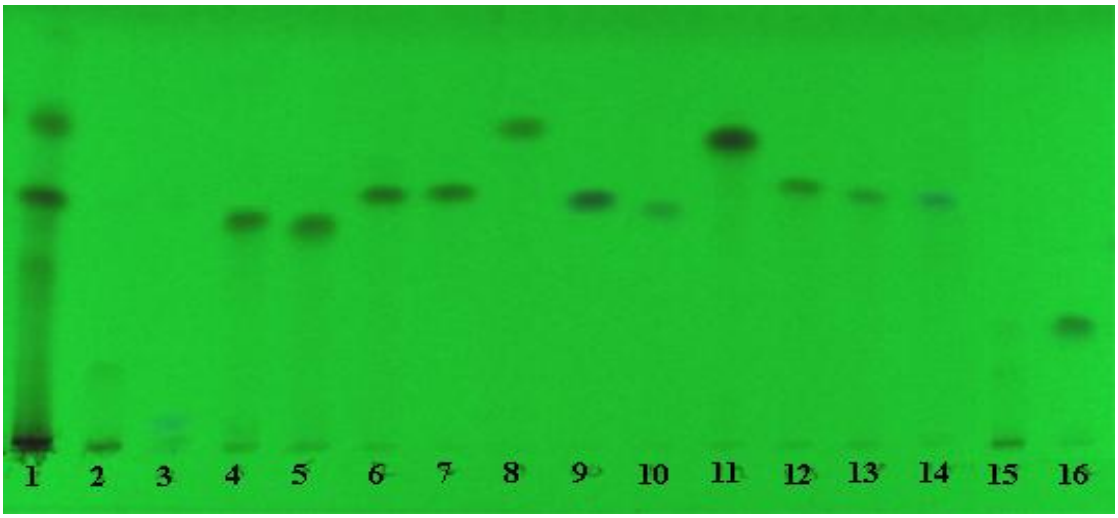


Figure 11 TLC pattern of isolated 13 compounds from *A. lakoocha* callus cultures

1 = callus extract
2 = CAL12 (–catechin)
3 = CAL13 (aesculin)
4 = CAL7 (albanin A)
5 = CAL8 (isoartocarpesin)
6 = CAL10 (norartocarpin)
7 = CAL9 (cudraflavone C)
8 = CAL11 (cudraflavone B)
9 = CAL6 (artotonkin)
10 = CAL5 (chlorophorin)
11 = CAL 1
12 = CAL 2
13 = CAL 3
14 = CAL 4
15 = 50 MeOH-F5 fraction
16 = authentic oxyresveratrol

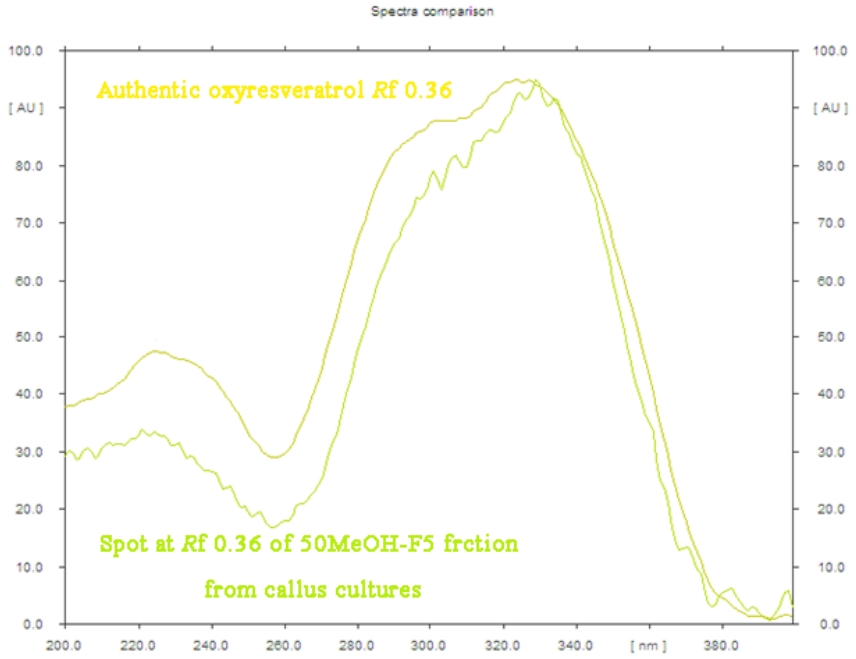


Figure 12 UV absorbtion spectrum of authentic oxyresveratrol and 50MeOH-F5 fraction of similar *R*f value to oxyresveratrol which was separated from callus extract

The major metabolites were found to be isoartocarpesin, norartocarpin and cudraflavone B, which were separated from other compounds in callus extract. These compounds were subjected to comparison of their UV–Vis spectra with reference standards. (Figures 13-15).

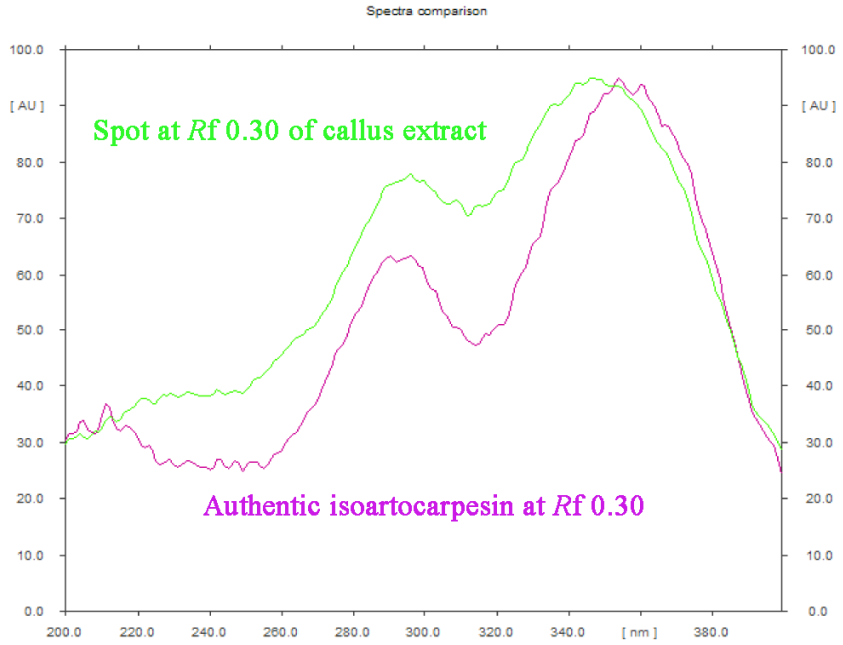


Figure 13 UV absorbtion spectrum of authentic isoartocarpesin and compound of similar *R*f value to isoartocarpesin which was separated from callus extract

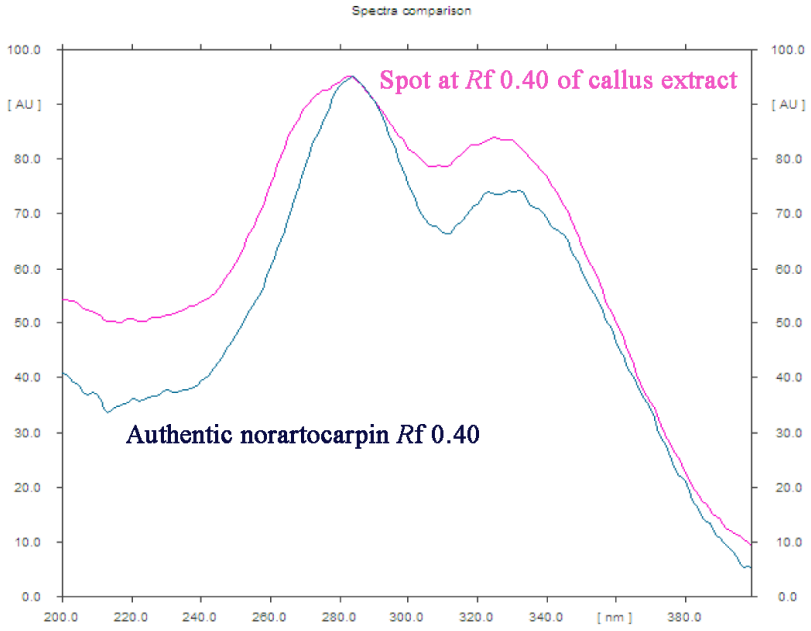


Figure 14 UV absorbtion spectrum of authentic norartocarpin and compound of similar *R*f value to norartocarpin which was separated from callus extract

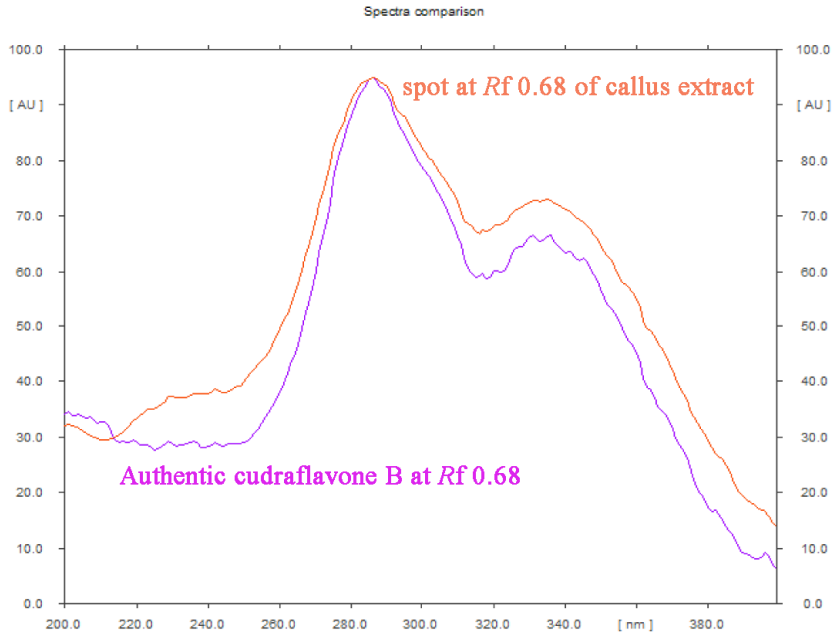


Figure 15 UV absorbtion spectrum of authentic cudraflavone B and compound of similar *R*f value to cudraflavone B which was separated from callus extract

7.2 Preparation of Standard Solutions

Isoartocarpin, norartocarpin and cudraflavone B were isolated from callus of *A. lakoocha*. For norartocarpin and cudraflavone B, the standard solution of each compound with an accurate concentration of 0.16 mg/ml was prepared by dissolving the compound (1.6 mg) in methanol (10 ml). From this solution, 4 additional standard solutions (0.08, 0.04, 0.02 and 0.01 mg/ml) were prepared by serial dilution. For isoartocarpin, a standard solution with an accurate concentration of 0.16 mg/ml was prepared by dissolving the sample (1.6 mg) in methanol (10 ml). From this solution, 7 additional standard solutions (0.08, 0.04, 0.02, 0.01, 0.005, 0.0025 and 0.00125 mg/ml) were obtained by serial dilution.

7.3 TLC-densitometric Conditions

Chromatography was performed on a TLC aluminum sheet (silica gel 60 F_{254} plate, 20 × 10 cm) using methylene chloride/ methanol/toluene (75: 7: 18) with sulfuric acid 0.1 % as the mobile phase. Each sample (10 µl) was applied as a band (5 × 0.30 mm) in triplicate. Plates were developed in a glass tank preequilibrated with the mobile phase. The solvent was allowed to run up the plate to a height of 8 cm. Chromatograms were evaluated by measuring the peak area with the TLC scanner in the absorbance mode at 254 nm.

7.4 Standard Calibration Curve

A standard calibration curve of each compound was produced for TLC densitometric analysis. The calibration curve of standard norartocarpin showed linearity of the relationship from 0.01-0.16 mg/ml, and the correlation coefficient was 0.9981. The calibration curve of standard cudraflavone B showed linearity of the relationship from 0.1-1.6 mg/ml, and the correlation coefficient was 0.9906. The calibration curve of isoartocarpesin showed linearity of the relationship from 0.00125-0.04 mg/ml, and the correlation coefficient was 0.9957.

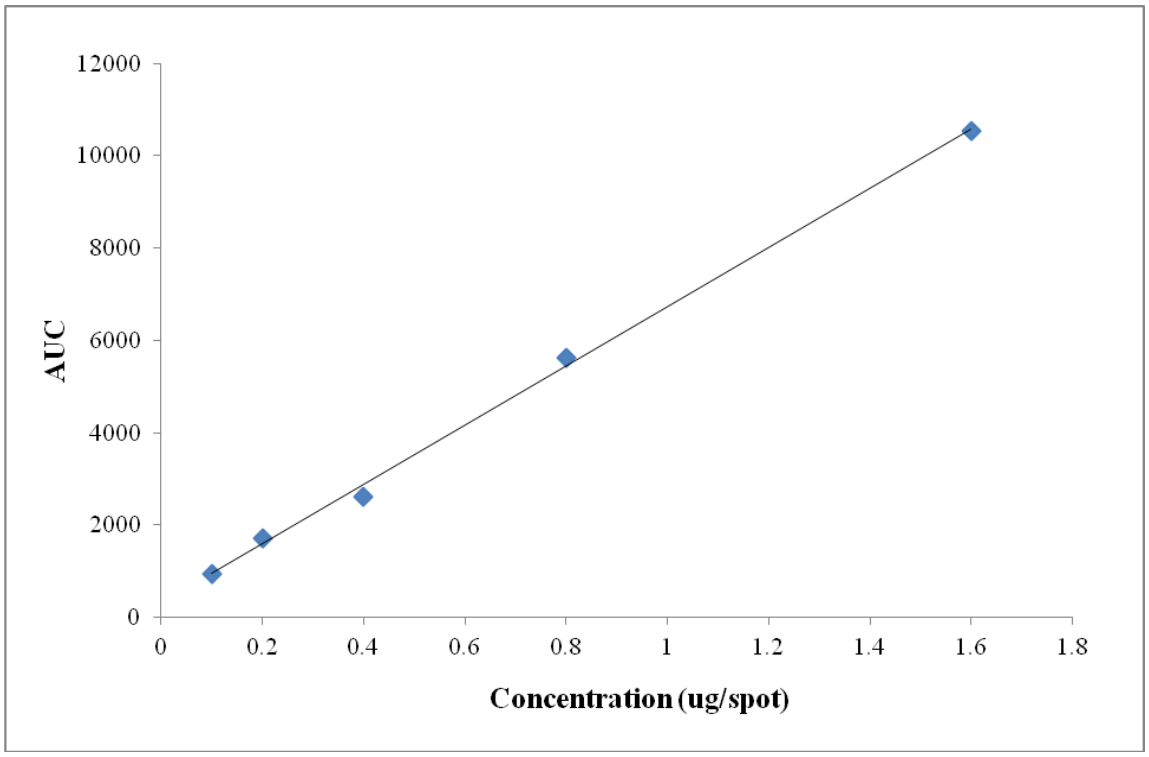


Figure 16 The Calibration curve of Norartocarpin

$Y = 6414.4x + 305.22$ (r^2=0.9981)

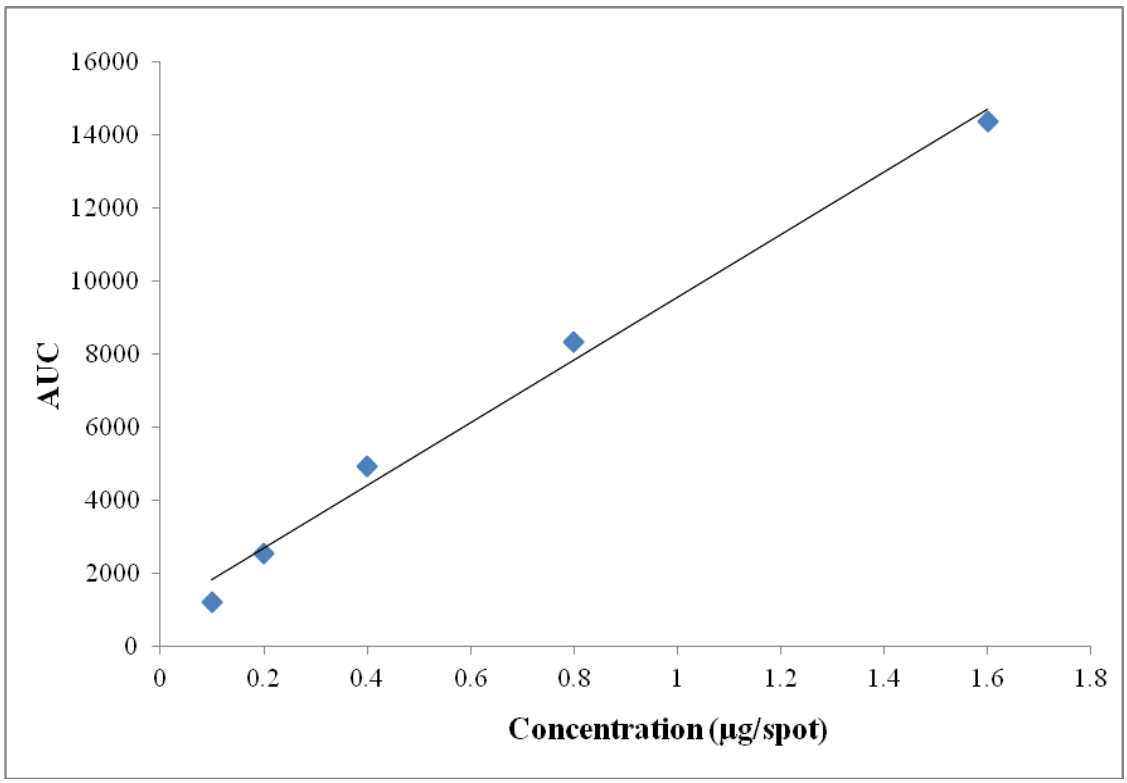


Figure 17 The calibration curve of Cudraflavone B

Y = 8586.40x + 950.31 (r^2=0.9906**)**

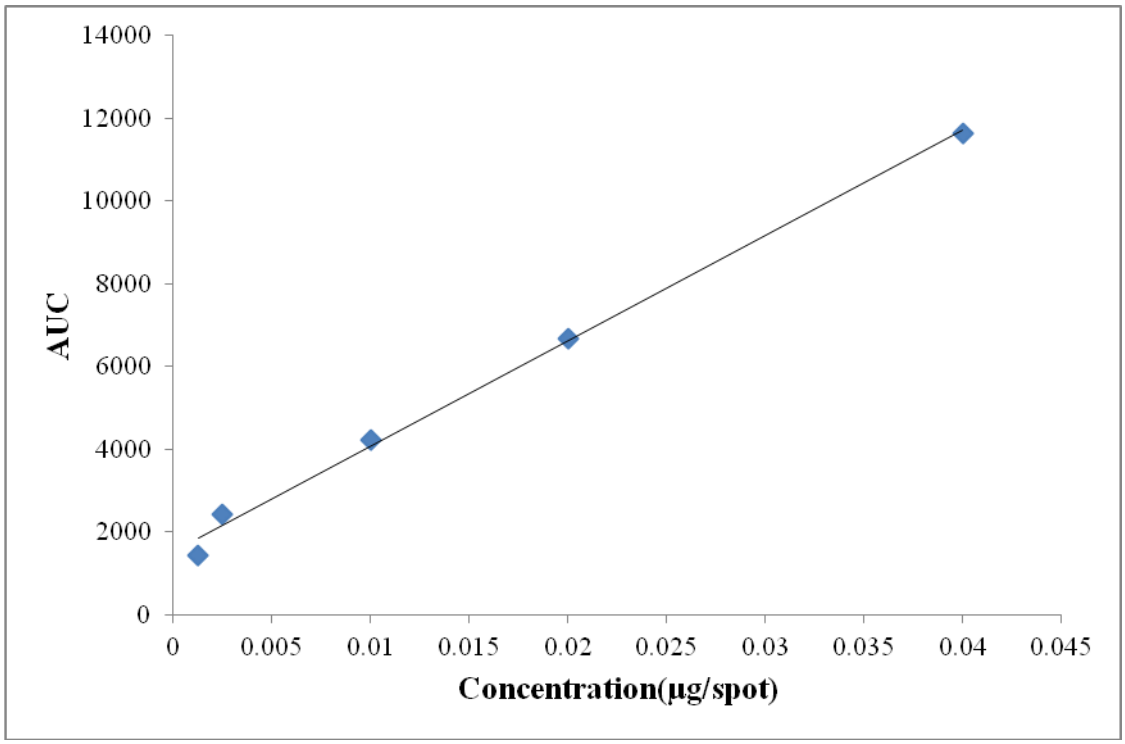


Figure 18 The calibration curve of Isoartocarpesin

Y = 254617x+ 1527.40 (r^2=0.9958**)**

8. Determination of Free Radical Scavenging Activity

8.1 Free Radical Scavenging Activity Assay

The assay was performed according to an established protocol (Braca *et al*., 2002).

8.2 Preparation of Test Sample

The test compound (1 mg) was dissolved in 1 ml of methanol (or suitable solvent) and diluted with methanol until a suitable range of concentration (mg/ml) was obtained. The concentration was expressed as μM in final concentration. For example, CAL7 (MW 354) at 1 mg/1ml was equal to 2825 μM [(1 mg × 10^3 × 1000 ml) /354]. For each well, 20 μl of test solution was added to the reaction mixture to furnish the total volume of 200 μl. The final concentration was calculated by the formula below.

$$N_1V_1 = N_2V_2$$

N_1 = Beginning concentration (μM)

V_1 = Beginning volume (μl)

N_2 = Final concentration (μM)

V_2 = Final volume (μl)

Thus, the final concentration of CAL7 solution = 2825 μM × 20 μl / 200 μl

= 282.5 μM

8.3 Preparation of DPPH Solution (100 μM)

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

8.4 Measurement of Activity

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

8.5 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_{50} evaluation of pure compounds, a graph showing concentration versus % DPPH reduction was plotted. The IC_{50} was calculated from the graph.

9. Determination of Tyrosinase Inhibitory Activity

In this study, tyrosinase inhibitory activity was determined by the dopachrome method using L-DOPA as the substrate. Dopachrome is one of the intermediate substances in the melanin biosynthesis. The red color of dopachrome can be detected by visible light. In this experiment a microplate reader (BIO-RAD, model 450) with 492 nm interference filter was used for detection. The potential tyrosinase inhibitor would show minimal dopachrome absorption. This method was modified from the methods of Masamoto (Masamoto, Iida, and Kubo, 1980; Iida *et al*., 1995 and Morita *et al*., 1994)

9.1 Preparation of the Reaction Mixture

9.1.1 Preparation of 20 mM phosphate buffer (pH 6.8)

Solution A: $NaH_2PO_4.2H_2O$ (312 mg) was dissolved in 100 ml of H_2O.

Solution B: Na_2HPO_4 (284 mg) was dissolved in 100 ml of H_2O.

Then, solutions A and B were mixed until pH 6.8 was reached.

9.1.2 Preparation of 0.8 mM L-DOPA

L-DOPA (0.8 mg) was dissolved in 5 ml of 20 mM phosphate buffer (pH 6.8).

9.1.3 Preparation of tyrosinase solution

Tyrosinase enzyme (0.5 mg) was dissolved in 5 ml of 20 mM phosphate buffer (pH 6.8).

9.1.4 Preparation of the test sample

One milligram of the test compound was dissolved in 3 ml of ethanol (or suitable solvent) and diluted with ethanol until a suitable range of concentrations (mg/ml) was obtained. The concentration was expressed as μM in the final calculation. For example, the concentration of compound CAL7 (MW 354) at 1 mg/3ml was equal to 942 μM [(1 mg $\times 10^3 \times$ 1000 ml) /(3 $\times$ 354)]. For each well, 20 μl of test solution was added to the reaction mixture to furnish the total volume of 200 μl. The final concentration was calculated by the formula below.

$$N_1V_1 = N_2V_2$$

N_1 = Beginning concentration (μM)

V_1 = Beginning volume (μl)

N_2 = Final concentration (μM)

V_2 = Final volume (μl)

Thus, the final concentration of CAL7 solution = 942 μM $\times$ 20 μl / 200 μl = 94.2 μM

9.2 Measurement of Activity

The reaction mixture (200 μl) was measured in four wells (A, B, C and D). In each well, the substance was added in the order of mixing, as follows;

A (control)	20 μl of mushroom tyrosinase solution (48 unit/ml)
	140 μl of 20 mM phosphate buffer (pH 6.8)
	20 μl of ethanol
B (blank of A)	160 μl of 20 mM phosphate buffer (pH 6.8)
	20 μl of ethanol
C (test sample)	20 μl of mushroom tyrosinase solution (48 unit/ml)
	140 μl of 20 mM phosphate buffer (pH 6.8)
	20 μl of test sample in ethanol
D (blank of C)	160 μl of 20 mM phosphate buffer (pH 6.8)
	20 μl of test sample in ethanol

After each well was mixed and preincubated at 25 ^{o}C for 10 min, 20 μl of 0.85 μM L-DOPA was added, and the mixture was incubated at 25 ^{o}C for 20 min. The absorbance of each well was measured at 492 nm with the microplate reader both before and after incubation.

9.3 Calculation of the Percent Inhibition of Tyrosinase Enzyme

The percent inhibition of tyrosinase reaction was calculated as follows.

$$\% \text{ Tyrosinase inhibition} = 100 \times [(A-B)-(C-D)] / (A-B)$$

A = The difference of optical density before and afer incubation at 492 nm without test sample

B = The difference of optical density before and afer incubation at 492 nm without test sample and enzyme

C = The difference of optical density before and afer incubation at 492 nm with test sample

D = The difference of optical density before and afer incubation at 492 nm with test sample, but without enzyme

9.4 Calculation of IC_{50}

After the % tyrosinase inhibition of the test solution in each concentration was obtained, a graph showing concentration against % tyrosinase inhibition was plotted. The IC_{50} (concentration at 50% tyrosinase inhibition) of each pure compound was then obtained from the graph.

CHAPTER IV

RESULTS AND DISCUSSION

1. Quantitative Analysis of Oxyresveratrol Content in *A. lakoocha* Heartwood and Puag-haad

Three samples of *A. lakoocha* heartwood (ALH-1 to ALH-3) were collected from natural habitats, while 11 samples (ALH-4 to ALH-14) were purchased from drugstores as indicated in Table 11. Two samples of the traditional drug Puag-Haad (PH-1 and PH-2) were obtained from drugstores (Table 11).

Table 11 Oxyresveratrol content in *A. lakoocha* heartwood and Puag-haad

Sample No.	Locality (site of collection)	Oxyresveratrol (mg/g) ± SD
Natural samples of *A. lakoocha* heartwood		
ALH-1	Chiang Rai (Northern province)	182.3 ± 0.18
ALH-2	Kanchanaburi (Western)	166.0 ± 0.80
ALH-3	Bangkok (Central)	49.0 ± 0.24
Commercial samples of *A. lakoocha* heartwood		
ALH-4	Nakhon Sawan (Central)	29.6 ± 0.11
ALH-5	Kamphaeng phet (Central)	31.6 ± 0.14
ALH-6	Nakhon Prathom (Central)	28.6 ± 0.12
ALH-7	Bangkok (Central)	45.2 ± 0.20
ALH-8	Bangkok (Central)	50.4 ± 0.23
ALH-9	Trang (Southern)	26.2 ± 0.11
ALH-10	Songkhla (Southern)	33.4 ± 0.17
ALH-11	Nakhon Sri Thammarat (Southern)	51.0 ± 0.18
ALH-12	Chon Buri (Eastern)	24.6 ± 0.12
ALH-13	Nakhon Ratchasima (Northeastern)	23.4 ± 0.11
ALH-14	Ubon Ratchathani (Northeastern)	69.6 ± 0.33
Samples of traditional drug ‘Puag-Haad’		
PH-1	Chiang Mai (Northern)	837.5 ± 4.04
PH-2	Chiang Rai (Northern)	780.1 ± 3.52

SD = standard deviation

The amounts of oxyresveratrol in 3 samples of *A. lakoocha* heartwood collected from natural habitats were 49.0–182.3 mg/g, whereas those in 11 commercial samples were in the range of 23.4–69.6 mg/g. The oxyresveratrol contents in 2 samples of traditional drug Puag-Haad were 780.1 and 837.5 mg/g

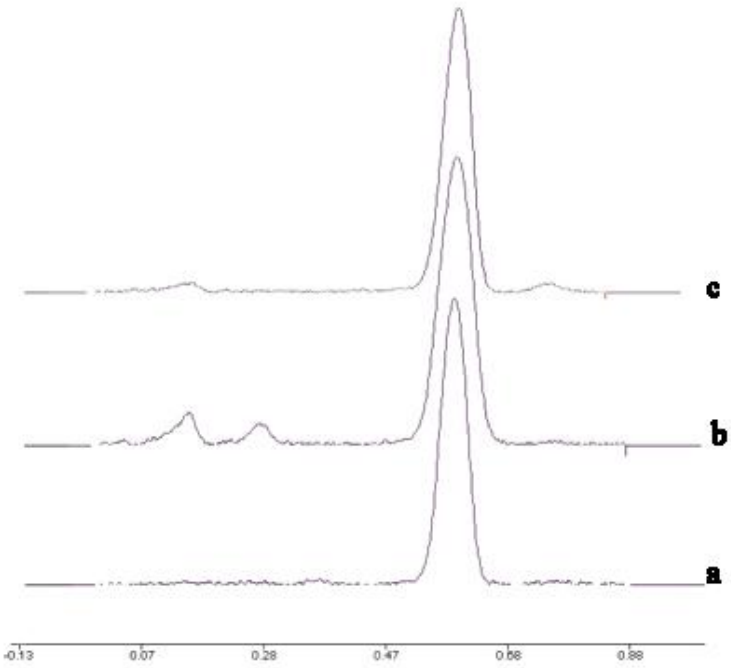


Figure 19 Representative TLC densitometric chromatograms.
a Standard sample of oxyresveratrol (0.80 μg/spot).
b Sample of *A. lakoocha* heartwood extract (1.60 μg/spot).
c Sample of Puag-Haad (2.00 μg/spot).

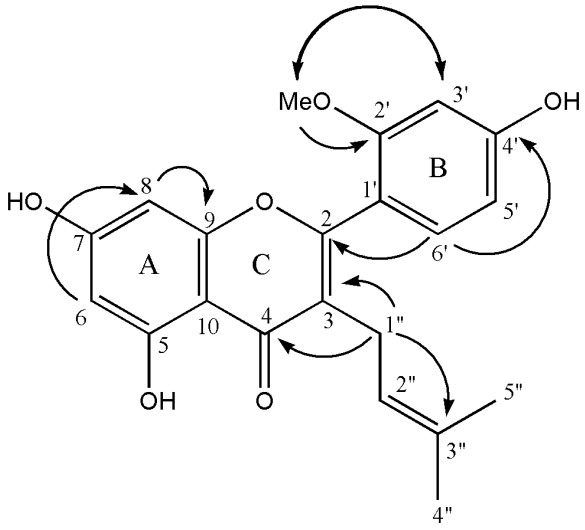
2. Structure Determination of Isolated Compounds

The structure determination of each isolates compound was performed by interpretation of their UV, MS and NMR data, and then comfirmed by comparison with previously reported values.

2.1 Structure Determination of Compound CAL1

Compound CAL1, a yellow amorphous powder, was analyzed for $C_{21}H_{20}O_6$ from its $[M+H]^+$ at *m/z* 369.1340 (calcd for $C_{21}H_{21}O_6$, 369.1333) in the HR-ESI-MS (Figure 29). The UV spectrum exhibited maxima at 209, 242, 258 and 303 nm, suggestive of a flavone skeleton (Figure 30). The ^{1}H NMR spectrum (Figure 31) showed the presence of an isoprenyl group with two methyl groups at δ 1.58 (3H, s, H-4″), δ 1.36 (3H, s, H-5″), an olefinic proton at δ 5.04 (1H, m, H-2″) and a pair of methylene protons at δ 3.00 (2H, d, *J* = 7 Hz, H-1″). It also showed a methoxy signal at δ 3.77 (CH_3O-C-2′). In addition, it exhibited an ABX-spin system of aromatic protons (B-ring) at δ 6.54 (1H, d, *J* = 2 Hz, H-3′), δ 6.48 (1H, dd, *J* = 8, 2 Hz, H-5′) and δ 7.12 (1H, d, *J* = 8 Hz, H-6′), and an AX-spin system of *meta*-coupled aromatic protons (A-ring) at δ 6.18 (1H, d, *J* = 2 Hz, H-8) and δ 6.23 (1H, d, *J* = 2 Hz, H-6).

The ^{13}C NMR spectrum contained a signal for a ketone carbonyl carbon at δ 183.6 (Figure 32). Interpretation of the HMBC and HMQC spectra (Figures 33 and 34) revealed the substitution pattern. The HMBC correlations from H-6' (δ 7.12) to C-2 (δ 163.3), C-4′ (δ 162.3) and C-2′ (δ 159.8), from H-1″ (δ 3.0) to C-2 (163.3), C-3 (δ 122.0), C-4 (δ 183.6) and C-3″ (δ 132.6), supported the location of the prenyl group at C-3. The HMBC experiment also showed the connectivity between the methoxy protons at δ 3.77 (MeO-2′) and C-2, and an NOE experiment further assigned the methoxy group at C- 2′, since irradiation of these protons (δ 3.78) enhanced H-3′ (Figure 35).



[342]

Figure 20 Important NOE (H↔H) and HMBC (H→C) correlations of CAL1

Based on the above spectral evidence, the structure of CAL1 was characterized as a new compound, namely 5,7,4′-trihydroxy-2′-methoxy-3-(γ,γ-dimethylallyl)-flavone **[342].**

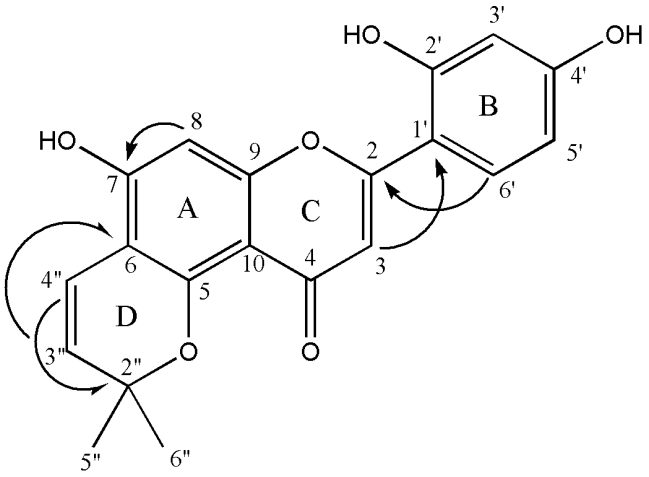
Table 12 NMR Spectral data of compound CAL1 (methanol-d_4)

position	compound CAL1		HMBC
	^{1}H (mult., *J* in Hz)	^{13} C	(correlation with ^{1}H)
2		163.3	6′, 1″
3		122.0	1″
4		183.6	1″
5		160.0	-
6	6.18, d (2.0)	99.6	8
7		165.6	-
8	6.23, d (2.0)	94.5	6
9		159.8	8
10		105.4	6, 8
1′		114.7	3′, 5′
2′		159.8	6′, OMe
3′	6.54, d (2.4)	100.2	5′
4′		162.3	3′, 6′
5′	6.48, dd (8.4, 2.4)	108.3	3′
6′	7.11, d (8.4)	132.3	-
1″	3.00, d (7), 2H	24.8	-
2″	5.04, m	122.8	4″, 5″
3″		132.6	1″, 4″, 5″
4″	1.58, s	25.8	5″
5″	1.36, s	17.6	4″
OMe	3.77	56.1	-

2.2 Structure Determination of Compound CAL2

Compound CAL2**,** a pale yellow amorphous powder, was analyzed for $C_{20}H_{16}O_6$ from its $[M+H]^+$ ion at *m/z* 353.1015 (calcd for 353.1020) in the HR-ESI-MS (Figure 36). The UV spectrum exhibited absorptions at 221, 287 and 346 nm (Figure 37). The ^{1}H NMR spectrum (Figure 38) showed the presence of a 2,2-dimethyl-3,4-dehydropyran ring at δ 1.46 (6H, s, H-5″, H-6″), δ 5.73 (1H, d, *J* = 10 Hz, H-3″) and δ 6.66 (1H, d, *J* = 10 Hz, H-4″). It also exhibited an ABX-spin system of aromatic protons at δ 6.60 (1H, d, *J* = 2 Hz, H-3′), δ 6.56 (1H, dd, *J* = 9, 2 Hz, H-5′) and δ 7.83 (1H, d, *J* = 9 Hz, H-6′), and an aromatic proton at δ 6.43 (1H, br s, H-8). The ^{13}C NMR spectrum contained a signal for a ketone carbonyl carbon at δ 183.6 (Figure 39). Examination of the HMBC and HMQC spectra (Figures 40 and 41) revealed the substitution pattern. The HMBC correlations from H-4″ (δ 5.73) to C-7 (δ 159.3) and H-3″ (δ 6.66) to C-6 (δ 105.8) supported the location of the 2,2-dimethyl-

3,4-dehydropyran ring at C-5 and C-6. The HMBC experiment showed the connectivities from the proton at C-6′ (δ 7.83) to C-2 (δ 108.6), and from H-3 (δ 7.07) to C-1′ (δ 110.8).



[343]

Figure 21 Important HMBC (H→C) correlations of CAL2

Based on the above spectral evidence, the structure of compound CAL2 was elucidated as 7,2′,4′-trihydroxy-3″,4″-dehydropyrano [1″,4″:5,6] flavone.

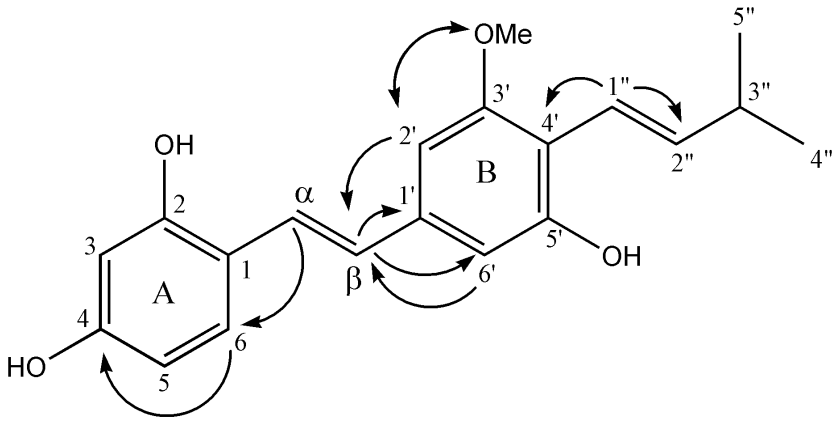
Table 13 NMR Spectral data of compound CAL2 (acetone-d_6)

position	compound CAL2		HMBC
	^{1}H (mult., *J* in Hz)	^{13}C	(correlation with ^{1}H)
2		163.0	6′
3	7.07, s	108.6	1′
4		183.6	-
5		158.0	-
6		105.8	8, 3″
7		159.3	8
8	6.43, s	95.5	7
9		157.4	-
10		105.8	-
1′		110.8	3
2′		159.3	-
3′	6.60, br s	104.4	-
4′		162.6	-
5′	6.56, dd (9, 2)	109.2	-
6′	7.83, d (9)	131.0	2
1″	-	-	-
2″		78.6	-
3″	6.66, d (10)	115.9	3″
4″	5.73, d (10)	129.2	3″, 4″
5″	1.46, s	28.4	-
6″	1.46, s	28.4	2″, 3″

2.3 Structure Determination of Compound CAL3

Compound CAL3, a yellow amorphous solid, was analyzed for $C_{20}H_{22}O_4$ from its $[M+H]^+$ ion at *m/z* 327.1621 (calcd for 327.1597) in the HR-ESI-MS (Figure 42). The UV spectrum exhibited absorptions at 218 and 346 nm (Figure 43). The ^{1}H NMR spectrum (Figure 44) showed the presence of a *trans*-olefin at δ 7.36 and 6.93 (1H each, d, *J* = 16 Hz, H-α and H-β). The ^{1}H NMR spectrum showed signals for an ABX-spin system of aromatic protons (A-ring) at δ 6.45 (1H, d, *J* = 2 Hz, H-3), δ 6.38 (1H, dd, *J* = 9, 2 Hz, H-5) and δ 7.41 (1H, d, *J* = 9 Hz, H-6) typical of substitution at the 2- and 4-positions on the A ring. An AX-spin system of *meta*-coupled aromatic protons (B-ring) at δ 6.71 (1H, d, *J* = 2 Hz, H-2′) and δ 6.67 (1H, d, *J* = 2 Hz, H-6′) suggested that the ring B was tetrasubstituted. The ^{1}H NMR spectrum showed the presence of a prenyl group at δ 1.07 (6H, d, *J* = 6.8 Hz, H-4″, 5″), δ 6.64 (1H, d, *J* = 8 Hz, H-1″), δ 6.63 (1H, d, *J* = 8 Hz, H-2″) and δ 2.40 (1H, m, H-3″). In addition, the ^{1}H NMR

spectrum showed a signal for a methoxyl group at δ 3.86 (3H, s). Interpretation of the ^{13}C NMR, HMBC and HMQC spectra (Figures 45, 46 and 47) revealed the substitution for this compound. The HMBC correlations from H-6′ (δ 6.67) to C-β (δ 126.3), and H-2′ (δ 6.71) to C-β (δ 126.3), and H-β (6.93) to C-1′ (δ 138.7), C-6′ (δ 101.8), and H-α (δ 7.36) to C-1 (δ 117.4), C-6 (δ 128.3), and H-1″ (δ 6.64) to C-4′ (δ 113.4), and C-2″ (δ 141.4), supported the presence of the prenyl group at C-4′. The HMBC experiment showed the connectivity between the methoxy protons at δ 3.86 (MeO-3′) and C-3′. The result from an NOE experiment assigned the methoxy group at C- 3′ since irradiation the proton at δ 3.86 enhanced the H-2′ signal (Figure 48). These results provided support for the presence of a prenyl substituent at C-4′ and the methoxyl group at C-3′.



[**344**]

Figure 22 NOE experiment (H↔H) and important HMBC (H→C) correlations of CAL3

Compound CAL3 was characterized as a new compound, namely *trans*-2,4,5′-trihydroxy-3′-methoxy-4′-(3-methyl-*E*-but-1-enyl)-stilbene.

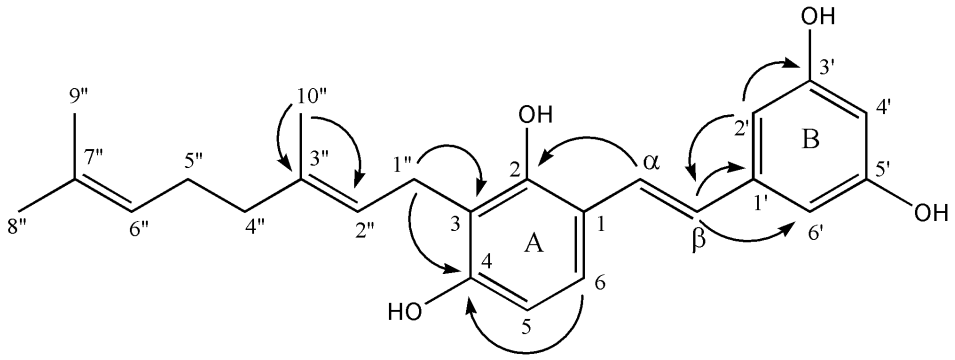
Table 14 NMR Spectral data of compound CAL3 (acetone-d_6)

position	compound CAL3		HMBC
	^{1}H (mult., *J* in Hz)	^{13} C	(correlation with ^{1}H)
1		117.4	3, 5, β
2		156.9	6
3	6.45, d (2)	103.7	5
4		159.1	3, 6
5	6.38, dd (9,2)	108.5	3
6	7.41, d (9)	128.3	α
α	7.36, d (16)	124.3	6
β	6.93, d (16)	126.3	6′
1′		138.7	α, β
2′	6.71,d (2)	107.1	β, 6′
3′		156.8	OMe
4′		113.4	1″
5′		159.6	6′
6′	6.67, d (2)	101.8	β
1″	6.64, br s	119.0	2″
2″	6.64, br s	141.4	1″
3″	2.40, m	34.0	1″, 4″, 5″
4″	1.07, d (6.8)	23.2	5″, 3″, 2″
5″	1.07, d (6.8)	23.2	4″, 3″
OMe	3.86, s	56.0	-

2.4 Structure Determination of Compound CAL4

Compound CAL4 was obtained as a yellow amorphous solid. It was analyzed for $C_{24}H_{28}O_4$ from its $[M+H]^+$ ion at *m/z* 381.2076 (calcd for 381.2067) in the HR-ESI-MS (Figure 49). The UV spectrum exhibited absorptions at 209, 242, 258 and 303 nm, suggestive of a flavone skeleton (Figure 50). Its ^{1}H NMR spectrum (Figure 51) also showed the presence of a *trans*-olefin at δ 6.79, 7.36 (1H each, d, *J* = 16 Hz, H-α and H-β). The ^{1}H NMR spectrum showed signals for an AX-spin system of *ortho*-coupled aromatic protons (A-ring) at δ 6.48 (1H, d, *J* = 8 Hz, H-5), δ 7.25 (1H, d, *J* = 8 Hz, H-6) typical of 1,2,3,4-tetrasubstitution. An ABX-spin system of aromatic protons (B-ring) at δ 6.52 (2H, d, *J* = 2 Hz, H-2′, H-6′) and δ 6.25 (1H, t, H-4′) suggested that ring B was symmetrically trisubstituted. The ^{1}H NMR spectrum also showed the presence of a geranyl group [δ 3.44 (2H, d, *J* = 7 Hz, H-1″), δ 5.30 (1H, m, H-2″), δ 2.08 (1H, m, H-4″), δ 1.98 (1H, m, H-5″), δ 5.09 (1H, m, H-6″), δ 1.57 (3H, s, H-8″), δ 1.63 (3H, s, H-9″) and δ 1.79 (3H, s, H-10″)]. Interpretation of the ^{13}C NMR, HMBC and HMQC spectra (Figures 52, 53 and 54) revealed the substitution pattern. The HMBC correlations from H-2′ (δ 6.52) to C-β (δ 127.2), C-3′

(δ 159.5); H-β (6.79) to C-1′ (δ 141.5), C-6′ (δ 105.7); H-α (δ 7.36) to C-2 (δ 154.4); H-1″ (δ 3.44) to C-3 (δ 116.4) , C-4 (δ 156.7); H-10″ (δ 1.79) to C-2″ (δ 123.5) and C-3″ (δ 135.7) supported the location of geranyl substituted at C-3.



[**345**]

Figure 23 Important HMBC (H→C) correlations of CAL4

Compound CAL4 was characterized as a new compound, namely 3-geranyl-2,4,3′,5′-tetrahydroxystilbene.

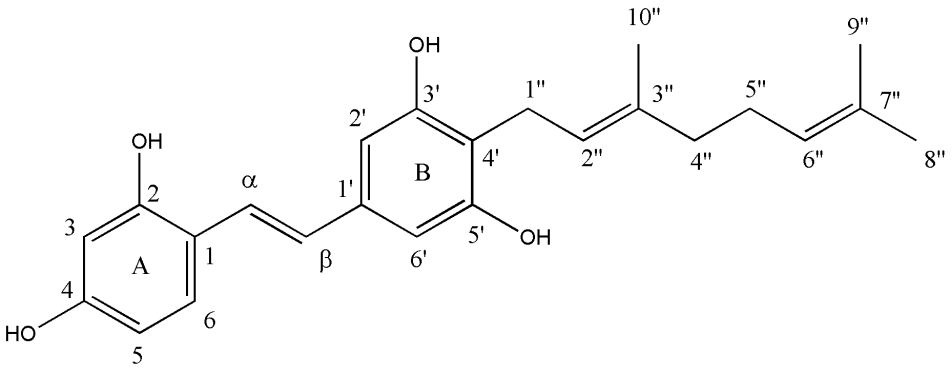
Table 15 NMR Spectral data of compound CAL4 (acetone-d_6)

position	compound CAL 4		HMBC
	^{1}H (mult., *J* in Hz)	^{13}C	(correlation with ^{1}H)
1		118.2	5, β
2		154.4	6, α, 1″
3		116.4	1″
4		156.7	6, 1″
5	6.48, d (8)	108.9	-
6	7.25, d (8)	124.8	α
α	7.36, d (16)	124.6	6
β	6.79 , d (16)	127.2	2′, 6′
1′		141.5	α, β
2′	6.52, d (2)	105.7	β
3′		159.5	2′
4′	6.25, t, 2	102.4	2′, 6′
5′		159.5	2′, 6′
6′	6.52, d (2)	105.7	β
1″	3.44, d (7), 2H	23.1	2″
2″	5.30, m	123.5	10″
3″		135.7	10″
4″	1.98, m	40.6	5″
5″	2.08, m	27.4	4″
6″	5.09, m	125.2	9″
7″		131.7	-
8″	1.63, s	25.8	6″, 9″, 10″
9″	1.57, s	17.7	6″, 8″
10″	1.79, s	16.3	4″, 8″

2.5 Structure Determination of Compound CAL5

Compound CAL5 was obtained as a yellow amorphous solid. The ESI-MS revealed a molecular ion at *m/z* 381.21 (Figure 55). The UV spectrum exhibited absorptions at 220, 261 and 328 nm (Figure 56). The ^{1}H NMR spectrum (Figure 57) showed the presence of a *trans*-olefin at δ 6.82, 7.37 (1H each, *J* = 16 Hz, H-α and H-β). The ^{1}H NMR spectrum showed signals for an ABX-spin system of aromatic protons (A-ring) at δ 6.42 (1H, d, *J* = 2 Hz, H-3), δ 6.36 (1H, dd, *J* = 8, 2 Hz, H-5) and δ 7.37 (1H, d, *J* = 8 Hz, H-6) typical of 2, 4- trisubstitution on the A ring. The aromatic protons (B-ring) at δ 6.56 (2H, s, H-2′, 6′) suggested that ring B was symmetrically tetrasubstituted. The ^{1}H NMR spectrum showed signals for the presence of a geranyl group at δ 3.36 (2H, d, *J* = 7 Hz, H-1″), δ 5.33 (1H, m, H-2″), δ 1.98 (2H, m, H-4″), δ 2.10 (2H, m, H-5″), δ 5.10 (1H, m, H-6″), δ 1.57 (3H, s, H-8″), δ 1.62 (3H, s, H-9″) and δ 1.78 (3H, s, H-10″).

This compound was identified as chlorophorin **[346]** through comparison of its ^{1}H and ^{13}C-NMR (Figure 58) and MS data with those reported in the literature (Table 16). The compound was first isolated from *Chlorophora excels* (Christensen *et al*., 1987).



Chlorophorin **[346]**

Table 16 NMR Spectral data of compound CAL5 (acetone-d_6) and chlorophorin

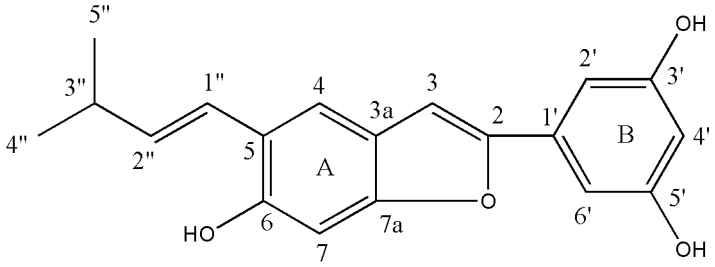
Position	compound CAL5		chlorophorin in CD_3CN	
	^1H (mult., *J* in Hz)	^{13}C	^1H (mult., *J* in Hz)	^{13}C
1		117.5		115.9
2		156.9		154.5
3	6.42, d (2)	103.6	6.44, d (2.4)	101.8
4		158.9		156.7
5	6.36, dd (2, 8)	108.4	6.38, dd (2.4, 8.5)	107.1
6	7.37, d (8)	123.5	7.39, d (8.5)	121.6
1′		134.5		134.1
2′	6.56, s	105.6	6.58, s	104.1
3′		156.7		154.8
4′		114.8		113.2
5′		156.7		154.8
6′	6.56, s	105.6	6.58, s	104.1
α	6.82, d (16)	126.5	6.83, d (16.4)	124.9
β	7.25, d (16)	128.0	7.27, d (16.4)	126.7
1″	3.36, m	23.0	3.37, d (7)	21.3
2″	5.33, m	124.3	5.34, m	121.9
3″		138.1		136.4
4″	1.98, m	40.6	1.93, m	38.8
5″		27.5		25.8
6″	5.10, m	124.3	5.10, m	123.5
7″		131.6		130.4
8″	1.62, s	25.8	1.62, s	24.3
9″	1.57, s	17.7	1.57, s	16.2
10″	1.78, s	16.3	1.79, s	14.7

2.6 Structure Determination of Compound CAL6

Compound CAL6 was obtained as a yellow amorphous solid and its molecular formula was analyzed as $C_{19}H_{18}O_4$ from its $[M+H]^+$ ion at *m/z* 311.13 in the ESI-MS (Figure 59). The UV absorptions at 228 and 339 nm (Figure 60) were indicative of a 2-arylbenzofuran skeleton (Yenesew *et al*., 2002; Kapche *et al*., 2009), and this was supported by the ^1H-NMR signal at δ 7.01 (1H, br s, H-3) and the ^{13}C-NMR signals at δ 102.3 (C-3) and δ 155.8 (C-2) (Figures 61 and 62) (Yenesew *et al*., 2002). The ^1H NMR spectrum showed the presence of an isoprenyl group [two methyl groups at δ 1.10, 1.12 (6H, d, *J* = 6 Hz, H-4″, H-5″), an olefinic proton at δ 2.48 (1H, m, H-3″) and a pair of methylene protons at δ 6.77 (1H, dd, *J* = 16, 1 Hz, H-1″), 6.21 (1H, dd, *J* = 16, 2 Hz, H-2″)]. An AX_2 -spin system of aromatic protons at δ 6.86 (2H, d, *J* = 2

Hz, H-2′ and H-6′), δ 6.32 (1H, d, *J* = 2 Hz, H-4′) was observed for ring B. Two aromatic protons of ring A were observed at δ 7.60 (1H, s, H-4) and δ 7.01 (1H, s, H-7). The ^{13}C NMR spectrum showed that there were five oxgenated sp^2 carbons δ 155.8 (C-2), δ 153.8 (C-6), δ 155.7 (C-7a), δ 159.8 (C-3′, 5′). The signals for the prenyl group appeared in the ^{13}C NMR spectrum at δ 123.0 (C-1″), δ 137.5 (C-2″), δ 32.6 (C-3″), δ 22.9(C-4″, 5″).

This compound was identified as artotonkin [**341**] through comparison of its ^{1}H-and ^{13}C-NMR and MS data with those reported in the literature (Table 17). The flavonoid was first isolated from *Artocarpus tonkinensis* (Moraceae) (Lien *et al*., 1997).



[**341**]

Table 17 NMR Spectral data of compound CAL6 and artotonkin (acetone-d_6)

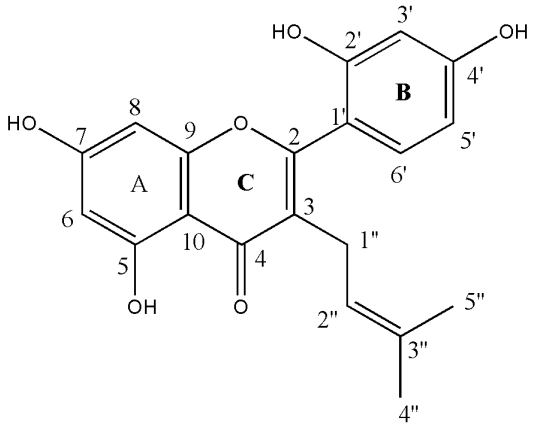
Position	compound CAL6		artotonkin	
	^{1}H (mult., *J* in Hz)	^{13}C	^{1}H (mult., *J* in Hz)	^{13}C
2	-	155.8	-	155.6
3	7.01, s	102.3	7.03, br s	102.3
3a	-	133.4	-	133.2
4	7.60, s	118.5	7.62, s	118.3
5	-	123.4	-	123.3
6	-	153.8	-	153.7
7	7.01, s	98.3	7.01, br s	98.3
7a	-	155.7	-	155.7
1′	-	122.9	-	122.9
2′	6.86, d (2)	103.8	6.85, d (2.2)	103.8
3′	-	159.8	-	159.8
4′	6.37, t (2)	103.6	6.37, t (2.2)	103.8
5′	-	159.8	-	159.8
6′	6.86, d (2)	102.3	6.85, d (2.2)	103.5
1″	6.77, dd (16, 1)	123.0	6.78, dd (15.9, 1.1)	123.0
2″	6.21, dd (16, 7)	137.5	6.22, dd (16.1, 7.0)	137.3
3″	2.48, m	32.6	2.49, dd (1.2, 6.7)	32.6
4″	1.10, d (6)	22.9	1.11, d (6.9)	23.0
5″	1.12, d (6)	22.9	1.11, d (6.9)	23.0

2.7 Structure Determination of Compound CAL7

Compound CAL7 was obtained as a yellow amorphous solid and its molecular formula was analyzed as $C_{20}H_{18}O_6$ from its $[M+H]^+$ ion at *m/z* 355.12 in the ESI-MS (Figure 63). The UV spectrum exhibited absorptions at 210, 241 and 256 nm, suggestive of a flavone skeleton (Figure 64).

The ^{1}H NMR spectrum (Figure 65) showed the presence of an isoprenyl group [two methyl groups at δ 1.43 (3H, s, H-4″), δ 1.57 (3H, s, H-5″), an olefinic proton at δ 5.12 (1H, m, H-2″) and a pair of methylene protons at δ 3.11 (2H, d, *J* = 7 Hz, H-1″)]. The AX-spin system of *meta*-coupled aromatic protons (A-ring) at δ 6.32 (1H, d, *J* = 2 Hz, H-6), δ 6.24 (1H, d, *J* = 2 Hz, H-8) suggested that the A ring was tetrasubstituted. An ABX -spin system of aromatic protons at δ 6.55 (1H, d, *J* = 2 Hz, H-3′), δ 6.50 (1H, dd, *J*= 8, 2 Hz, H-5′) and δ 7.18 (1H, d, *J* = 8 Hz, H-6) was observed for the B ring.

This compound was identified as albanin A [**132**] through comparison of its ^{1}H-and ^{13}C-NMR (Figure 66) and MS data with those reported in the literature (Table 18). (Ferrari *et al*., 1989).



[**132**]

Table 18 NMR Spectral data of compound CAL7 (acetone-d_6) and albanin A (methanol-d_4)

Position	compound CAL7		albanin A	
	^{1}H (mult., *J* in Hz)	^{13}C	^{1}H (mult., *J* in Hz)	^{13}C
2		162.4		163.3
3		121.8		121.6
4		183.0		182.9
5		163.4		162.0
6	6.24, d (2)	99.2	6.25, d (2)	99.1
7		164.7		164.6
8	6.32, d (2)	94.2	6.32, d (2)	94.0
9		159.3		159.2
10		104.0		105.3
1′		113.1		113.0
2′		157.2		157.1
3′	6.55, d (2)	103.1	6.56, d (2.5)	103.9
4′		161.5		161.3
5′	6.50, dd (2, 8)	108.1	6.51, dd (2.5, 8)	108.0
6′	7.18, d (8)	132.1	7.18, d (8)	132.1
1″	3.11, d (7.1)	24.6	3.10, d (7)	24.4
2″	5.12, m	122.7	5.13, m	122.5
3″		132.3		131.9
4″	1.57, s	25.8	1.43, s	25.6
5″	1.43, s	17.6	1.57, s	17.5

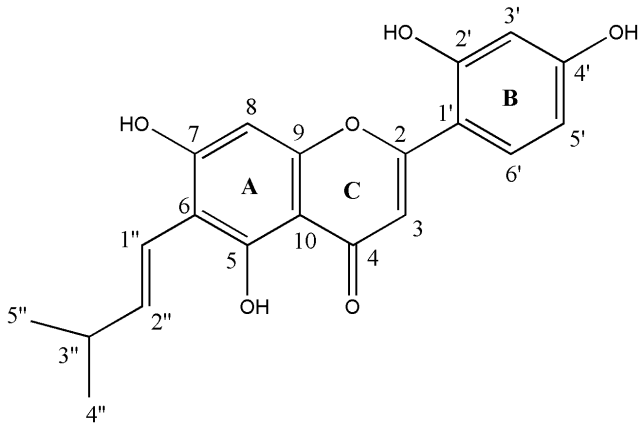
2.8 Structure Determination of Compound CAL8

Compound CAL8 was obtained as a yellow amorphous solid and its molecular formula was analyzed as $C_{20}H_{18}O_6$ from its $[M+H]^+$ ion at *m/z* 355.12 in the ESI-MS (Figure 67). The UV absorptions at 211, 287 and 347 nm (Figure 68) were suggestive of a flavone skeleton.

The ^{1}H NMR spectrum (Figure 69) showed the presence of an isoprenyl group [two methyl groups at δ 1.11 (3H, s, H-4″), δ 1.09 (3H, s, H-5″), an methine proton at δ 2.43 (1H, m, H-3″) and a pair of olefinic protons at δ 6.55 (1H, d, *J* = 16 Hz, H-1″), 6.69 (1H, d, *J* = 16 Hz, H-2″)]. An ABX -spin system of aromatic protons at δ 6.42 (1H, d, *J* = 2 Hz, H-3′), δ 6.45 (1H, dd, *J* = 9, 2 Hz, H-5′) and δ 7.73 (1H, d, *J* = 9 Hz, H-6′) was observed for ring B. Aromatic protons at δ 6.46 (1H, s, H-8) and δ 7.12 (1H, s, H-3) were also observed.

This compound was identified as isoartocarpesin [**58**] through comparison of its ^{1}H- and ^{13}C-NMR (Figure 70) and MS data with those reported in the literature

(Table 19). The flavonoid was first isolated from *Artocarpus incisus* (Moraceae) (Shimizu *et al*., 1998).



[**58**]

Table 19 NMR Spectral data of compound CAL8 (methanol-*d*4) and isoartocarpesin (acetone-d_6)

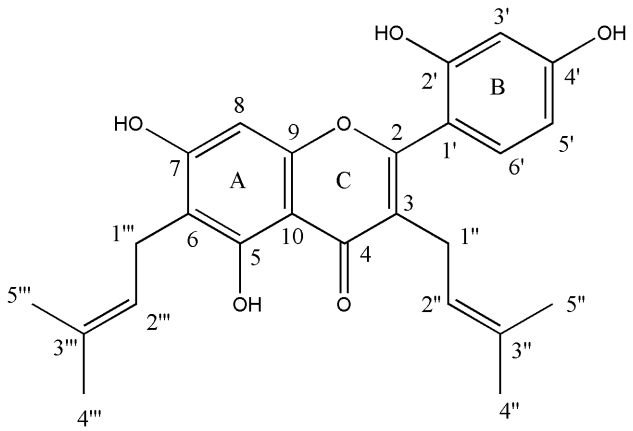
Position	compound CAL8		isoartocarpesin	
	^{1}H (mult., *J* in Hz)	^{13}C	^{1}H (mult., *J* in Hz)	^{13}C
2		163.86		163.09
3	7.12, s	108.32	7.03,s	108.91
4		184.52		184.14
5		163.24		161.19
6		110.19		109.88
7		163.26		162.65
8	6.46, s	94.25	6.54, s	94.63
9		157.28		157.0
10		105.02		105.48
1′		110.84		111.17
2′		160.31		159.72
3′	6.42, d (2)	104.22	6.56	104.78
4′		163.31		162.99
5′	6.45, dd (9, 2.5)	109.09	6.51, dd (8.55, 2.45)	109.60
6′	7.73, d (9)	130.90	7.77,d (8.55)	131.36
1″	6.55, d (16)	117.41	6.58, dd (0.78, 16.12)	117.75
2″	6.69, dd (16, 7)	142.63	6.72, dd (7.08, 16.12)	142.40
3″	2.43, m	34.31	2.39, m	34.38
4″	1.10, d (6.4)	23.20	1.04, d (6.8)	23.61
5″	1.10, d (6.4)	23.20	1.04, d (6.8)	23.61

2.9 Structure Determination of Compound CAL9

Compound CAL9 was obtained as a yellow amorphous solid and its molecular formula was analyzed as $C_{25}H_{26}O_6$ from its $[M+H]^+$ ion at *m/z* 423.18 in the ESI-MS (Figure 71). The UV absorbtions at 212, 262 and 312 nm (Figure 72) were suggestive of a flavone skeleton.

The ^{1}H-NMR spectrum (Figure 73) exhibited signals for two prenyl groups. The first prenyl group [δ 1.77 (3H, br s, H-5‴), δ 1.64 (3H, br s, H-4‴), δ 3.36 (2H, m, H-1‴) and δ 5.29 (1H, m, H-2‴)] was located on C-6 of ring A. An aromatic proton signal for H-8 was observed at δ 6.39. The second prenyl group [δ 1.43 (3H, s, H-4″), δ 1.56 (3H, s, H-5″), δ 3.11 (2H, d, *J* = 6.9 Hz, H-1″) and δ 5.14 (1H, m, H-2″)] was situated at C-3 of ring C. For ring B, the presence of an ABM spin system at δ 6.52 (1H, dd, *J* = 2, 8 Hz, H-5′), δ 6.57 (1H, d, *J* = 2 Hz, H-3′), 7.19 (1H, d, *J* = 8 Hz, H-6′) indicated 2′,4′-dihydroxy substitution. The ^{13}C-NMR spectrum (Figure 74) displayed 25 signals for one carbonyl group, four methyl groups, six methine carbons, two methylene carbons and twelve quaternary carbons. The signals δ 162.3 (C-2), δ 121.6 (C-3) and δ 183.1 (C-4) confirmed the flavone structure.

This compound was identified as cudraflavone C [**34**] through comparison of its ^{1}H-and ^{13}C-NMR and MS data with those reported in the literature (Table 20). The flavonoid was first isolated from *Cudrania tricuspidata* (Moraceae) (Hano *et al*., 1990c).



[**34**]

Table 20 NMR Spectral data of compound CAL9 and cudraflavone C (acetone-d_6)

Position	compound CAL9		cudraflavone C	
	^{1}H (mult., *J* in Hz)	^{13}C	^{1}H (mult., *J* in Hz)	^{13}C
2	-	162.3	-	162.0
3	-	121.6	-	121.5
4	-	183.1	-	183.0
5	-	160.1	-	160.0
6	-	111.8	-	111.8
7	-	162.3	-	162.3
8	6.39, s	93.6	6.40, s	93.5
9	-	157.1		157.0
10	-	105.1	-	105.0
1′	-	113.2	-	113.1
2′	-	157.1	-	157.1
3′	6.57,d (2)	103.9	6.57, d, (2.0)	103.9
4′	-	161.4	-	161.3
5′	6.52, dd (2, 8)	108.1	6.52, dd, (8.0, 2.0)	108.1
6′	7.19, d (8)	132.8	7.19, d (8.0)	132.2
1″	3.11, d (7), 2H	24.7	3.12, d (7.0), 2H	24.6
2″	5.14, m	122.8	5.14, m	122.7
3″	-	131.9	-	132.0
4″	1.43, s	17.6	1.43, s	17.9
5″	1.56, s	25.8	1.57, s	25.8
1‴	3.36, d (7), 2H	22.0	3.41, d (6.9), 2H	22.0
2‴	5.29, m	123.5	5.29, m	123.3
3‴	-	131.5	-	131.4
4‴	1.64, s	25.9	1.65, s	25.9
5‴	1.77, s	17.6	1.78, s	17.6

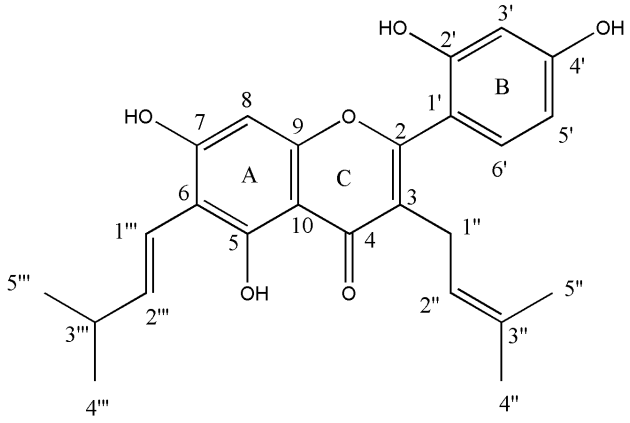
2.10 Structure Determination of Compound CAL10

Compound CAL10 was obtained as a yellow amorphous solid and its molecular formula was analyzed as $C_{25}H_{26}O_6$ from its $[M+H]^+$ ion at *m/z* 423.18 in the ESI-MS (Figure 75). The UV spectrum showed absorbtions at 210, 279 and 325 nm (Figure 76).

The ^{1}H-NMR spectrum (Figure 77) exhibited signals for two prenyl groups. The first prenyl group [δ 1.08 (3H, d, *J* = 6 Hz, H-4‴), δ 1.10 (3H, d, *J* = 6 Hz, H-5‴), δ 2.45 (1H, m, H-3‴] was located on ring A. The second prenyl group [δ 1.43 (3H, s, H-4″), δ 1.57 (3H, s, H-5″), δ 3.11 (2H, d, *J* = 7, H-1″) and δ 5.12 (1H, m, H-2″)] was situated at C-3 of ring C. An aromatic singlet signal appeared at δ 6.41 for H-8. For ring B, the presence of an ABM spin system at δ 6.50 (1H, dd, *J* = 2, 8 Hz, H-5′), δ 6.57 (1H, d, *J* = 2 Hz, H-3′), δ 7.18 (1H, d, *J* = 8 Hz, H-6′ indicated 2′,4′-dihydroxy

substitution. The ^{13}C-NMR spectrum (Figure 78) showed signals at δ 162.12 (C-2), δ 121.62 (C-3) and δ 183.28 (C-4) confirmed flavone structure.

This compound was identified as norartocarpin [**128**] through comparison of its ^{1}H-and ^{13}C-NMR and MS data with those reported in the literature (Table 21).



[**128**]

Table 21 NMR Spectral data of compound CAL10 and norartocarpin(acetone-d_6)

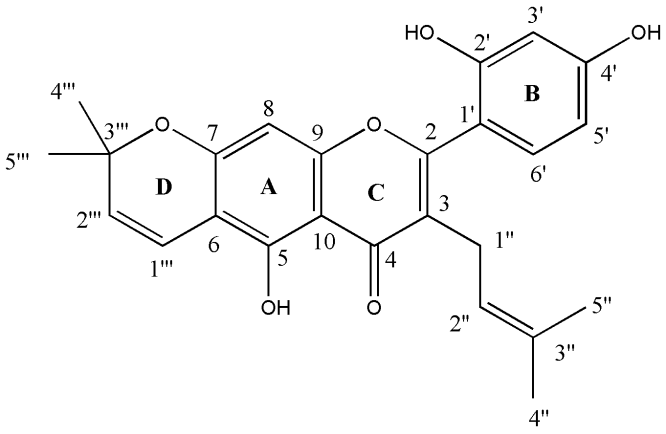
Position	compound CAL10		norartocarpin in methanol-d_4	
	^{1}H (mult., *J* in Hz)	^{13}C	^{1}H (mult., *J* in Hz)	^{13}C
2		162.1		163.2
3		121.6		121.7
4		183.3		183.8
5		160.8		160.8
6		109.2		109.7
7		163.2		163.1
8	6.41, s	93.8	6.30, s	93.8
9		157.0		157.7
10		105.1		105.1
1′		113.0		113.3
2′		161.8		161.8
3′	6.57, d (2)	103.9	6.58,d (2)	103.7
4′		157.0		157.5
5′	6.50, dd (8, 2)	108.1	6.38, dd (8, 2)	107.9
6′	7.18, d (8)	132.1	7.04, d (8)	132.3
1″	3.11, d (7), 2H	24.6	3.07, d (6.8), 2H	24.2
2″	5.12, m	122.7	5.08, m	122.9
3″		132.3		132.6
4″	1.38, s	17.6	1.43, s	17.6
5″	1.54, s	25.8	1.57, s	25.8
1‴	6.64, d (16)	117.3	6.56, d (16)	117.5
2‴	6.76, dd (16, 7)	141.9	6.69, d (16, 7.04)	142.2
3‴	2.45, m	33.9	2.41, m	34.4
4‴	1.09, d (6)	23.1	1.08, d (6)	23.2
5‴	1.11, d (6)	23.1	1.10, d (6)	23.2

2.11 Structure Determination of Compound CAL11

Compound CAL11 was obtained as a yellow amorphous solid and its molecular formula was analyzed as $C_{25}H_{24}O_6$ from its $[M+Na]^+$ ion at *m/z* 423.18 in the ESI-MS (Figure 79). The UV spectrum showed absorbtions at 223, 262 and 314 nm (Figure 80).

The ^{1}H-NMR spectrum (Figure 81) exhibited the signals of prenyl groups signals at [δ 1.45 (3H, s, H-4″), δ 1.57 (3H, s, H-5″),δ 3.12 (2H, d, *J* = 7 Hz, H-1″) and δ 5.12 (1H, m, H-2″)] which was located at C-3 of ring C. The second prenyl group [δ 1.43 (6H, s, H-4‴, 5‴), δ 5.73 (1H, d, *J* = 10 Hz, H-1″) and 6.68 (1H, d, *J* = 10 Hz, H-2″) was substituted at C-6 of ring D. An aromatic singlet signal appeared at δ 6.26 (H-8). For ring B, the presence of an ABM spin system at δ 6.51 (1H, dd, *J* = 2, 8 Hz, H-5′), δ 6.56 (1H, d, *J* = 2, H-3′), δ 7.19 (1H, d, J = 8 Hz, H-6′) indicated 2′,4′-dihydroxy substitution. The ^{13}C-NMR spectrum (Figure 82) showed signals at δ 158.5 (C-2), δ 121.9 (C-3) and δ 183.2 (C-4) confirmed a flavone structure.

This compound was identified as cudraflavone B **[347]** through comparison of its ^{1}H-and ^{13}C-NMR and MS data with those reported in the literature (Table 22). The flavonoid was first reported from *Cudrania tricuspidata* (Moraceae) (Fujimoto *et al*., 1984).



[347]

Table 22 NMR Spectral data of compound CAL11 and cudraflavone B (acetone-d_6)

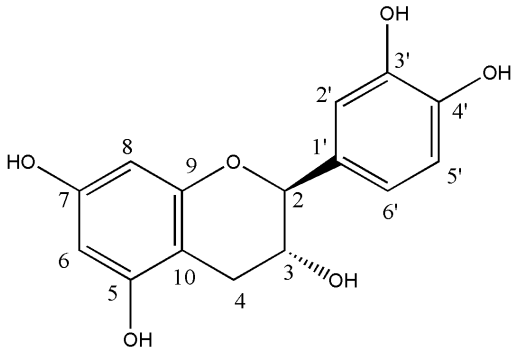
Position	compound CAL11		cudraflavone B	
	^{1}H (mult., *J* in Hz)	^{13}C	^{1}H (mult., *J* in Hz)	^{13}C
2		158.5		158.4
3		121.9		121.7
4		183.2		183.2
5		157.2		157.2
6		105.7		105.7
7		162.7		162.8
8	6.26, s	95.2	6.24, s	95.2
9		160.1		160.6
10		105.7		105.6
1′		112.9		112.7
2′		157.3		157.3
3′	6.56, d (2)	103.9	6.55, d (2)	103.8
4′		161.5		161.6
5′	6.51, dd (2, 8)	108.1	6.49, dd (2, 8)	108.0
6′	7.19, d (8)	132.3	7.16, d (8)	132.2
1″	3.12, d (7), 2H	24.6	3.15, d (6.5), 2H	24.6
2″	5.12, t (7)	122.6	5.08, t	122.5
3″		132.2		132.2
4″	1.45, s	25.8	1.42, s	25.8
5″	1.57, s	17.6	1.52, s	17.7
1‴	5.73, d (10)	115.9	5.71, d (10)	115.9
2‴	6.68, d (10)	129.1	6.66, d (10)	129.1
3‴		78.6		78.6
4‴	1.43, s	28.4	1.42, s	28.3
5‴	1.43, s	28.4	1.42, s	28.3

2.12 Structure determination of compound CAL12

Compound CAL12 was obtained as a brown amorphous power and its molecular formula was analyzed as $C_{15}H_{14}O_6$ from its $[M+H]^+$ ion at *m/z* 291.09 in the ESI-MS (Figure 83). The UV spectrum exhibited absorption at 211 and 280 nm (Figure 84).

The ^{1}H-NMR spectrum (Figure 85) showed proton signals for a flavan skeleton at δ 2.85 (1H, dd, *J* = 16, 7 Hz, H-4a) and δ 2.52 (1H, dd, *J* = 16, 6 Hz, H-4b), aromatic proton doublets at 5.93 (1H, d, *J* = 2 Hz,H-6), δ 5.86 (1H, d, *J* = 2 Hz,H-8) and an ABX system at δ 6.84 (1H, d, *J* = 2 Hz, H-2′), δ 6.71 (1H, dd, *J* = 8, 2 Hz, H-6′).

This compound was identified as **(–)** catechin [**108**] through comparison of its ^{1}H-and ^{13}C-NMR (Figure 86), optical rotation (– 48) and MS data with those reported in the literature (Table 23). The compound was isolated from *Carapa guianensis* (Meliaceae) (Shu-Hua *et al*., 2003).



[**108**]

Table 23 NMR Spectral data of compound CAL12 and (–) catechin (methanol-d_4)

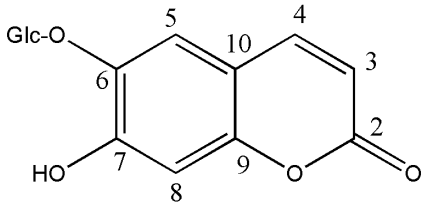
Position	compound CAL12		(–) catechin	
	^{1}H (mult., *J* in Hz)	^{13}C	^{1}H (mult., *J* in Hz)	^{13}C
2	4.75, d (7.5)	82.9	4.57, d (7.6)	82.3
3	3.97, ddd (5, 8, 7)	68.8	4.01, ddd (5.4, 8.2, 7.6)	68.3
4a	2.85, dd (16, 7)	28.5	2.88, dd (16.1, 5.4)	28.2
4b	2.51, dd (16, 6)	28.5	2.52, dd (16, 2)	28.2
5		157.6		156.9
6	5.93, d (2)	96.4	5.95, d (1.8)	96.2
7		157.8		157.0
8	5.86, d (2)	95.6	5.88, d (1.8)	95.4
9		156.9		156.3
10		100.9		100.7
1'		132.3		131.6
2'	6.84, d (2)	115.3	6.83, d (1.8)	115.0
3'		146.3		145.6
4'		146.3		145.7
5'	6.76, d (8)	116.1	6.77, d (8)	116.0
6'	6.71, dd (8, 2)	120.0	6.72, dd (8.0, 1.8)	119.9

2.13 Structure determination of compound CAL13

Compound CAL6 was obtained as a yellow amorphous solid and its molecular formula was analyzed as $C_{15}H_{16}O_9$ from its $[M+H]^+$ ion at *m/z* 341.08 in the ESI-MS (Figure 87). The UV spectrum exhibited absorptions at 207, 221 and 331 nm (Figure 88), characteristic of a coumarin structure. (Matsuda *et al*., 1995).

The ^{1}H and ^{13}C-NMR spectra (Figures 89 and 90) indicated the presence of a coumarin skeleton by the signals at δ 6.21 (1H, d, *J*=10 Hz, H-3), δ 7.83 (1H,d, *J*= 10 Hz, H-4) and δ 113.0 (C-3), δ 146.0 (C-4). The two signals at δ 6.80 and δ 7.43, showed that substituents were present at C-6 and C-7 of the B-ring.

This compound was identified as aesculin **[348]** through comparison of its ^{1}H- and ^{13}C-NMR and MS data with those reported in the literature (Table 24) (Matsuda *et al*., 1995).



[348]

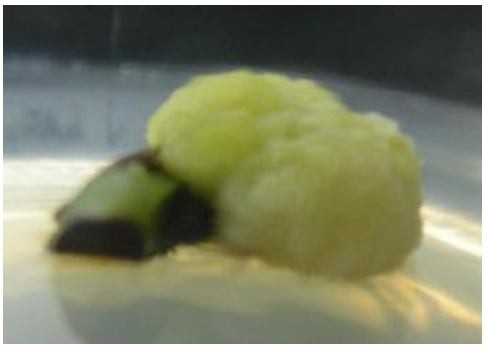
Table 24 NMR Spectral data of compound CAL13 and aesculin (methanol-d_4)

Position	compound CAL13		aesculin	
	^{1}H (mult., *J* in Hz)	^{13}C	^{1}H (mult., *J* in Hz)	^{13}C
2		163.7		163.7
3	7.83, d (10)	113.0	7.89, d (9.5)	113.1
4	6.21, d (10)	146.0	6.17, d (9.5)	145.9
5	7.43, s	116.9	7.39, d (2)	116.9
6		153.7		153.4
7		144.5		144.4
8	6.80, s	104.6	6.77, d (2)	104.6
9		152.7		152.6
10		112.8		112.8
Glc-1		104.4		104.4
Glc-2		74.9		74.8
Glc-3		78.5		78.5
Glc-4		71.4		71.4
Glc-5		77.7		77.7
Glc-6		62.6		62.6

3 Tissue Culture of *A. lakoocha*

3.1 Establisment of callus cultures

Callus formation was induced successfully on WPM (Woody Plant Medium) supplemented with 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 1 mg/l benzyladenine (BA).



24 a

24 b

24 c

Figure 24 Induction of callus from the explants of *A. lakoocha* on WPM agar medium contain 1 mg/l 2, 4-D and 1 mg/l BA

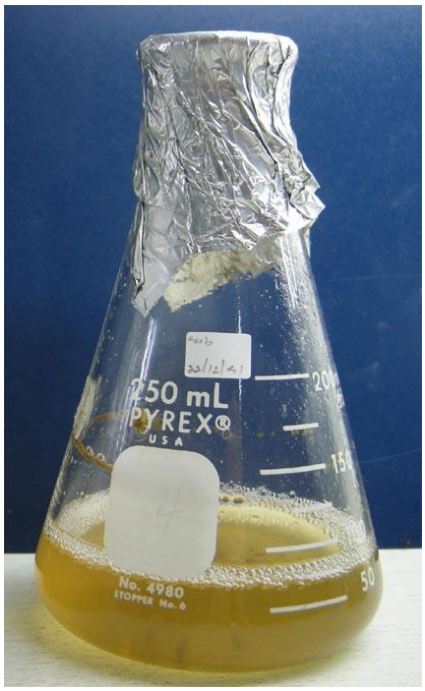
24 a Induction of callus from the cotyledon explants

24 b Induction of callus from the stem explants

24 c Induction of callus from the leaf explants

3.2 Establisment of cell suspension cultures

Cell suspension cultures of *A lakoocha* were obtained from the callus which were maintained by a regular subculturing as described earlier. The callus tissues were separated into small aggregates before transfers into WPM liquid medium culture and rotated at 120 rpm. on a rotary shaker. Cell suspension was maintained in the same medium by subculture every three weeks.



25 a **25 b**

Figure 25 Cell suspension cultures of *A. lakoocha* maintained in WPM liquid medium contain 1 mg/l 2,4-D and 1 mg/l BA.

25 a The apparent friable callus cultures

25 b cell suspension cultures

3.3 Relationship between cell growth and secondary metabolites production

Figure 26 shows the culture growth of the callus cultures of *A. lakoocha* during a period of 25 days. It can be seen that the starting callus cultures had a lag phase of almost 10 days before entering a rapid growth for a period of 7 days (day 10-day 17) to obtain its highest peak at day 17, with about 0.2 g dry weight.

During this growth cycle, the formation of secondary metabolites was monitored by TLC-densitometric method (Figure 27). Various compounds isolated from the callus cultures with structures elucidated were used as standard. Figure 27 shows that the TLC pattern of the ethanolic extract obtained from various ages of the callus tissue. The standard mixture and individual 11 pure compounds were also chromatographed in the same TLC condition. It can be seen that three major secondary products, i.e. isoartocarpesin, norartocarpin and cudraflavone B were present all the period of the culture growth, gradually increased during the first 17 days and declined rapidly when the culture entered the stationary phase until day 25.

It should be noted that no sequential formation of these metabolites was observed. All of the compounds were formed and changed parallel and in the same period of growth curve.

Based on these results, it was concluded that the whole profile of secondary metabolites of *A. lakoocha* was expressed all over the growth period of the callus culture until entering the stationary phase of the fully mature tissues.

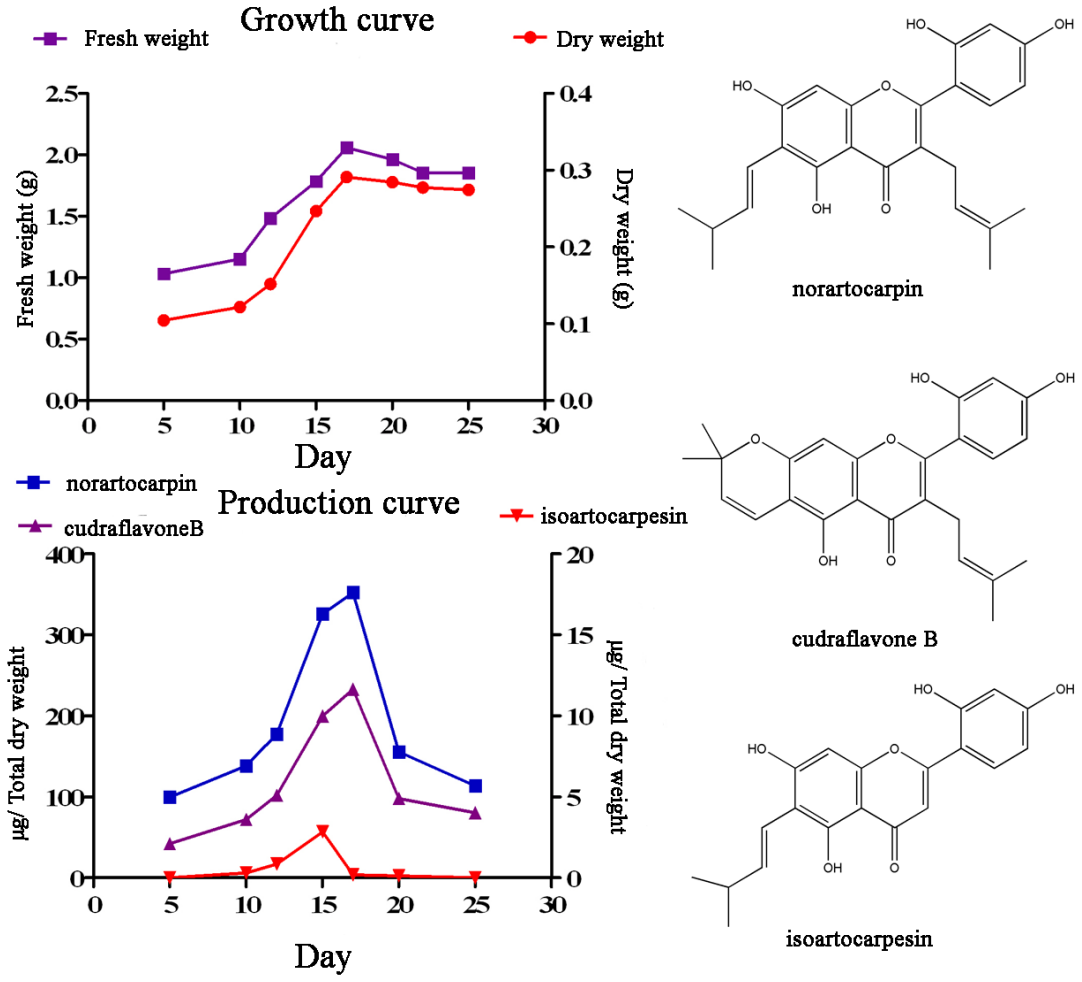


Figure 26 Growth curve and production curve of *A. lakoocha* callus culture

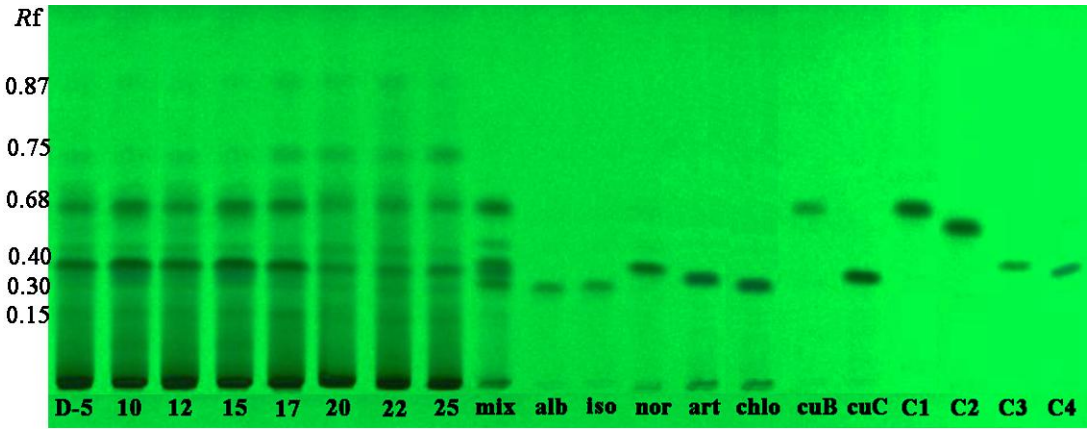


Figure 27 TLC pattern of ethanol extract from *A. lakoocha* callus cultures and time-courses of cell growth under 254 nm UVlight with authentic compounds

D-5 = callus day 5
D-10= callus day 10
D-12 = callus day 12
D-15 = callus day 15
D-17 = callus day 17
D-20 = callus day 20
D-22 = callus day 22
D-25 = callus day 25
Mix = mix standard
alb = albanin A
iso = isoartocarpesin
nor = norartocarpin
art = artotonkin
chlo = chlorophorin
cuB = cudraflavone B
cuC = cudraflavone C
C1 = CAL 1
C2 = CAL 2
C3 = CAL 3
C4 = CAL 4

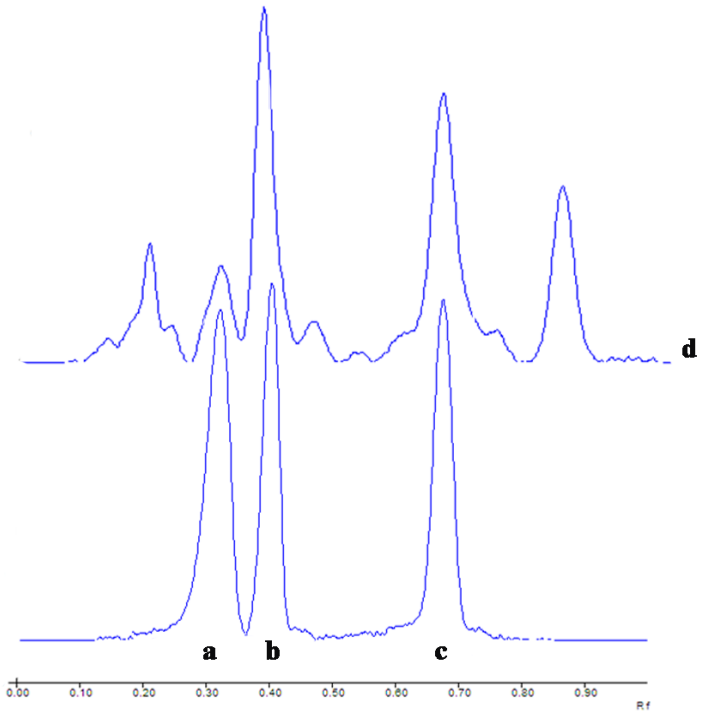


Figure 28 Representative TLC densitometric chromatograms.
a isoartocarpesin b norartocarpin
c cudraflavone B d chromatogram of callus extract

4. Free Radical Scavenging Activity

Free radicals are products of normal cellular metabolism that can be defined as an atom or molecules containing one or more unpaired electrons in its atomic or molecular orbitals. Reactive oxygen species (ROS) represent the most important class of radical species generated in living systems. They include superoxide ($O_2^{\bullet-}$), peroxyl ($ROO^{\bullet}$), alkoxyl ($RO^{\bullet}$), hydroxyl ($HO^{\bullet}$) and hydrogen peroxide (H_2O_2). ROS can be either deleterious or beneficial, but the excess ROS can damage cellular lipids, proteins and DNA (Valko *et al*., 2007). These pathophysiological conditions can cause violent chronic diseases including cancer, artherosclerosis, stroke, rheumatoid arthritis, neurodegeneration and diabetes (Fang, Yang, and Wu, 2002). In this study, ten compounds from *A. lakoocha* cultures were evaluated for their DPPH scavenging activity. Quercetin was employed as positive control. The results are summarized in Table 25.

Table 25 Percentage of DPPH reduction by compounds isolated from *A. lakoocha* calli.

compound	DPPH 1 mg/ml	DPPH IC_{50}(μM)
1. norartocarpin (CAL10)	46.52738	-
2. cudraflavone C (CAL9)	34.18487	-
3. albanin A (CAL7)	42.73605	-
4. aesculin (CAL13)	17.95114	-
5. artotonkin (CAL6)	95.34351	12.5
6. chlorophorin (CAL5)	95.53859	38.63
7. cudraflavone B (CAL11)	29.2228	-
8. isoartocarpesin (CAL8)	38.89575	-
9. *trans*-2,4,5′-trihydroxy-3′-methoxy-4′-(3-methyl-*E*-but-1-enyl)-stilbene (CAL3)	95.6152	37.47
10. 3-geranyl-2,4,3′,5′-tetrahydroxystilbene (CAL4)	94.48479	34.29
11. quercertin*	95.67253	10.3

* positive control

As shown in Table 25, ten pure compounds were tested for their DPPH free radical scavenging activity. It can be seen that all compounds exhibited recognizable DPPH scavenging activity. The most active compound was artotonkin [**315**], but its activity was lower than that of the positive control quercetin.

5. Tyrosinase Inhibitory Activity

Tyrosinase is a copper monooxygenase enzyme widely distributed in nature. It has been found in plants, fungi, insects and animals. A number of physiological functions of this

enzyme have been studied (Gelder *et al*., 1997). Tyrosinase is one of the important key enzymes involved in the molting process of insects (Kubo, Yokokawa and Kinst-Hori, 1995). A search for its inhibitors may therefore lead to discovery of insect control agents. In plants, tyrosinase has been found to be responsible for browning in fruits and vegetables. In mammals and humans, the biosynthesis of melanin has been studied intensively by Raper, and subsequently by Manson (Britton, 1983), which led to the proposal of Raper-Manson scheme of melanogenesis and the function of tyrosinase in the biosynthesis of the skin pigment melanin is well-established (Gelder *et al*., 1997). Thus, the study of tyrosinase inhibitors should be useful for the treatment of localized hyperpigmentation in human. Moreover, tyrosinase inhibitors are becoming more popular in the development of cosmetic products (Kubo *et al*., 1995).

In this study, the tyrosinase inhibitory activity of each pure compound was determined by a modified dopachrome method (Masamoto *et al*., 1980; Iida *et al*., 1995 and Morita *et al*., 1994). The activity of each of these compounds was expressed as IC_{50} value (concentration of 50% inhibition) in comparison with oxyresveratrol and kojic acid, known inhibitors of tyrosinase. The results are summarized in Table 26.

Table 26 Percentage of tyrosinase inhibition by compounds isolated from *A. lakoocha* calli.

compound	Tyrosinase Inhibitory 0.33 mg/ml	Tyrosinase Inhibitory IC_{50} (μM)
1. norartocarpin (CAL10)	22.68657	-
2. cudraflavone C (CAL9)	35.52239	-
3. albanin A (CAL7)	11.64179	-
4. aesculin (CAL13)	43.58209	-
5. artotonkin (CAL6)	79.40299	46.2
6. chlorophorin (CAL5)	85.97015	7.90
7. cudraflavone B (CAL11)	20.59701	-
8. isoartocarpesin (CAL8)	26.56716	-
9. *trans*-2,4,5′-trihydroxy-3′-methoxy-4′-(3-methyl-*E*-but-1-enyl)-stilbene (CAL3)	87.16418	12.8
10. 3-geranyl-2,4,3′,5′-tetrahydroxystilbene (CAL4)	66.26866	66.0
11. Kojic acid*	57.31343	62.29
12. Oxyresveratrol*	83.28358	1.73

* positive control

Among the ten pure compounds tested for tyrosinase inhibitory activity, it can be seen that stilbenoids showed recognizable tyrosinase inhibitory activity, whereas other compounds showed weak activity.

CHAPTER V

CONCLUSION

The TLC densitometric method developed in this study is a simple, convenient, sensitive and reliable procedure. It was an effective analytical tool for the evaluation of oxyresveratrol content in both *A. lakoocha* heartwood and the traditional drug Puag-Haad and was modified for analysis of secondary metabolite production in cell cultures.

Callus cultures of *A. lakoocha* can be establishted from aerial part explants on WPM medium which contained 20 g/l sucrose, 1 mg/l 2,4-D, 1 mg/l BA and 0.8 % (w/v) agar and subculture on this medium.

From cell cultures of *A lakoocha*, thirteen compounds were characterized. Most of them found in Genus *Artocarpus*, but thirteen isolates except norartocarpin were found for the first time from this plant. It should be noted that a trace amount of oxyresveratrol was detected by TLC densitometry and comparison of the UV–Vis spectra with reference standards. However, full identification could not be done. The major metabolites were isoartocarpesin, norartocarpin and cudraflavone B. These compounds were present all the period of the culture growth, gradually increased during the first 17 days and declined rapidly when the culture entered the stationary phase until day 25. All of the compounds were formed and changed parallel and in the same period of growth curve. Based on these results, it was concluded that the whole profile of secondary metabolites of *A. lakoocha* was expressed all over the growth period of the callus culture until entering the stationary phase of the fully mature tissues.

The study also focused on the production of secondary metabolites from callus cultures of *A. lakoocha*, and this led to the isolation of four new compounds together with the nine known compounds. The isolated compounds were evaluated for their free radical scavenging and antityrosinase activity. Artotonkin, chlorophorin, *trans*-2,4,5′-trihydroxy-3′-methoxy-4′-(3-methyl-*E*-but-1-enyl)-stilbene and 3-geranyl-2,4,3′,5′-tetrahydroxystilbene showed appreciable free radical scavenging activity and antityrosinase activity, while the other compounds were weakly active. In previous studies, norartocarpin, cudraflavone C, artotonkin and albanin A showed a strong inhibitory

effect on pancreatic lipase (Zhao *et al*., 2009), whereas cudraflavone C had a strong inhibitory effect on murine leukemia P-388 (Syah *et al*., 2006). In this study cudraflavone C and norartocarpin were found as main compounds from callus cultures. Further studies should also be carried out on developing techniques for large scale production. These bioactive compounds from *A. lakoocha* provide possibilities for application in medicine and cosmetics.

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APPENDIX

High resolution report

Analysis Name	D:\Data\customer\75MF2_5_II.d	Acquisition Date	5/3/2011 2:11:24 PM
Method	NaFormate_pos_infusion .m	Operator	Sutichai Ext: 3560
Sample Name	75MF2_5_II	Instrument	micrOTOF Bruker
		Calibrate by	Sodium Formate

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Not active			Set Dry Heater	150 °C
Scan Begin	100 m/z	Set Capillary	5000 V	Set Dry Gas	2.0 l/min
Scan End	1500 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

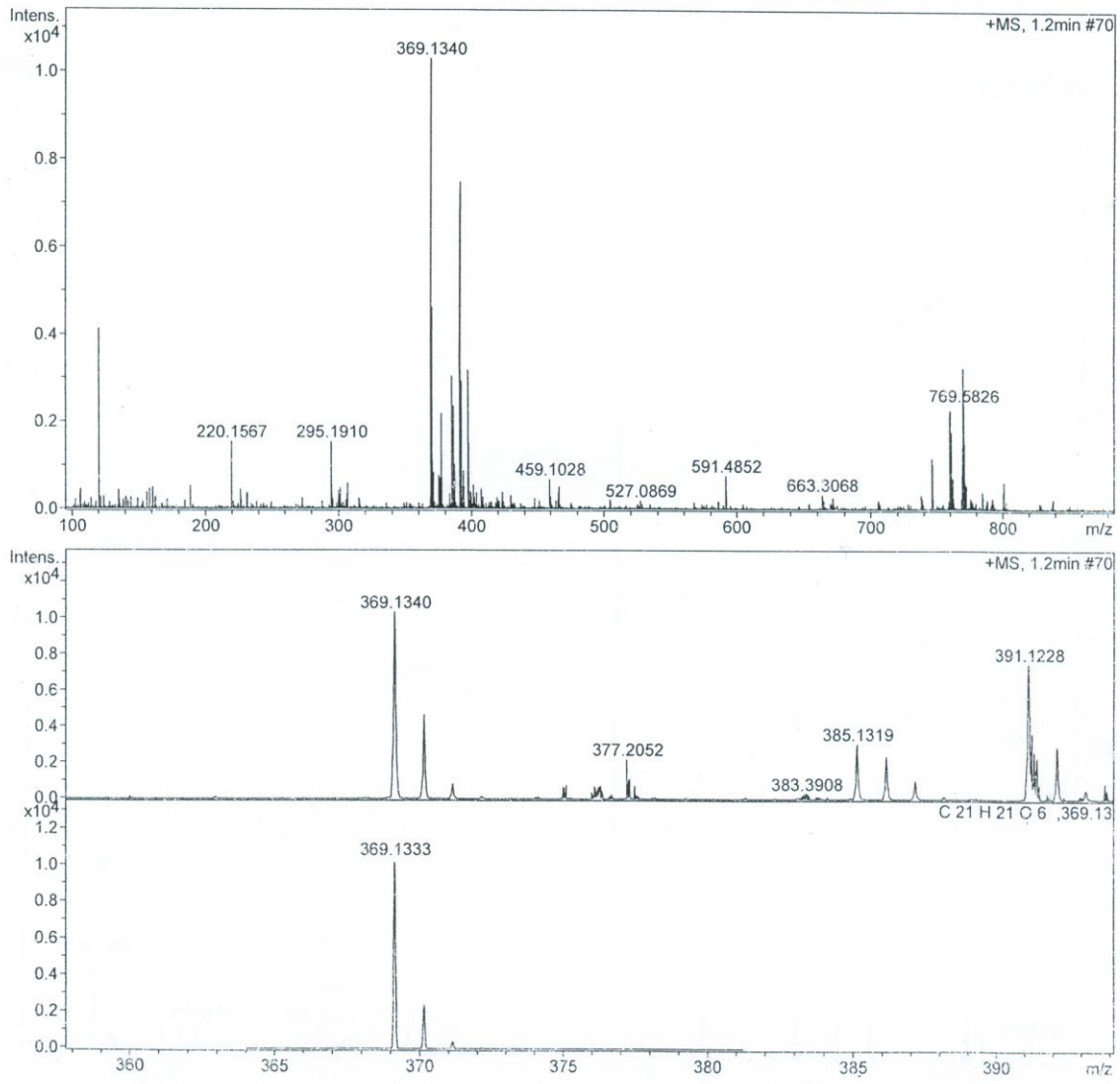

Figure 29 HRESI Mass Spectrum of compound CAL1

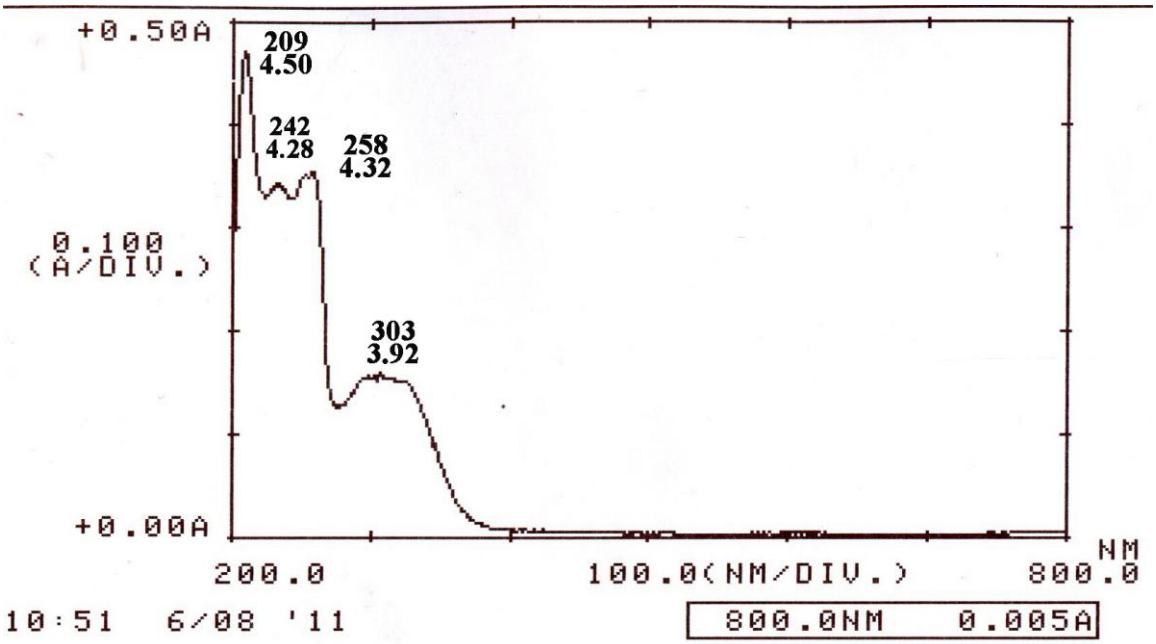

Figure 30 UV Spectrum of compound CAL1 (methanol)

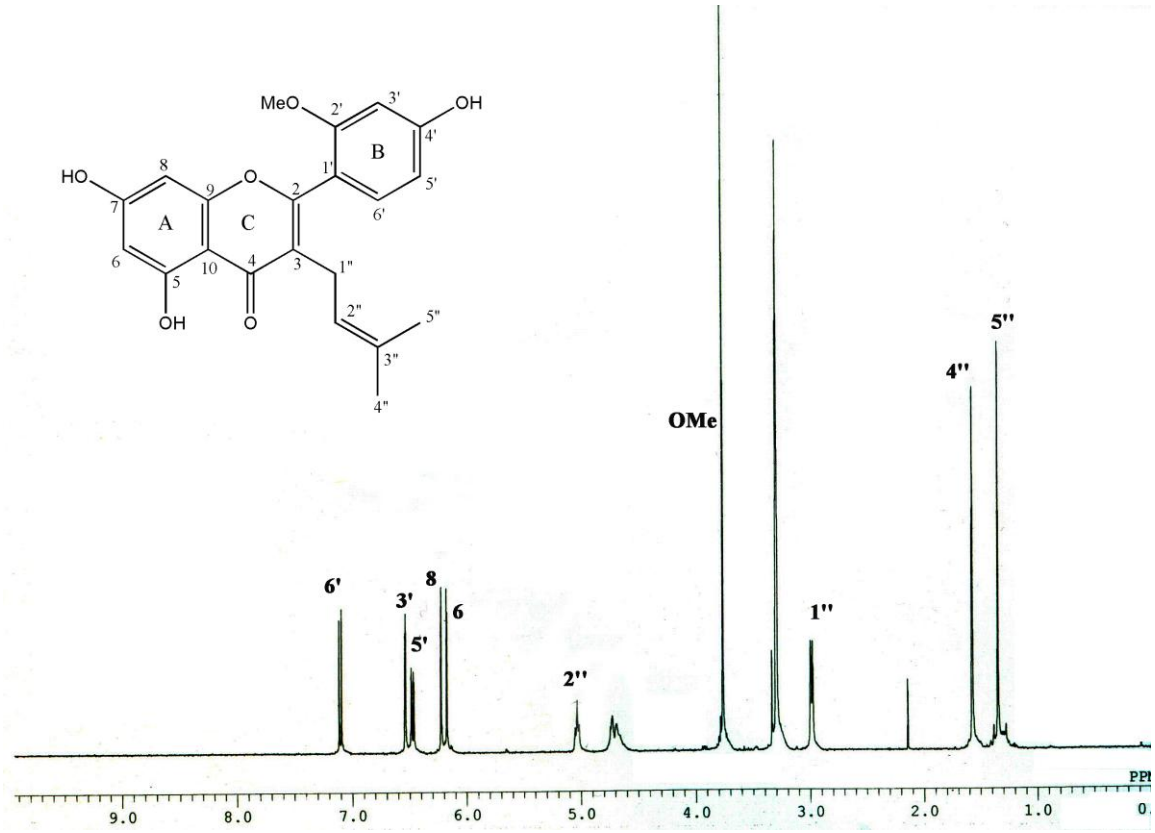

Figure 31 ^{1}H-NMR (400 MHz) Spectrum of compound CAL1 (methanol-d_4)

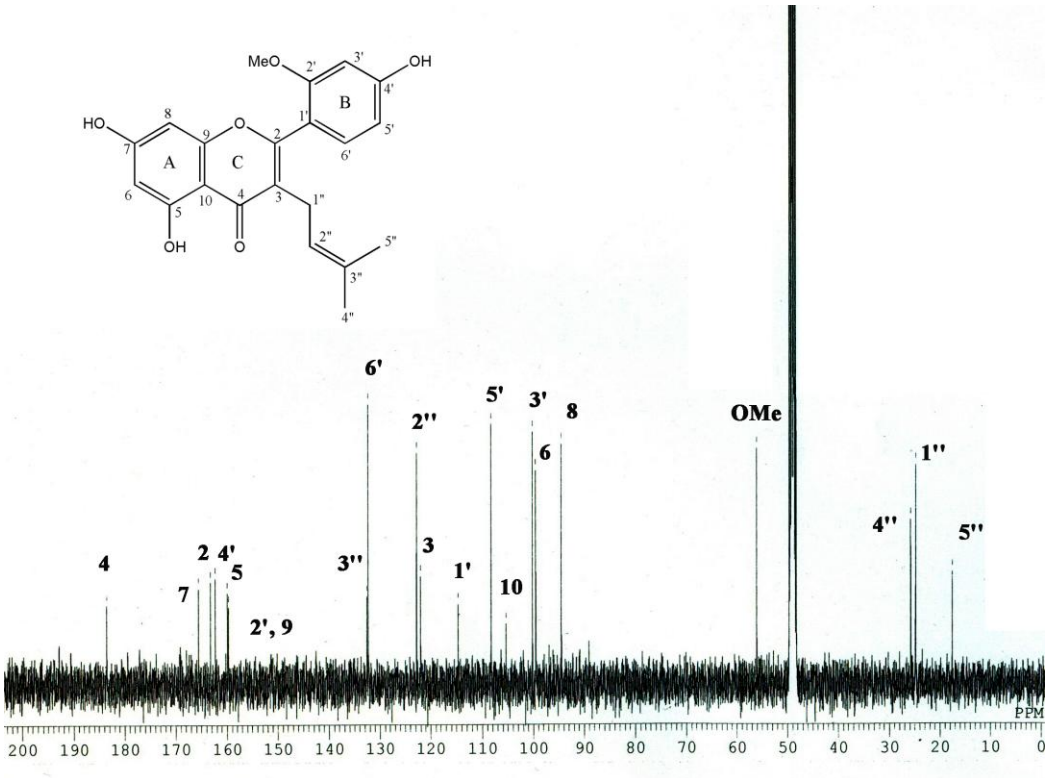

Figure 32 ^{13}C-NMR (100 MHz) Spectrum of compound CAL1 (methanol-d_4)

Figure 33 HMQC Spectrum of compound CAL1 (methanol-d_4)

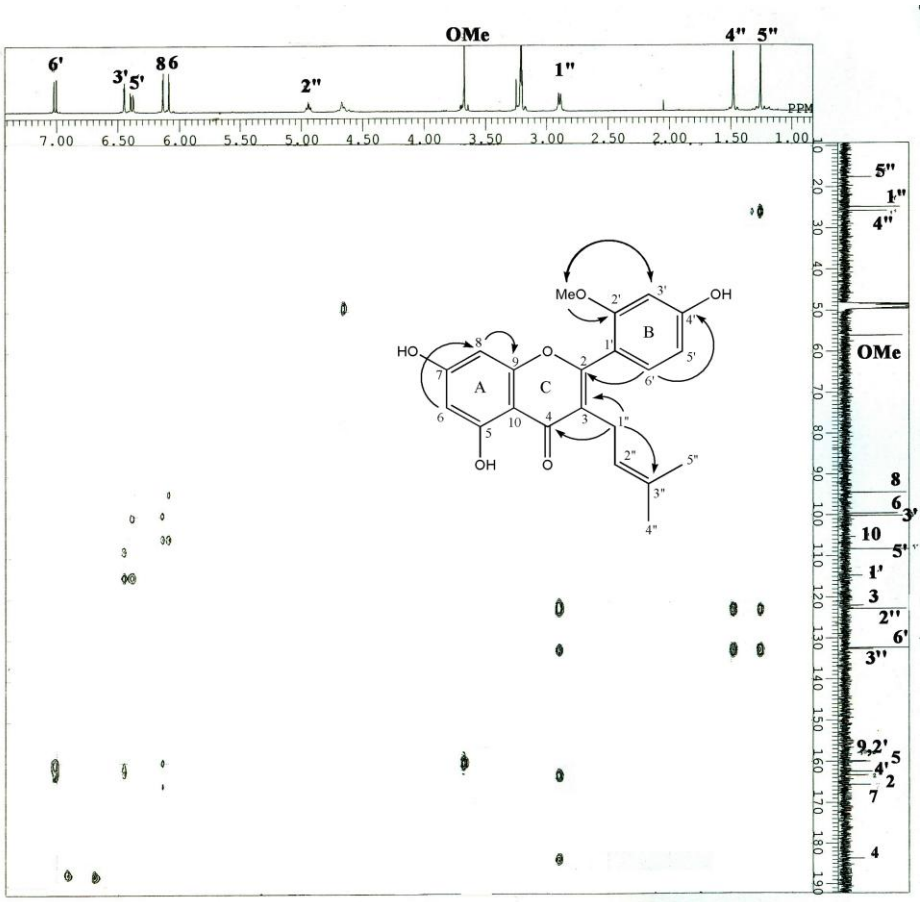

Figure 34 HMBC Spectrum of compound CAL1 (methanol-d_4)

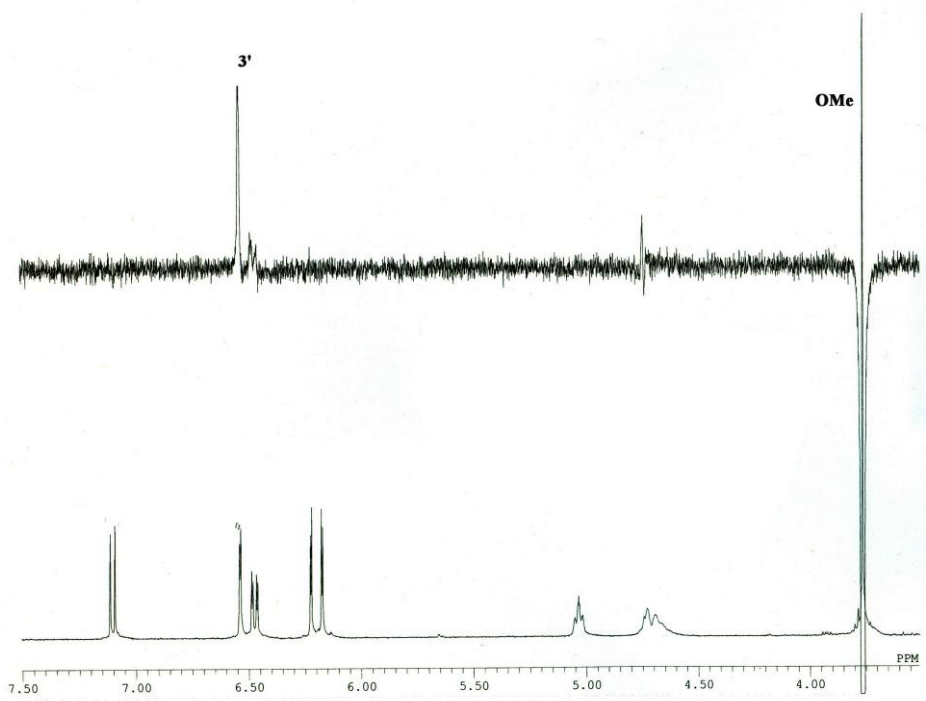

Figure 35 NOE Spectrum of compound CAL1 (methanol-d_4)

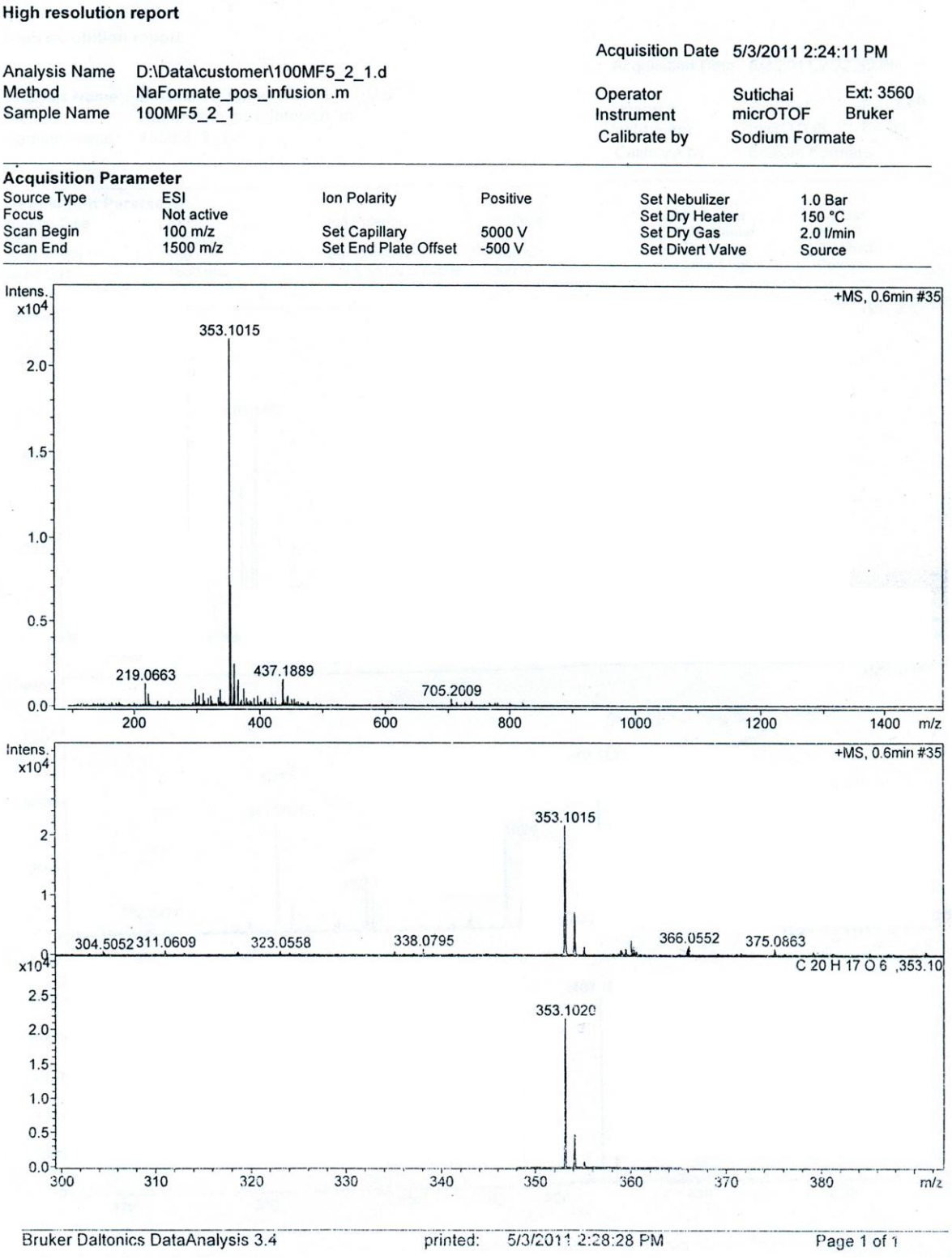

Figure 36 HRESI Mass Spectrum of compound CAL2

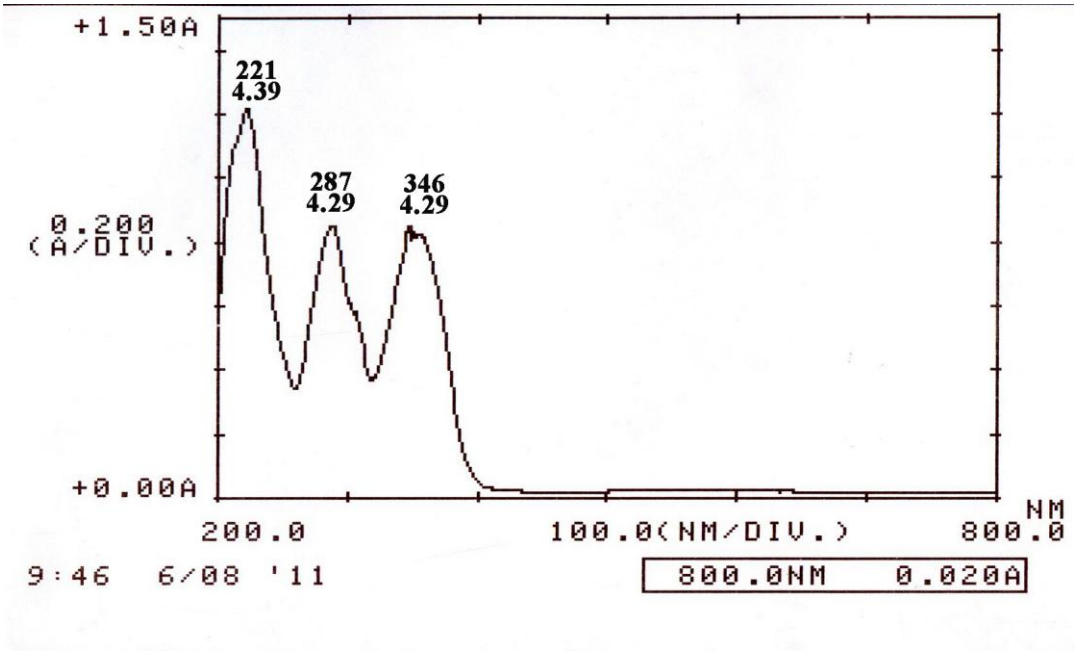

Figure 37 UV Spectrum of compound CAL2 (methanol)

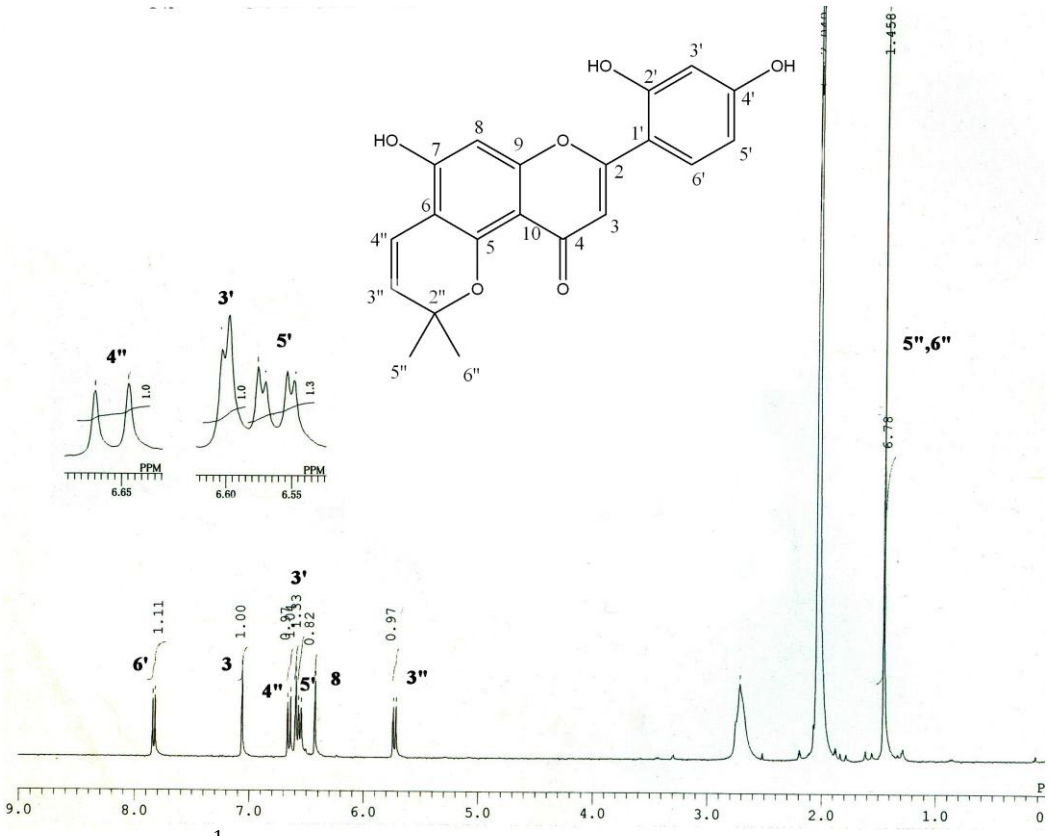

Figure 38 ^{1}H-NMR (400 MHz) Spectrum of compound CAL2 (acetone-d_6)

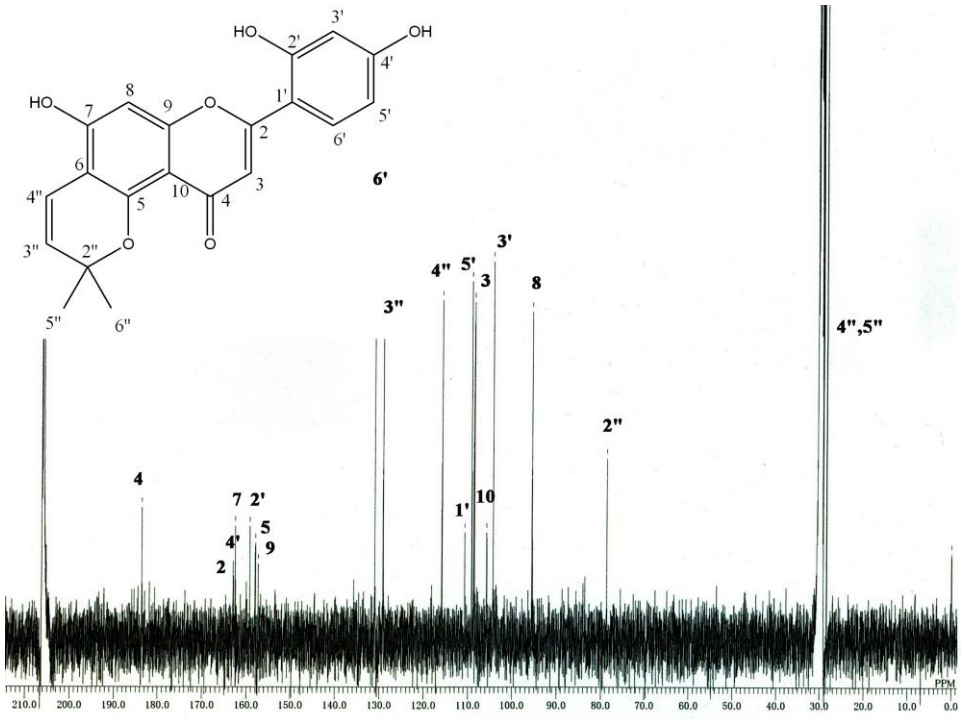

Figure 39 ^{13}C-NMR (100 MHz) Spectrum of compound CAL2 (acetone-d_6)

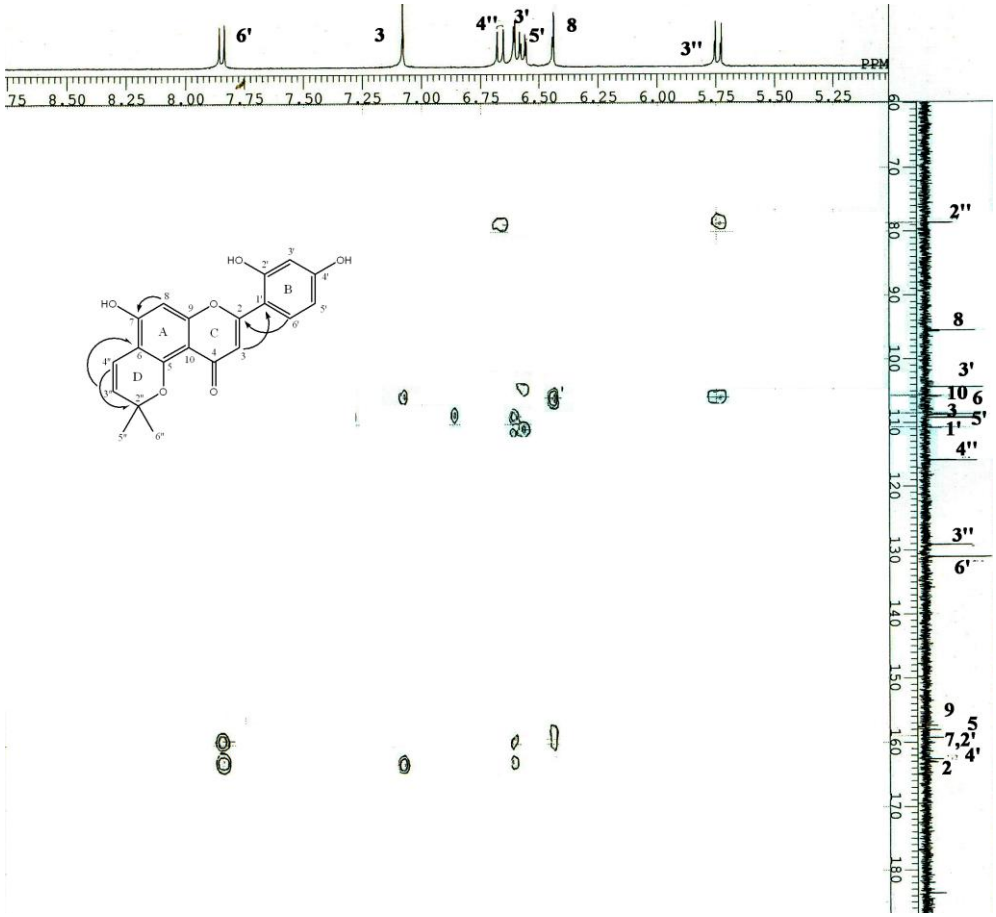

Figure 40 HMBC Spectrum of compound CAL2 (acetone-d_6)

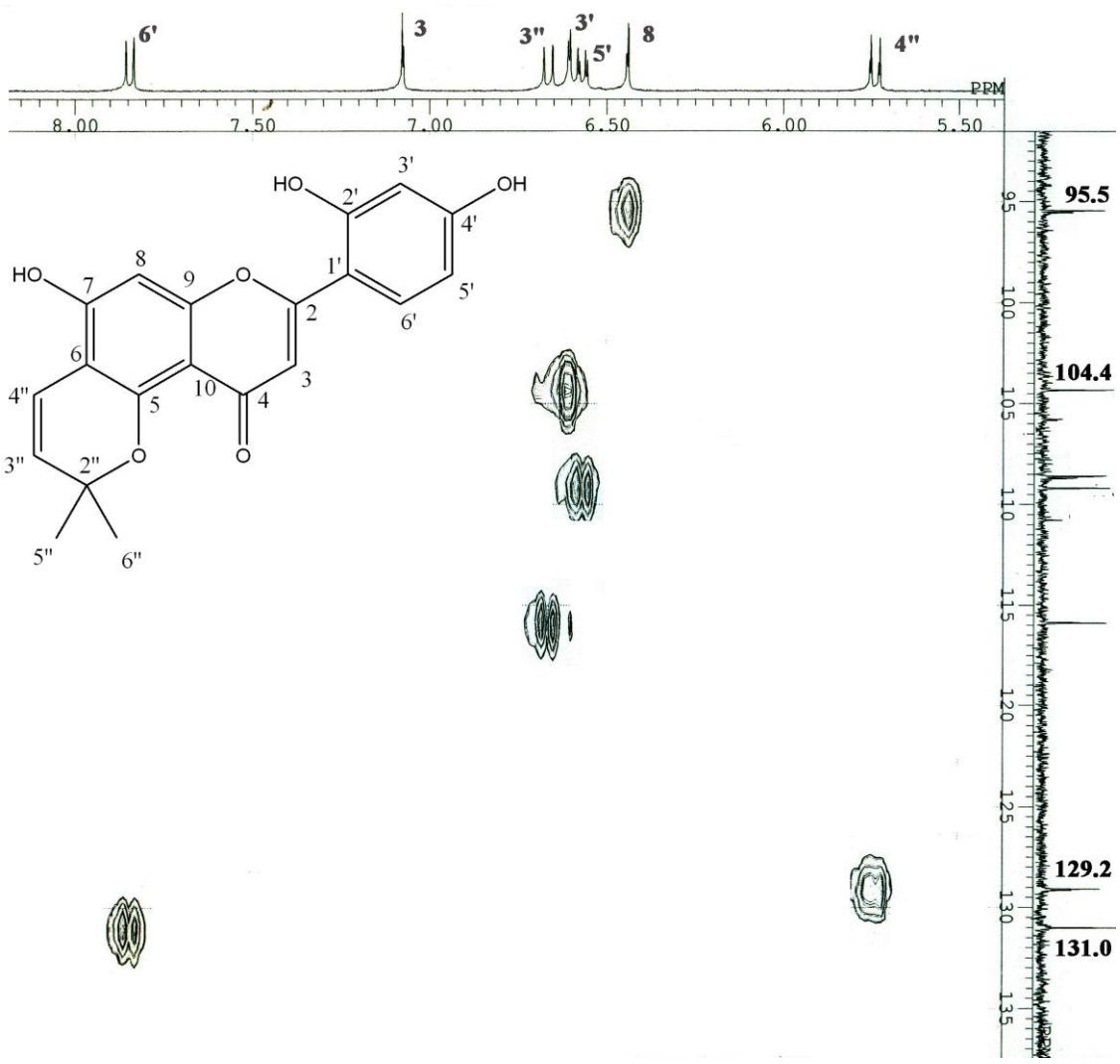

Figure 41 HMQC Spectrum of compound CAL2 (acetone-d_6)

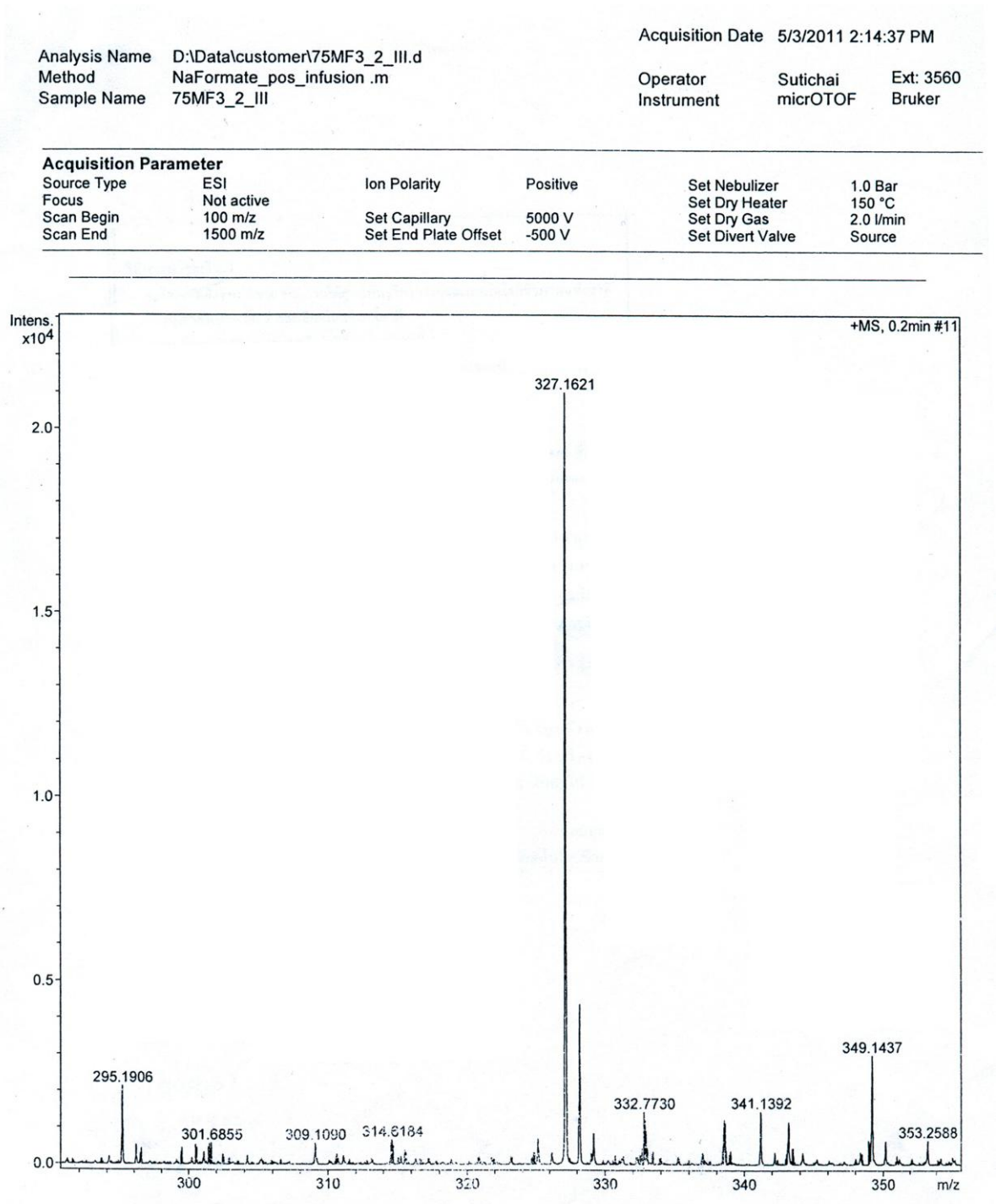

Figure 42 HRESI Mass Spectrum of compound CAL3

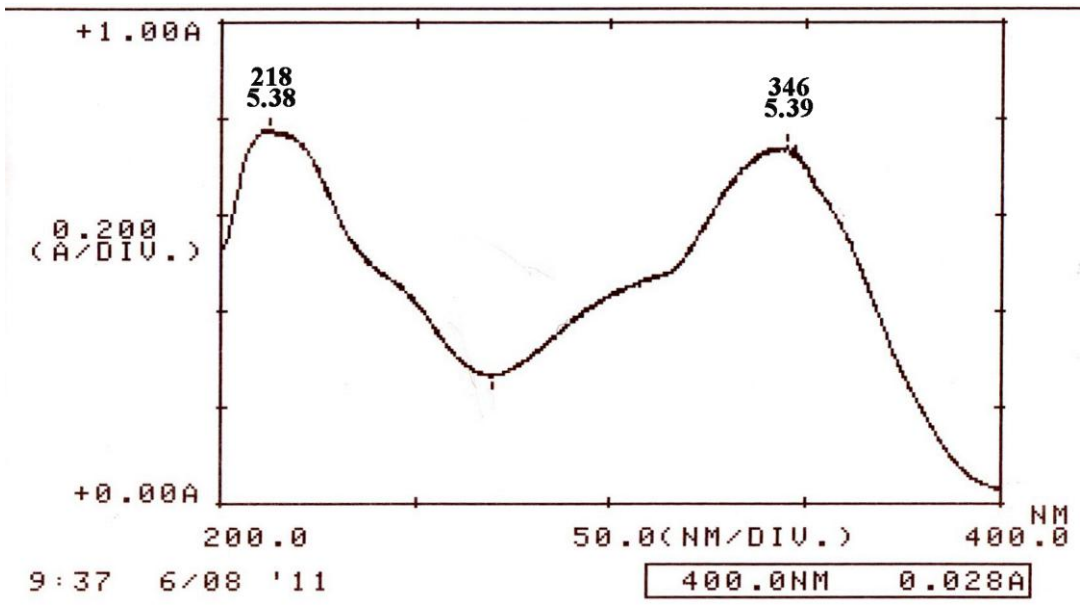

Figure 43 UV Spectrum of compound CAL3 (methanol)

Figure 44 ^{1}H-NMR (400 MHz) Spectrum of compound CAL3 (acetone-d_6)

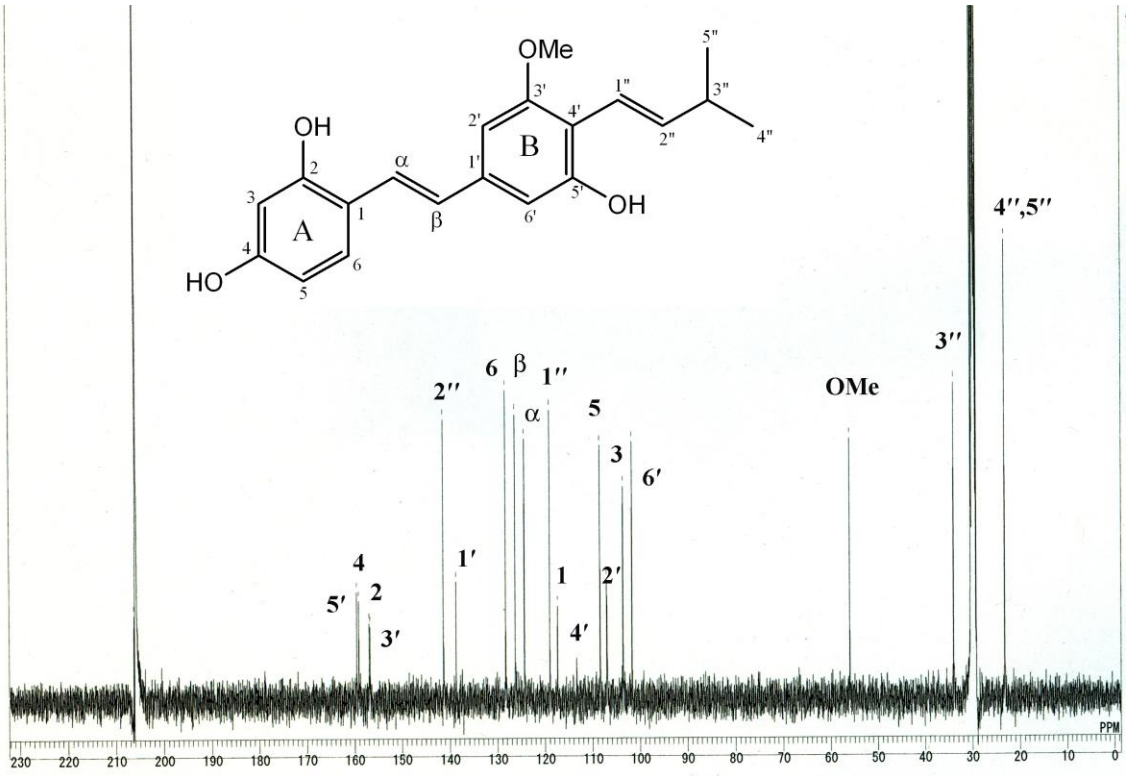

Figure 45 ^{13}C-NMR (100 MHz) Spectrum of compound CAL3 (acetone-d_6)

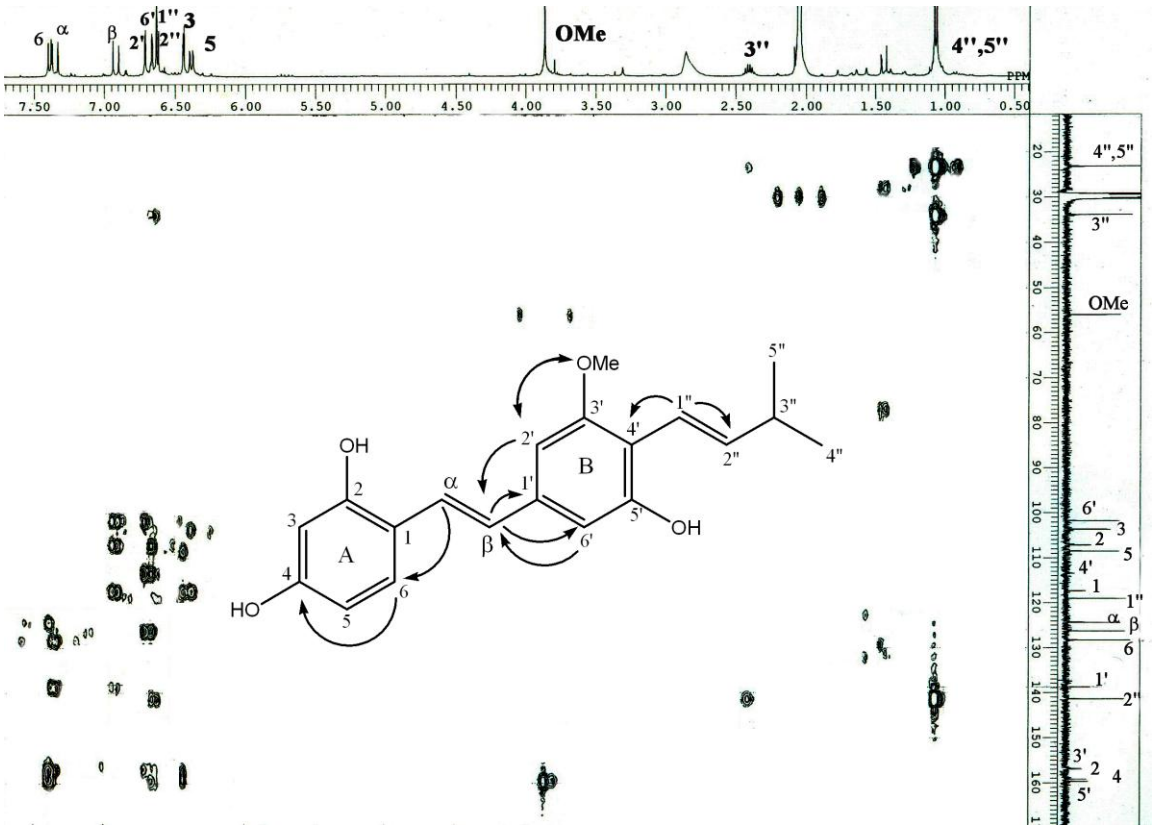

Figure 46 HMBC Spectrum of compound CAL3 (acetone-d_6)

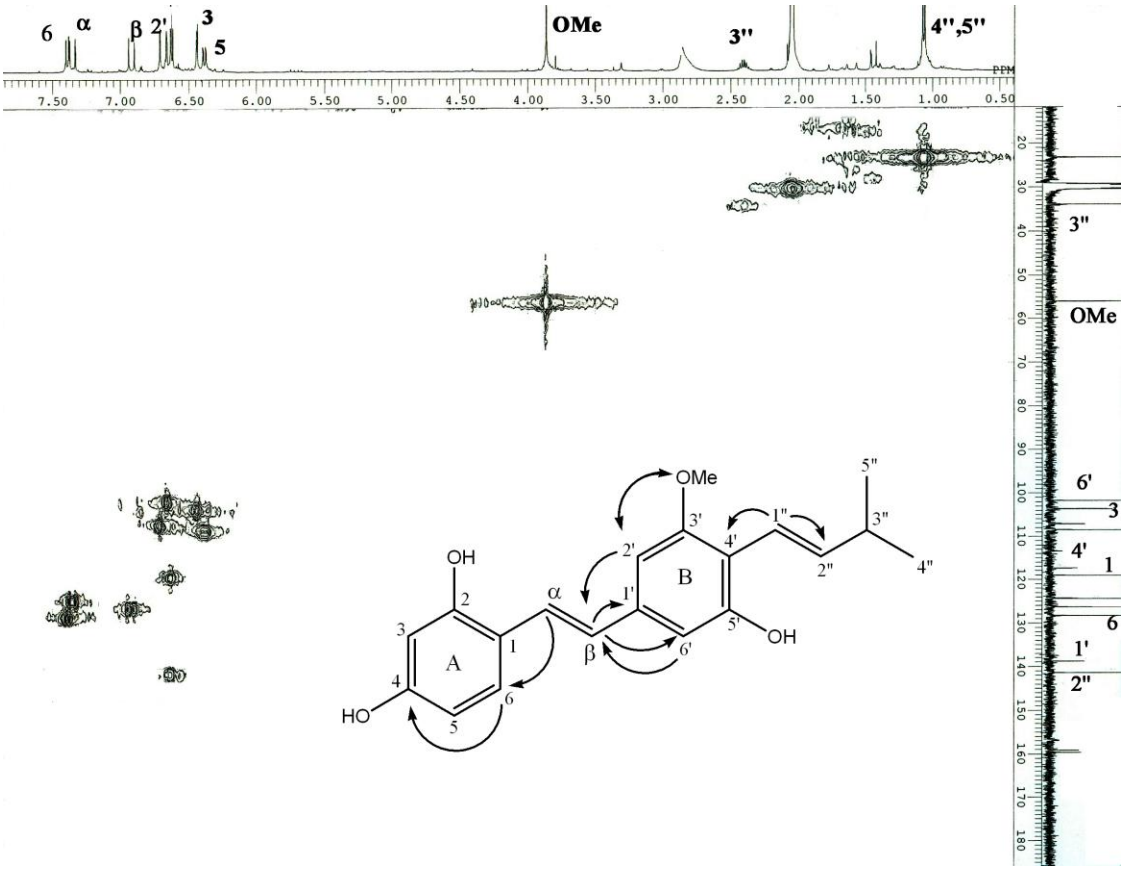

Figure 47 HMQC Spectrum of compound CAL 3 (acetone-d_6)

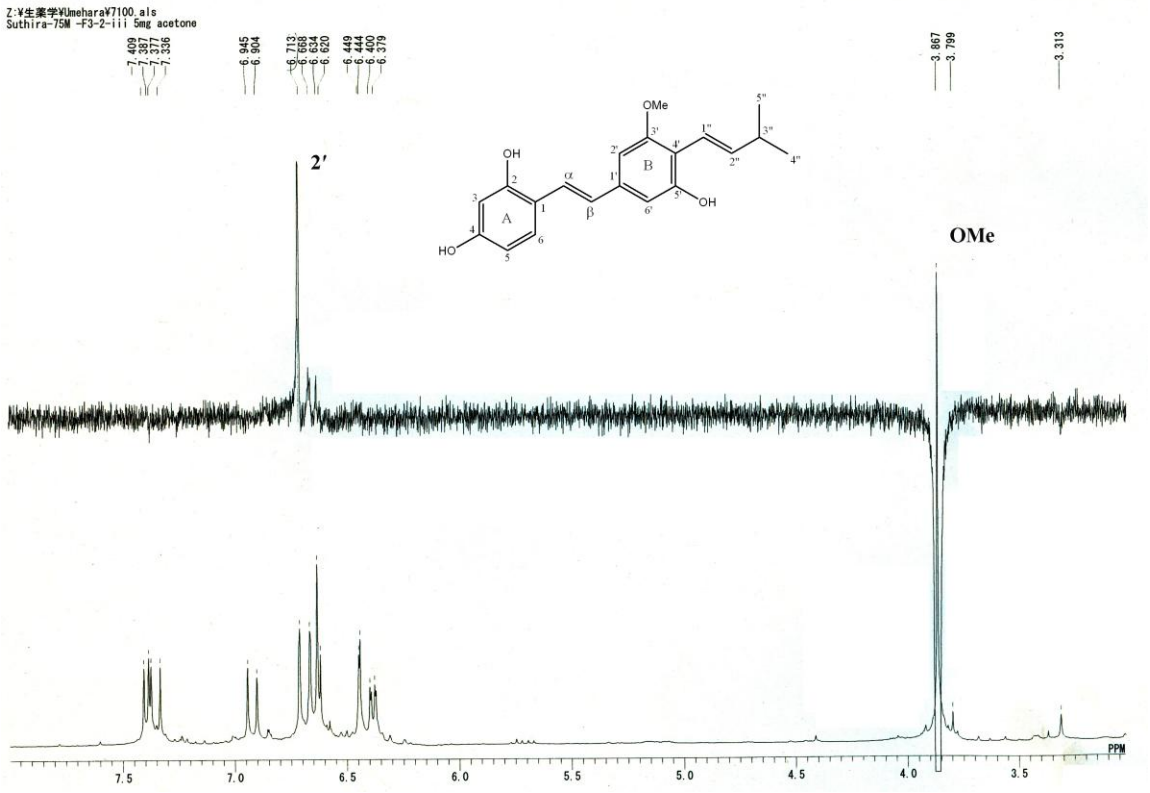

Figure 48 NOE Spectrum of compound CAL3 (acetone-d_6)

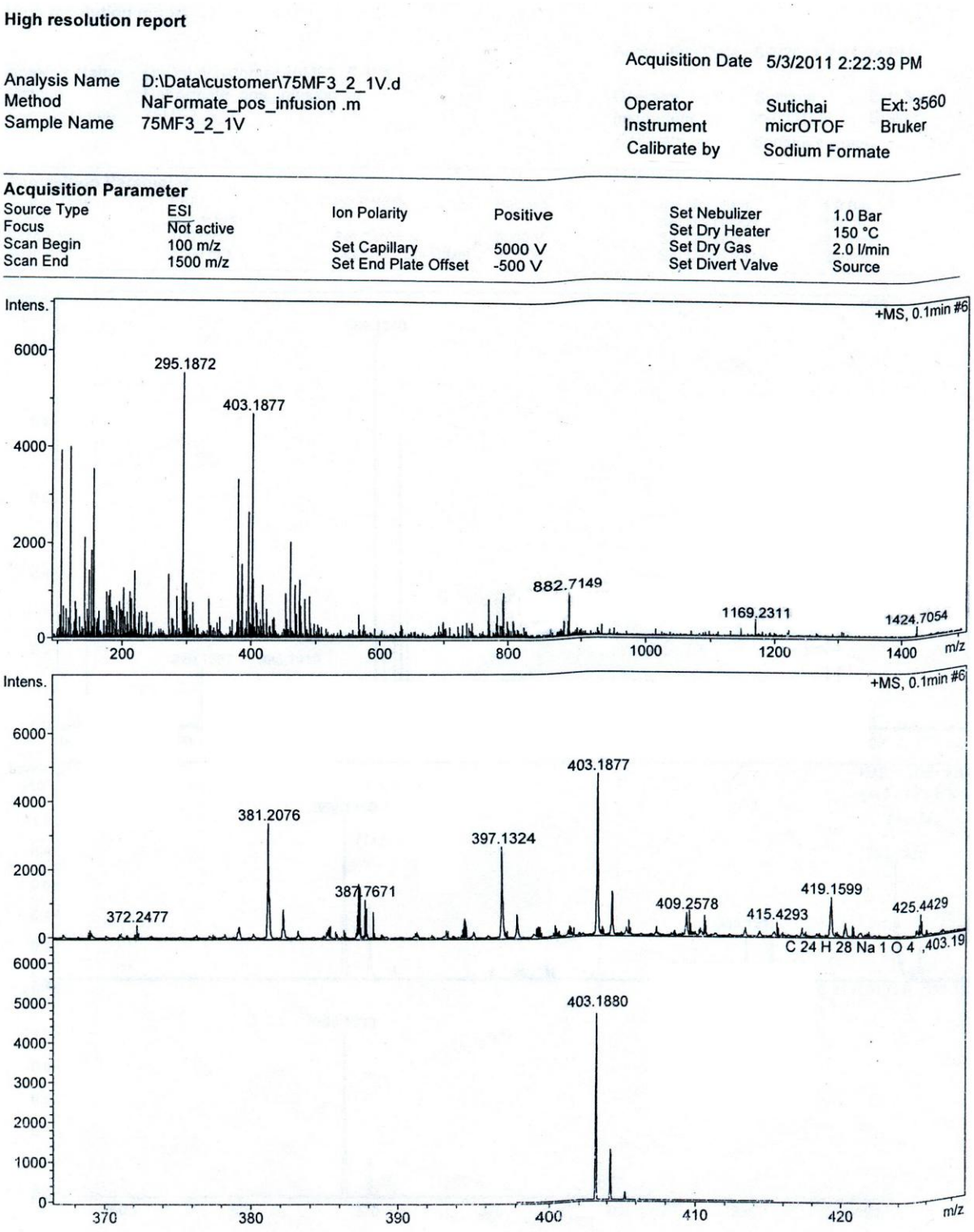

Figure 49 HRESI Mass Spectrum of compound CAL4

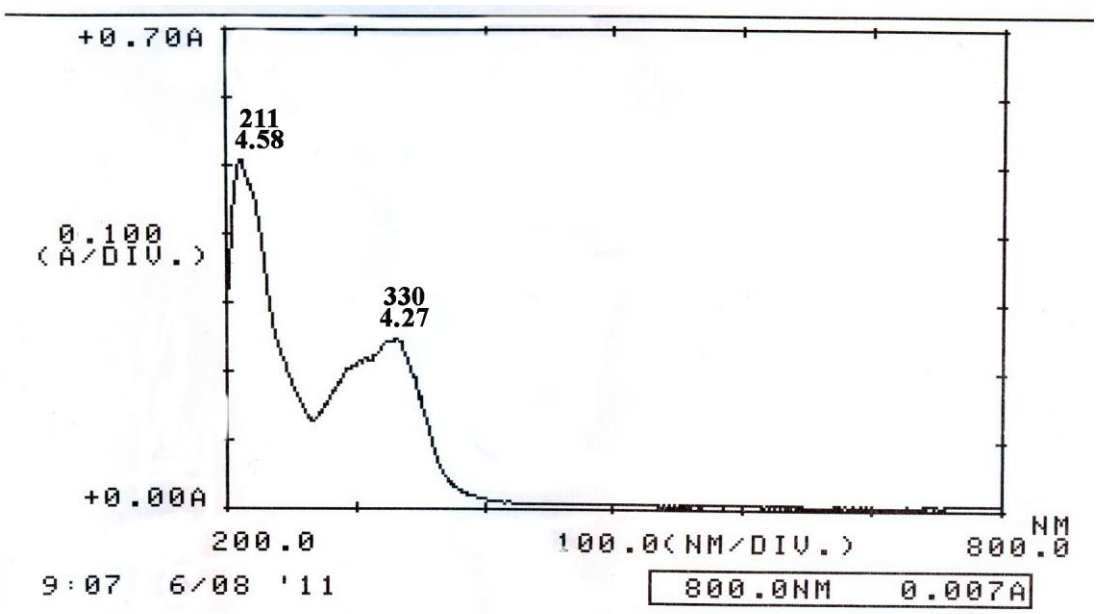

Figure 50 UV Spectrum of compound CAL4 (methanol)

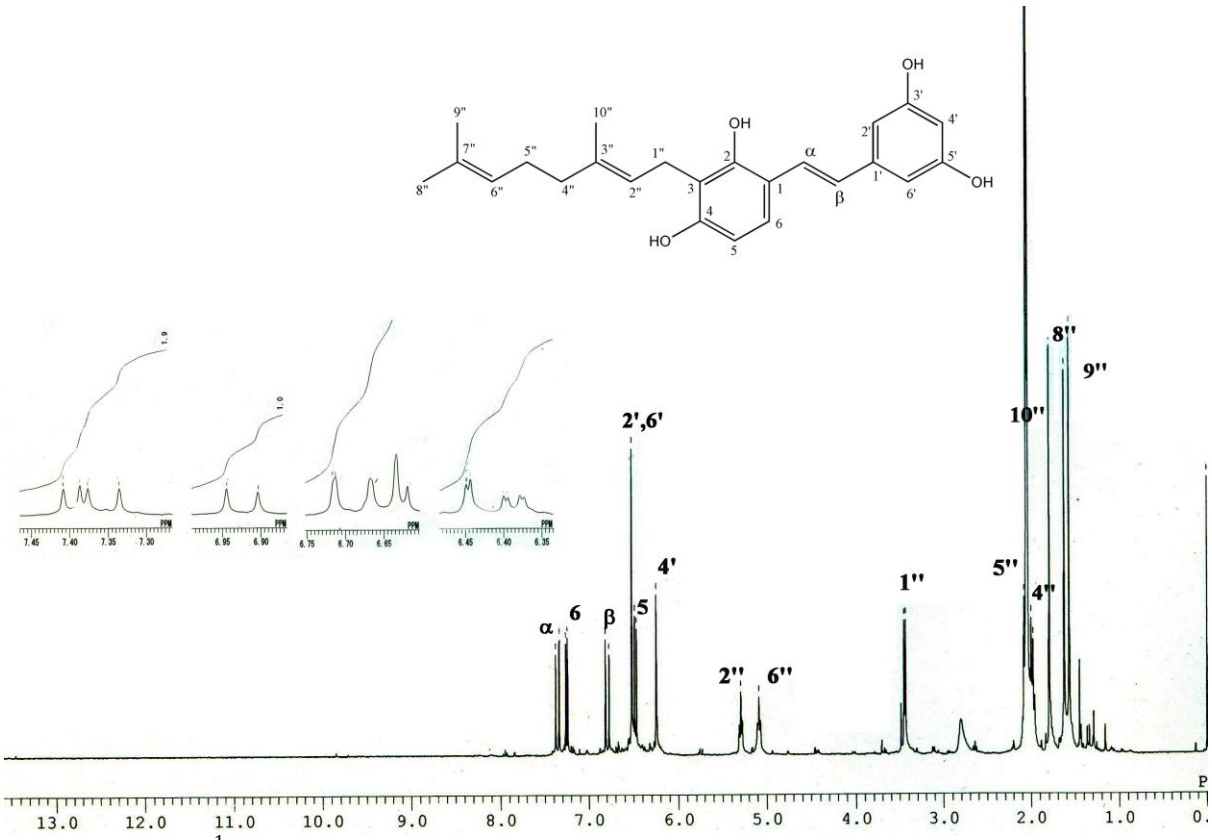

Figure 51 ^{1}H-NMR (400 MHz) Spectrum of compound CAL4 (acetone-d_6)

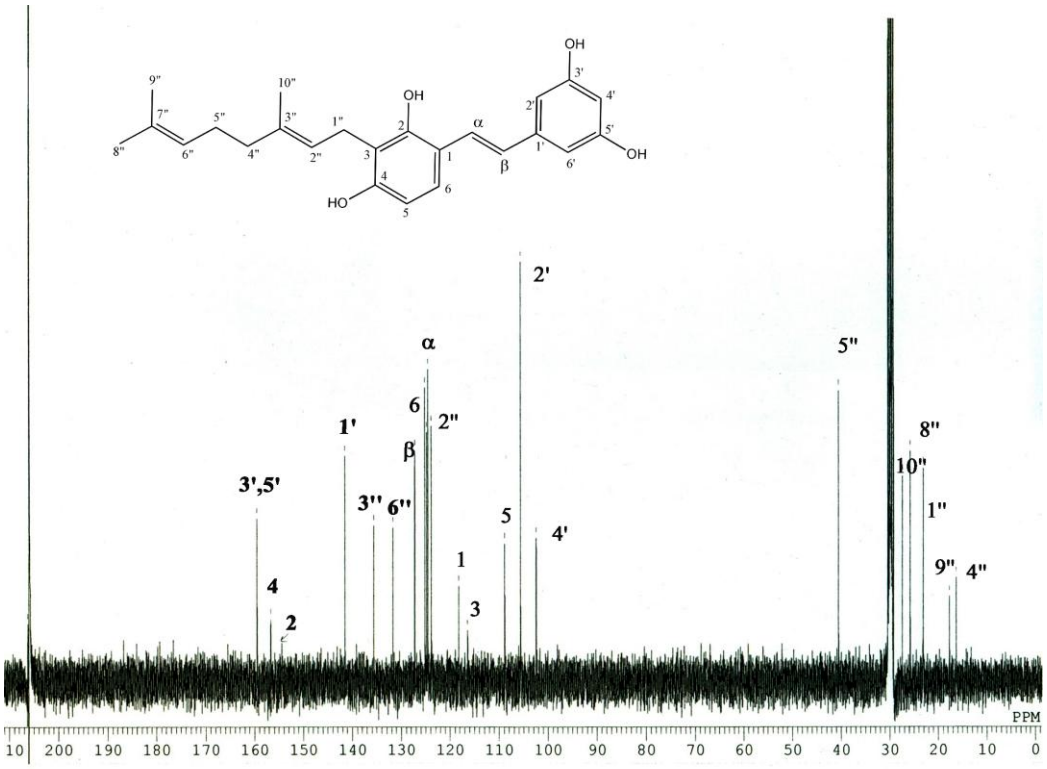

Figure 52 ^{13}C-NMR (100 MHz) Spectrum of compound CAL4 (acetone-d_6)

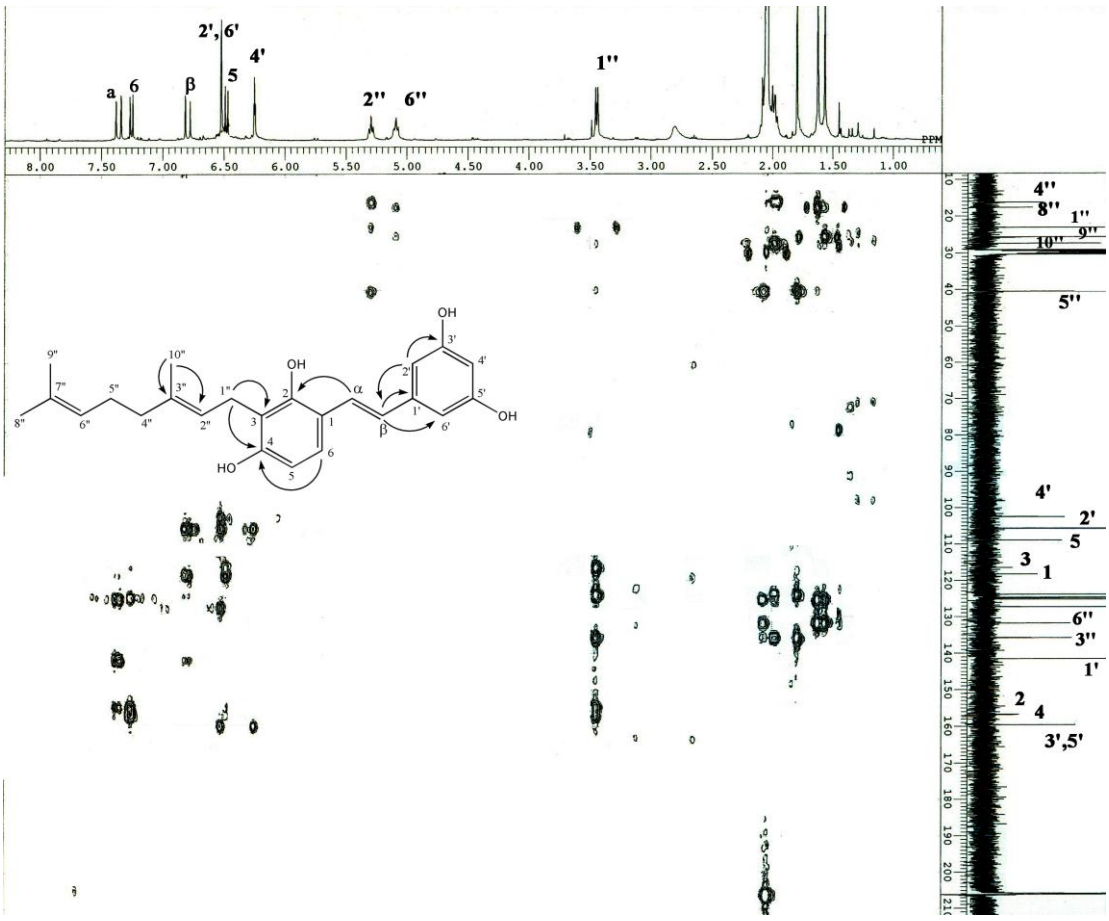

Figure 53 HMBC Spectrum of compound CAL4 (acetone-d_6)

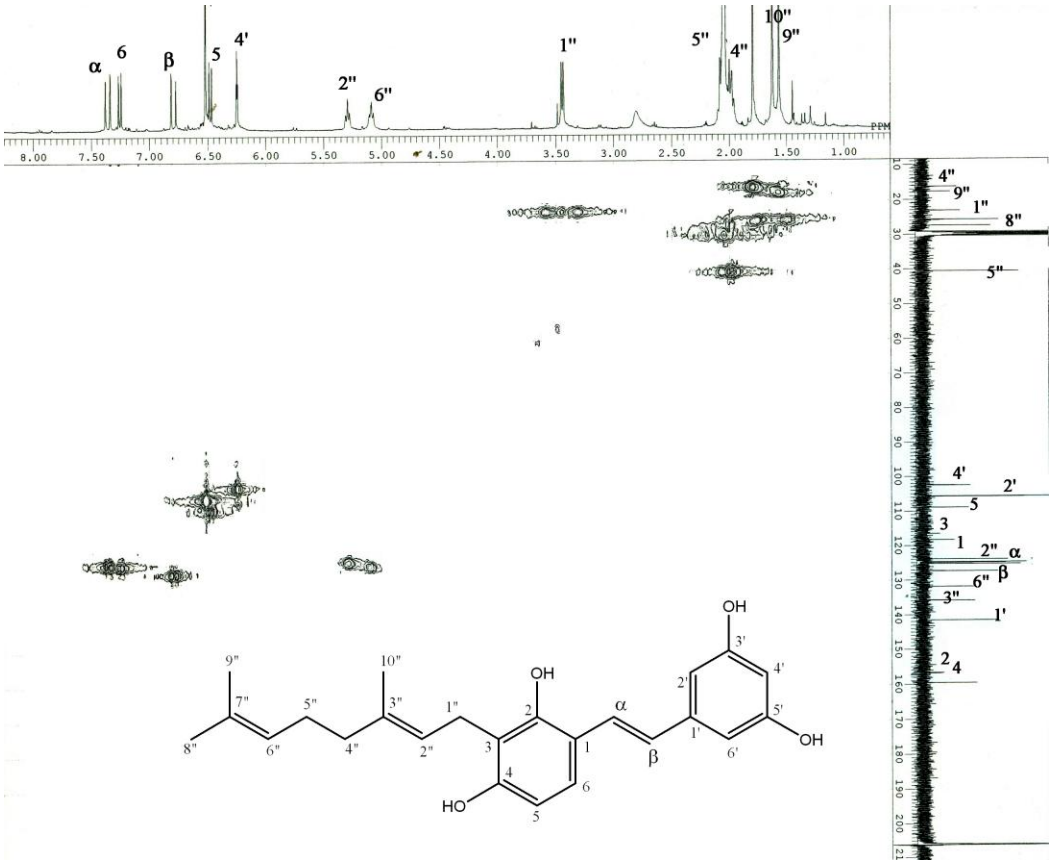

Figure 54 HMQC Spectrum of compound CAL 4 (acetone-d_6)

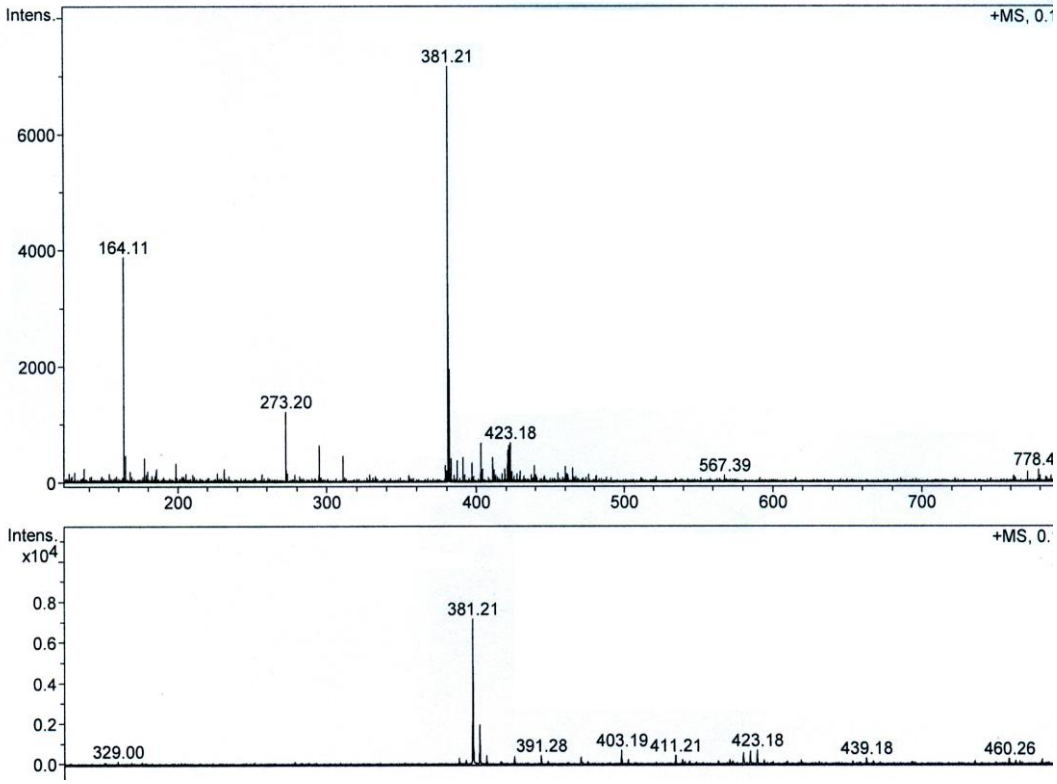

Figure 55 ESI Mass Spectrum of compound CAL5

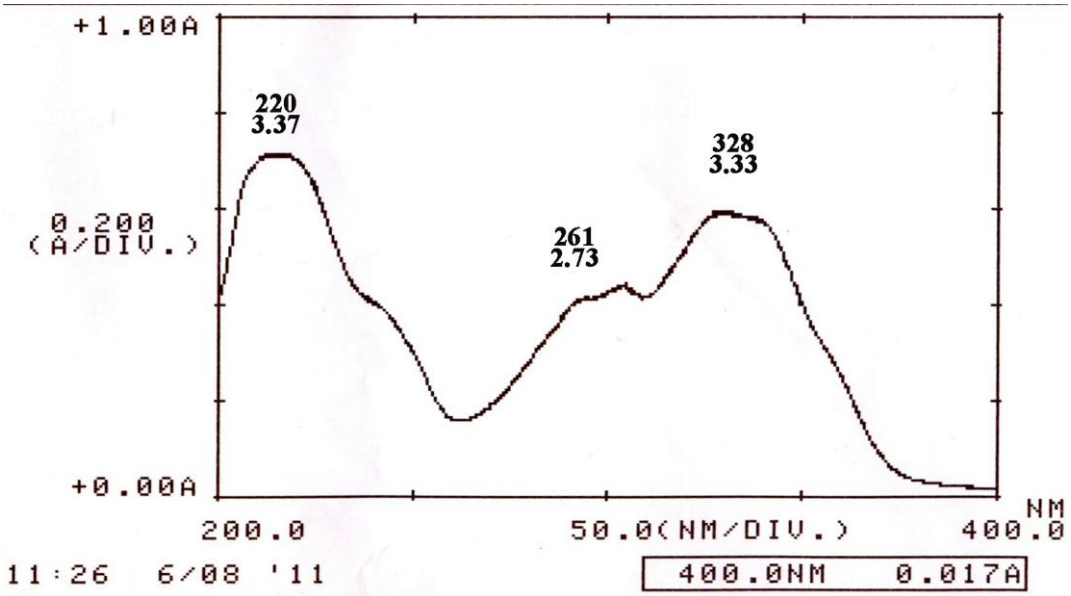

Figure 56 UV Spectrum of compound CAL5 (methanol)

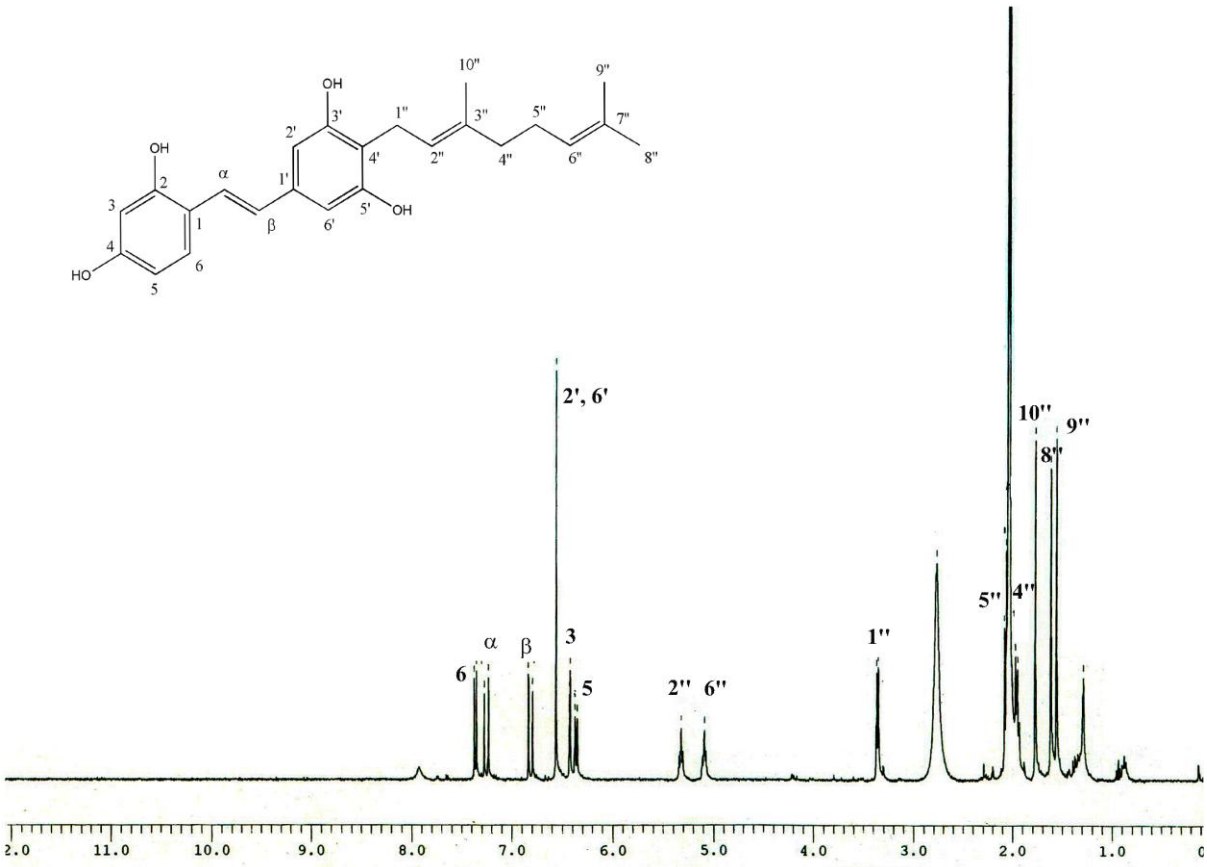

Figure 57 ^{1}H-NMR (400 MHz) Spectrum of compound CAL5 (acetone-d_6)

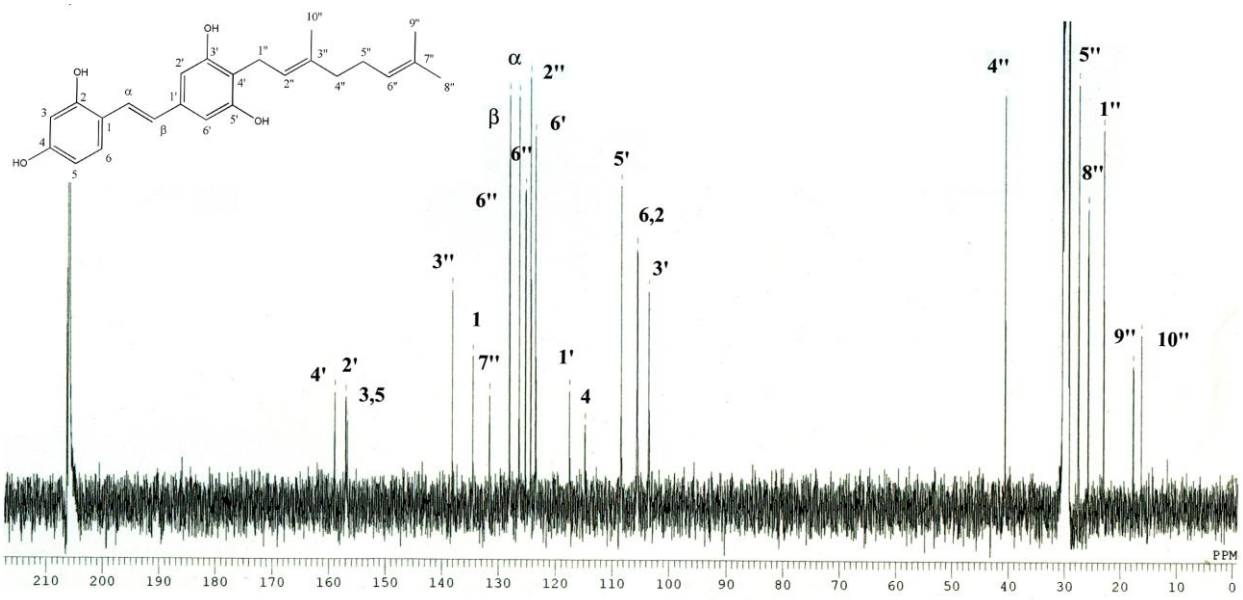

Figure 58 ^{13}C-NMR (100 MHz) Spectrum of compound CAL5 (acetone-d_6)

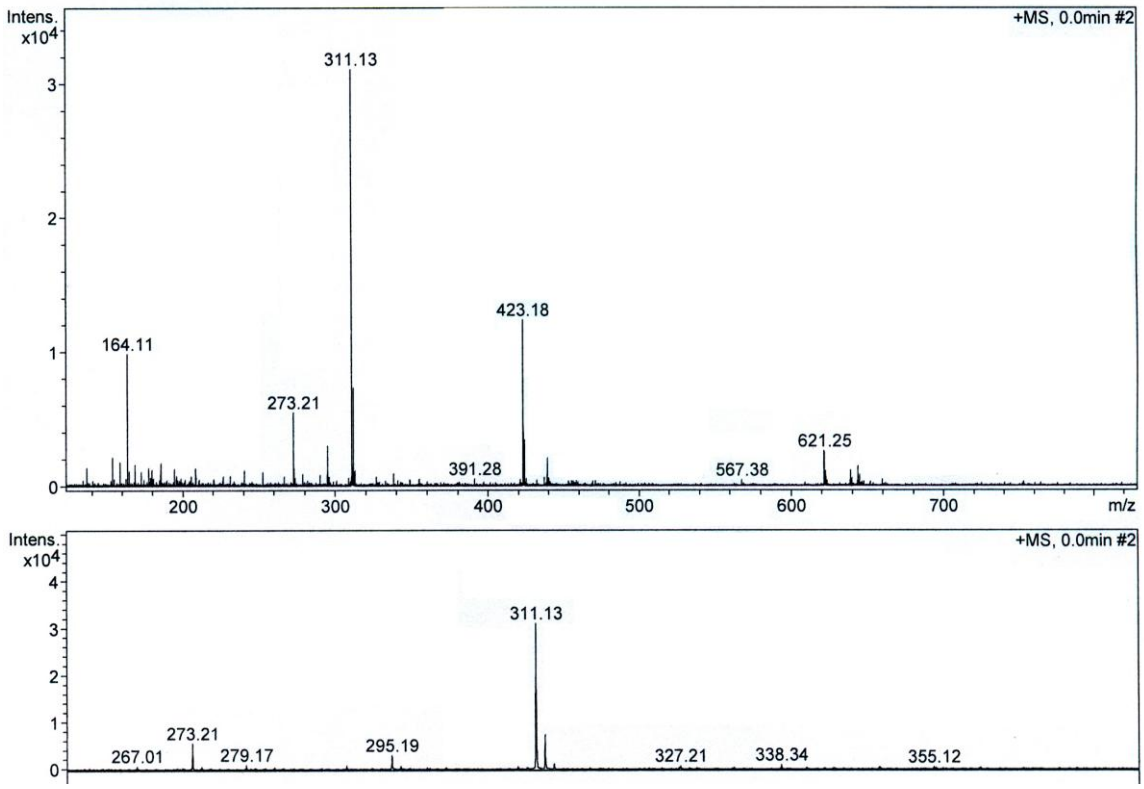

Figure 59 ESI Mass Spectrum of compound CAL6

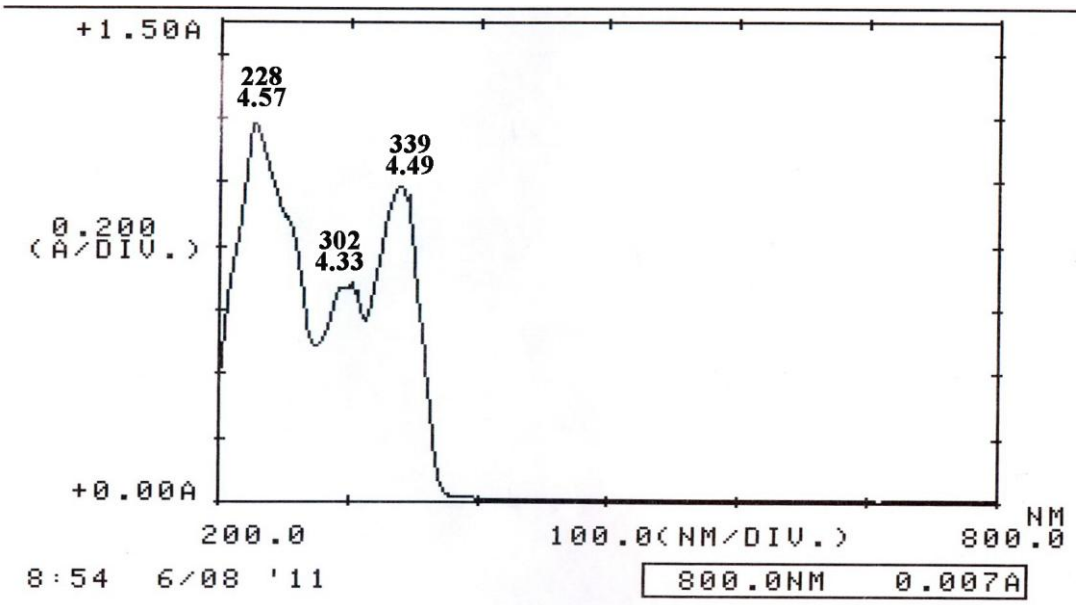

Figure 60 UV Spectrum of compound CAL6 (methanol)

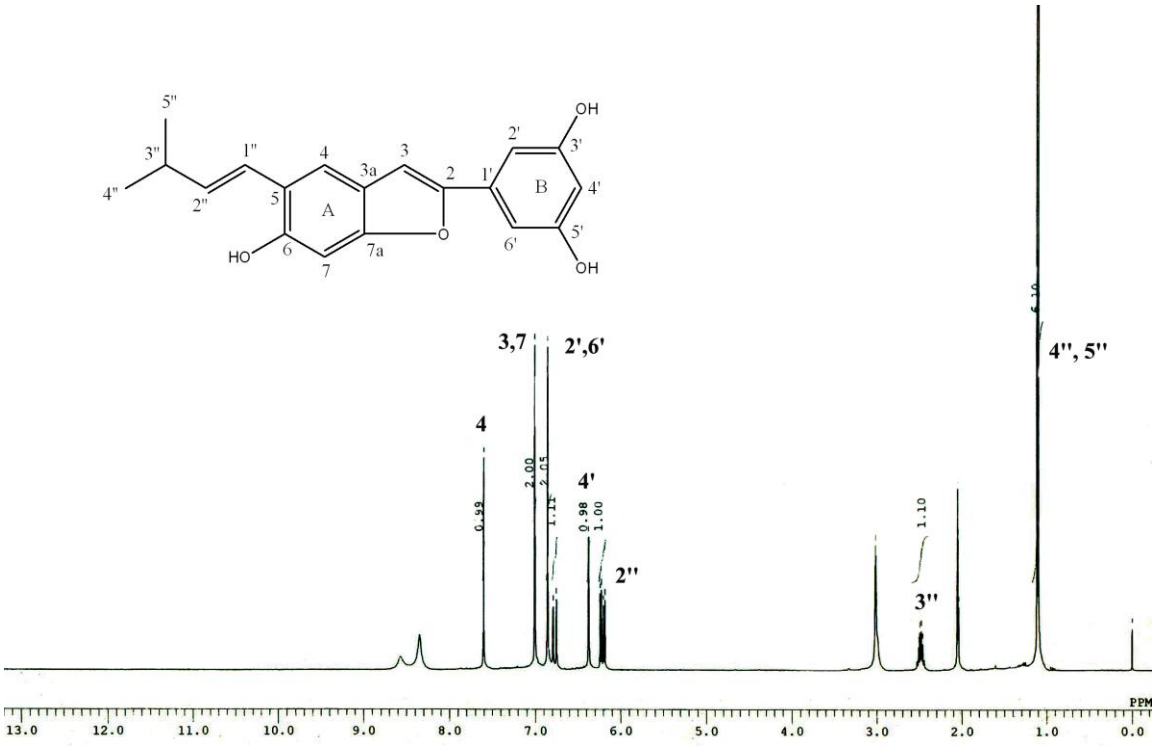

Figure 61 ^{1}H-NMR (400 MHz) Spectrum of compound CAL6 (acetone-d_6)

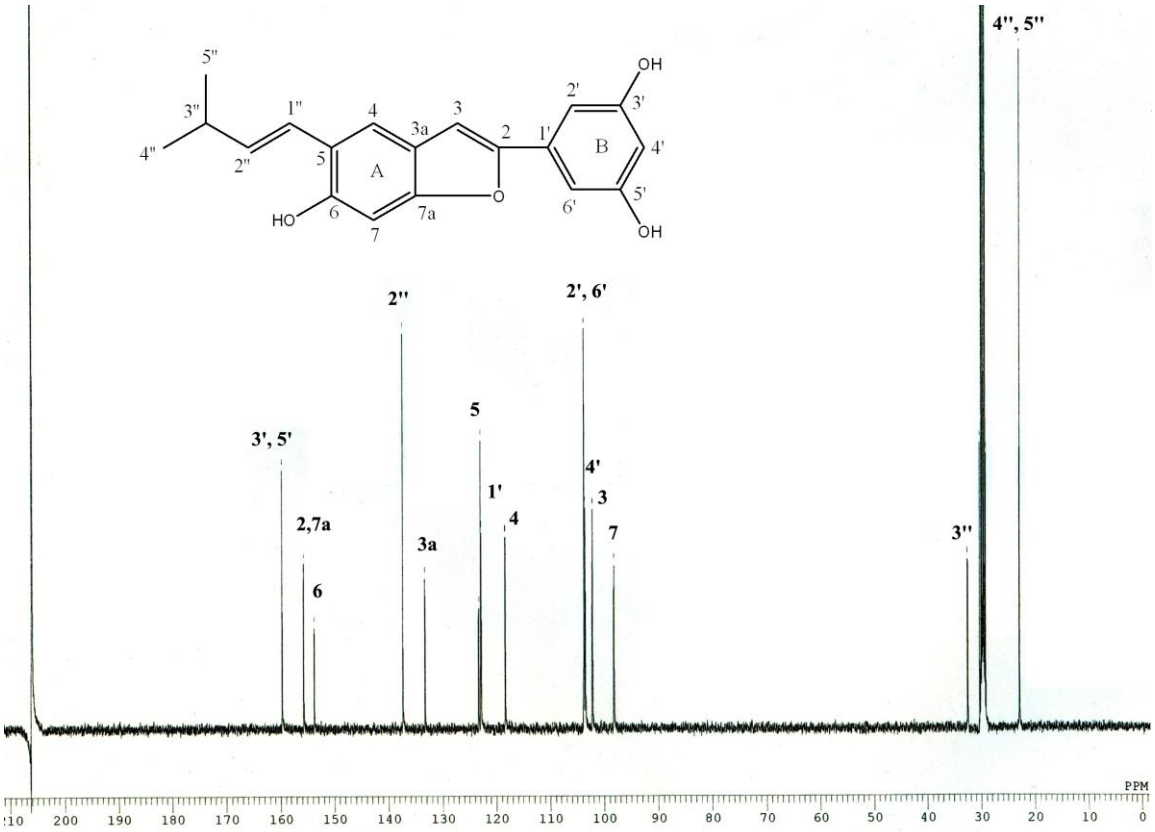

Figure 62 ^{13}C-NMR (100 MHz) Spectrum of compound CAL6 (acetone-d_6)

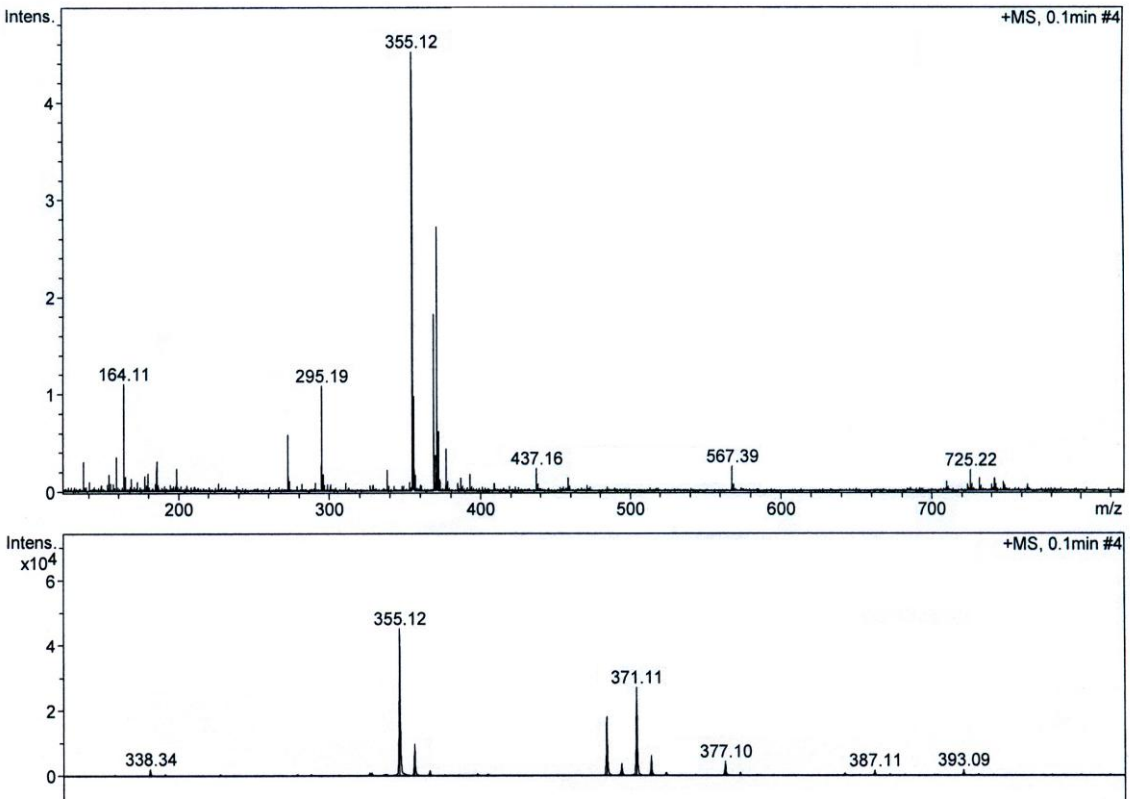

Figure 63 ESI Mass Spectrum of compound CAL7

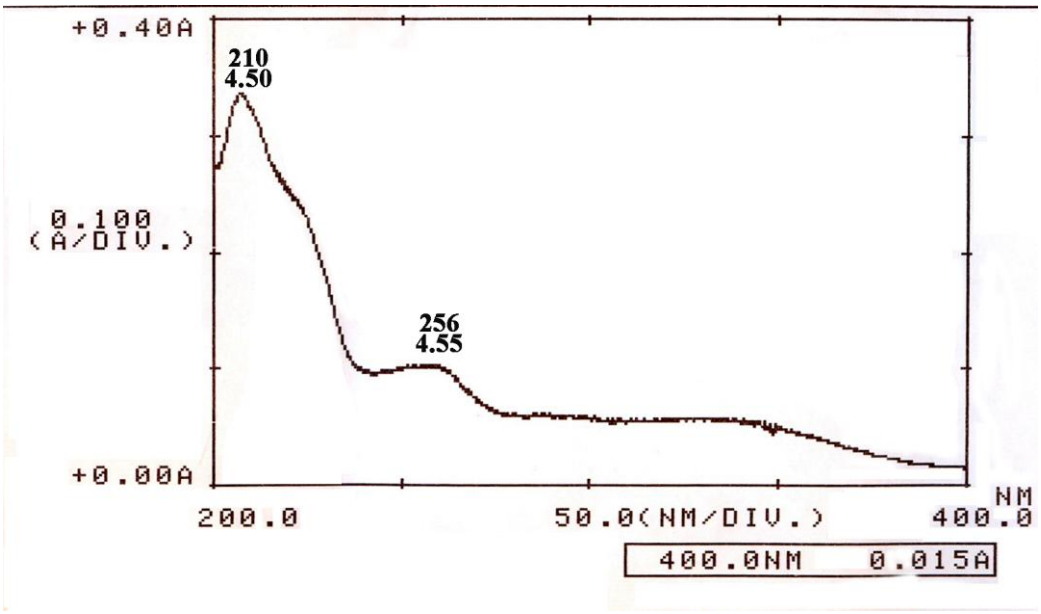

Figure 64 UV Spectrum of compound CAL7 (methanol)

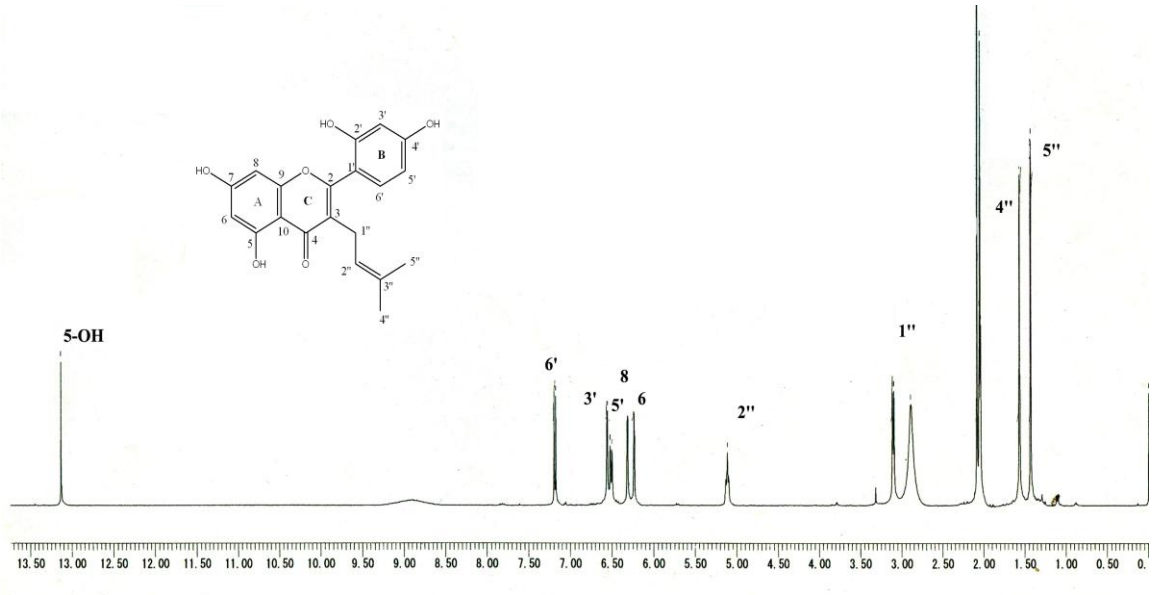

Figure 65 ^{1}H-NMR (400 MHz) Spectrum of compound CAL7 (acetone-d_6)

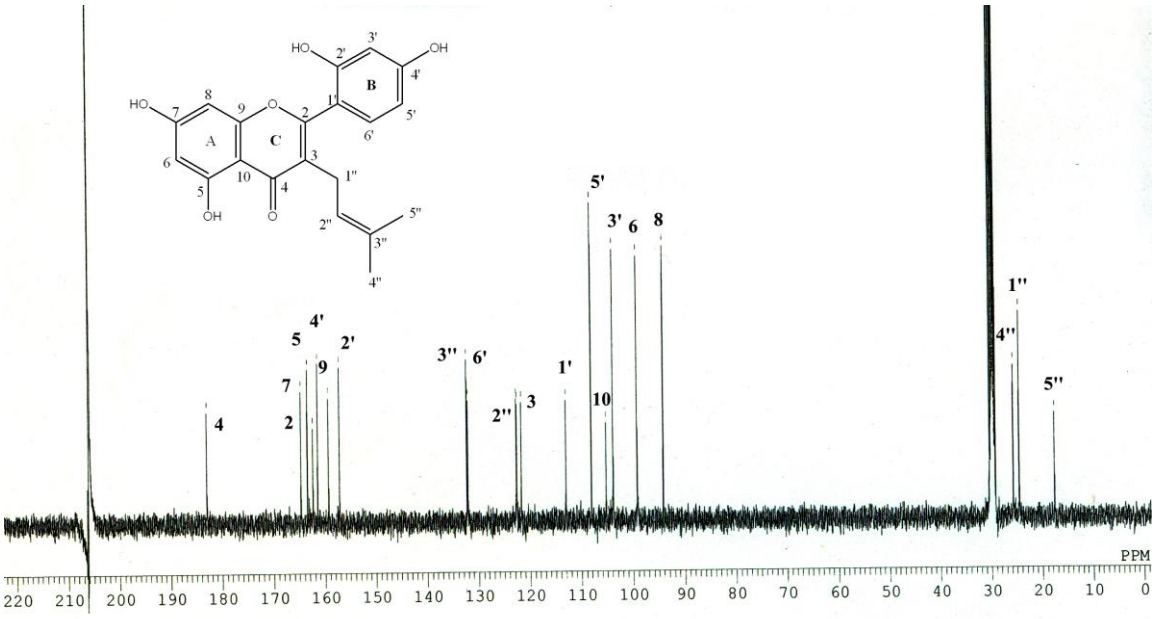

Figure 66 ^{13}C-NMR (100 MHz) Spectrum of compound CAL7 (acetone-d_6)

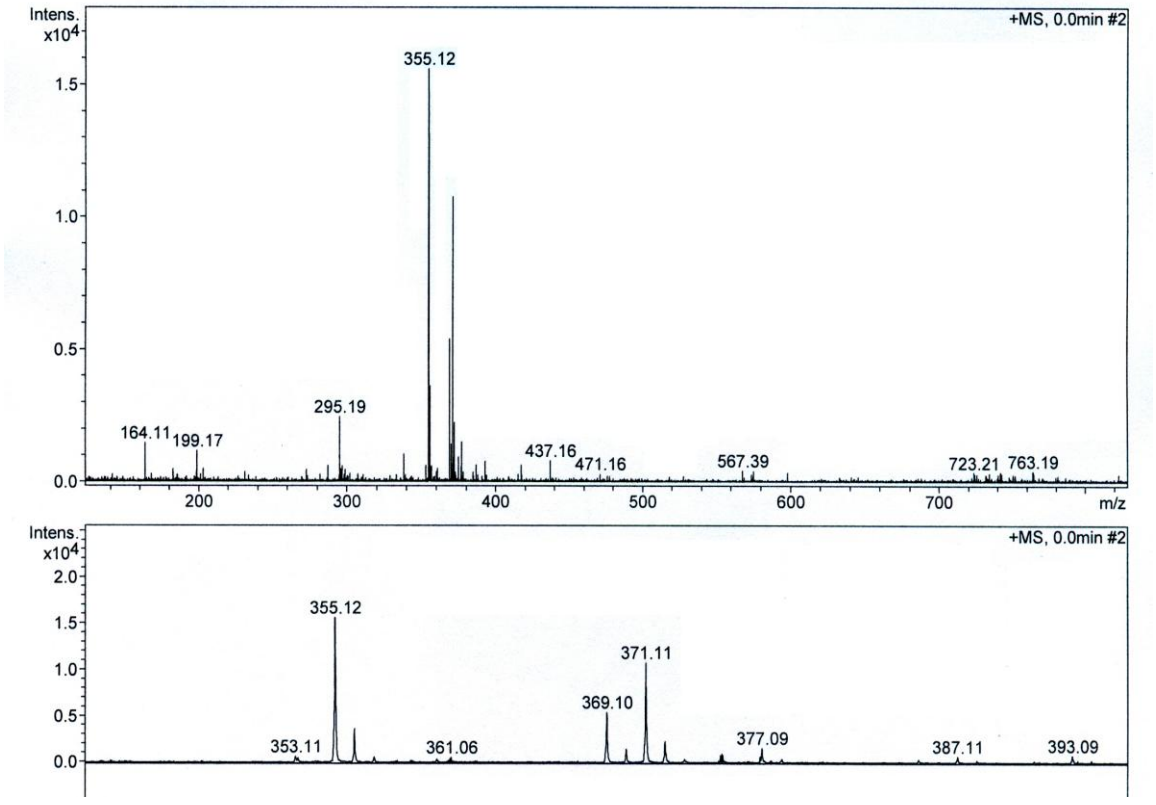

Figure 67 ESI Mass Spectrum of compound CAL8

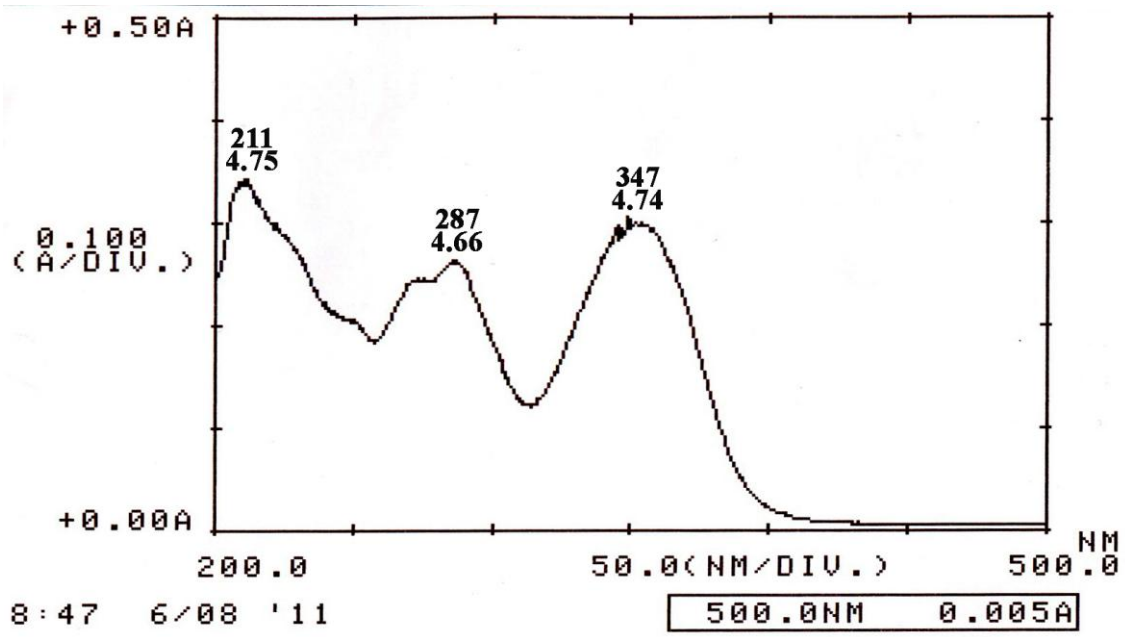

Figure 68 UV Spectrum of compound CAL8 (methanol)

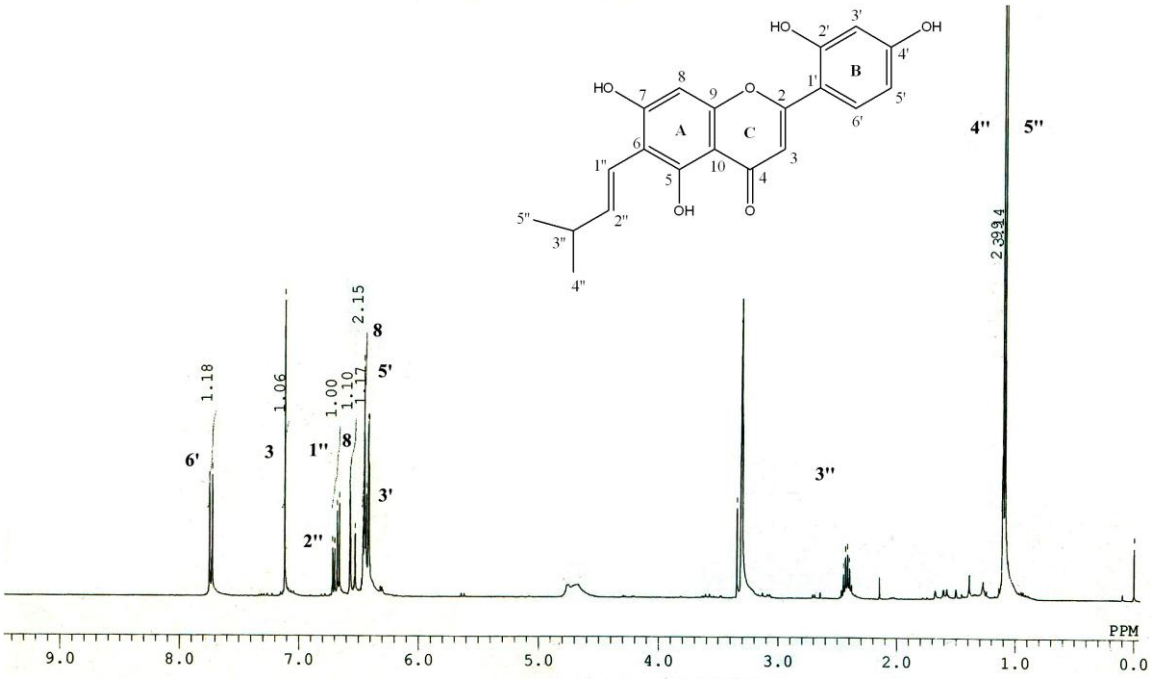

Figure 69 ^{1}H-NMR (400 MHz) Spectrum of compound CAL8 (methanol-d_4)

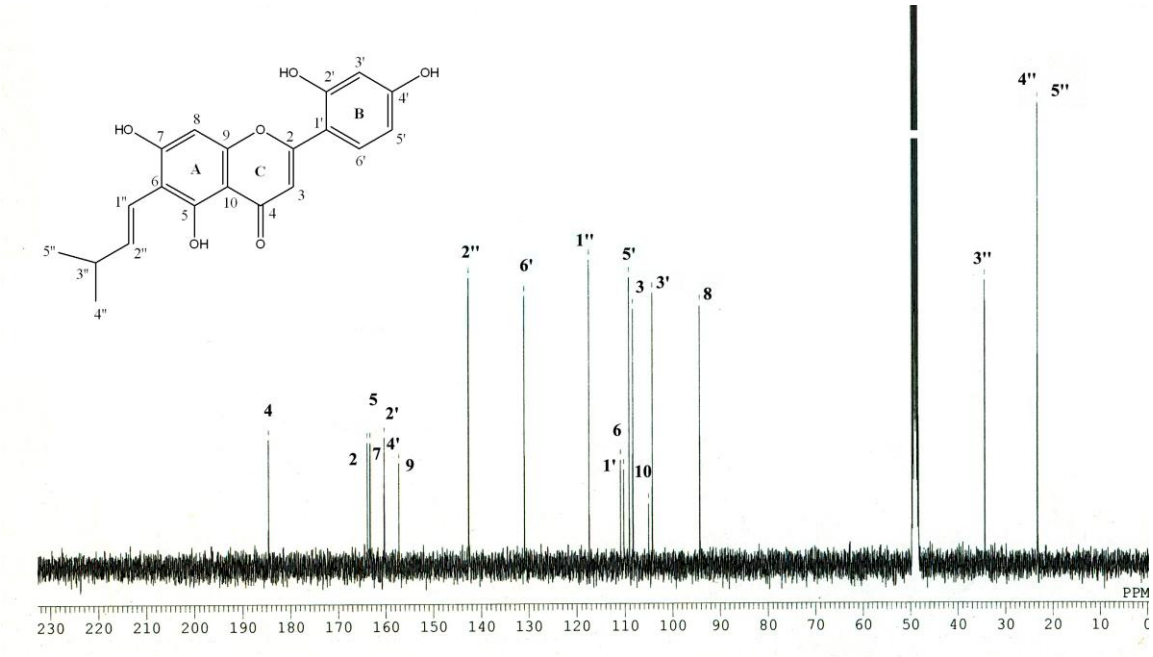

Figure 70 ^{13}C-NMR (100 MHz) Spectrum of compound CAL8 (methanol-d_4)

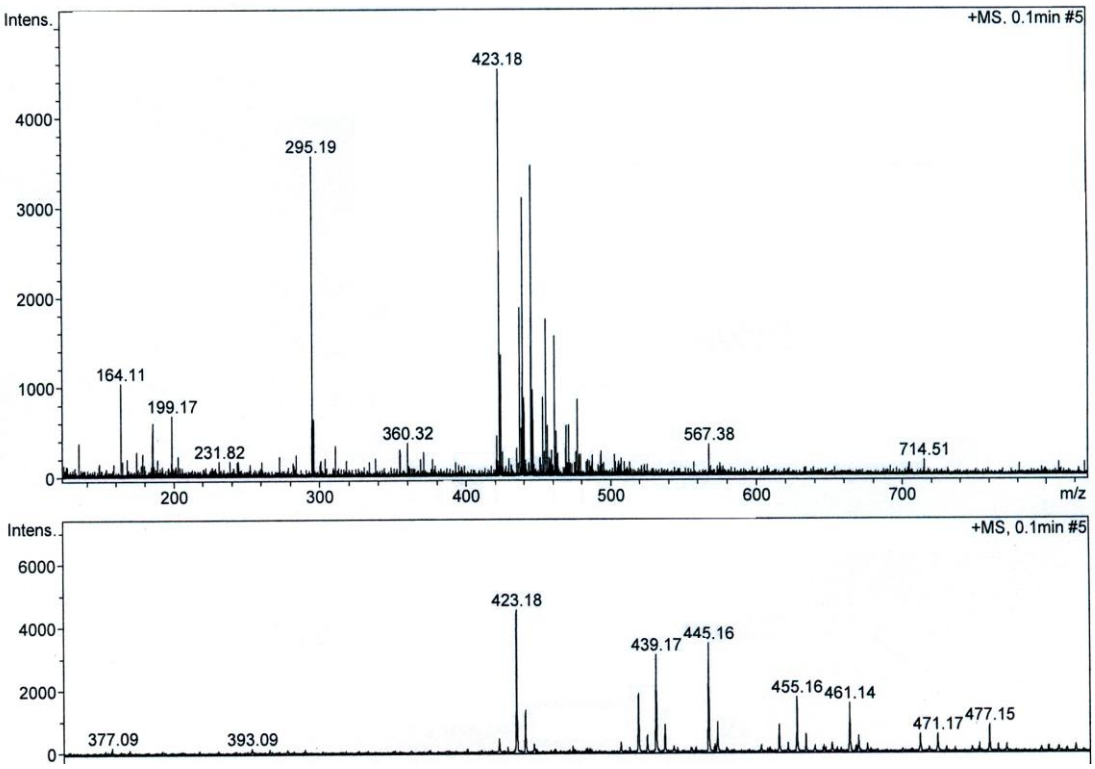

Figure 71 ESI Mass Spectrum of compound CAL9

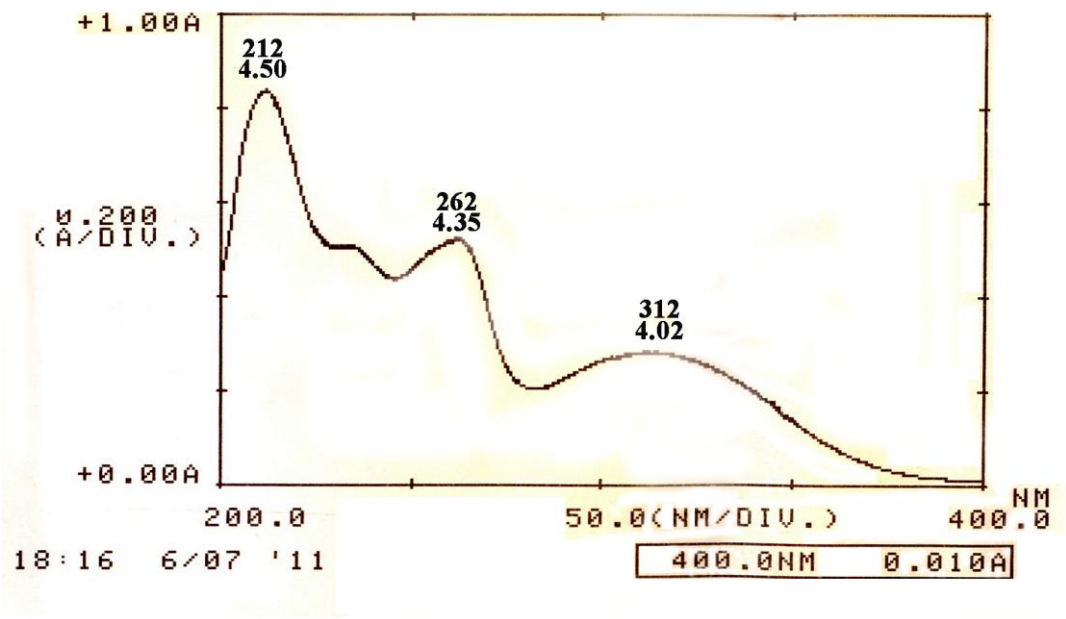

Figure 72 UV Spectrum of compound CAL9 (methanol)

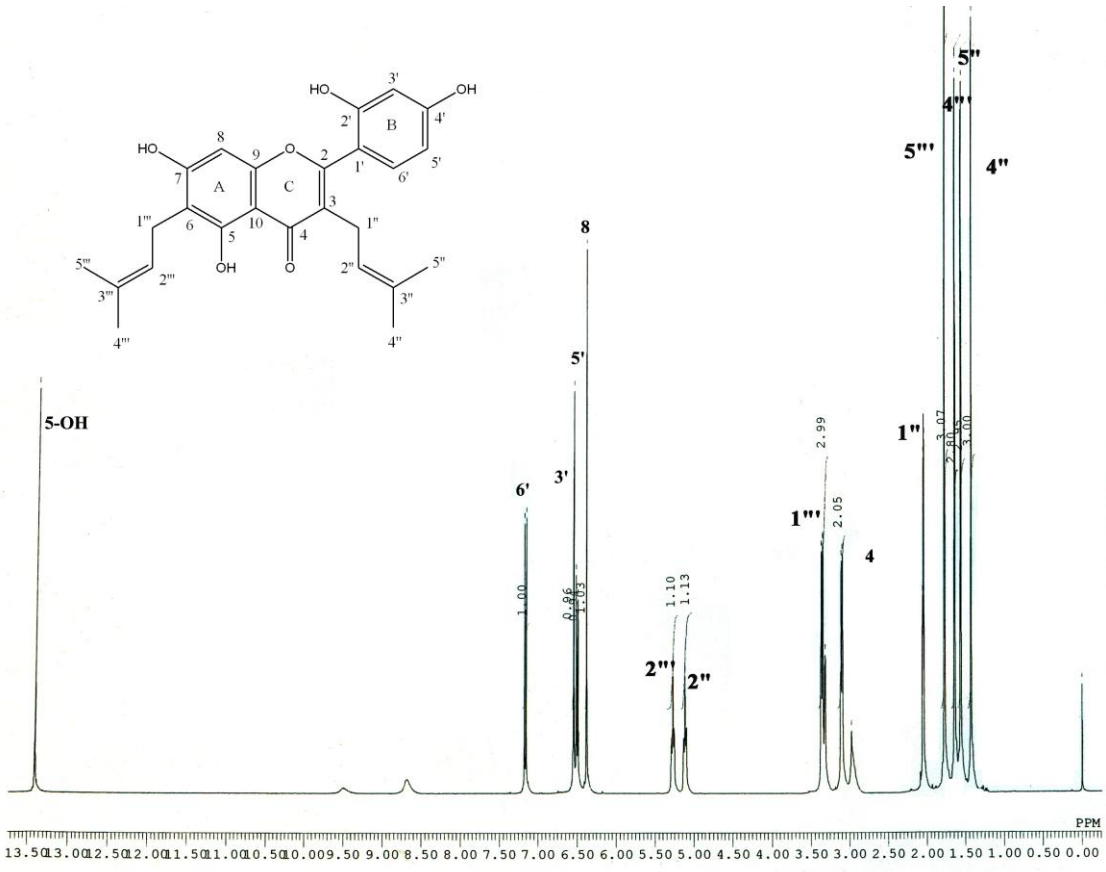

Figure 73 ^{1}H-NMR (400 MHz) Spectrum of compound CAL9 (acetone-d_6)

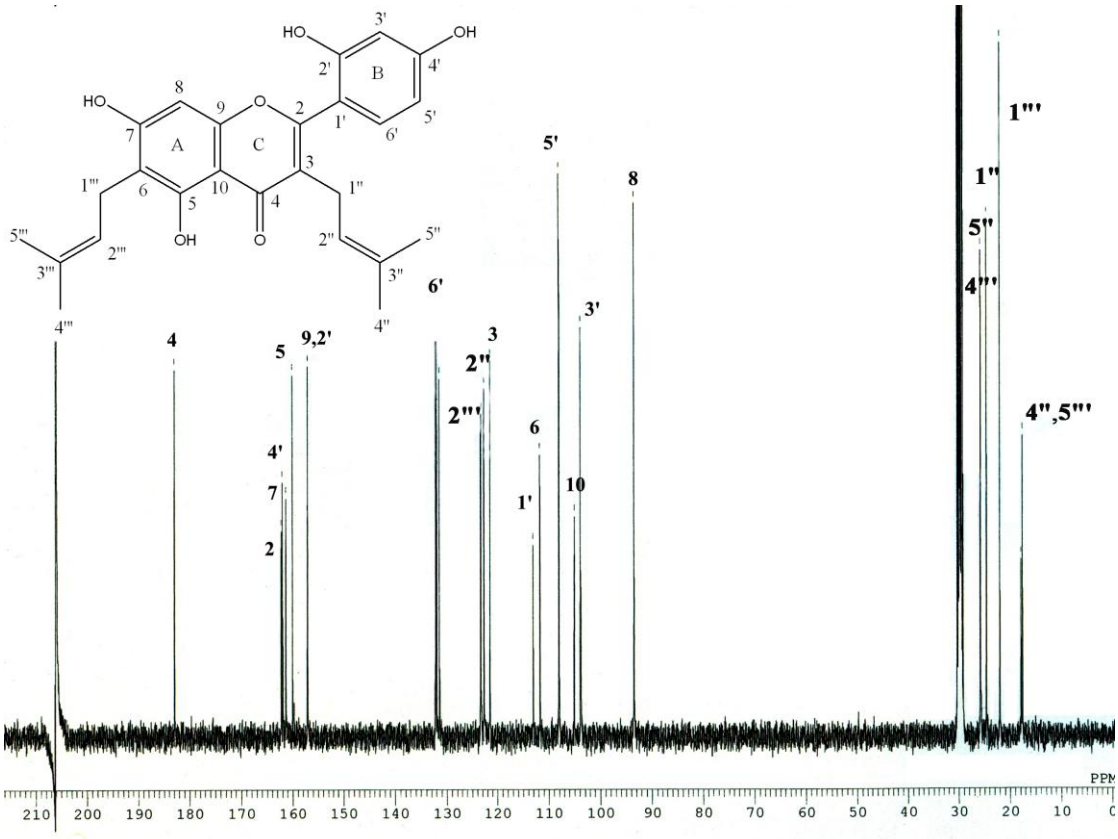

Figure 74 ^{13}C-NMR (100 MHz) Spectrum of compound CAL9 (acetone-d_6)

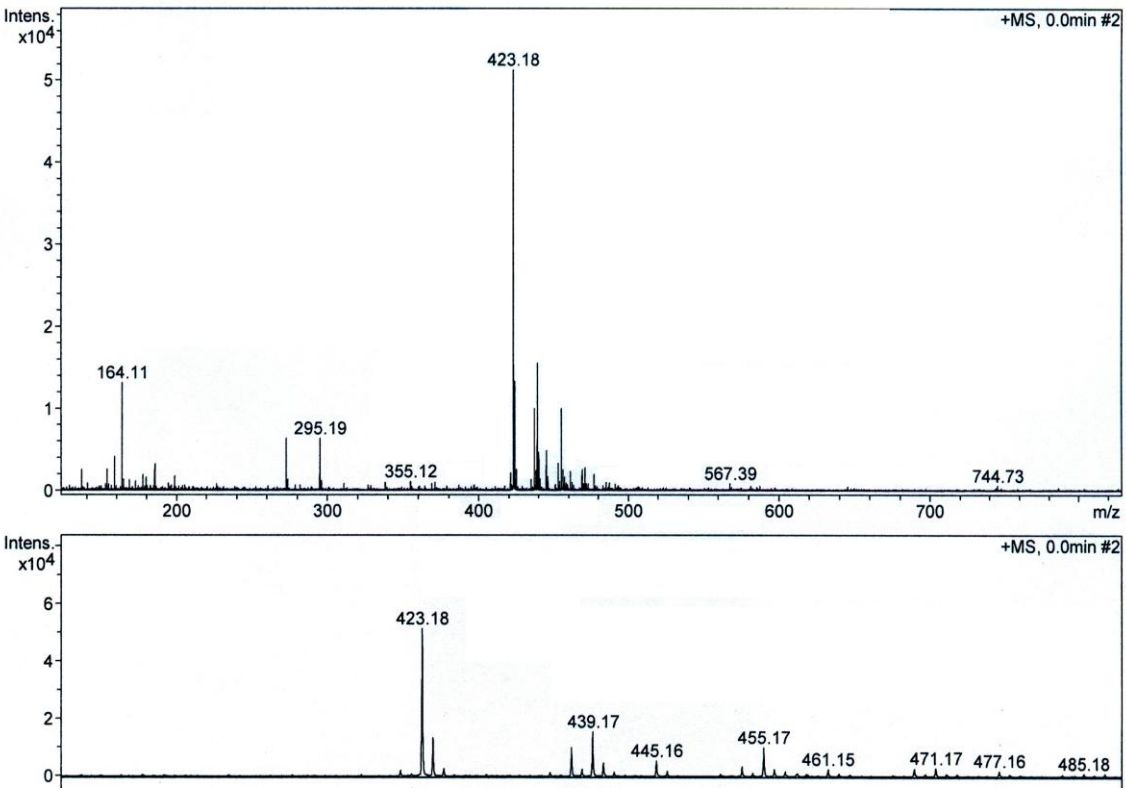

Figure 75 ESI Mass Spectrum of compound CAL10

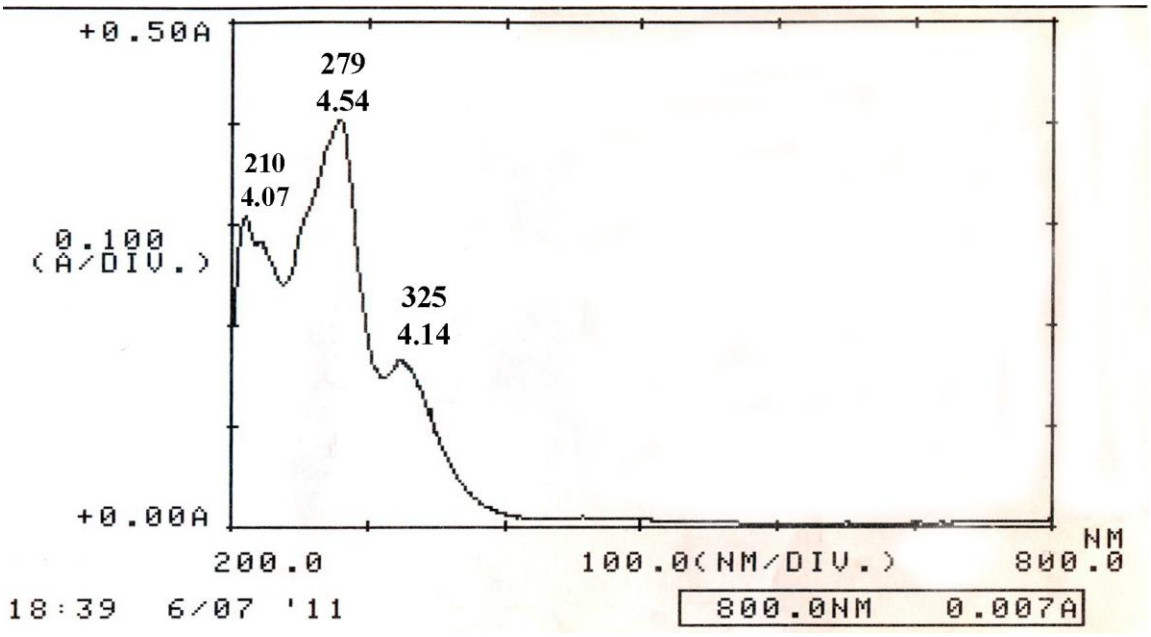

Figure 76 UV Spectrum of compound CAL10 (methanol)

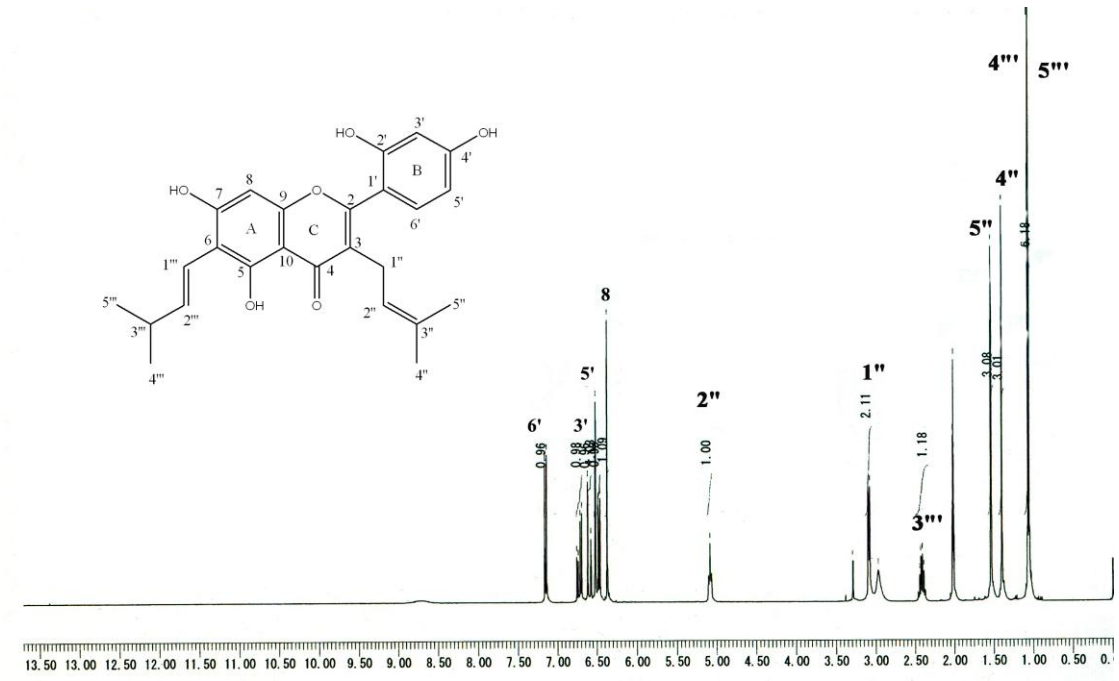

Figure 77 ^{1}H-NMR (400 MHz) Spectrum of compound CAL10 (acetone-d_6)

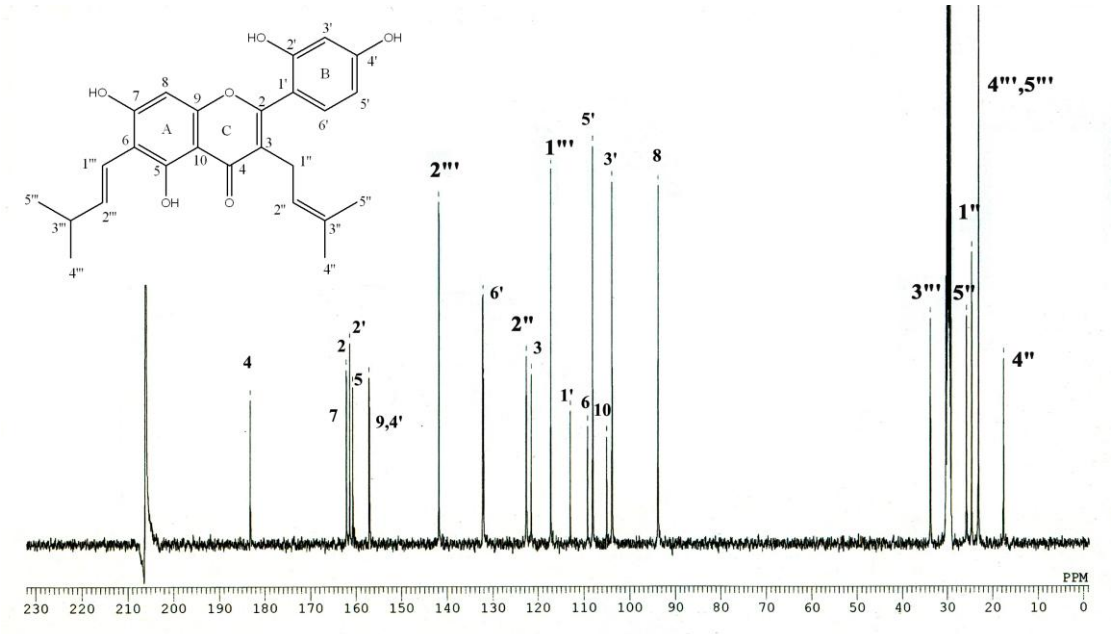

Figure 78 ^{13}C-NMR (100 MHz) Spectrum of compound CAL10 (acetone-d_6)

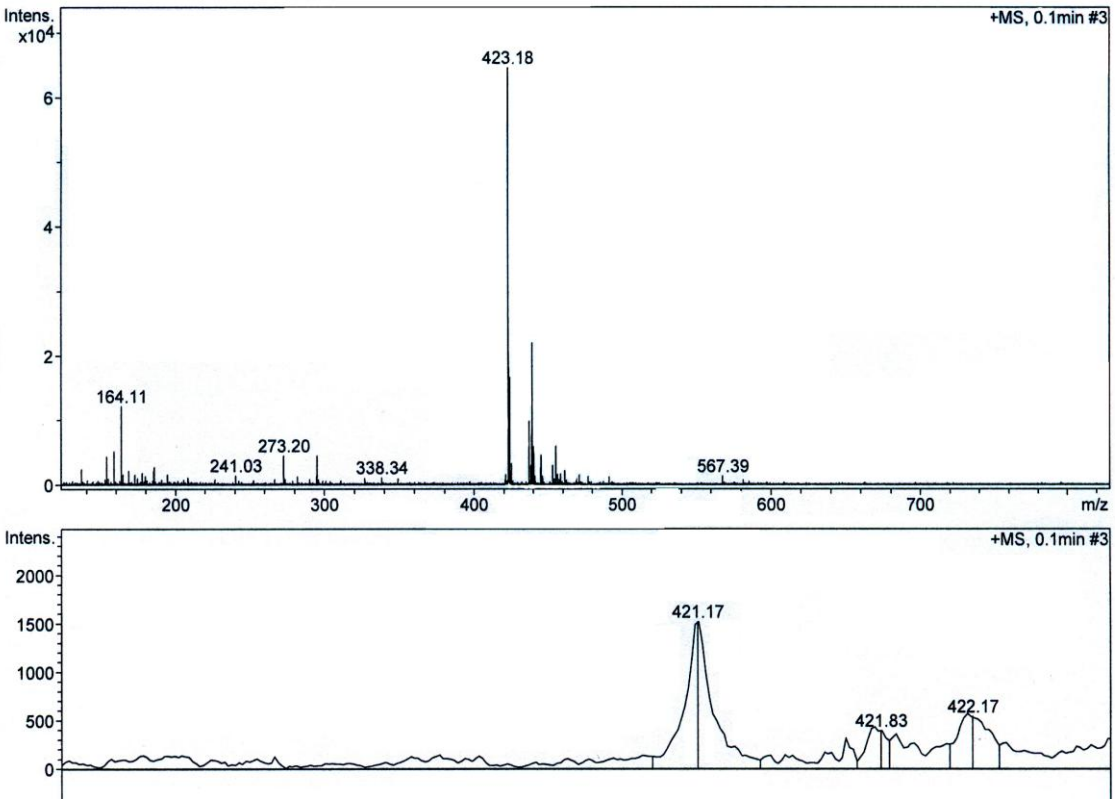

Figure 79 ESI Mass Spectrum of compound CAL11

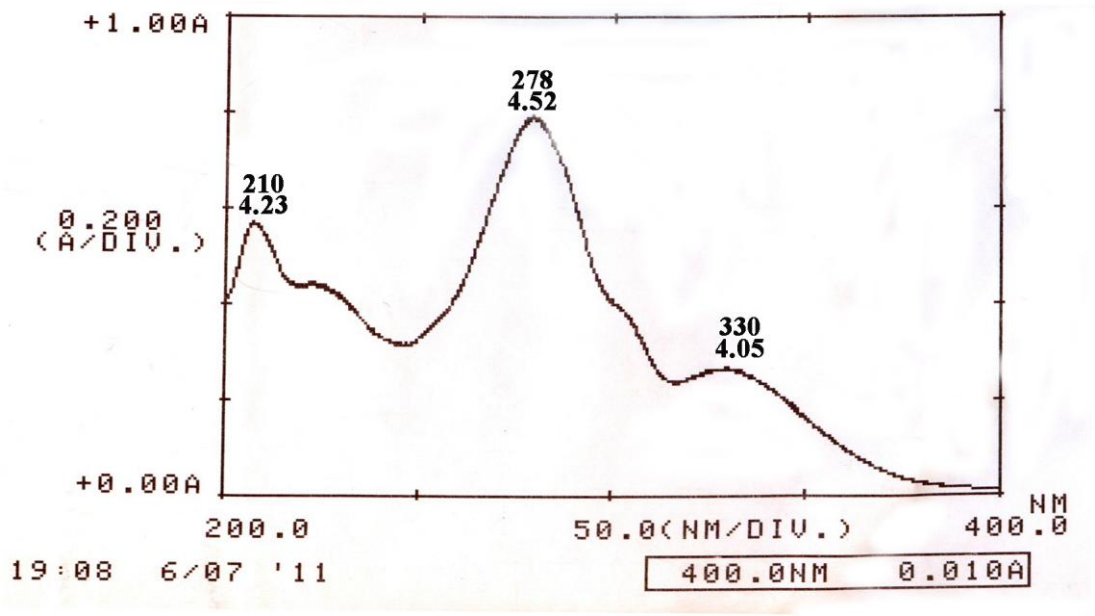

Figure 80 UV Spectrum of compound CAL11 (methanol)

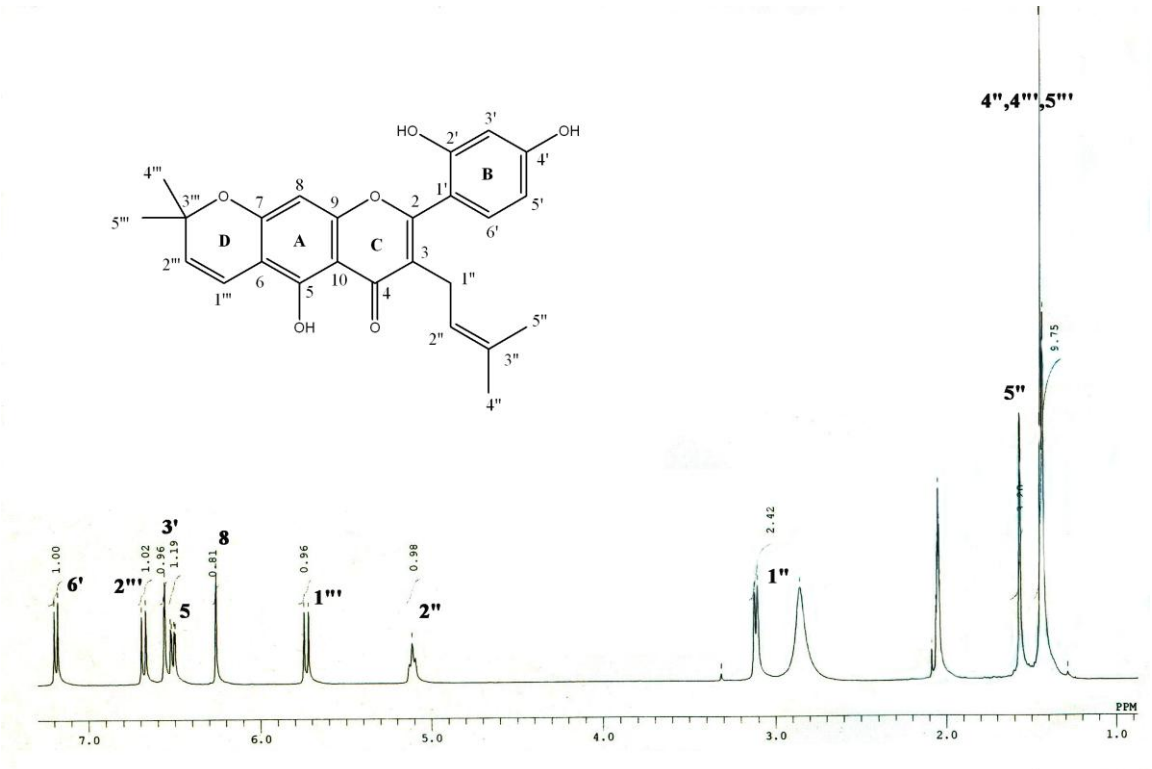

Figure 81 ^{1}H-NMR (400 MHz) Spectrum of compound CAL11 (acetone-d_6)

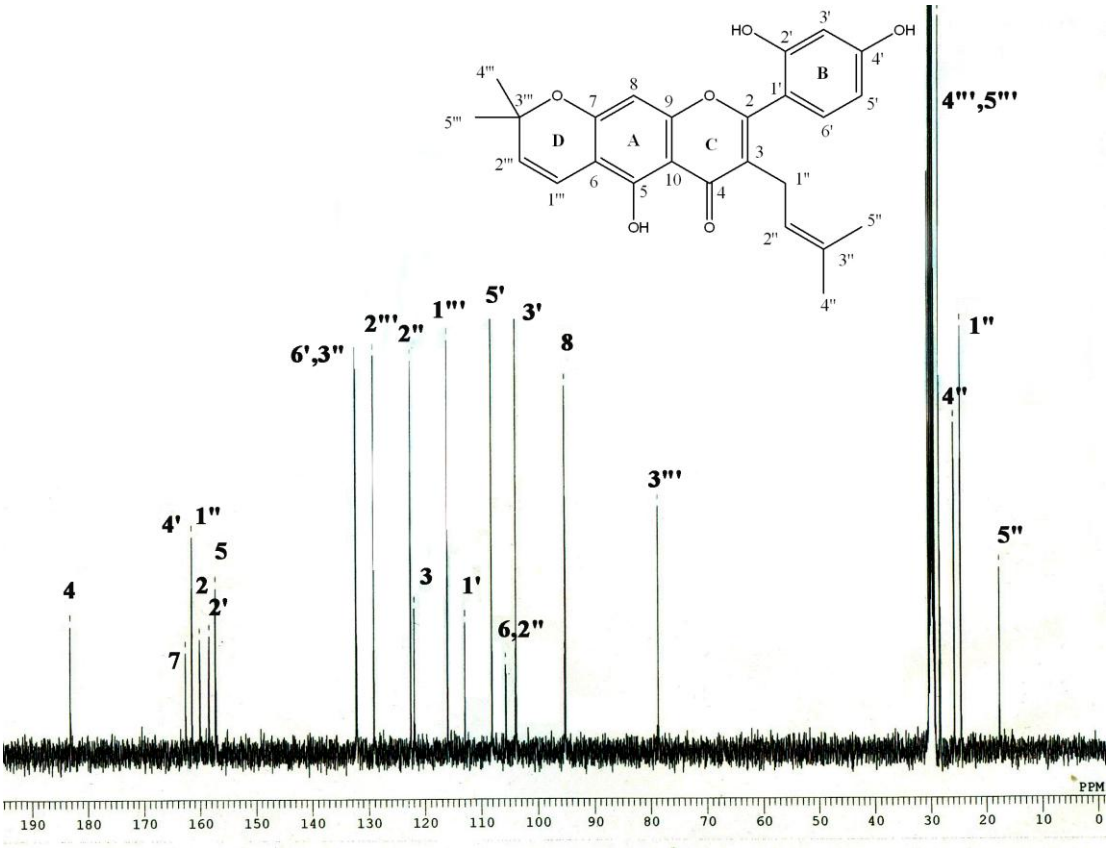

Figure 82 ^{13}C-NMR (100 MHz) Spectrum of compound CAL11 (acetone-d_6)

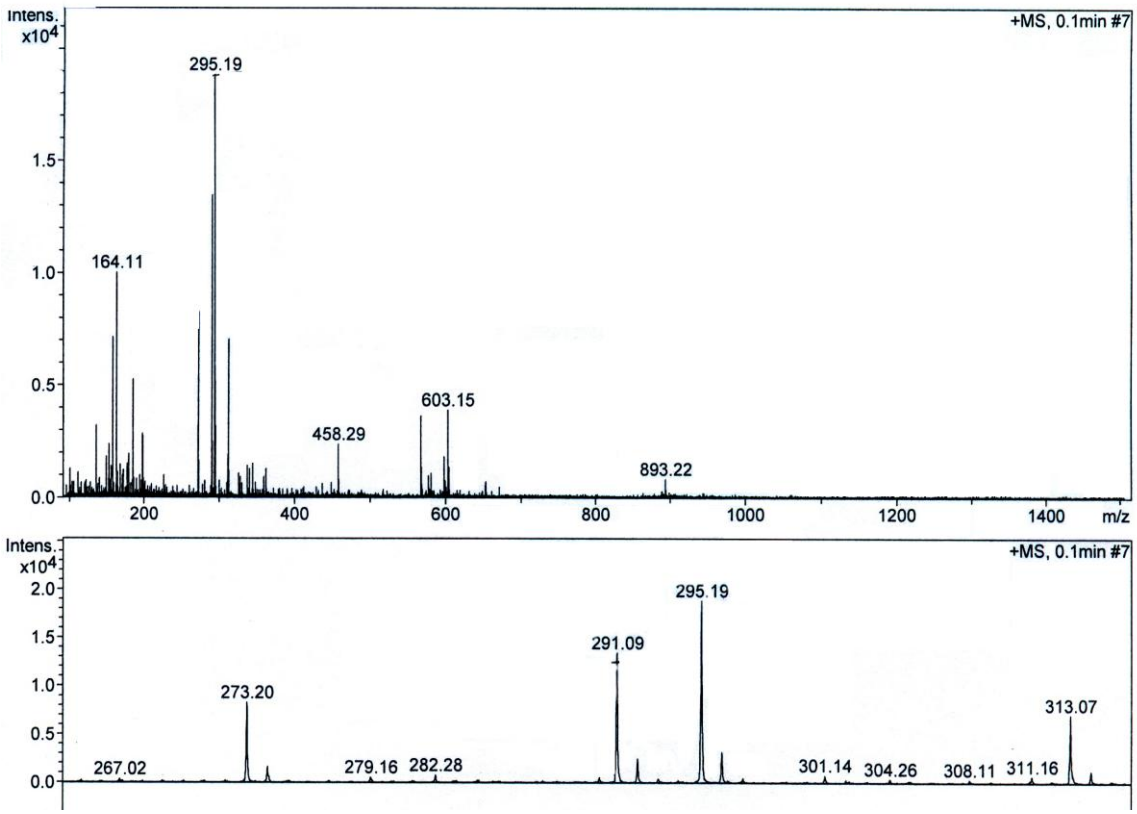

Figure 83 ESI Mass Spectrum of compound CAL12

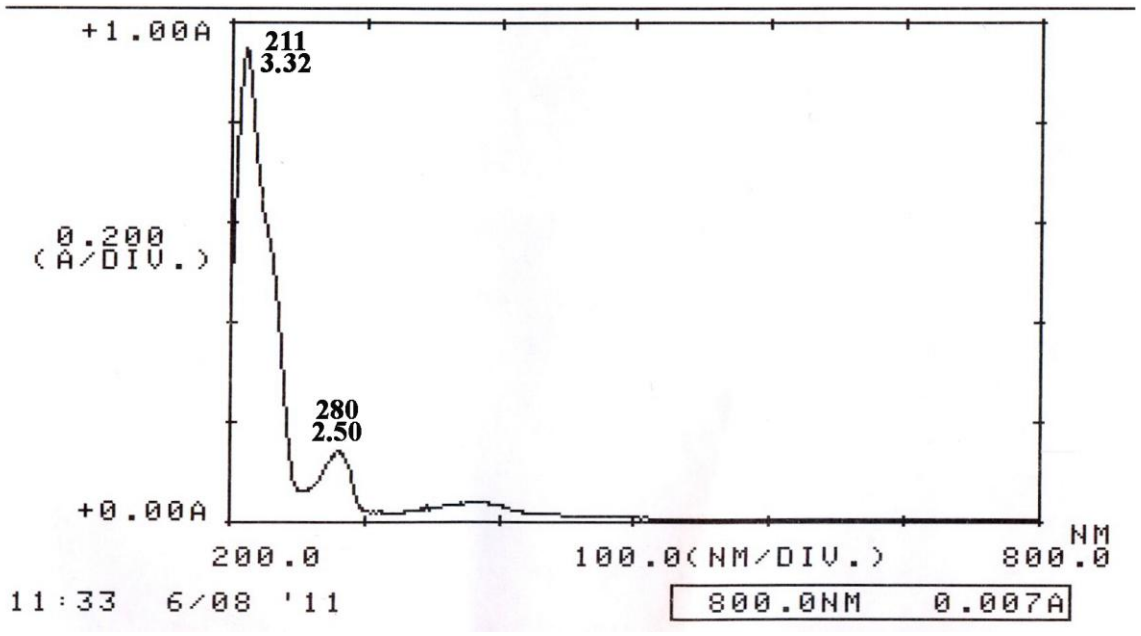

Figure 84 UV Spectrum of compound CAL12 (methanol)

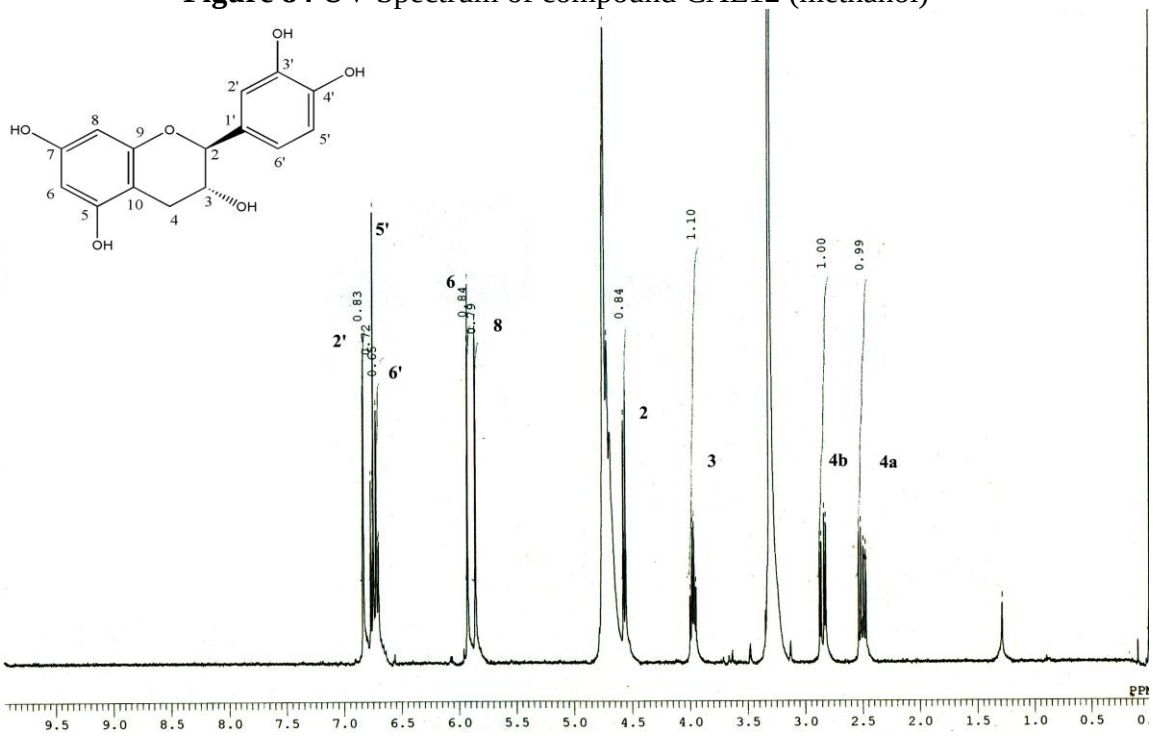

Figure 85 ^{1}H-NMR (400 MHz) Spectrum of compound CAL12 (methanol-d_4)

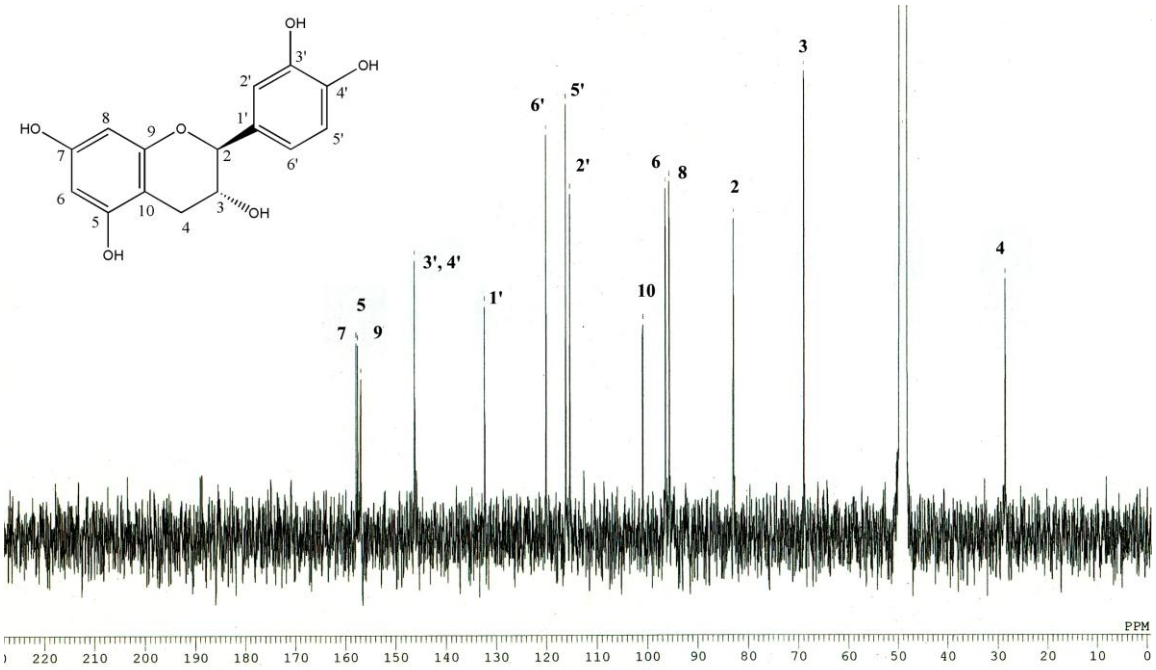

Figure 86 ^{13}C-NMR (100 MHz) Spectrum of compound CAL12 (methanol-d_4)

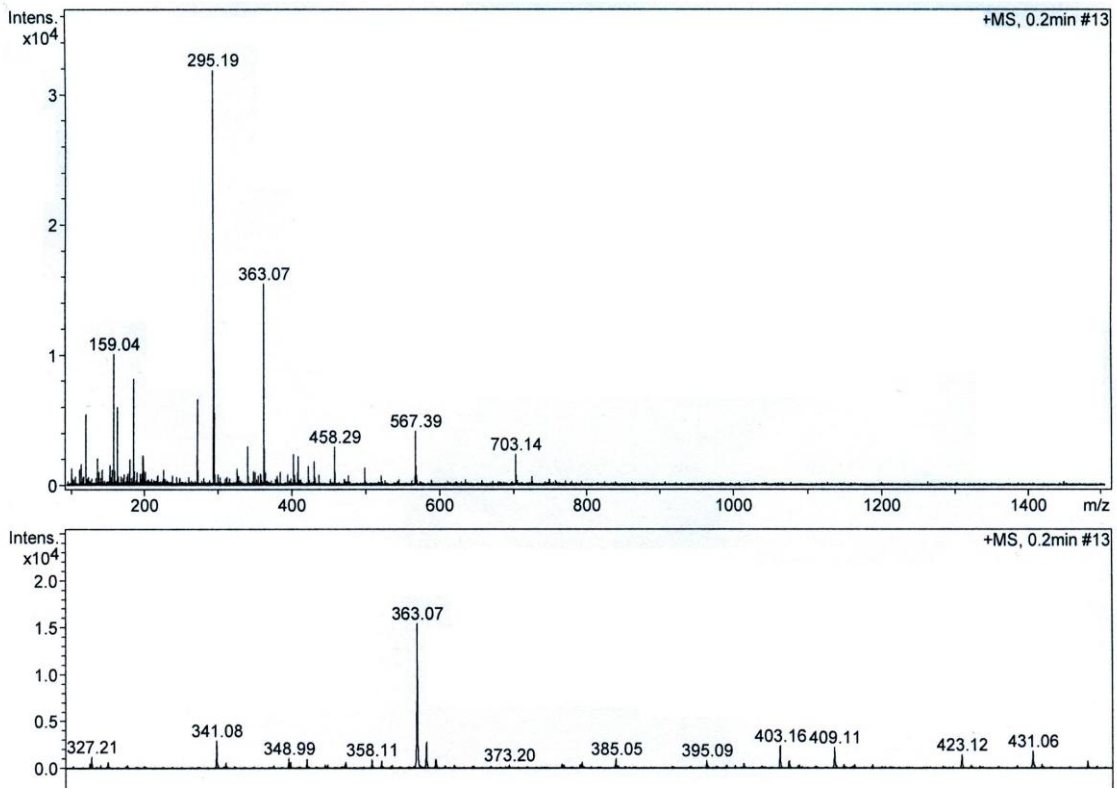

Figure 87 ESI Mass Spectrum of compound CAL13

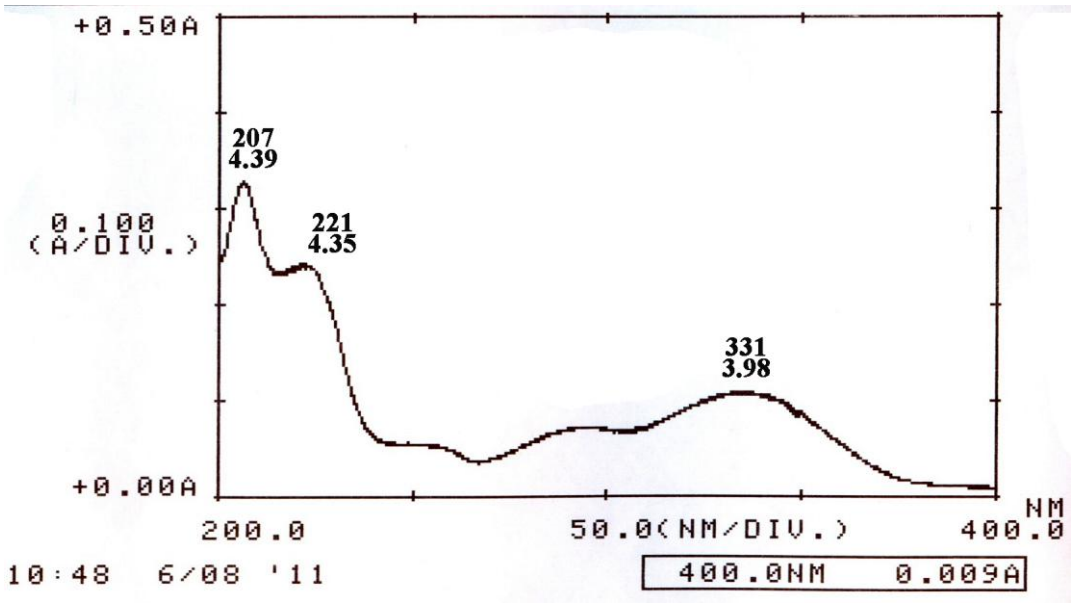

Figure 88 UV Spectrum of compound CAL13 (methanol)

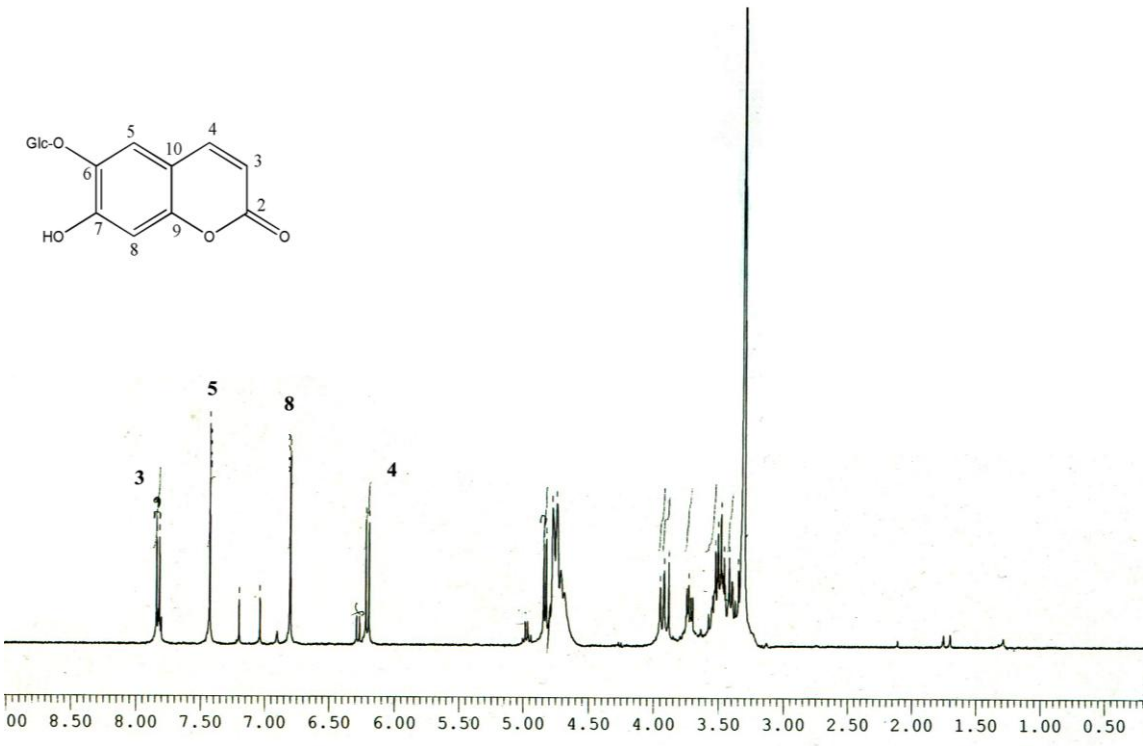

Figure 89 ^{1}H-NMR (400 MHz) Spectrum of compound CAL13 (methanol-d_4)

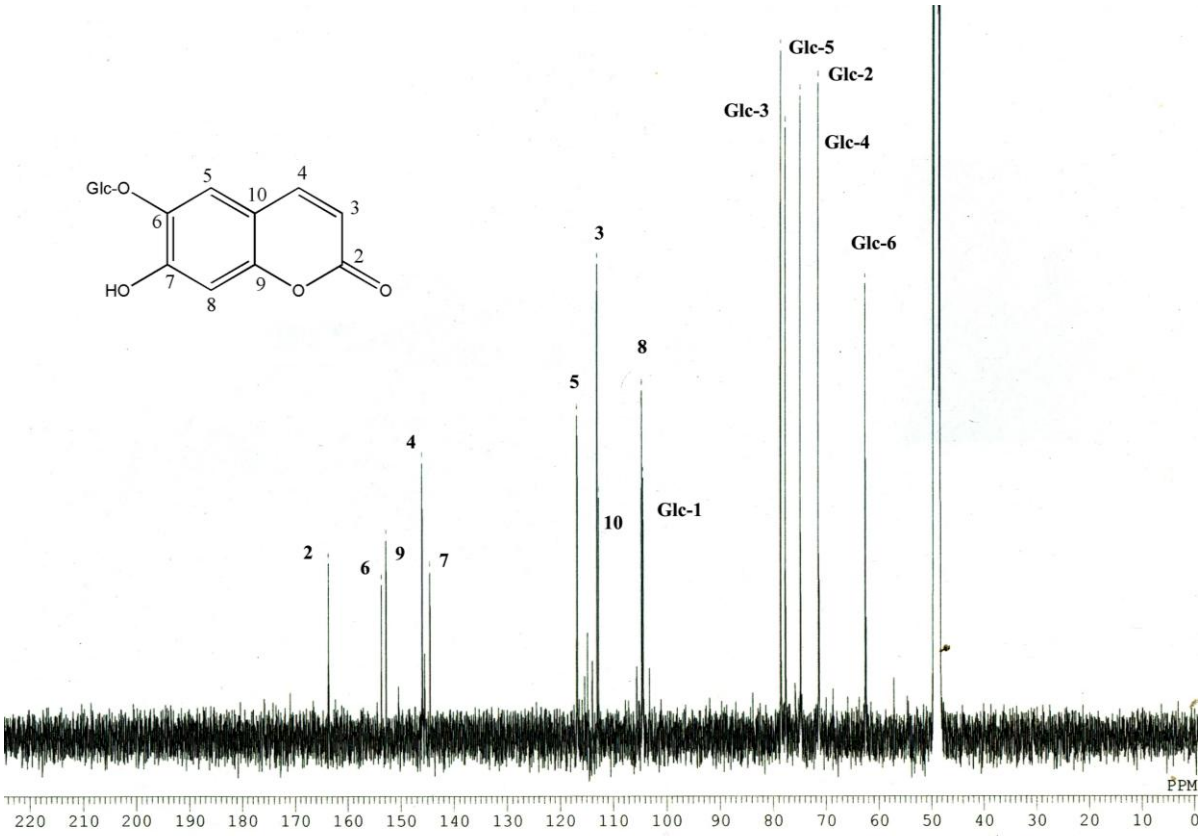

Figure 90 ^{13}C-NMR (100 MHz) Spectrum of compound CAL13 (methanol-d_4)

VITA

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Publications

Maneechai, S., Likhitwitayawuid, K., Sritularak, B., Palanuvej, C., Ruangrungsi, N. and Sirisa-ard, P. 2009. Quantitative Analysis of Oxyresveratrol Content in *Artocarpus lakoocha* and 'Puag-Haad'. Medical Principal and Practice. 18: 223–227.