Chemical Constituents from the Rhizomes of *Curcuma zedoaria* (Christm.) Rosc. and the Stems of *Citrus medica* Linn.

Subaidah Pomkeua

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Chemical Studies
Prince of Songkla University
2010

Thesis Title Chemical Constituents from the Rhizomes of *Curcuma zedoaria* (Christm.) Rosc. and the Stems of *Citrus medica* Linn.
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The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Science Degree in Chemical Studies.

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(Prof. Dr. Amornrat Phongdara)
Dean of Graduate School

ชื่อวิทยานิพนธ์ องค์ประกอบทางเคมีจากเหง้าขมิ้นอ้อยและลำต้นมะนาวควาย
ผู้เขียน นางสาวสุใบด๊ะ ผอมเกื้อ
สาขาวิชา เคมีศึกษา
ปีการศึกษา 2553

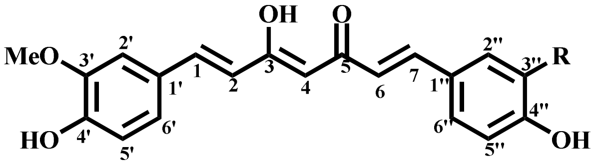
บทคัดย่อ

ตอนที่ 1 องค์ประกอบทางเคมีจากเหง้าขมิ้นอ้อย

การศึกษาองค์ประกอบทางเคมีของส่วนสกัดหยาบอะซีโตนจากเหง้าขมิ้นอ้อย สามารถแยกสารที่มีการรายงานแล้วจำนวน 7 สาร ซึ่งประกอบด้วยสารประเภท curcuminoids 2 สาร คือ curcumin (**CC1**) และ demethoxycurcumin (**CC2**), สารประเภท gingerdiones 1 สาร คือ 1-dehydrogingerdione (**CC3**), และสารประเภท sesquiterpenes 4 สาร คือ germacrone (**CC4**), (+)-germacrone-4,5-epoxide (**CC5**), zederone (**CC6**) และ comosone II **(CC7)** โครงสร้างของสารประกอบเหล่านี้วิเคราะห์โดยใช้ข้อมูลทางสเปกโทรสโกปีและเปรียบเทียบกับสารที่มีรายงานการวิจัยแล้ว

ตอนที่ 2 องค์ประกอบทางเคมีจากลำต้นมะนาวควาย

การศึกษาองค์ประกอบทางเคมีของส่วนสกัดหยาบอะซีโตนจากลำต้นมะนาวควาย สามารถแยกสารที่มีรายงานแล้วจำนวน 15 สาร ซึ่งประกอบด้วยสารประเภท acridone alkaloids 3 สาร คือ citrusinine-I **(MNC1)**, *N*-methylataphyllinine **(MNC2)** และ citracridone I **(MNC3)**, สารประเภทอนุพันธ์ของเบนซีน 3 สาร คือ valencic acid **(MNC4)**, vanillin **(MNC5)** และ 4-hydroxybenzaldehyde **(MNC6)** สารประเภท coumarins 1 สาร คือ xanthyletin **(MNC7)** สารประเภท flavonoids 2 สาร คือ erythrisenegalone **(MNC8)** และ citrusinol **(MNC9)** สารประเภท lignans 2 สาร คือ (+)-syringaresinol **(MNC10)** และ dihydrodehydrodiconifenyl alcohol **(MNC11)** สารประเภท limonoids 2 สาร คือ nomilin **(MNC12)** และ limonin **(MNC13)** และ สารประเภท steroids 2 สาร คือ สารผสมของ *β*-sitosterol (**MNC14**) และ stigmasterol (**MNC15**) โครงสร้างของสารประกอบเหล่านี้วิเคราะห์โดยใช้ข้อมูลทางสเปกโทรสโกปีและเปรียบเทียบกับสารที่มีรายงานการวิจัยแล้ว



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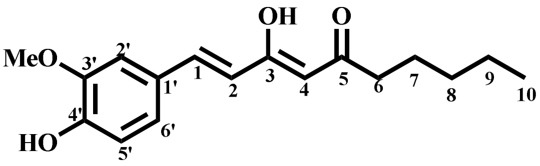

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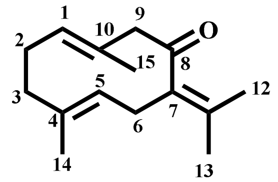

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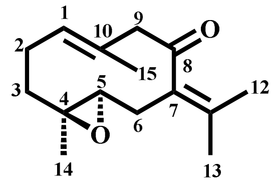

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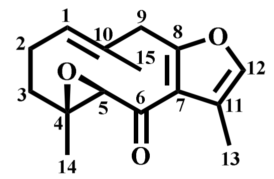

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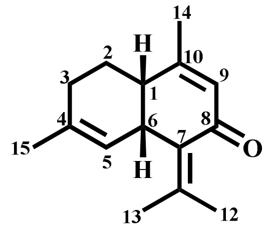

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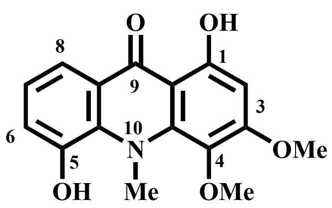

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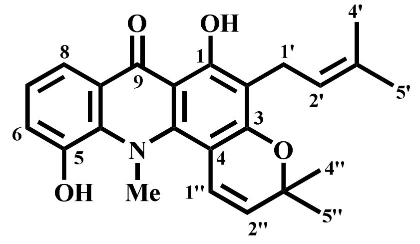

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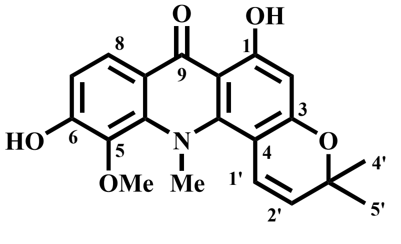

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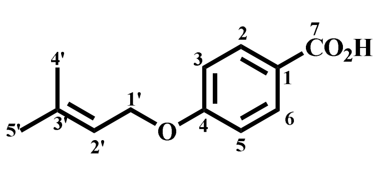

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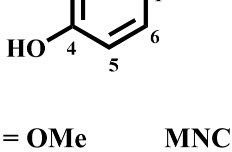

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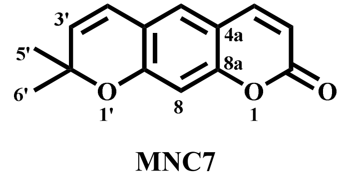

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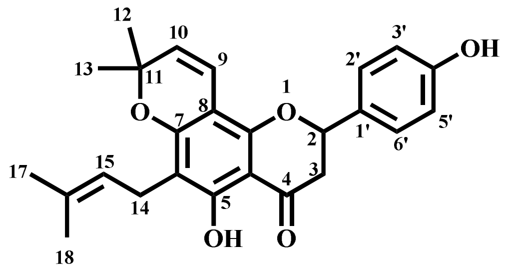

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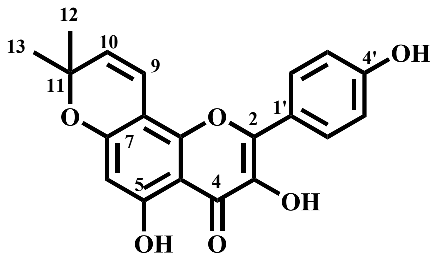

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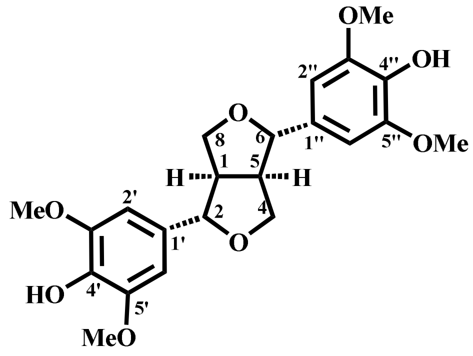

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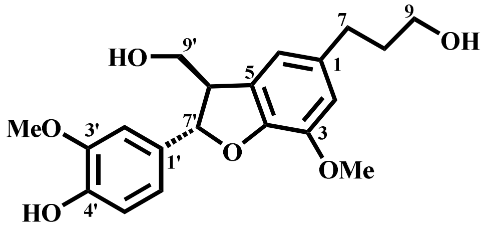

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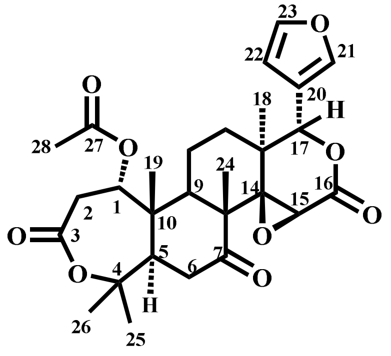

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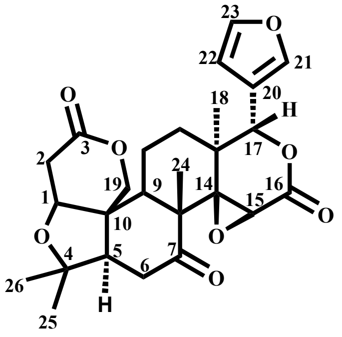

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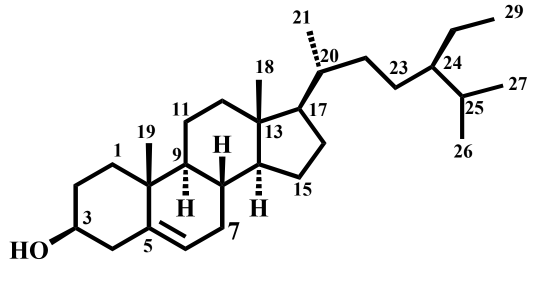

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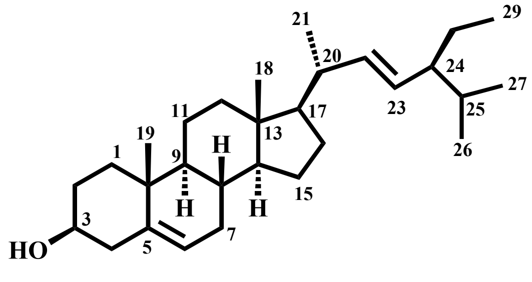

MNC15

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Author Miss Subaidah Pomkeua
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Academic Year 2010

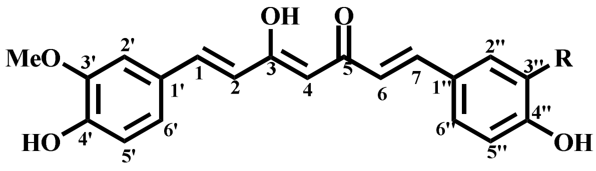
ABSTRACT

Part I Chemical Constituents from the Rhizomes of Curcuma zedoaria

Investigation of the crude acetone extract of the rhizomes of *Curcuma zedoaria*, yielded 7 known compounds; two curcuminoids: curcumin (**CC1**) and demethoxycurcumin (**CC2**), one gingerdione: 1-dehydrogingerdione (**CC3**), together with four sesquiterpenes: germacrone (**CC4**), (+)-germacrone-4,5-epoxide (**CC5**), zederone (**CC6**) and comosone II **(CC7**). Their structures were elucidated by spectroscopic methods and comparison with those reported in the literatures.

Part II Chemical Constituents from the Stems of Citrus medica

Investigation of the crude acetone extract of the stems of *Citrus medica*, yielded 15 known compounds; three acridone alkaloids: citrusinine-I **(MNC1**), *N*-methylataphyllinine **(MNC2)** and citracridone I **(MNC3)**, three benzene derivatives: valencic acid **(MNC4)**, vanillin **(MNC5)** and 4-hydroxybenzaldehyde, **(MNC6)**, a coumarin: xanthyletin **(MNC7)**, two flavonoids: erythrisenegalone **(MNC8)** and citrusinol **(MNC9)**, two lignans: (+)-syringaresinol **(MNC10)** and dihydrodehydrodiconifenyl alcohol **(MNC11)**, two limonoids: nomilin **(MNC12)** and limonin **(MNC13)** and two steroids: a mixture of *β*-sitosterol (**MNC14**) and stigmasterol (**MNC15**). Their structures were elucidated by spectroscopic methods and comparison with those reported in the literaturs.



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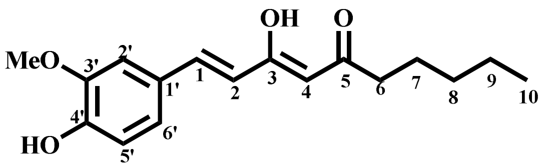

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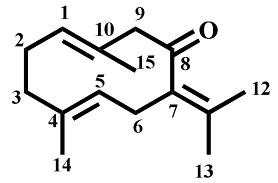

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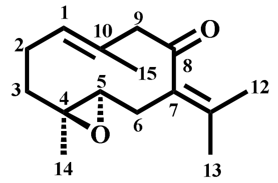

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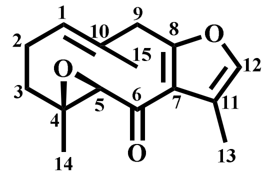

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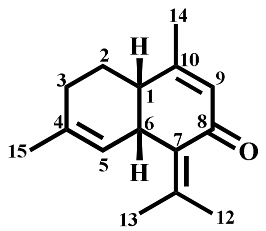

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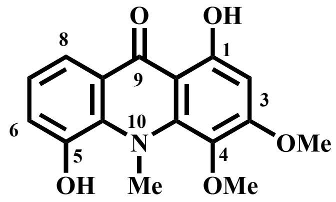

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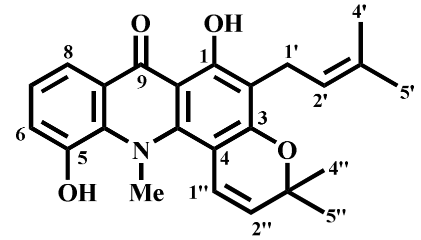

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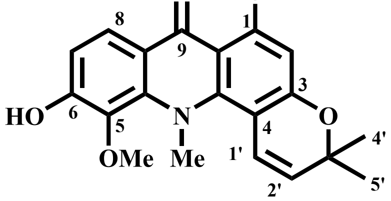

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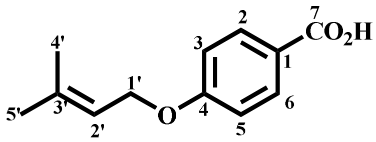

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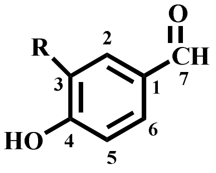

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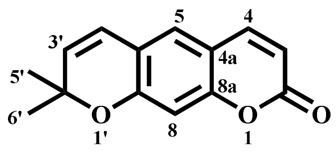

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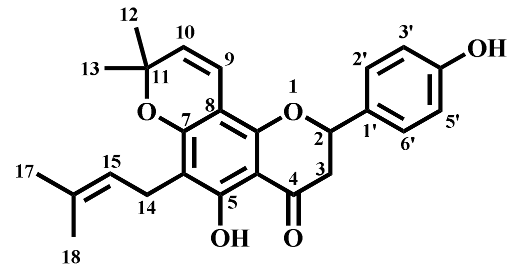

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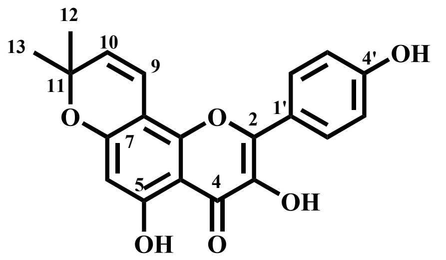

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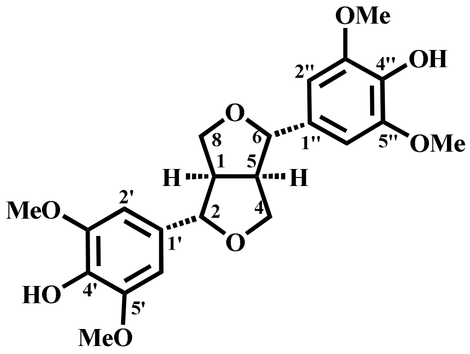

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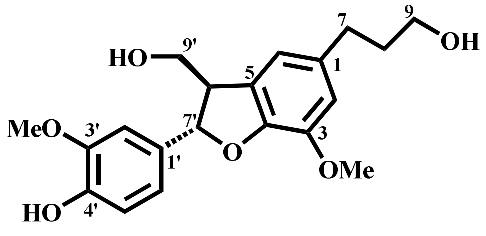

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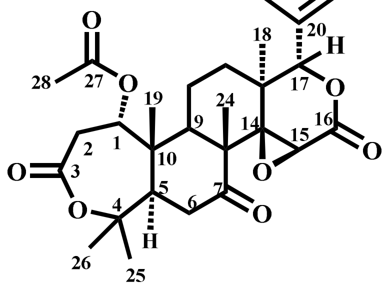

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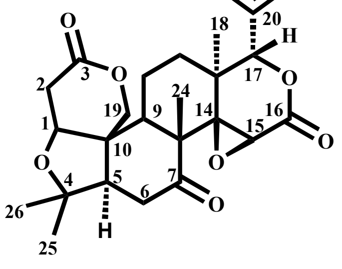

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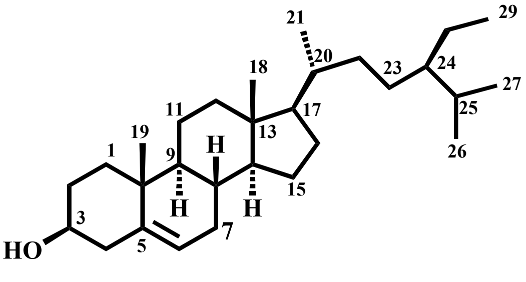

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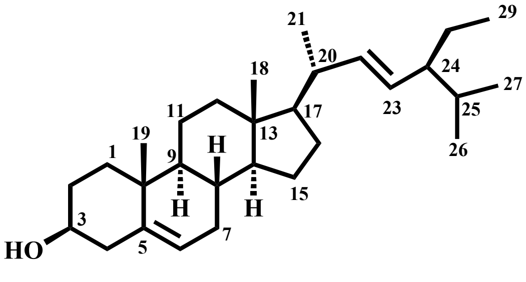

MNC15

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Subaidah Pomkeua

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

The purpose of this research is to investigate the chemical constituents from the rhizome of *Curcuma zedoaria* and the stems of *Citrus medica*. They are part of the basic researches on the Thai medicinal plants. Seven compounds and fifteen compounds have been isolated from the rhizomes of *C. zedoaria* and the stems of *C. medica*, respectively.

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PART I Chemical constituents from the rhizomes of *C. zedoaria*

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CHAPTER 1.2 EXPERIMENTAL

CHAPTER 1.3 RESULTS AND DISCUSSION

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CHAPTER 2.1 INTRODUCTION

CHAPTER 2.2 EXPERIMENTAL

CHAPTER 2.3 RESULTS AND DISCUSSION

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LIST OF ABBREVIATIONS AND SYMBOLS

s = singlet
d = doublet
t = triplet
m = multiplet
dd = doublet of doublet
dt = doublet of triplet
br s = broad singlet
br d = broad doublet
g = gram
nm = nanometer
m.p. = melting point
cm^{-1} = reciprocal centimeter (wave number)
δ = chemical shift relative to TMS
J = coupling constant
$[\alpha]_D$ = specific rotation
λ_{max} = maximum wavelength
ν = absorption frequencies
ε = molar extinction coefficient
°C = degree celcius
MHz = Megahertz
ppm = part per million
c = concentration
IR = Infrared
UV = Ultraviolet
NMR = Nuclear Magnetic Resonance
DEPT = Distortionless Enhancement by Polarization Transfer
HMBC = Heteronuclear Multiple Bond Correlation
NOESY = Nuclear Overhauser Effect Spectrosopy
CC = Column Chromatography

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

QCC = Quick Column Chromatography
PLC = Preparative Thin Layer Chromatography
TMS = tetramethylsilane
Acetone-d_6 = deuteroacetone
DMSO-d_6 = deuterodimethyl sulphoxide
$CDCl_3$ = deuterochloroform
CD_3OD = deuteromethanol
EtOAc = ethyl acetate
MeOH = methanol

CHAPTER 1.1
INTRODUCTION

1.1.1 Introduction

Curcuma zedoaria (Christm.) Rosc. (family Zingiberaceae) has been widely cultivated as a vegetable or spice in South and Southeast Asian countries. It is locally called “Khamin oi” (Smitinand, 2001). The rhizomes of this plant are used as stimulant, stomachic, carminative, diuretic, anti-diarrheal, anti-emetic, anti-pyretic, depurator, and also to clean and cure ulcers, wounds, and other kinds of skin disorders in India and Southeast Asian countries (Matsuda *et al.*, 2001).

According to Smitinand (2001), there are thirteen species of genus Curcuma found in Thailand as follows.

1. *aeruginosa* Roxb.
2. *aromatica* Salisb.
3. *mangga* Valeton&Zijp
4. *sessilis* Gage
5. *zedoaria* (Christm.) Roscoe
6. *alismatifolia* Gagnep.
7. *comosa* Roxb.
8. *parviflora* Wall.
9. *sparganifolia* Gagnep.
10. *longa* L.
11. *roscoeana* Wall.
12. *xanthorrhiza* Roxb.
13. *amarissima* Roscoe

In Thailand, *C. zedoaria* has been found in every part of the country. It has many local Thai names: Khamin khuen (ขมิ้นขึ้น) Northern, Khamin oi (ขมิ้นอ้อย) Central, Haeo dam (แฮ้วดำ) Chiang Mai (Smitinand, 2001).

Trees

Flowers

Rhizome

Figure 1 Different parts of *Curcuma zedoaria* (Christm.) Rosc.

1.1.2 Review of literatures

The chemical constituents isolated from the *Curcuma zedoaria* (Christm.) Rosc. were summarized in **Table 1**. Information obtained from SciFinder Scholar copyright in 2010 will be presented and classified into groups: Curcuminoids, monoterpenoids and sesquiterpenoids.

Table 1 Compounds from the *Curcuma zedoaria* (Christm.) Rosc.

a. Curcuminoids
b. Monoterpenoids
c. Sesquiterpenoids

Scientific name	Part	Compounds	Bibliography
Curcuma zedoaria (Christm.) Rosc.	Rhizome	Furanogermenone, **c1** Germacrone, **c2**	Rongbao *et al.*, 1991
		Curcumin, **a1** Demethoxycurcumin, **a3** Bisdemethoxycurcumin, **a2** Curcolonol, **c38** Guaidiol, **c39** Curcarabranol A, **c3**	Syu *et al.*, 1998
		Curcarabranol B, **c4** 7α,11α-Epoxy-5β-hydroxy-9-guaiaen-8-one, **c5** Aerugidiol,**c6** Zedoarondiol, **c7** Isozedoarondiol, **c8**	Matsuda *et al.*, 2001

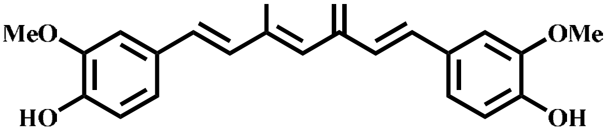
Table 1 (Continued)

Scientific name	Part	Compounds	Bibliography
		Zedoalactone B, **c9** Alismoxide, **c10** Bisacumol, **c11** Bisacurone, **c12** *β*-Eugesmol, **c13** *β*-Dictyopterol**, c14** Curzerenone, **c15** Curcumadione, **c16** Curcumenone, **c17** 4*S*-Dihydrocurcumenone, **c18** Isofuranodienone, **c19** Zederone, **c20** 13-Hydroxygermacrone, **c21** Glechomanolide, **c22** (+)-Germacrone-4,5-epoxide, **c23** Curdione, **c25** Neocurdione, **c24** Dehydrocurdione, **c26** Curcumenol, **c28** 4-Epicurcumenol, **c27** Isocurcumenol, **c29** Neocurcumenol, **c30** Procurcumenol, **c31** Isoprocurcumenol, **c32** Furanodiene, **c33** Curcumin, **a1** Bisdemethoxycurcumin, **a2**	
		Curcumenolactone A, **c34**	Matsuda *et al.*, 2001

Table 1 (Continued)

Scientific name	Part	Compounds	Bibliography
		Curcumenolactone B, **c35**	
		Curcumenolactone C, **c36**	
		1,8-Cineole, **b1**	Gurdip *et al.*, 2003
		Terpinolene, **b2**	
		o-Cymene, **b3**	
		α-Pinene, **b4**	
		β-Pinene, **b5**	
		Germacrone, **c2**	Quy Bao *et al.*, 2004
		β-Pinene, **b5**	
		1,8-Cineole, **b1**	
		Camphor, **b6**	
		Isoborneol, **b7**	
		Zingiberene, **c37**	

a. Curcuminoids



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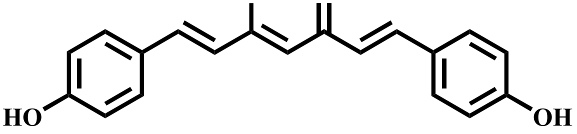
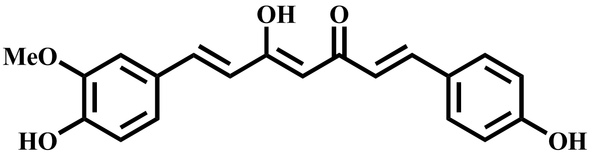
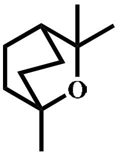


Bisdemethoxycurcumin, **a2**

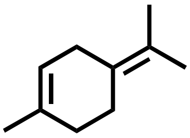


Demethoxycurcumin, **a3**

b. Monoterpenoids



1,8-Cineole, **b1**



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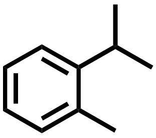

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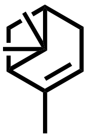

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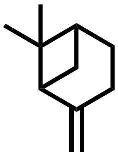

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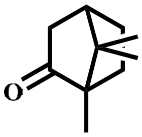

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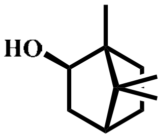
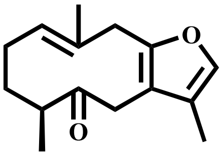


Isoborneol, **b7**

c. Sesquiterpenoids



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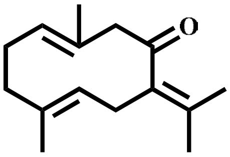

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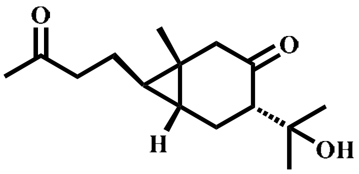

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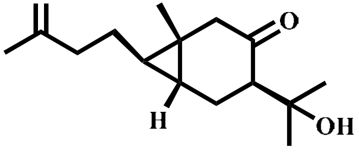

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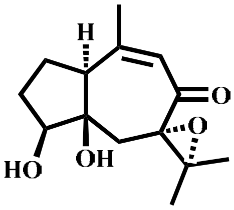
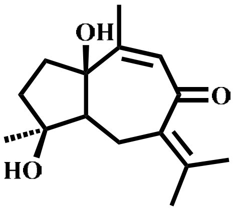


7α,11α-Epoxy-5β-hydroxy-9-guaiaen-8-one, **c5**



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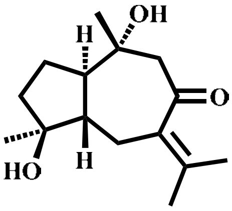

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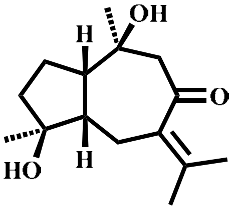

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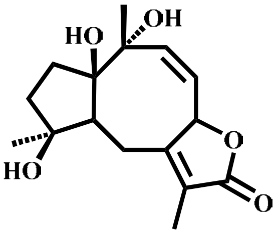

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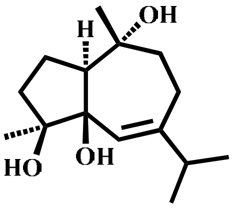

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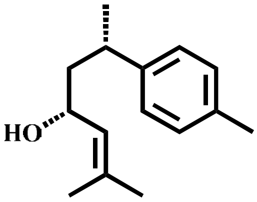

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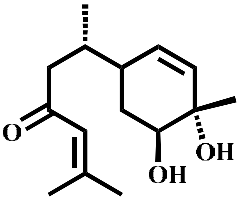

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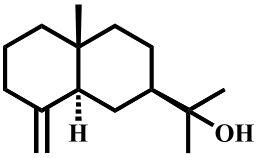

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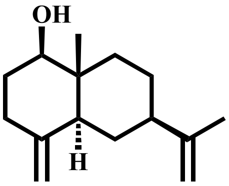

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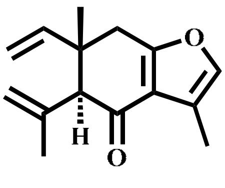

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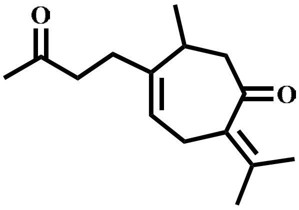

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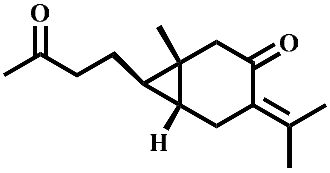
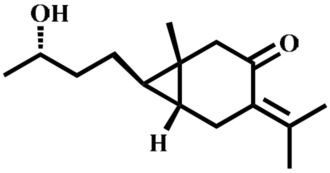
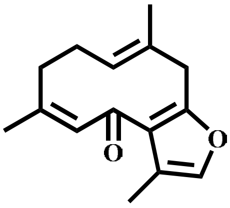


Curcumenone, **c17**



4*S*-Dihydrocurcumenone, **c18**



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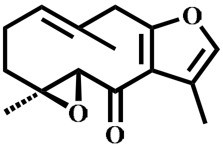

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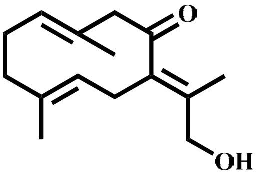

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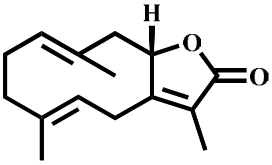
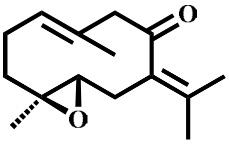
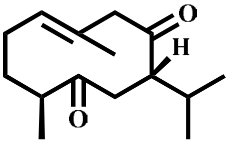


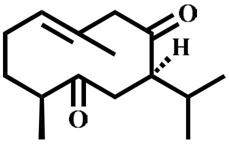
Glechomanolide, **c22**



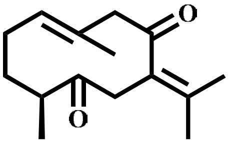
(+)-Germacrone-4,5-epoxide, **c23**



Neocurdione, **c24**



Curdione, **c25**



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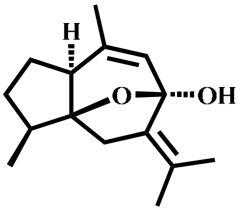
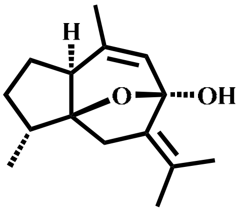
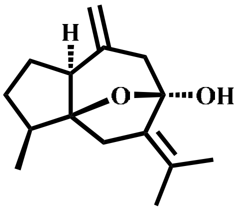


4-Epicurcumenol, **c27**



Curcumenol, **c28**



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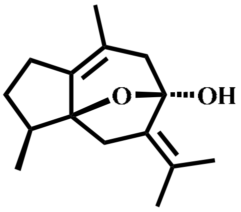

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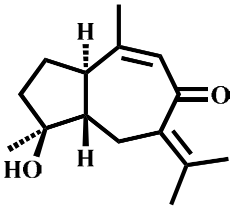

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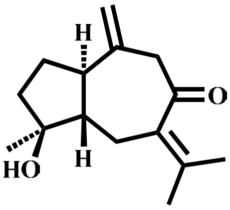
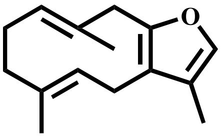
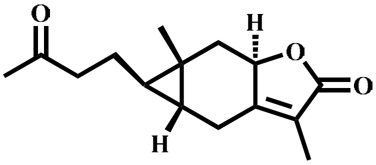


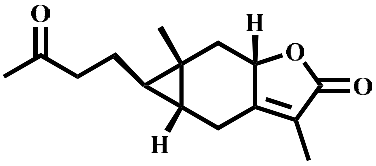
Isoprocurcumenol, **c32**



Furanodiene, **c33**



Curcumenolactone A, **c34**



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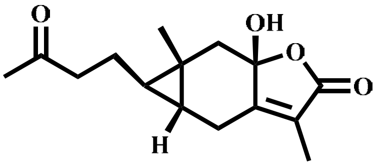

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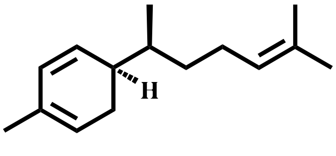

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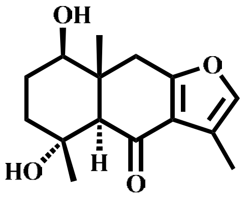

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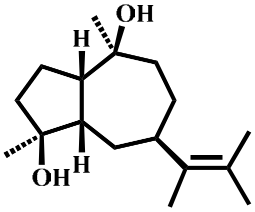


Guaidiol, **c39**

1.1.1 Objective

This part of research work is to investigate the chemical constituents from the rhizomes of *Curcuma zedoaria* (Christm.) Rosc.. It involved isolation, purification and structure elucidation.

CHAPTER 1.2
EXPERIMENTAL

1.2.1 Instruments and chemicals

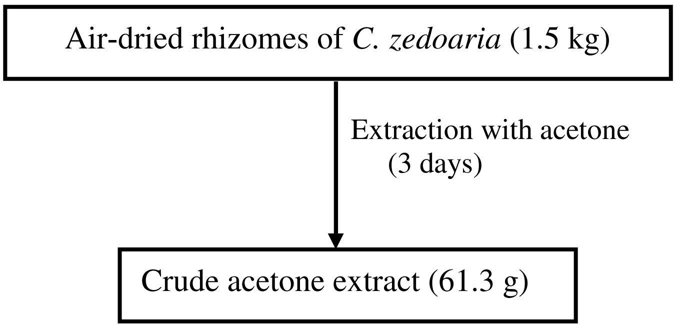
Melting point was recorded in ^{o}C on a digital Electrothermal 9100 Melting Point Apparatus. Ultraviolet spectra were measured with a UV-160A spectrophotometer (SHIMADZU) and principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in methanol solution. The optical rotation $[\alpha]_D$ was measured in chloroform, acetone and methanol solution with Sodium D line (590 nm) on a JASCO P-1020 digital polarimeter. The IR spectra were measured with a Perkin-Elmer 783 FTS165 FT-IR spectrophotometer. ^{1}H and ^{13}C – Nuclear magnetic resonance spectra were recorded on a FT-NMR Bruker Ultra Shield^{TM} 300 and 500 MHz spectrometer at Department of Chemistry, Faculty of Science, Prince of Songkla University and spectra were recorded in deuterochloroform and deuteroacetone as δ value in ppm downfield from TMS (internal standard δ 0.00) and coupling constant (*J*) are expressed in hertz. Quick column chromatography (QCC) and column chromatography was performed by using silica gel 60 H (Merck) and silica gel 100 (70-230 Mesh ASTM, Merck) respectively. For thin-layer chromatography (TLC), aluminum sheets of silica gel 60 F_{254} (20×20 cm, layer thickness 0.2 mm, Merck) were used for analytical purposes and the compounds were visualized under ultraviolet light. Solvents for extraction and chromatography were distilled at their boiling ranges prior to use except chloroform was analytical grade reagent.

1.2.2 Plant material

The rhizomes of *C. zedoaria* were collected from Pattalung province in the Southern part of Thailand, in May, 2008. Identification was made by Mr. Ponlawat Pattarakulpisutti, Department of Biology, Faculty of Science, Prince of Songkla University. The specimen (0013171) has been deposited in the Herbarium of Department of Biology, Faculty of Science, Prince of Songkla University, Thailand.

1.2.3 Extraction and isolation

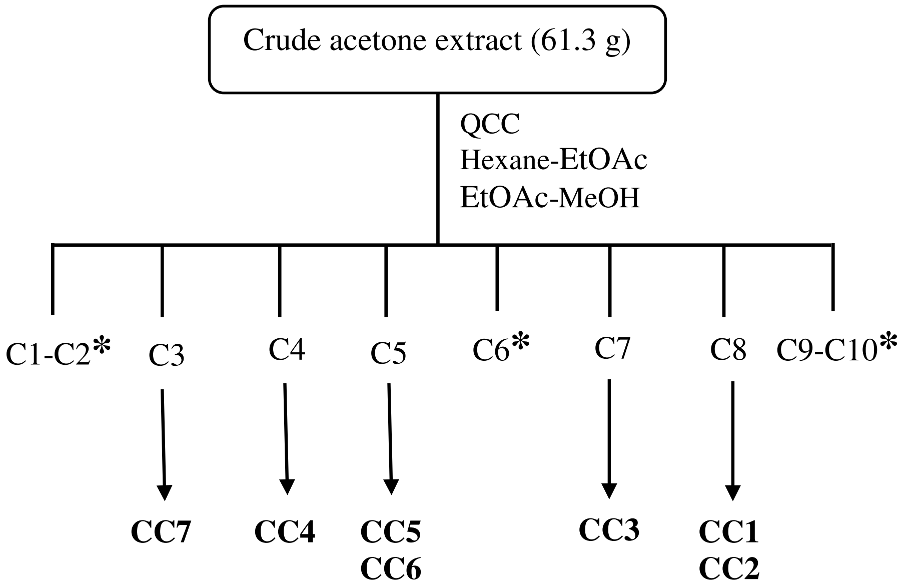
Air-dried rhizomes of *C. zedoaria* (1.5 kg) were immersed in acetone at room temperature for 3 days. After evaporation, a dark orange gum of acetone extract (61.3 g) was obtained. The process of extraction was shown in **Scheme 1**.



Scheme 1 Isolation of crude extract from the rhizomes of *C. zedoaria*

1.2.4 Isolation and chemical investigation of the crude acetone extract from the rhizomes of *C. zedoaria*

The acetone extract (61.3 g) was subjected to quick column chromatography using silica gel as stationary phase and eluted with a gradient of hexane, hexane-EtOAc, EtOAc-MeOH and finally with pure MeOH. On the basis of their TLC characteristics, the fractions which contained the same major components were combined to give fractions C1-C10, which were further purified to afford seven pure compounds as shown in **Scheme 2**.



* No further investigation

Scheme 2 Isolation of compounds **CC1-CC7** from acetone extract

Fraction C3 (4.94 g) was subjected to QCC with a gradient of EtOAc-hexane and followed by CC eluting with acetone–hexane (1:20, v/v) to give **CC7:** comosone II (11.3 mg). Fraction 4 (120.0 mg) was purified by CC eluting with methylene chloride to give **CC4:** germacrone (15.0 mg) as a white solid.

Fraction C5 (13.39 g) was purified by QCC with a gradient of acetone–hexane to afford eight fractions (C5A-C5H).

Subfraction C5E (4.52 g) was filtered and washed with hexane to give white crystals of **CC6:** zederone (1.26 g) and the mother liquor as yellow viscous oil after evaporation of the solvent.

Subfraction C5G (377.8 mg) was purified by CC eluting with acetone-hexane (1:10, v/v) to afford seven fractions (5G1-5G7).

Subfraction C5G3 (45.0 mg) was purified by CC eluting with EtOAc-hexane (2:10, v/v) to give **CC5:** (+)-germacrone-4,5-epoxide (12.0 mg).

Fraction C7 (4.32 g) was purified by QCC with a gradient of EtOAc-hexane to afford six fractions (C7A-C7F).

Subfraction C7A (85.0 mg) was purified by CC eluting with EtOAc-hexane (1:5, v/v) to give **CC3:** 1-dehydrogingerdione (8.0 mg).

Fraction C8 (17.06 g) was purified by QCC with a gradient of acetone-hexane to afford fourteen fractions (C8A-C8N).

Subfraction C8G (50.0 mg) was purified by CC eluting with acetone-hexane (2:10 v/v) to give an orange solid which was further washed with hexane to give **CC1:** curcumin (28.0 mg) as an orange solid.

Subfraction C8N (8.7 g) was purified by CC eluting with acetone-hexane (1:10 v/v) to give **CC2:** demethoxycurcumin (5.13 g) as an orange solid.

Compound CC1: curcumin, orange solid, m.p. 174-175 °C; UV λ_{max} (MeOH) (log ε): 421 (4.42) nm; IR (KBr) ν_{max} 3385 (O-H stretching), 1625 (C=O stretching); For ^{1}H NMR (acetone-d_6, 300 MHz) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 2**.

Compound CC2: demethoxycurcumin, orange solid, UV λ_{max} (MeOH) (log ε): 419 (4.67) nm; IR (KBr) ν (cm^{-1}): 3360 (O-H stretching) and 1637 (C=O stretching); For ^{1}H NMR (acetone-d_6, 300 MHz) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 4**.

Compound CC3: 1-dehydrogingerdione, yellow crystals, m.p. 83-84°C; UV λ_{max} (MeOH) (log ε): 371 (4.62) nm; IR (Neat) ν (cm^{-1}): 3422 (O-H stretching) and 1639 (C=O stretching); For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 6**.

Compound CC4: germacrone, white solid, m.p. 49-51°C; UV λ_{max} (MeOH) (log ε): 242 (3.63); IR (Neat) ν (cm^{-1}): 1671 (C=O stretching); For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 8**.

Compound CC5: (+)-germacrone-4,5-epoxide, white solid, , $[\alpha]^{27}_D$ +327° (c 1.0, $CHCl_3$), m.p. 58-60°C; UV λ_{max} (MeOH) (log ε): 239 (3.59) nm; IR (Neat) ν (cm^{-1}): 1677 (C=O stretching); For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 10**.

Compound CC6: zederone, white crystals, $[\alpha]^{27}_D$ +274° (c 1.0, $CHCl_3$), m.p. 148-150°C; UV λ_{max} (MeOH) (log ε): 241 (3.76) nm; IR (KBr) ν (cm^{-1}):

1662 (C=O stretching); For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 12**.

Compound CC7: comosone II, colorless oil, $[\alpha]^{28}_D$ +6° (*c* 0.2, $CHCl_3$); UV λ_{max} (MeOH) (log ε): 239 (3.77) nm; IR (Neat) ν (cm^{-1}): 1676 (C=O stretching); For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 14**.

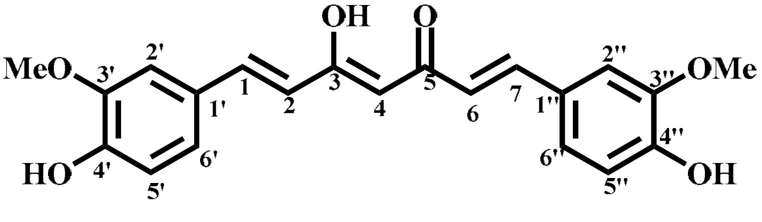
CHAPTER 1.3
RESULTS AND DISCUSSION

1.3.1 Structure elucidation of compounds from the rhizomes of *C. zedoaria*

The crude acetone extract from the rhizomes of *C. zedoaria* was subjected to repeated quick column and column chromatography over silica gel to furnish seven known compounds of two curcuminoids: curcumin (**CC1**) and demethoxycurcumin (**CC2**), one gingerdione: 1-dehydrogingerdione (**CC3**), together with four sesquiterpenes: germacrone (**CC4**), (+)-germacrone-4,5-epoxide (**CC5**), zederone (**CC6**) and comosone II (**CC7**).

Their structures were elucidated mainly by 1D and 2D NMR spectroscopic data: ^{1}H, ^{13}C NMR, DEPT 135°, DEPT 90°, HMQC, HMBC, COSY and NOESY. The physical data of the known compounds were also compared with the reported values.

1.3.1.1 Compound CC1



Compound **CC1** was obtained as an orange solid, m.p. 174-175 °C. The UV-Vis spectrum exhibited the absorption bands at $\lambda_{\max}$ 421 nm. The IR spectrum of compound **CC1** indicated the presence of hydroxyl at 3385 cm^{-1} and conjugated carbonyl at 1625 cm^{-1}.

The ^1H NMR spectrum (**Table 2**) displayed signals of two doublets of the *trans* double bonds at δ 6.70 and 7.60 ppm (each 2H) with a coupling constant of 15.9 Hz, a 1,3,4-trisubstituted benzene [δ 6.88 (2H, *d*, J = 8.1 Hz), 7.17 (2H, *d*, J = 8.1 Hz) and 7.32 (2H, *s*)], a methoxyl signal at δ 3.91 (6H, *s*) and a hydroxyl signal at δ 7.32 (*s*). This result was also supported by a HMBC experiment (**Figure 2**), (**Table 2**).

The ^{13}C NMR spectral data showed 11 signals for 21 carbons. Analysis of DEPT 135° of this compound suggested the presence of one methyl (δ 55.4), six methine (δ 100.8, 110.7, 115.4, 121.4, 123.0 and 140.6) and four quaternary carbons (δ 127.3, 148.0, 149.2 and 183.6). On the basis of the above results and comparison with the reported data of curcumin [Masuda *et al.*, 1992], compound **CC1** was assigned as curcumin.

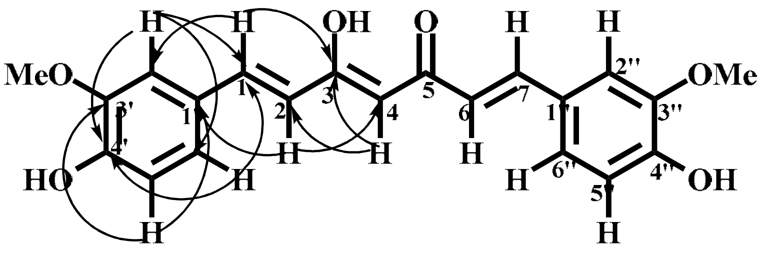


Figure 2 Selected HMBC correlations of **CC1**

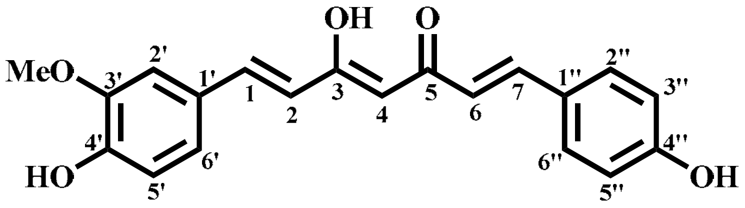
Table 2 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CC1** (acetone-d_6)

Position	δ_C		δ_H (mult, J, Hz)	HMBC
1/7	140.6	CH	7.60 (*d*, J = 15.9 Hz)	C-3/5, C- 2′/2″
2/6	121.4	CH	6.70 (*d*, J = 15.9 Hz)	C-4, C-1′/1″
3/5	183.6	C	-	-
4	100.8	CH	5.98 (*s*)	C-2/6, C-3/5
1′/1″	127.3	C	-	-
2′/2″	110.7	CH	7.32 (*s*)	C-1/7, C-4′/4″, C-6′/6″
3′/3″	148.0	C	-	-
4′/4″	149.2	C	-	-
5′/5″	115.4	CH	6.88 (*d*, J = 8.1 Hz)	C-1′/1″, C-3′/3″
6′/6″	123.0	CH	7.17 (*d*, J = 8.1 Hz)	C-1/7, C-2′/2″, C-4′/4″
OMe	55.4	CH_3	3.91 (*s*)	C-3′/3″
OH	-	-	7.32 (*s*)	-

Table 3 Comparison of ^{1}H NMR and spectral data between compounds **CC1** (acetone-d_6) and curcumin (**R**, $CDCl_3$)

Position	CC1 δ_H (mult, J, Hz)	R δ_H (mult, J, Hz)
1/7	7.60 (*d*, J = 15.9 Hz)	7.59 (*d*, J = 15.9 Hz)
2/6	6.70 (*d*, J = 15.9 Hz)	6.47 (*d*, J = 15.9 Hz)
3/5	-	-
4	5.98 (*s*)	5.80 (*s*)
1′/1″	-	-
2′/2″	7.32 (*s*)	7.05 (*d*, J = 1.9 Hz)
3′/3″	-	-
4′/4″	-	-
5′/5″	6.88 (*d*, J = 8.1 Hz)	6.93 (*d*, J = 8.0 Hz)
6′/6″	7.17 (*d*, J = 8.1 Hz)	7.12 (*dd*, J = 8.0, 1.9 Hz)
OMe	3.91 (*s*)	3.95 (*s*)

1.3.1.2 Compound CC2



Compound **CC2** was obtained as orange solid. The UV-Vis spectrum exhibited the absorption bands at λ_{max} 419 nm. The IR spectrum of compound **CC2** indicated the presence of hydroxyl at 3360 cm^{-1} and conjugated carbonyl at 1637 cm^{-1}.

The ^{1}H NMR spectrum (**Table 4**) displayed the presence of two *trans* double bonds as four doublet signals at δ 6.67, 6.72, 7.60 and 7.61 ppm with the same coupling constants of 15.6 Hz, two sets of aromatic signals due to a 1,4-disubstituted benzene [δ 6.90 (2H, *d*, J = 8.7 Hz), 7.57 (2H, *d*, J = 8.7 Hz)] and a 1,3,4-trisubstituted benzene [δ 6.89 (1H, *d*, J = 8.1 Hz), 7.18 (1H, *dd*, J = 8.1, 1.8 Hz), 7.34 (1H, *d*, J = 1.8 Hz)]. The presence of one methoxyl group was also shown by three-proton singlet signal at δ 3.91 ppm. This result was also supported by a HMBC experiment (**Figure 3**), (**Table 4**).

The ^{13}C NMR spectral data showed 17 signals for 20 carbons. Analysis of DEPT 135° and DEPT 90° spectra of this compound suggested the presence of one methoxyl (δ 55.6), ten methine (δ 100.8, 110.6, 115.3, 115.9, 121.1, 121.4, 123.0, 130.1, 140.2, 140.5) and six quaternary carbons (δ 126.7, 127.3, 147.9, 149.2, 159.7 and 183.7). On the basis of the above results and comparison with the reported data of demethoxycurcumin [Masuda *et al.*, 1992], compound **CC2** was assigned as demethoxycurcumin.

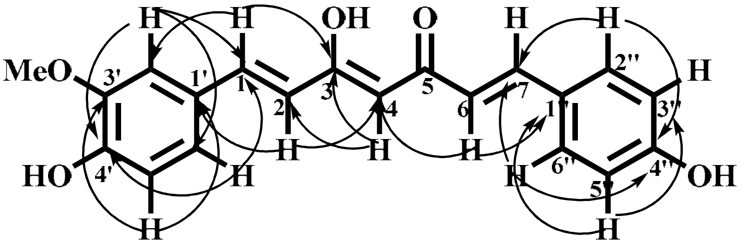


Figure 3 Selected HMBC correlations of **CC2**

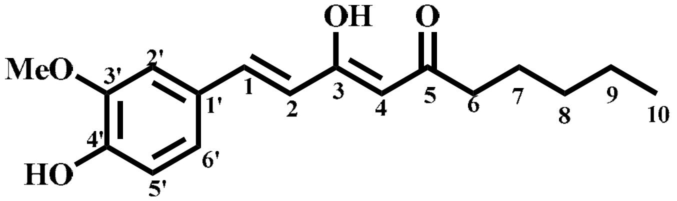
Table 4 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CC2** (acetone-d_6)

Position	δ_C		δ_H **(mult, *J*, Hz)**	**HMBC**
1	140.5	CH	7.60 (*d*, *J* = 15.6 Hz)	C-3, C-2′
2	121.1	CH	6.67 (*d*, *J* = 15.6 Hz)	C-4, C-1′
3	183.7	C	-	-
4	100.8	CH	5.85 (*s*)	C-2, C-3, C-5, C-6
5	183.7	C	-	-
6	121.4	CH	6.72 (*d*, *J* = 15.6 Hz)	C-4, C-1″
7	140.2	CH	7.61 (*d*, *J* = 15.6 Hz)	C-5, C-2″
1′	127.3	C	-	-
2′	110.6	CH	7.34 (*d*, *J* = 1.8 Hz)	C-1, C-4′, C-6′
3′	147.9	C	-	-
4′	149.2	C	-	-
4′-OH				
5′	115.3	CH	6.89 (*d*, *J* = 8.1 Hz)	C-1′, C-3′
6′	123.0	CH	7.18 (*d*, *J* = 8.1, 1.8 Hz)	C-1, C-2′, C-4′
1″	126.7	C	-	-
2″/6″	130.1	CH	7.57 (*d*, *J* = 8.7 Hz)	C-7, C-4″, C-6″
3″/5″	115.9	CH	6.90 (*d*, *J* = 8.7 Hz)	C-1″, C-5″
4″	159.7	C	-	-
4″-OH				
OMe	55.6	CH_3	3.91 (*s*)	-

Table 5 Comparison of ^{1}H NMR spectral data between compounds **CC2** (acetone-d_6) and demethoxycurcumin (**R**, CDCl$_3$)

Position	**CC2** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**
1	7.60 (*d*, *J* = 15.6 Hz)	7.61 (*d*, *J* = 15.9 Hz)
2	6.67 (*d*, *J* = 15.6 Hz)	6.48 (*d*, *J* = 15.9 Hz)
3	-	-
4	5.85 (*s*)	5.80 (*s*)
5	-	-
6	6.72 (*d*, *J* = 15.6 Hz)	6.47 (*d*, *J* = 15.9 Hz)
7	7.61 (*d*, *J* = 15.6 Hz)	7.59 (*d*, *J* = 15.9 Hz)
1′	-	-
2′	7.34 (*d*, *J* = 1.8 Hz)	7.05 (*d*, *J* = 1.8 Hz)
3′	-	-
4′	-	-
4′-OH		5.86 (*s*)
5′	6.89 (*d*, *J* = 8.1 Hz)	6.93 (*d*, *J* = 7.8 Hz)
6′	7.18 (*d*, *J* = 8.1, 1.8 Hz)	7.12 (*d*, *J* = 7.8, 1.8 Hz)
1″	-	-
2″/6″	7.57 (*d*, *J* = 8.7 Hz)	7.45 (*d*, *J* = 8.1 Hz)
3″/5″	6.90 (*d*, *J* = 8.7 Hz)	6.88 (*d*, *J* = 8.1 Hz)
4″	-	-
4″-OH		5.86 (*s*)
OMe	3.91 (*s*)	3.95 (*s*)

1.3.1.3 Compound CC3



Compound **CC3** was obtained as a yellow solid, m.p. 83-84 °C. The UV-Vis spectrum exhibited the absorption bands at $\lambda_{\max}$ 371 nm. The IR spectrum of compound **CC3** indicated the presence of hydroxyl at 3422 cm^{-1} and conjugated carbonyl at 1639 cm^{-1}.

The ^{1}H NMR spectrum (**Table 6**) displayed a *trans* double bond as evidenced by two doublet signals at δ 6.34 and 7.53 ppm with a coupling constant of 15.9 Hz. The ^{1}H NMR signals at δ 6.92 (*d*, J = 8.4 Hz), δ 7.10 (*dd*, J = 8.4, 1.8 Hz) and δ 7.02 (*d*, J = 1.8 Hz) established the presence of three aromatic protons with ortho, ortho/meta and meta coupling, respectively. The presence of one methoxyl group was also shown by a three–proton singlet signal at δ 3.94 ppm. The ^{1}H NMR spectrum displayed signals of methylene protons at δ 2.38 (2H, *t*, J = 7.5 Hz, H-6), a multiplet at δ 0.94 (3H, H-10), and two broad multiplet signals at δ 1.26-1.41. This result was also supported by a HMBC experiment (**Figure 4**, **Table 6**).

The ^{13}C NMR spectral data showed 17 signals for 17 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of two methyl (δ 13.0 and 55.9), four methylene (δ 22.4, 25.3, 31.5 and 43.1), six methine (δ 100.1, 109.5, 114.8, 120.6, 122.6 and 139.8), four quaternary carbons (δ 127.7, 146.8, 147.7 and 178.1) and a carbonyl (δ 200.2). On the basis of the above results and comparison with the reported data of 1-dehydrogingerdione [Charles *et al.*, 2000], compound **CC3** was assigned as 1-dehydrogingerdione.

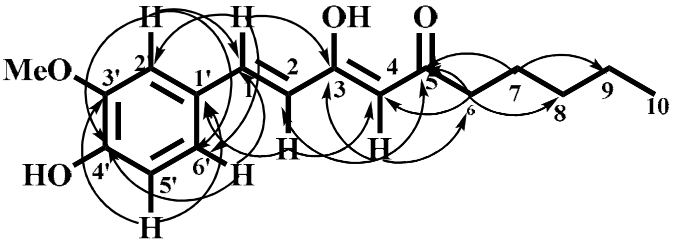


Figure 4 Selected HMBC correlations of **CC3**

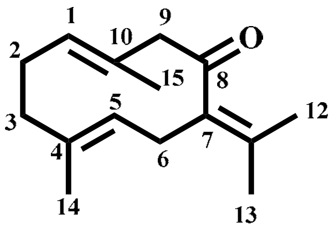
Table 6 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CC3** (CDCl$_3$)

Position	δ_C		δ_H **(mult, *J*, Hz)**	**HMBC**
1	139.8	CH	7.53 (*d*, *J* = 15.9 Hz)	C-3, C-2′
2	120.6	CH	6.34 (*d*, *J* = 15.9 Hz)	C-4, C-1′
3	178.1	C	-	-
4	100.1	CH	5.63 (1H, *s*, H-4 Hz)	C-2, C-3, C-5, C-6
5	200.2	C	-	-
6	43.1	CH$_2$	2.38 (*t*, *J* = 7.5)	C-4, C-5, C-8
7	25.3	CH$_2$	1.61-1.72 (*m*)	C-5, C-9
8	31.5	CH$_2$	1.26-1.41 (*m*)	C-6, C-7, C-10
9	22.4	CH$_2$	1.26-1.41 (*m*)	C-6, C-7, C-10
10	13.0	CH$_3$	0.94 (*m*)	C-8, C-9
1′	127.7	C	-	-
2′	109.5	CH	7.02 (*d*, *J* = 1.8 Hz)	C-1, C-4′, C-6′
3′	146.8	C	-	-
4′	147.7	C	-	-
5′	114.8	CH	6.92 (*d*, *J* = 8.4 Hz)	C-1′, C-3′
6′	122.6	CH	7.10 (*dd*, *J* = 8.4, 1.8 Hz)	C-1, C-2′, C-4′
OH	-	-	5.87 (*s*)	-
OMe	55.9	CH$_3$	3.94 (*s*)	C-3′

Table 7 Comparison of ^{1}H NMR and ^{13}C NMR spectral data between compounds **CC3** ($CDCl_3$) and 1-dehydrogingerdione (**R**, $CDCl_3$)

Position	**CC3** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**	**δ_C, CC3**	**δ_C, R**
1	7.53 (*d*, *J* = 15.9 Hz)	7.52 (*d*, *J* = 16.0 Hz)	139.8	140.2
2	6.34 (*d*, *J* = 15.9 Hz)	6.32 (*d*, *J* = 16.0 Hz)	120.6	120.4
3	-	-	178.1	177.5
4	5.63 (*s*)	5.62 (*s*)	100.1	100.1
5	-	-	200.2	197.5
6	2.38 (*t*, *J* = 7.5 Hz)	2.37 (*m*)	43.1	41.3
7	1.61-1.72 (*m*)	1.65 (*m*)	31.5	33.3
8	1.26-1.41 (*m*)	1.32 (*m*)	25.3	28.8
9	1.26-1.41 (*m*)	1.32 (*m*)	22.4	24.6
10	0.94 (*m*)	0.91 (*t*, *J* = 7.0 Hz)	13.0	15.8
1′	-	-	127.7	126.1
2′	7.02 (*d*, *J* = 1.8 Hz)	7.01 (*d*, *J* = 1.5 Hz)	109.5	112.7
3′	-	-	146.8	149.5
4′	-	-	147.6	147.1
5′	6.92 (*d*, *J* = 8.4 Hz)	6.93 (*d*, *J* = 8.5 Hz)	114.8	115.3
6′	7.1 (*dd*, *J* = 8.4, 1.8 Hz)	7.1 (*dd*, *J* = 8.5, 1.5 Hz)	122.6	122.3
OH	5.87 (*s*)	-	-	-
OMe	3.94 (*s*)	3.94 (*s*)	55.9	56.8

1.3.1.4 Compound CC4



Compound **CC4** was obtained as a white solid, m.p. 49-51°C. The IR spectrum of compound **CC4** indicated the presence of conjugated carbonyl absorption at 1671 cm^{-1}.

The ^{1}H NMR spectrum (**Table 8**) displayed signals assignable to four tertiary methyl at δ 1.42 (*s*, Me-14), 1.61 (*s*, Me-15), 1.73 (*s*, Me-13) and 1.77 (*s*, Me-12), two olefinic methine protons at δ 4.71 (1H, *dd, J* = 11.1, 3.3 Hz, H-5) and 4.98 (1H, *br d, J* = 10.8, H-1) together with four methylene protons at δ 2.17-2.29 (4H, *m,* H-2, H-3), 2.91 (2H, *m,* H-6), 2.95 (1H, *d, J* = 10.5 Hz, H-9a) and 3.41 (1H, *d, J* = 10.5 Hz, H-9b). The locations of the four methyl groups (Me-12, Me-13, Me-14, Me-15) at C-11, C-11, C-4 and C-10, respectively, were deduced from HMBC correlations of Me-12 (δ 1.77) and Me-13 (δ 1.73) with C-11 (δ 137.1), of Me-14 (δ 1.42) with C-4 (δ 126.5) and of Me-15 (δ 1.61) with C-10 (δ 134.8). The lack of NOESY cross peaks between H-5 and Me-14 and between H-1 and Me-15 suggested *E*-configurations of both double bonds. This result was also supported by a HMBC experiment (**Figure 5**, **Table 8**).

The ^{13}C NMR spectral data displayed 15 signals for 15 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound supported the presence of four methyl (δ 15.4, 16.6, 19.7 and 22.2), four methylene (δ 23.9, 29.0, 37.9 and 55.7), two methine (δ 125.3, and 132.5), four quaternary carbons (δ 126.5, 129.3, 134.8, 137.1) and a carbonyl (δ 207.6). On the basis of the above results and comparison with the reported data of germacrone [Lee *et al.*, 2006], compound **CC4** was assigned as germacrone.

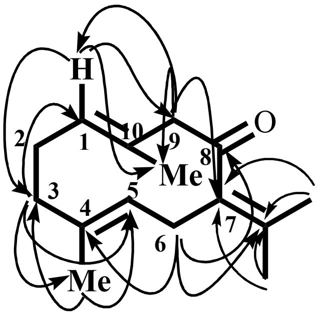


Figure 5 Selected HMBC correlations of **CC4**

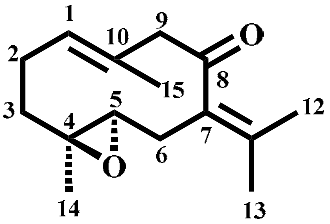
Table 8 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CC4** ($CDCl_3$)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**
1	132.5	CH	4.98 (*br d, J* = 10.8 Hz)	C-3, C-9
2	23.9	CH_2	2.17-2.29 (*m*)	C-4, C-10
3	37.9	CH_2	2.17-2.29 (*m*)	C-1, C-5, C-14
4	126.5	C	-	-
5	125.3	CH	4.71 (*dd, J* = 11.1, 3.3 Hz)	C-3, C-7, C-14
6	29.0	CH_2	2.91 (*m*)	C-4, C-8, C-11
7	129.3	C	-	-
8	207.6	C	-	-
9	55.7	CH_{2a}	2.95 (*d, J* = 10.5 Hz)	C-1, C-7, C-15
		CH_{2b}	3.41 (*d, J* = 10.5 Hz)	C-1, C-7, C-15
10	134.8	C	-	-
11	137.1	C	-	-
12	19.7	CH_3	1.77 (*s*)	C-7, C-11, C-13
13	22.2	CH_3	1.73 (*s*)	C-7, C-11, C-12
14	15.4	CH_3	1.42 (*s*)	C-3, C-5
15	16.6	CH_3	1.61 (*s*)	C-1, C-9

Table 9 Comparison of ^{1}H NMR and ^{13}C NMR spectral data between compounds **CC4** ($CDCl_3$) and germacrone (**R**, $CDCl_3$)

Position	**CC4** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**	**δ_C, CC4**	**δ_C, R**
1	4.98 (*br d, J* = 10.8 Hz)	4.99 (*dd, J* = 12.2, 3.5 Hz)	132.5	132.2
2	2.17-2.29 (*m*)	2.0-2.4 (*m*)	23.9	24.0
3	2.17-2.29 (*m*)	2.0-2.4 (*m*)	37.9	37.9
4	-	-	126.5	126.8
5	4.71 (*dd, J* = 11.1, 3.3 Hz)	4.71 (*dd, J* = 11.5, 4.0 Hz)	125.3	125.5
6	2.91 (*m*)	2.86 (*dd, J* = 11.5, 13.5 Hz)	29.0	28.9
	2.91 (*m*)	2.97 (*dd, J* = 13.5, 4.0 Hz)	29.0	28.9
7	-	-	129.3	129.0
8	-	-	207.6	207.2
9	2.95 (*d, J* = 10.5 Hz)	2.95 (*d, J* = 10.5 Hz)	55.7	55.9
	3.41 (*d, J* = 10.5 Hz)	3.41 (*d, J* = 10.5 Hz)	55.7	55.9
10	-	-	134.8	134.9
11	-	-	137.1	137.0
12	1.77 (*s*)	1.71 (*s*)	19.7	19.8
13	1.73 (*s*)	1.76 (*s*)	22.2	22.2
14	1.42 (*s*)	1.43 (*s*)	15.4	15.5
15	1.61 (*s*)	1.61 (*s*)	16.6	16.7

1.3.1.5 Compound CC5



Compound **CC5** was obtained as a white solid, m.p. 58-60°C, $[\alpha]^{27}_{D}$ +327° (*c* 1.0, $CHCl_3$) IR spectrum of compound **CC5** indicated the presence of conjugated carbonyl absorptions at 1677 cm^{-1}.

The ^1H NMR spectrum (**Table 10**) displayed signals assignable to four tertiary methyl at δ 1.04 (*s*, Me-14), 1.73 (*s*, Me-15), 1.82 (*s*, Me-13) and 1.83 (*s*, Me-12) together with four methylene protons.

The ^{13}C NMR spectral data displayed 15 signals for 15 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of four methyl (δ 15.8, 17.0, 20.4 and 22.7), four methylene (δ 24.6, 29.6, 37.6 and 55.5), two methine (δ 64.5, and 129.7,) four quaternary carbons (δ 126.7, 60.8, 133.7, 134.5) and a carbonyl (δ 204.6).

The ^1H and ^{13}C NMR spectral data of compound **CC5** were closely related to those of compound **CC4** suggesting the same sesquiterpene skeleton. The differences were shown at positions 4 and 5 in which an olefinic methine proton H-5 at δ_H 4.71 in **CC4** was replaced by an oxymethine proton at δ_H 2.44 in **CC5** and the chemical shifts of C-4 (δ 126.5) and C-5 (δ 125.3) which were those of sp^2 carbons in **CC4** were replaced by those of C-4 (δ 60.8) and C-5 (δ 64.5) in **CC5** whose values suggested an epoxide functionality.

The stereochemistry at C-4 and C-5 was deduced by NOESY experiment, from which there was no cross peak between H-5/Me-14. This result indicated that Me-14 and H-5 were on the opposite side. The ^1H and ^{13}C NMR spectral data of 4 and 5 positions agreed well with those reported by Yoshihara *et al.*, 1984. The optical rotation of compound **CC5** is dextrorotatory ($[\alpha]^{27}_{D}$ +327°, *c* 1.0, $CHCl_3$), similar to a (+)-germacrone-4,5-epoxide ($[\alpha]^{16}_{D}$ +399°, *c* 1.05, $CHCl_3$), (Yoshihara *et al.*, 1984), therefore compound **CC5** was identified as (+)-germacrone-4,5-epoxide.

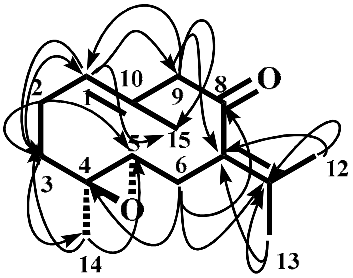


Figure 6 Selected HMBC correlations of **CC5**

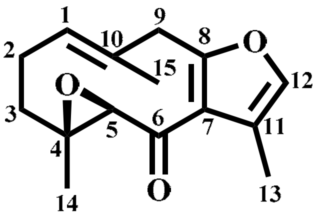
Table 10 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CC5** ($CDCl_3$)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**
1	129.7	CH	5.20 (*br d, J* = 9.9 Hz)	C-3, C-9
2	24.6	CH_2	2.24, 2.32 (*m*)	C-4, C-10
3	37.6	CH_2a	2.13 (*m*)	C-1, C-5, C-14
		CH_2b	1.15 (*td, J* = 12.6, 6.9 Hz)	C-1, C-5, C-14
4	60.8	C	-	-
5	64.5	CH	2.44 (*dd, J* = 10.8, 2.4 Hz)	C-3, C-7, C-14
6	29.6	CH_2a	2.06 (*dd, J* = 13.8, 10.8 Hz)	C-4, C-8, C-11
		CH_2b	2.87 (*br d, J* = 13.8 Hz)	C-4, C-8, C-11
7	133.7	C	-	-
8	204.6	C	-	-
9	55.5	CH_2a	3.04 (*br s*)	C-1, C-7, C-15
		CH_2b	3.44 (*br s*)	C-1, C-7, C-15
10	126.7	C	-	-
11	134.5	C	-	-
12	20.4	CH_3	1.83 (*s*)	C-7, C-11, C-13
13	22.7	CH_3	1.82 (*s*)	C-7, C-11, C-12
14	15.8	CH_3	1.04 (*s*)	C-3, C-5
15	17.0	CH_3	1.73 (*s*)	C-1, C-9

Table 11 Comparison of ^{1}H NMR and ^{13}C NMR spectral data between compounds **CC5** ($CDCl_3$) and (+)-germacrone-4,5-epoxide (**R**, $CDCl_3$)

Position	**CC5** δ_H **(mult, *J*, Hz)**	**R** δ_H **(mult, *J*, Hz)**	δ_C**, CC5**	δ_C**, R**
1	5.20 (*br d, J* = 9.9 Hz)	5.21 (*br d, J* = 9.6 Hz)	129.7	129.6
2	2.24, 2.32 (*m*)	2.13, 2.26 (*m*)	24.6	24.5
3	2.13 (*m*)	2.13 (*m*)	37.6	37.6
	1.15 (*td, J* = 12.6, 6.9 Hz)	1.15 (*ddd, J* = 12.6, 12.6, 6.9 Hz)		
4	-	-	60.8	60.5
5	2.44 (*dd, J* = 10.8, 2.4 Hz)	2.44 (*dd, J* = 10.8, 2.4 Hz)	64.5	64.3
6	2.06 (*dd, J* = 13.8, 10.8 Hz)	2.06 (*dd, J* = 14.4, 10.8 Hz)	29.6	29.6
	2.87 (*br d, J* = 13.8 Hz)	2.87 (*br d, J* = 14.4 Hz)		
7	-	-	126.7	126.6
8	-	-	204.6	204.4
9	3.04 (*br s*)	3.04 (*br s*)	55.5	55.4
	3.44 (*br s*)	3.44 (*br s*)		
10	-	-	133.7	133.7
11	-	-	134.5	134.3
12	1.83 (*s*)	1.83 (*s*)	20.4	20.3
13	1.82 (*s*)	1.82 (*s*)	22.7	22.7
14	1.04 (*s*)	1.04 (*s*)	15.8	15.8
15	1.73 (*s*)	1.73 (*s*)	17.0	16.9

1.3.1.6 Compound CC6



Compound **CC6** was obtained as a white solid, m.p. 148-150°C. The IR spectrum of compound **CC6** indicated the presence of conjugated carbonyl absorption at 1662 cm^{-1}.

The ^{1}H NMR spectrum (**Table 12**) displayed a doublet at δ 7.09 (1H, *br s*, H-12) assignable for a trisustituted furan ring, and a vinylic proton signal at δ 5.47 (1H, *dd, J* = 10.8, 3.6 Hz, H-1). The presence of an oxymethine proton at δ_H 3.81 (δ_C 66.3) and an oxyquaternary carbon at δ_C 63.7 confirmed the presence of an epoxide in the molecule. In addition there methyl signals were evident at δ 1.35 (*s*, CH_3-14), 1.60 (*s*, CH_3-15) and 2.11 (*s*, CH_3-13).

The ^{13}C NMR spectral data displayed a total of 15 carbons while the DEPT-135° and HMQC experiments indicated that 9 out of 15 carbons had attached protons. Analysis of the ^{13}C and DEPT-135 spectra allowed discernment of the carbon resonances into three methyls (δ 10.4, 14.9 and 15.5), three methylenes (δ 24.4, 37.8 and 41.7), three methines (δ 66.3, 131.0 and 137.8) and six quaternary carbons, including a carbonyl group (δ 192.0). In the HMBC spectrum H-12 at δ 7.09 showed correlations with C-7 (δ 122.0), C-8 (δ 156.9) and C-13 (δ 10.4), supporting the attachment of a methyl furan ring at C-7, C-8 of the main skeleton. The oxymethine proton H-5 did not show NOESY correlation with Me-14 suggesting their *trans* relationship. The optical rotation of compound **CC6** is dextrorotatory ($[\alpha]^{27}_D$ +274°, *c* 1.0, $CHCl_3$), similar to zederone ($[\alpha]^{31}_D$ +290°, *c* 1.14, $CHCl_3$), (Giang *et al.*, 2000). Thus on the basis of its spectroscopic data and comparison with the previously reported data of zederone (Giang *et al.*, 2000), compound **CC6** was therefore, assigned as zederone.

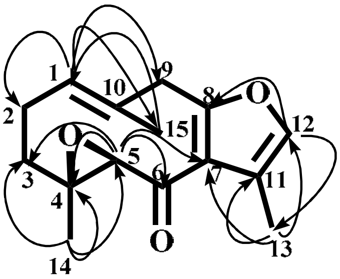


Figure 7 Selected HMBC correlations of **CC6**

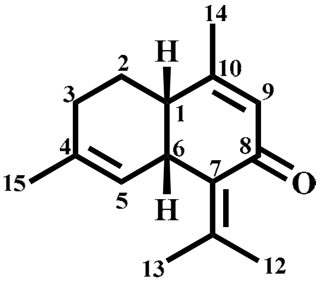
Table 12 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CC6** ($CDCl_3$)

Position	δ_C		δ_H **(mult, *J*, Hz)**	**HMBC**
1	131.0	CH	5.47 (*dd, J* = 10.8, 3.6 Hz)	C-3, C-9, C-15
2	24.4	CH_2	2.28-2.52 (*m*)	C-4, C-10
3	37.8	CH_{2a}	2.32 (*m*)	C-1, C-5, C-14
		CH_{2b}	1.28 (*dd, J* = 13.5, 3.6 Hz)	C-1, C-5, C-14
4	63.7	C	-	-
5	66.3	CH	3.81 (*s*)	C-3, C-7, C-14
6	192.0	C	-	-
7	122.0	C	-	-
8	156.9	C	-	-
9	41.7	CH_{2a}	3.68 (*d, J* = 16.5 Hz)	C-1, C-7, C-15
		CH_{2b}	3.76 (*d, J* = 16.5 Hz)	C-1, C-7, C-15
10	130.8	C	-	-
11	123.0	C	-	-
12	137.8	CH	7.09 (*br s*)	C-7, C-8, C-13
13	10.4	CH_3	2.11 (*s*)	C-7, C-11, C-12
14	14.9	CH_3	1.35 (*s*)	C-3, C-5
15	15.5	CH_3	1.60 (*s*)	C-1, C-9

Table 13 Comparison of ^{1}H NMR and ^{13}C NMR spectral data between compounds **CC6** ($CDCl_3$) and zederone (**R**, $CDCl_3$)

Position	**CC6** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**	**δ_C, CC6**	**δ_C, R**
1	5.47 (*dd, J* = 10.8, 3.6 Hz)	5.46 (*dd, J* = 12.0, 4.0 Hz)	131.0	131.1
2	2.28 (*m*)	2.21 (*d, J* = 13.5 Hz)	24.4	24.6
	2.52 (*m*)	2.51 (*dddd, J* = 13.5, 13.5, 12.0, 3.5 Hz)		
3	2.32 (*m*)	2.28 (*ddd, J* = 13.0, 3.5, 3.5 Hz)	37.8	37.9
	1.28 (*dd, J* = 13.5, 3.6 Hz)	1.27 (*ddd, J* = 13.5, 13.0, 4.0 Hz)		
4	-	-	63.7	63.9
5	3.81 (*s*)	3.79 (*s*)	66.3	66.5
6	-	-	192.0	192.1
7	-	-	122.0	122.1
8	-	-	156.9	157.0
9	3.68 (*d, J* = 16.5 Hz)	3.66 (*d, J* = 16.0 Hz)	41.7	41.8
	3.76 (*d, J* = 16.5 Hz)	3.73 (*d, J* = 16.0 Hz)		
10	-	-	130.8	131.0
11	-	-	123.0	123.2
12	7.09 (*br s*)	7.06 (*br s*)	137.8	138.0
13	2.11 (*s*)	2.09 (*br s*)	10.4	10.2
14	1.35 (*s*)	1.32 (*s*)	14.9	15.1
15	1.60 (*s*)	1.58 (*s*)	15.5	15.7

1.3.1.7 Compound CC7



Compound **CC7** was obtained as colorless oil, $[\alpha]^{28}_{D}$ +6° (*c* 0.2, $CHCl_3$). IR spectrum of compound **CC7** indicated the presence of conjugated carbonyl absorption at 1676 cm^{-1}.

The ^1H NMR spectrum (**Table 14**) displayed signals assignable to four vinyl methyls at δ 1.60, 1.88, 1.95 and 2.08, two methylenes at δ 1.81 (2H, *m*, H-3) and δ 1.77-2.22 (2H, *m*, H-2), two methines at δ 2.77 (1H, *br s*, H-1) and δ 3.78 (1H, *br s*, H-6), and two olefinic methine protons at δ 4.94 (1H, *br s*, H-5) and δ 5.92 (1H, *br s*, H-9).

The ^{13}C NMR spectral data displayed a total of 15 carbons while the DEPT-135° and HMQC experiments indicated that 10 out of 15 carbons had attached protons. Analysis of the ^{13}C and DEPT-135° spectra allowed discernment of the carbon resonances into four methyls (δ 20.8, 21.8, 23.0 and 23.4), two methylenes (δ 25.2 and 26.0), four methines (δ 38.3, 39.8, 122.0 and 130.8) and five quaternary carbons, including a carbonyl group (δ 191.9).

The stereochemistry at C-1 and C-6 was deduced from NOESY experiment. Cross peak was observed between H-1 and H-6, whose result indicated the *cis*-fused ring of **CC7**. The optical rotation of compound **CC7** is dextrorotatory ($[\alpha]^{28}_{D}$ +6°, *c* 0.2, $CHCl_3$), similar to comosone II ($[\alpha]^{27}_{D}$ +10.1°, *c* 0.70, $CHCl_3$), (Xu *et al.*, 2008). Thus on the basis of its spectroscopic data and comparison with the previously reported data of comosone II (Xu *et al.*, 2008), compound **CC7** was therefore, assigned as comosone II.

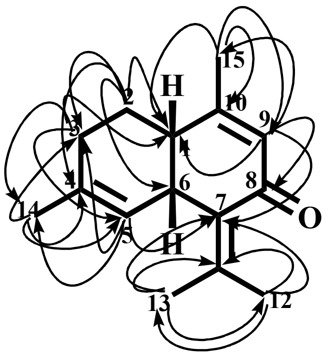


Figure 8 Selected HMBC correlations of **CC7**

Table 14 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CC7** (CDCl$_3$)

Position	δ_C		δ_H **(mult, *J*, Hz)**	**HMBC**
1	38.3	CH	2.77 (*br s*)	-
2	25.2	CH$_2$	1.77-2.22 (*m*)	C-1, C-3, C-4, C-6
3	26.0	CH$_2$	1.81 (*m*)	C-1, C-5, C-14
4	135.0	C	-	-
5	122.0	CH	4.94 (*br s*)	C-3, C-7, C-14
6	39.8	CH	3.78 (*br s*)	-
7	133.5	C	-	-
8	191.9	C	-	-
9	130.8	CH	5.92 (*br s*)	C-1, C-7, C-8, C-15
10	158.6	C	-	-
11	141.5	C	-	-
12	23.0	CH$_3$	2.08 (*s*)	C-7, C-11, C-13
13	21.8	CH$_3$	1.88 (*s*)	C-7, C-11, C-12
14	23.4	CH$_3$	1.60 (*s*)	C-3, C-5
15	20.8	CH$_3$	1.95 (*s*)	C-1, C-9

Table 15 Comparison of ^{1}H NMR and ^{13}C NMR spectral data between compounds **CC7** ($CDCl_3$) and comosone II (**R**, $CDCl_3$)

Position	**CC7** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**	**δ_C, CC7**	**δ_C, R**
1	2.77 (*br s*)	2.75 (*m*)	38.3	38.3
2	1.77-2.22 (*m*)	1.83-2.20 (*m*)	25.2	25.3
3	1.81 (*m*)	1.82 (*m*)	26.0	26.0
4	-	-	135.0	135.1
5	4.94 (*br s*)	4.92 (*br s*)	122.0	122.0
6	3.78 (*br s*)	3.76 (*br s*)	39.8	39.8
7	-	-	133.5	133.5
8	-	-	191.9	191.8
9	5.92 (*br s*)	5.90 (*br s*)	130.8	130.8
10	-	-	158.6	158.6
11	-	-	141.5	141.8
12	2.08 (*s*)	2.06 (*s*)	23.0	23.0
13	1.88 (*s*)	1.87 (*s*)	21.8	21.9
14	1.60 (*s*)	1.58 (*s*)	23.4	23.5
15	1.95 (*s*)	1.93 (*s*)	20.8	20.8

CHAPTER 2.1
INTRODUCTION

2.1.1 Introduction

Citrus medica Linn., belongs to the family Rutaceae. It is a small to medium-sized, shrubby tree, 3-10 m tall. The bark is distinct ridges and many prickles that is grey brown color. The stem has the character of rut twists. Leaves are single arrange alternate oval, with concave curly end, width 3-5 cm, length 7-12 cm. The flowers are white gathering in a bouquet. It is a large fruit-sized with the thick rough skin and an oval-shaped seed. They are found in the mixed forest and seaside forest.

According to Smitinand (2001), there are seventeen species of genus *Citrus* found in Thailand as follows:

1. *aurantifolia* (Christm.) Swingle
2. *hystrix* DC.
3. *limon* (L.) Burm.f.
4. *medica* L. var. *sarcodactylis.* Swing
5. *reticulata* Blanco
6. *japonica* Thunb
7. *aurantium* L var.*aurantium*
8. *ichangensis* Swingle
9. *macroptera* Mont.
10. *medica* Linn.
11. *latipes* Swingle
12. *semperflorens* Lush.
13. *maxima* Merr.
14. *halimii* B.C. Stone
15. *madurensis* Lour.
16. *nobilis* Lour.
17. *sinensis* (L.) Osbeck

In Thailand, *C. medica* has been found in every part of the country. It has many local Thai names: Manao khwai (มะนาวควาย) Pattani-Yala, Manao ripon (มะนาวริปน), Ma wo yao (มะโว่ยาว), Som o malako (ส้มโอมะละกอ) Chiang Mai, Som ma ngua (ส้มมะงั่ว) Central (Smitinand, 2001).

Trees

Leaves

Stem

Flowers

Fruits

Figure 9 Different parts of *Citrus medica* Linn.

2.1.2 Review of Literatures

The chemical constituents isolated from the four species of genus *Citrus* were summarized in **Table 16**. Information obtained from SciFinder Scholar copyright in 2010 will be presented and classified into groups: Acridone alkaloids, Aromatics, Coumarins, Flavonoids, Limonoids, Sesquiterpenoids and Steroids.

Table 16 Compounds from plants of *Citrus* genus

- **a**. Acridone alkaloids
- **b**. Aromatics
- **c**. Coumarins
- **d**. Flavonoids
- **e**. Limonoids
- **f**. Sesquiterpenoids
- **g**. Steroids

Scientific name	Part	Compounds	Bibliography
Citrus limonia	Stem	Imperatorin, **c1** Xanthotoxin, **c2** Bergapten, **c3** Isoimpinellin, **c4** Limettin, **c5** Scopoletol, **c6** Umbelliferone, **c7** Xanthoxol, **c8** Aesculetin, **c9** Stigmasterol, **g1** *β*-Sitosterol-3-*O*-*β*-glucoside, **g2**	Abdel-Fattah *et al*., 2003
	fruit peels	Limonflavonyl lactone A, **d5** Limonflavonyl lactone B, **d6**	Sultana *et al*., 2008

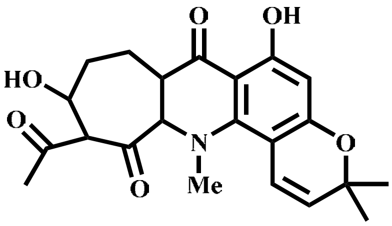
Table 16 (Continued)

Scientific name	Part	Compounds	Bibliography
Citrus nobilis	Seeds	Citrobilin, **e1** Limonin, **e2** Nomilin, **e3** Deacetyl nomilin, **e4** Obacunon, **e5** Limonexic acid, **e6** *β*-Sitosterol-3-*O*-*β*-D-glucoside, **g2** 2,2-Dimethylpyranoflavanol, **d1**	Bui *et al*., 2004
	Root bark	Elemol, **f1** Suberosin, **c10** Suberenol, **c12** Crenyllatin, **c13** Xanthyletin, **c11** Xanthoxyletin, **c14** Nordentatin, **c15** Citropone A, **a1** 5-Hydroxynoracronycine, **a2** Citrusinine I, **a3** Citracridone I, **a4**	Wu *et al*., 1987
Citrus maxima	Root bark	5-Hydroxynoracronycine, **a2** Natsucitrine-II, **a5** Grandisine-I, **a6** Citracridone-III, **a7**	Teng *et al*., 2005

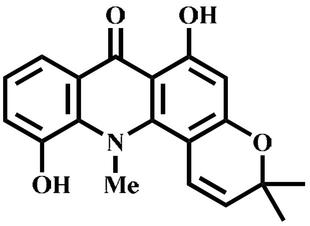
Table 16 (Continued)

Scientific name	Part	Compounds	Bibliography
Citrus medica L.Var. *sarcodactylis*	Aerial Part	3,5,6-Trihydroxy-4',7-dimethoxyflavone, **d2**	He *et al.*, 1985
		3,5,6-Trihydroxy-3',4',7-trimethoxyflavone, **d3**	
		Diosmetin, **d4**	Yin *et al.*, 2004
		Diosmin, **d7**	
		Aviprin, **c16**	
		Obcumone, **e5**	
		3,4-Dihydroxybenzoic acid, **b1**	
		3-(3-Methoxy-4-hydroxyphenyl)-acrylic acid, **b2**	
		Vanillic acid, **b3**	
		Limettin, **c5**	
		Scopoletol, **c6**	
		Umbelliferone, **c17**	
		7-Hydroxy-5-methoxycoumarin, **c18**	Yin *et al.*, 2004
		6,7-Dimethoxycoumarin, **c19**	
		p-Coumaric acid, **c20**	
		Limonin, **e2**	
		Nomilin, **e3**	
		Stigmasterol, **g1**	
		β-Sitosterol-3-*O*-*β*-D-glucoside, **g2**	

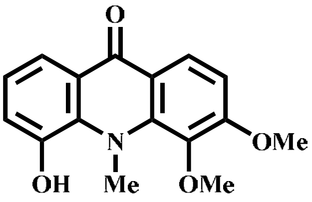
a. Acridone alkaloids



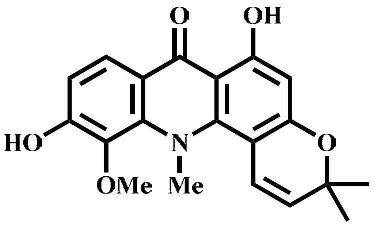
Citropone A, **a1**



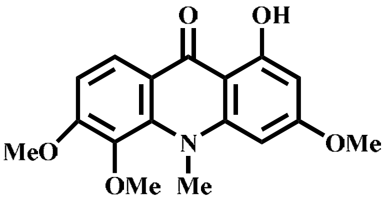
5-Hydroxynoracronycine, **a2**



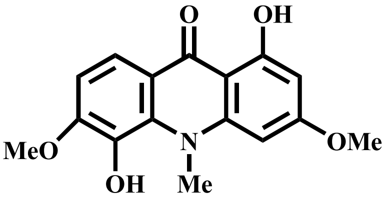
Citrusinine I, **a3**



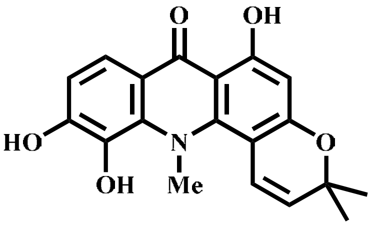
Citracridone I, **a4**



Natsucitrine-II, **a5**

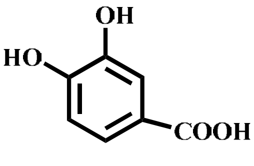


Grandisine-I, **a6**

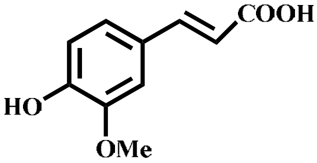


Citracridone-III, **a7**

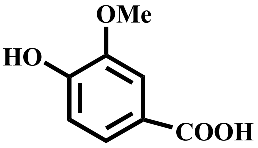
b. Aromatics



3,4-Dihydroxybenzoic acid, **b1**

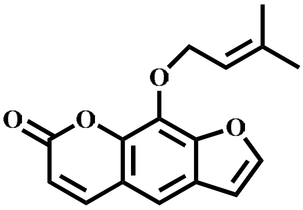


3-(3-Methoxy-4-hydroxyphenyl)acrylic acid, **b2**

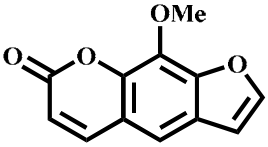


Vanillic acid, **b3**

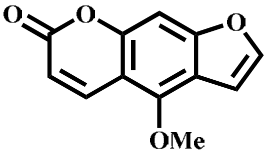
c. Coumarins



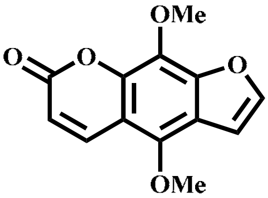
Imperatorin, **c1**



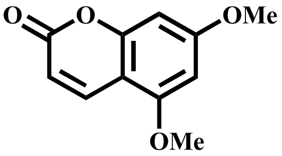
Xanthotoxin, **c2**



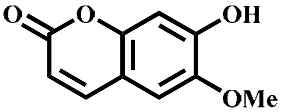
Bergapten, **c3**



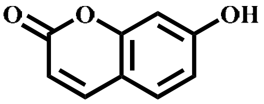
Isoimpinellin, **c4**



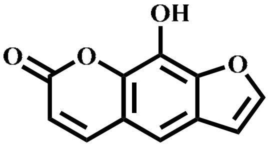
Limettin , **c5**



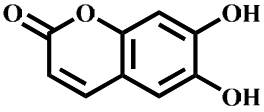
Scopoletol, **c6**



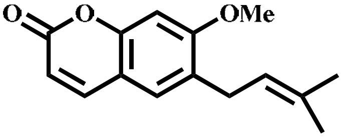
Umbelliferone, **c7**



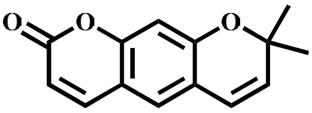
Xanthoxol, **c8**



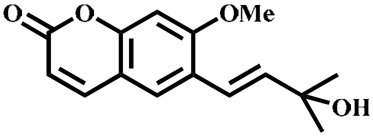
Aesculetin, **c9**



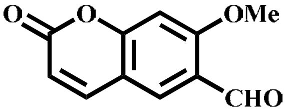
Suberosin, **c10**



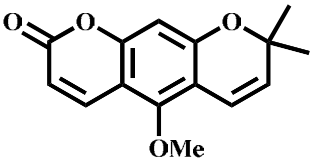
Xanthyletin, **c11**



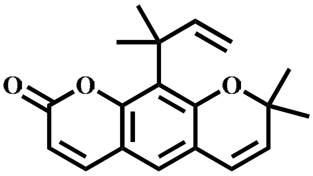
Suberenol, **c12**



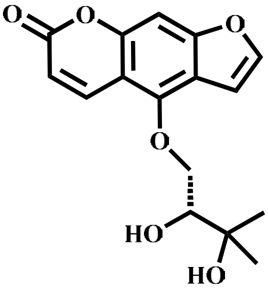
Crenyllatin, **c13**



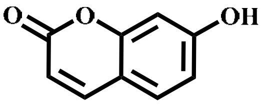
Xanthoxyletin, **c14**



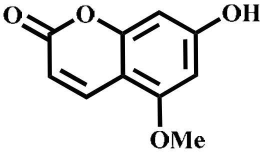
Nordentatin, **15**



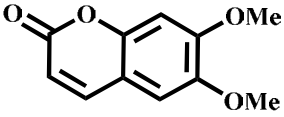
Aviprin, **c16**



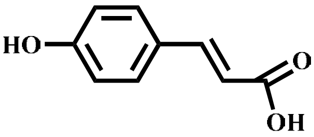
Umbelliferone, **c17**



7-Hydroxy-5-methoxycoumarin, **c18**

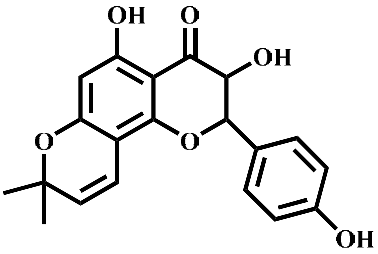


6,7-Dimethoxycoumarin, **c19**

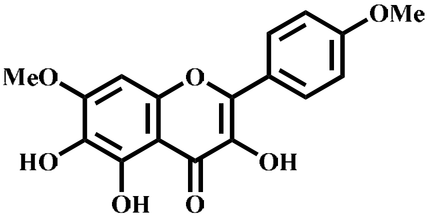


p-Coumaric acid, **c20**

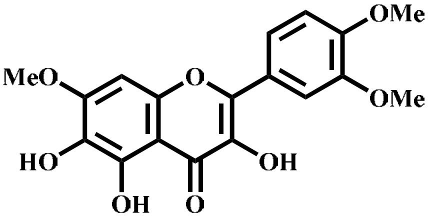
d. Flavonoids



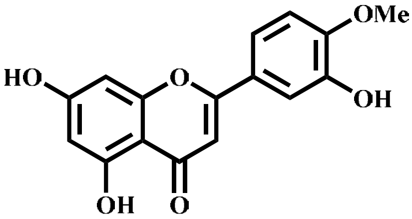
2,2-Dimethylpyranoflavanol, **d1**



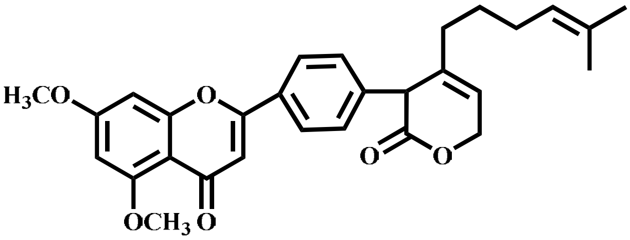
3,5,6-Trihydroxy-4',7-dimethoxyflavone, **d2**



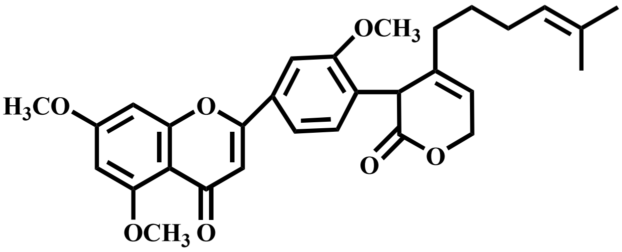
3,5,6-Trihydroxy-3',4',7-trimethoxyflavone, **d3**



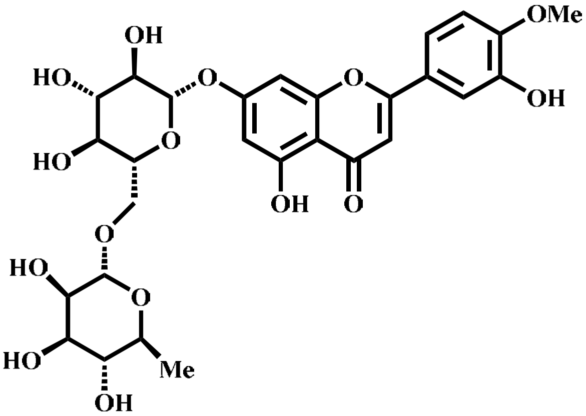
Diosmetin, **d4**



Limonflavonyl lactone A, **d5**

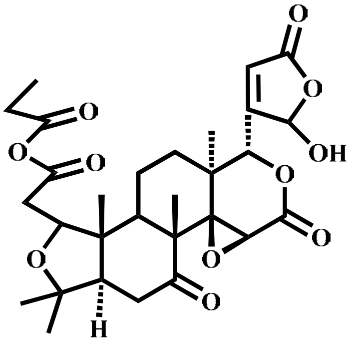


Limonflavonyl lactone B, **d6**

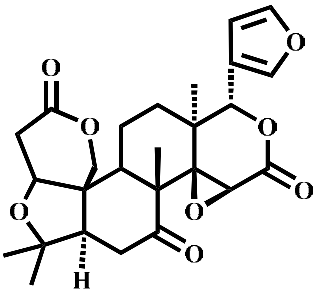


Diosmin, **d7**

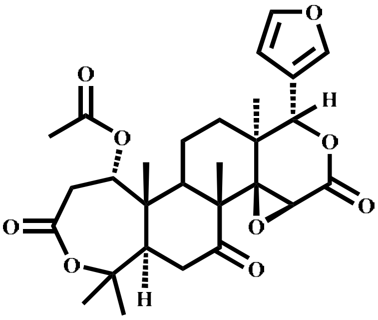
e. Limonoids



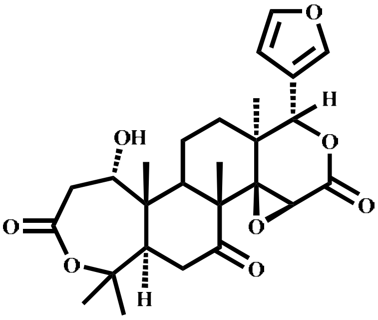
Citrobilin, **e1**



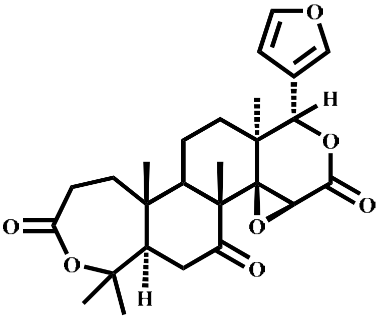
Limonin, **e2**



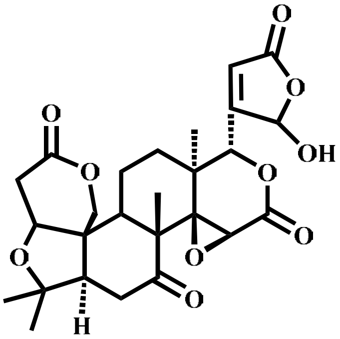
Nomilin, **e3**



Deacetyl nomilin, **e4**

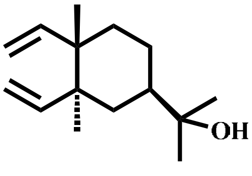


Obacunon, **e5**



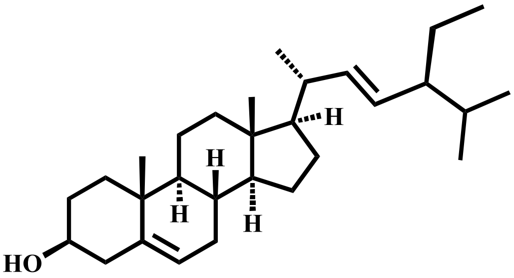
Limonexic acid, **e6**

f. Sesquiterpenoids

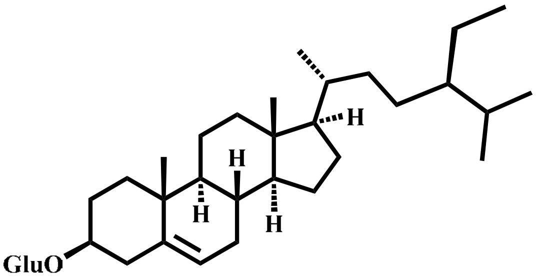


Elemol, **f1**

g. Steroids



Stigmasterol, **g1**



β-Sitosterol-3-O-*β*-D-glucoside, **g2**

2.1.3 Objective

This part of research work is to investigate the chemical constituents from the stems of *Citrus medica* Linn. It involved isolation, purification and structure elucidation.

CHAPTER 2.2
EXPERIMENTAL

2.2.1 Instruments and chemicals

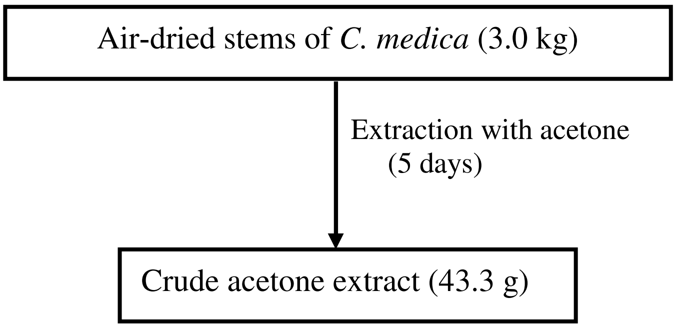
Melting point was recorded in ^{o}C on a digital Electrothermal 9100 Melting Point Apparatus. Ultraviolet spectra were measured with a UV-160A spectrophotometer (SHIMADZU) and principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in methanol solution. The optical rotation $[\alpha]_D$ was measured in chloroform, acetone and methanol solution with Sodium D line (590 nm) on a JASCO P-1020 digital polarimeter. The IR spectra were measured with a Perkin-Elmer 783 FTS165 FT-IR spectrophotometer. ^{1}H and ^{13}C – Nuclear magnetic resonance spectra were recorded on a FT-NMR Bruker Ultra Shield^{TM} 300 and 500 MHz spectrometer at Department of Chemistry, Faculty of Science, Prince of Songkla University and spectra were recorded in deuterochloroform and deuteroacetone as δ value in ppm downfield from TMS (internal standard δ 0.00) and coupling constant (J) are expressed in hertz. Quick column chromatography (QCC) and column chromatography was performed by using silica gel 60 H (Merck) and silica gel 100 (70-230 Mesh ASTM, Merck) respectively. For thin-layer chromatography (TLC), aluminum sheets of silica gel 60 F_{254} (20×20 cm, layer thickness 0.2 mm, Merck) were used for analytical purposes and the compounds were visualized under ultraviolet light. Solvents for extraction and chromatography were distilled at their boiling ranges prior to use except chloroform was analytical grade reagent.

2.2.2 Plant material

The stems of *C. medica* were collected from Pattalung province in the Southern part of Thailand, in August, 2009. Identification was made by Mr. Ponlawat Pattarakulpisutti, Department of Biology, Faculty of Science, Prince of Songkla University. The specimen (0013595) has been deposited in the Herbarium of Department of Biology, Faculty of Science, Prince of Songkla University, Thailand.

2.2.3 Extraction and isolation

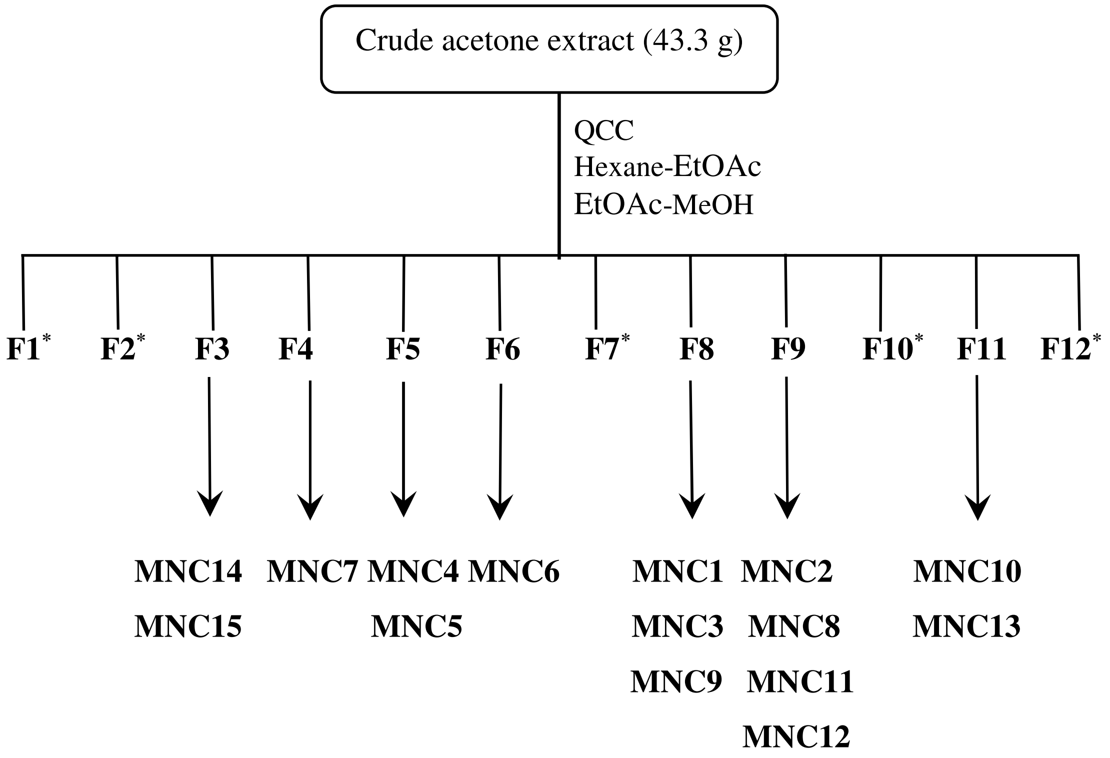
Air-dried stems of *C. medica* (3.0 kg) were immersed in acetone at room temperature for 5 days. After evaporation, a dark green gum of acetone extract (43.3 g) was obtained. The process of extraction was shown in **Scheme 3**.



Scheme 3 Isolation of crude extract from the stems of *C. medica*

2.2.4 Isolation and chemical investigation of the crude acetone extract from the stems of *C. medica*

The acetone extract (43.3 g) was subjected to quick column chromatography using silica gel as stationary phase and eluted with a gradient of hexane, hexane-EtOAc, EtOAc-MeOH and finally with pure MeOH. On the basis of their TLC characteristics, the fractions which contained the same major components were combined to give fractions F1-F12 which were further purified to afford fifteen pure compounds as shown in **Scheme 4**.



* No further investigation

Scheme 4 Isolation of compounds **MNC1- MNC15** from acetone extract

Fraction F3 (3.0 g) was filtered and washed with hexane to yield a mixture of **MNC14:** *β*-sitosterol and **MNC15:** stigmasterol (178.0 mg) as a white solid and the mother liquor as yellow viscous oil after evaporation of the solvent.

Fraction F4 (1.5 g) was subjected to QCC with a gradient of EtOAc–hexane and followed by CC with acetone–hexane (1:5, v/v) to give **MNC7:** xanthyletin (13 mg).

Fraction F5 (2.5 g) was purified by QCC with a gradient of acetone–hexane to afford eight fractions (F5A-F5H).

Subfraction F5C (154.0 mg) was separated by CC eluting with acetone-hexane (1:6, v/v) to give a white solid of **MNC4:** valencic acid (18 mg).

Subfraction F5G (30.2 mg) was further purified on preparative TLC and eluted with MeOH-CH_2Cl_2 (0.2:9.8) to give a colorless oil of **MNC5**: vanillin (6.6 mg).

Fraction F6 (2.3 g) was purified by QCC with a gradient of EtOAc–hexane to afford 6 fractions (F6A-F6F).

Subfraction F6E (56.7 mg) was further purified on preparative TLC and eluted with MeOH-CH_2Cl_2 (0.2:9.8) to give a colorless oil of **MNC6**: 4-hydroxybenzaldehyde (2.6 mg).

Fraction F8 (4.5 g) was purified by QCC with a gradient of acetone-hexane to afford fifteen fractions (F8A-F8O).

Subfraction F8I (250.0 mg) was purified by CC eluting with a gradient of acetone-CH_2Cl_2 and followed by CC with EtOAc-CH_2Cl_2 (1:10, v/v) to give **MNC1:** citrusinine-I (3.5 mg).

Subfraction F8K (615.0 mg) was purified by CC eluting with EtOAc-hexane (2:10, v/v) to give **MNC3:** citracridone-I (9.4 mg).

Subfraction F8L (60.2 mg) was purified by CC eluting with acetone-hexane (1:10, v/v) to give **MNC9:** citrusinol (2.5 mg).

Fraction F9 (6.1 g) was purified by QCC with a gradient of acetone-hexane to afford twelve fractions (9A-9L).

Subfraction F9F (1.5 g) was filtered and washed with hexane to give a white solid of **MNC12**: nomilin (80.0 mg) and the mother liquor as yellow viscous oil after evaporation of the solvent.

Subfraction F9H (115.0 mg) was purified by QCC with a gradient of acetone-CH_2Cl_2 and followed by CC eluting with EtOAc-CH_2Cl_2 (1:10, v/v) to give **MNC2:** *N*-methylataphyllinine (7.0 mg) and **MNC8:** erythrisenegalone (7.0 mg).

Subfraction F9L (30.2 mg) was purified by CC eluting with acetone–hexane (1:5, v/v) to give **MNC11:** dihydrodehydrodiconifenyl alcohol (6.3 mg).

Fraction F11 (4.5 g) was purified by QCC with a gradient of acetone–hexane to afford eight fractions (F11A-F11H).

Subfraction F11C (354.0 mg) was separated by CC eluting eluting with acetone-hexane (1:6, v/v) to give **MNC13:** limonin (45.0 mg).

Subfraction F11D (147.0 mg) was purified by CC with acetone-hexane (1:5, v/v) to give **MNC10:** (+)-syringaresinol (10.5 mg).

Compound MNC1: citrusinine-I, yellow solid, m.p. 206-207 $^{\circ}$C; UV λ_{max} (MeOH) (log ε): 203 (3.80), 221 (3.74), 263 (4.19), 319 (3.71) and 416 (3.27) nm; IR (Neat) ν (cm^{-1}); 3386 (O-H stretching), 1633 (C=O stretching) and 1604 (aromatic) cm^{-1}; For ^{1}H NMR (acetone-d_6, 300 MHz) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 17**.

Compound MNC2: *N*-methylataphyllinine, orange crystals, m.p. 195-196 °C; UV λ_{max} (MeOH) (log ε): 205 (3.97), 290 (4.16), 345 (3.60) and 422 (3.22) nm; IR (Neat) ν (cm^{-1}): 3374 (O-H stretching), 1635 (C=O stretching) and 1604 (aromatic) cm^{-1}; For ^{1}H NMR (acetone-d_6, 300 MHz) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 19**.

Compound MNC3: citracridone-I, orange solid, m.p. 274-276 °C; UV λ_{max} (MeOH) (log ε): 205 (3.54), 269 (3.92), 338 (3.19) and 392 (2.40) nm; IR (neat) ν_{max}: 3405 (O-H stretching), 1626 (C=O stretching) and 1604 (aromatic); For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 21**.

Compound MNC4: valencic acid, white solid, m.p. 189-190℃; UV λ_{max} (MeOH) (log ε): 202 (4.51) and 249 (4.43) nm; IR (Neat) ν (cm^{-1}): 3390 (O-H stretching), 1672 (C=O stretching) and 1250 (C-O stretching); For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 23**.

Compound MNC5: vanillin, colorless oil, UV λ_{max} (MeOH) (log ε): 233 (3.37), 291 (2.61) and 306 (2.82) nm; IR (Neat) ν (cm^{-1}): 3384 (O-H stretching), 1648 (C=O stretching) and 1621 (aromatic); For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 25**.

Compound MNC6: 4-hydroxybenzaldehyde, colorless oil, UV λ_{max} (MeOH) (log ε): 237 (3.39), 293 (2.64) and 306 (2.84) nm; IR (Neat) ν (cm^{-1}): 3367 (O-H stretching), 1684 (C=O stretching) and 1602 (aromatic); For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 26**.

Compound MNC7: xanthyletin, colorless crystals, m.p. 130-131°C; UV λ_{max} (MeOH) (log ε):): 223 (3.90), 265 (3.84), 304 (3.24), 348 (3.63) nm; IR (Neat) ν (cm^{-1}): 1707 (C=O stretching) and 1282 (C-O stretching); For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 27**.

Compound MNC8: erythrisenegalone, yellow oil, $[\alpha]^{27}_{D}$ -11.3° (*c* 0.2, MeOH), UV λ_{max} (MeOH) (log ε): 221 (4.23), 226 (4.19), 280 (4.45) and 316 (3.46) nm; IR (Neat) ν (cm^{-1}): 3418 (O-H stretching) and 1625 (C=O stretching); For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 29**.

Compound MNC9: citrusinol, yellow needles, m.p. 253-254℃; UV λ_{max} (MeOH) (log *ε*): 239 (3.84), 281 (3.82), 332 (3.49), 382 (3.49) nm; IR (Neat) ν (cm^{-1}): 3360 (O-H stretching), 1620 (C=O stretching); For ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) spectral data, see **Table 31**.

Compound MNC10: (+)-syringaresinol, colorless solid, m.p. 179-181℃, $[\alpha]^{27}_{D}$ +55.7°(*c* 0.17, $CHCl_3$); UV λ_{max} (MeOH) (log *ε*): 205 (4.04), 237 (3.43), 281 (3.30) nm; IR (Neat) ν (cm^{-1}): 3435 (O-H stretching) and 1611 (aromatic); For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 33**.

Compound MNC11: dihydrodehydrodiconifenyl alcohol, colorless oil, $[\alpha]^{27}_{D}$ -11.3° (*c* 0.2, MeOH); UV λ_{max} (MeOH) (log *ε*): 208 (4.83), 282 (3.80) nm; IR (Neat) ν (cm^{-1}): 3392 (O-H stretching), and 1628 (aromatic); For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 35**.

Compound MNC12: nomilin, white crystals, m.p. 189-190℃; $[\alpha]^{27}_{D}$ -79.3° (*c* 0.10, $CHCl_3$); UV λ_{max} (MeOH) (log *ε*): 209 (2.39) nm; IR (KBr) ν_{max}: 1730 (C=O stretching) and 875 (*β*-substituted furan); For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 37**.

Compound MNC13: limonin, white crystals, m.p. 285-286℃; $[\alpha]^{27}_{D}$ -139.5° (*c* 0.10, Me_2CO); UV λ_{max} (MeOH) (log *ε*): 206 (3.39) nm; IR (KBr) ν_{max}: 1730, 1709 (C=O stretching) and 883 (*β*-substituted furan); For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 39**.

Compound MNC14: *β*-sitosterol and **MNC15:** stigmasterol: a mixture, white solid; For ^{1}H NMR ($CDCl_3$, 300 MHz) spectral data, see **Fig. 103**.

CHAPTER 2.3

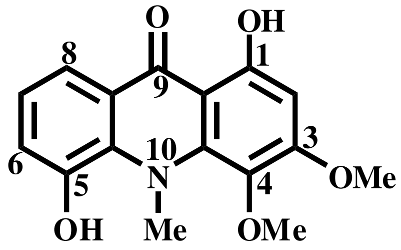
RESULTS AND DISCUSSION

2.3.1 Structure elucidation of compounds from the stems of *Citrus medica* Linn.

The crude acetone extract from the stems of *C. medica* was subjected to quick column chromatography and repeated column chromatography over silica gel to furnish fifteen known compounds; three acridone alkaloids: citrusinine-I (**MNC1**), *N*-methylataphyllinine **(MNC2)** and citracridone I (**MNC3**), three benzene derivatives: valencic acid **(MNC4)**, vanillin **(MNC5)** and 4-hydroxybenzaldehyde, **(MNC6)**, a coumarin: xanthyletin **(MNC7)**, two flavonoids: erythrisenegalone **(MNC8)** and citrusinol **(MNC9)**, two lignans: (+)-syringaresinol **(MNC10)** and dihydrodehydrodiconifenyl alcohol **(MNC11)**, two limonoids: nomilin **(MNC12)** and limonin **(MNC13)** and two steroids: a mixture of *β*-sitosterol (**MNC14**) and stigmasterol (**MNC15**).

Their structures were elucidated mainly by 1D and 2D NMR spectroscopic data: ^{1}H, ^{13}C NMR, DEPT 135°, DEPT 90°, HMQC, HMBC, COSY and NOESY. The physical data of the isolated compounds were also compared with the reported values.

2.3.1.1 Compound MNC1



Compound **MNC1** was obtained as a yellow solid, m.p. 206-207 °C. The UV-Vis spectrum exhibited the absorption bands at λ_{max} 203,221, 263, 319, and 416 nm characteristic of a 9-acridone chromophore. An infrared (IR) absorption indicated the presence of hydroxyl (3386 cm^{-1}) and chelated carbonyl (1633 cm^{-1}) groups.

The ^1H-NMR spectrum showed a singlet signal at δ 14.22 indicating the presence of a chelated phenolic hydroxyl group. Three sharp singlets (each 3H) at δ 3.76, 3.83, and 3.98 were due to a methoxyl, *N*-methyl and a methoxyl groups, respectively. Signals of three adjacent aromatic protons at δ 7.78 (1H, *d*, J = 7.8 Hz), 7.30 (1H, *br d*, J = 7.8 Hz) and 7.16 (1H, *t*, J = 7.8 Hz) were assigned to H-8, H-6, and H-7, respectively. The deshielding of H-8 is reasonable because it lies in the *peri*-position with respect to the 9-carbonyl moiety. A sharp one-proton singlet signal at δ 6.41 could be attributed to an aromatic proton at C-2 which was confirmed by HMBC correlation of H-2 (δ 6.41) with the carbons at δ 105.9 (C-9a), 160.0 (C-3) and 130.3 (C-4). Two singlet signals at δ 3.98 and δ 3.76 (each 3H) were assigned for methoxyl groups at C-3 and C-4 respectively due to HMBC correlations (**Figure 10**) of 3-OMe with the carbon at δ 160.0 (C-3) and 4-OMe with the carbon at δ 130.3 (C-4). On the basis of the above analysis and comparison with the literature, the structure of **MNC1** was identified as citrusinine-I (Wu and Furukawa, 1983).

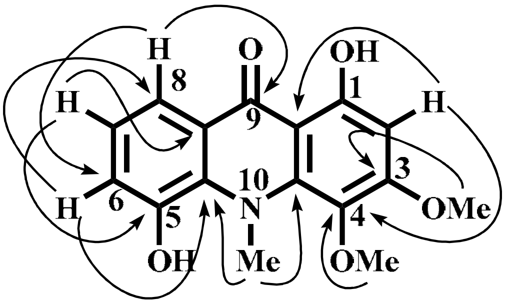


Figure 10 Selected HMBC correlations of **MNC1**

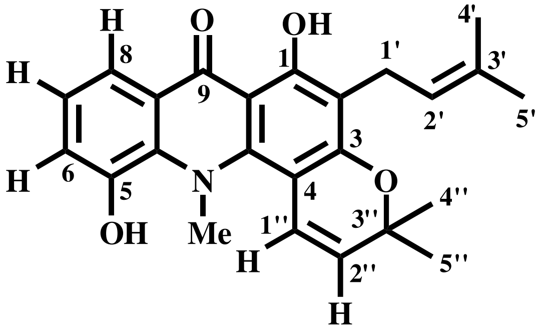
Table 17 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **MNC1** (acetone-d_6)

Position	**δ_C**		**δ_H (mult, J, Hz)**	**HMBC**
1	160.3	C	-	-
1-OH	-	-	14.22 (*s*)	-
2	93.7	CH	6.41 (*s*)	C-9a, C-4, C-3
3	160.0	C	-	-
3-OMe	55.7	CH_3	3.98 (*s*)	C-3
4	130.3	C	-	-
4-OMe	59.5	CH_3	3.76 (*s*)	C-4
5	148.0	C	-	-
5-OH	-	-	9.42 (*br s*)	-
6	119.9	CH	7.30 (*br d*, J = 7.8 Hz)	C-5a, C-8, C-5
7	122.5	CH	7.16 (*t*, J = 7.8 Hz)	C-5, C-8a
8	116.3	CH	7.78 (*d*, J = 7.8 Hz)	C-5a, C-9, C-6
9	182.2	C	-	-
4a	142.2	C	-	-
5a	137.4	C	-	-
8a	124.5	C	-	-
9a	105.9	C	-	-
10-NMe	45.9	CH_3	3.83 (*s*)	C-4a, C-5a

Table 18 Comparison of ^{1}H NMR and ^{13}C NMR spectral data between compounds **MNC1** (acetone-d_6) and citrusinine-I (**R**, DMSO-d_6+CDCl$_3$)

Position	**MNC1** δ_H **(mult, *J*, Hz)**	**R** δ_H **(mult, *J*, Hz)**	δ_C**, MNC1**	δ_C**, R**
1-OH	14.22 (*s*)	14.05 (*s*)	160.3	159.9
2	6.41 (*s*)	6.30 (*s*)	93.7	93.4
3	-	-	160.0	159.4
3-OMe	3.98 (*s*)	3.92 (*s*)	55.7	55.9
4	-	-	130.3	129.7
4-OMe	3.76 (*s*)	3.77 (*s*)	59.5	59.9
5-OH	9.42 (*br s*)	9.16 (*br s*)	148.0	148.1
6	7.30 (*br d*, *J* = 7.8 Hz)	7.19 (*dd*, *J* = 8.0, 2.0 Hz)	119.9	119.9
7	7.16 (*t*, *J* = 7.8 Hz)	7.04 (*t*, *J* = 8.0 Hz)	122.5	122.4
8	7.78 (*d*, *J* = 7.8 Hz)	7.68 (*dd*, *J* = 8.0, 2.0 Hz)	116.3	115.7
9	-	-	182.2	181.9
4a	-	-	142.2	141.8
5a	-	-	137.4	137.1
8a	-	-	124.5	124.1
9a	-	-	105.9	105.8
10-NMe	3.83 (*s*)	3.71 (*s*)	45.9	45.9

2.3.1.2 Compound MNC2



Compound **MNC2** was isolated as orange crystals, m.p. 195-196 °C. The UV-Vis spectrum exhibited the absorption bands at $\lambda_{\max}$ 205, 290, 345 and 422 nm characteristic of a 9-acridone chromophore which was supported by the presence of IR absorption of hydroxyl (3374 cm^{-1}) and chelated carbonyl (1635 cm^{-1}) groups.

The ^1H NMR spectral data (**Table 19**) of **MNC2** showed a signal of a chelated hydroxyl group which appeared at δ 14.43 (*s*, 1-OH) and three adjacent aromatic proton signals with ABX pattern were shown at δ 7.72 (1H, *dd*, J = 8.1, 1.5 Hz), 7.32 (1H, *dd*, J = 8.1, 1.5 Hz) and 7.16 (1H, *t*, J = 8.1 Hz) attributable to H-8, H-6, and H-7, respectively. A prenyl group was shown as signals at δ 3.27 (2H, *br d*, J = 7.0 Hz, H-1′), 5.20 (1H, *br t, J* = 7.0 Hz, H-2′), 1.75, 1.60 (each, *s*, Me-4′, Me-5′), whose HMBC correlation of H-1′ at δ 3.27 with the carbons at δ 160.2 (C-1) and 159.3 (C-3) indicated a connection of a prenyl group at C-2. Signals of a 2,2-dimethyl pyran ring were shown at δ 6.90 (1H, *d, J* = 9.5 Hz, H-1″), 5.43 (1H, *d, J* = 9.5 Hz, H-2″) and 1.43 (6H, *s*, Me-4″, Me-5″). HMBC correlation of H-1″ (δ 6.90) with the carbons at δ 159.3 (C-3) and 146.0 (C-4a), of H-2″ at δ 5.43 with the carbon at δ 102.6 (C-4) suggested that a 2,2-dimethylpyran ring was fused to the acridone nucleus with an angular orientation. The angular orientation was supported by NOESY experiment, from which cross peak was observed between H-1″/N-Me. Therefore, compound **MNC2** was assigned as *N*-methylataphyllinine (Auzi *et al.*, 1996).

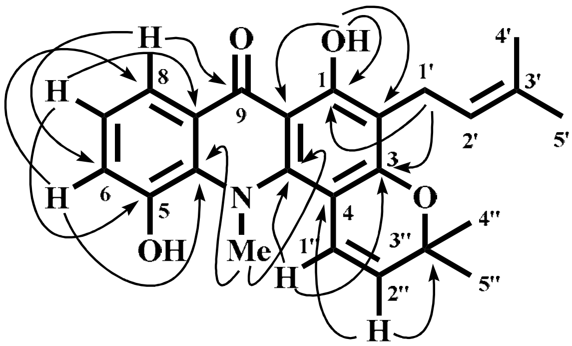


Figure 11 Selected HMBC correlations of **MNC2**

Table 19 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **MNC2** (acetone-d_6)

Position	δ_C	**Type of carbon**	δ_H **(mult, *J*, Hz)**	**HMBC**
1	160.2	C	-	-
1-OH	-	-	14.43 (*s*)	C-9a, C-1, C-2
2	106.4	C	-	-
3	159.3	C	-	-
4	102.6	C	-	-
5	147.8	C	-	-
5-OH		-	9.45 (*br s*)	-
6	119.5	CH	7.32 (*dd*, *J* = 8.1, 1.5 Hz)	C-8, C-5a
7	123.0	CH	7.16 (*t*, *J* = 8.1 Hz)	C-5, C-8a
8	116.5	CH	7.72 (*dd*, *J* = 8.1, 1.5 Hz)	C-5a, C-9
9	180.8	C	-	-
4a	146.0	C	-	-
5a	137.9	C	-	-
8a	124.9	C	-	-
9a	110.1	C	-	-
1′	21.2	CH_2	3.27 (*br d*, *J* = 7.0 Hz)	C-1, C-3, C-2′, C-3′
2′	122.5	CH	5.20 (*br t*, *J* = 7.0 Hz)	-
3′	131.0	C	-	-

Table 19 (Continued)

Position	**δ_C**	**Type of carbon**	**δ_H (mult, *J*, Hz)**	**HMBC**
4′	26.0	CH_3	1.75 (*s*)	C-2′, C-3′, C-5′
5′	18.0	CH_3	1.60 (*s*)	C-2′, C-3′, C-4′
1″	121.0	CH	6.90 (*d*, *J* = 9.5 Hz)	C-3″, C-3, C-4a
2″	123.5	CH	5.43 (*d*, *J* = 9.5 Hz)	C-3″, C-4, C-4″/5″
3″	76.2	C	-	-
4″/5″	27.3	CH_3×2	1.43 (*s*)	C-3″, C-2″
10-NMe	48.3	CH_3	3.66 (*s*)	C-4a, C-5a

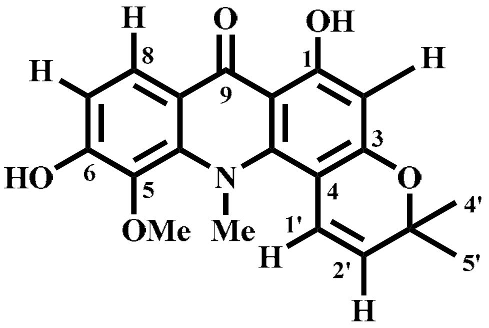
Table 20 Comparison of ^{1}H NMR spectral data between compounds **MNC2** (acetone-d_6) and *N*-methylataphyllinine (**R**, $CDCl_3$)

Position	**MNC2** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**
1-OH	14.43 (*s*)	14.32 (*s*)
2	-	-
3	-	-
4	-	-
5-OH	9.45 (*br s*)	-
6	7.32 (*dd*, *J* = 8.1, 1.5 Hz)	7.32 (*dd*, *J* = 7.0, 3.0 Hz)
7	7.16 (*t*, *J* = 8.1 Hz)	7.06 (*t*, *J* = 7.0 Hz)
8	7.72 (*dd*, *J* = 8.1, 1.5 Hz)	7.80 (*dd*, *J*= 7.0, 3.0 Hz)
9	-	-
4a	-	-
5a	-	-
8a	-	-
9a	-	-
1′	3.27 (*br d*, *J* = 7.0 Hz)	3.37 (*d*, *J* = 7.0 Hz)

Table 20 (Continued)

Position	**MNC2** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**
2′	5.20 (*br t*, *J* = 7.0 Hz)	5.30 (*m*)
3′	-	-
4′	1.75 (*s*)	1.82 (*s*)
5′	1.60 (*s*)	1.68 (*s*)
1″	6.90 (*d*, *J* = 9.5 Hz)	6.63 (*d*, *J* = 10.0 Hz)
2″	5.43 (*d*, *J* = 9.5 Hz)	5.51 (*d*, *J* = 10.0 Hz)
3″	-	-
4″/5″	1.43 (*s*)	1.52 (*s*)
10-NMe	3.66 (*s*)	3.78 (*s*)

2.3.1.3 Compound MNC3



Compound **MNC3** was isolated as an orange solid, m.p. 274-276 °C. The UV-Vis spectrum exhibited the absorption bands at λ_{max} 205, 269, 338 and 392 nm characteristic of a 9-acridone chromophore which was supported by the presence of IR absorption of hydroxyl (3405 cm^{-1}) and chelated carbonyl (1626 cm^{-1}) groups.

The ^{1}H NMR spectral data (**Table 21**) of **MNC3** indicated the presence of a chelated phenolic hydroxyl group at C-1 by the singlet signal at δ 14.23. The spectral data of **MNC3** were comparable to those of **MNC2**, except that a singlet signal of an aromatic proton at δ_H 6.26 in **MNC3** replaced signals of a prenyl group in **MNC2**. Its location was placed at C-2 due to HMBC correlations to δ 164.7 (C-1), 161.1 (C-3), 102.1 (C-4) and 106.8 (C-9a). In addition two methyl singlet signals at δ_H 3.70: δ_C 47.9 and δ_H 3.90: δ_C 60.0 were assigned for *N*-methyl and methoxyl groups, respectively. In the aromatic region, signals of AB pattern at δ 6.99 (1H, *d*, J = 8.7 Hz), and 8.06 (1H, *d*, J = 8.7 Hz) were attributed to H-7 and H-8, respectively. On the basis of the above results and comparison with the literature. The structure of **MNC3** was assigned as citracridone-I (Wu *et al.*, 1983).

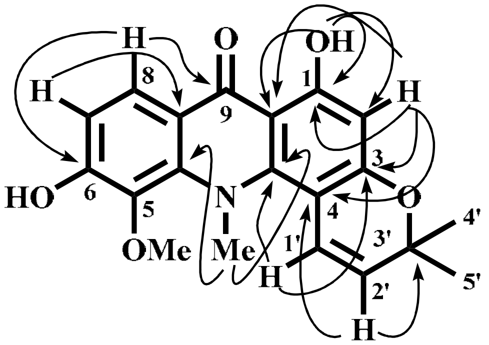


Figure 12 Selected HMBC correlations of **MNC3**

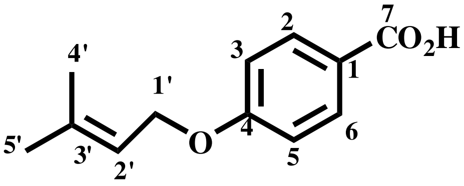
Table 21 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **MNC3** ($CDCl_3$)

Position	δ_C		δ_H (mult, *J*, Hz)	HMBC
1	164.7	C	-	-
1-OH	-	-	14.23 (*s*)	C-1, C-2, C-9a
2	98.7	C	6.26 (*s*)	C-1, C-3, C-4, C-9a
3	161.1	C	-	-
4	102.1	C	-	-
5	135.8	C	-	-
5-OMe	60.0	CH_3	3.90 (*s*)	-
6	154.4	C	-	-
7	112.0	CH	6.99 (*d*, *J* = 8.7 Hz)	C-5, C-8a
8	123.4	CH	8.06 (*d*, *J* = 8.7 Hz)	C-5a, C-9
9	181.5	C	-	-
4a	147.2	C	-	-
5a	141.5	C	-	-
8a	118.5	C	-	-
9a	106.8	C	-	-
1′	120.4	CH	6.54 (*d*, *J* = 9.9 Hz)	C-3, C-4a, C-3′
2′	124.7	CH	5.58 (*d*, *J* = 9.9 Hz)	C-4, C-3′, C-4′/5′
3′	77.2	C	-	-
4′/5′	27.2	$CH_3 \times 2$	1.52 (*s*)	C-3′, C-2′
10-NMe	47.9	CH_3	3.70 (*s*)	C-4a, C-5a

Table 22 Comparison of ^{1}H NMR spectral data between compounds **MNC3** ($CDCl_3$) and citracridone-I (**R**, $CDCl_3$+DMSO-d_6)

Position	**MNC3** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**
1	-	-
1-OH	14.23 (*s*)	14.52 (*s*)
2	6.26 (*s*)	6.23 (*d*, *J* = 1.0 Hz)
3	-	-
4	-	-
5	-	-
5-OMe	3.90 (*s*)	3.91 (*s*)
6-OH	-	9.33 (*s*)
7	6.99 (*d*, *J* = 8.7 Hz)	7.00 (*d*, *J* = 9.0 Hz)
8	8.06 (*d*, *J* = 8.7 Hz)	8.01 (*d*, *J* = 9.0 Hz)
9	-	-
4a	-	-
5a	-	-
8a	-	-
9a	-	-
1'	6.54 (*d*, *J* = 9.9 Hz)	6.63 (*dd*, *J* = 10.0, 1.0 Hz)
2'	5.58 (*d*, *J* = 9.9 Hz)	5.61 (*d*, *J* = 10.0 Hz)
3'	-	-
4'/5'	1.52 (*s*)	1.53 (*s*)
10-NMe	3.70 (*s*)	3.75 (*s*)

2.3.1.4 Compound MNC4



MNC4 was isolated as a white solid, m.p. 189-190℃. The UV spectrum exhibited the absorption bands at λ_{max} 202 and 249 nm. The IR spectrum showed absorption bands for hydroxyl at 3390 cm^{-1}, carbonyl group at 1672 cm^{-1}, and ether at 1250 cm^{-1}.

The ^{1}H NMR spectral data **(Table 23)** of **MNC4** showed the characteristic signals of a *para*-disubstituted benzene at δ_H 8.04 (2H, *d*, J = 8.9 Hz) and δ_H 6.94 (2H, *d*, J = 8.9 Hz) of H-2/H-6 and H-3/H-5 respectively. The substituent at C-4 was identified as an oxyprenyl group according to these signals: two singlets at δ_H 1.75 and 1.80 (3H each, *s*, H-4' and H-5', respectively) for two methyl protons, one doublet at δ_H 4.57 (2H, *d*, J = 6.7 Hz, H_2-1') for methylene protons and one triplet at δ_H 5.48 (1H, *t*, J = 6.7 Hz, H-2') for a methine proton.

The ^{13}C NMR spectral data **(Table 23)** exhibited 10 carbon signals, of which four [δ_C 121.6 (C-1), 132.2 (C-2/C-6), 114.3 (C-3/C-5), and 163.3 (C-4)] were attributed to aromatic ring, whereas five [δ_C 18.2 (C-5'), 25.8 (C-4'), 65.0 (C-1'), 118.9 (C-2') and 138.8 (C-3')] were characteristic of the carbons of an oxyprenyl side chain. A signal of a carboxyl carbon was shown at δ_C 171.6 (C-7). The location of an oxyprenyl side chain at C-4 was confirmed by HMBC correlations of H_2-1' (δ_H 4.57) with δ_C 163.3 (C-4), 118.9 (C-2') and 138.8 (C-3'), whereas that of a carboxyl group at C-1 was confirmed by HMBC correlations of H-6 (δ_H 8.04) with δ_C 171.6 (C-7), 121.6 (C-1), 114.3 (C-5) and 163.3 (C-4). Accordingly, the structure of **MNC4** was assigned as valencic acid (Ito *et al.*, 1988).

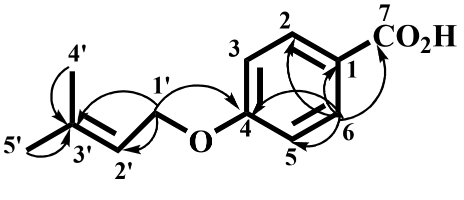


Figure 13 Selected HMBC correlations of **MNC4**

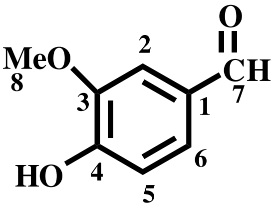
Table 23 ^{1}H, ^{13}C NMR and HMBC spectral data of **MNC4** ($CDCl_3$)

Position	δ_C		δ_H (mult, J, Hz)	HMBC
1	121.6	C	-	-
2/6	132.2	CH	8.04 (*d*, J = 8.9 Hz)	C-1, C-4, C-5, C-7
3/5	114.3	CH	6.94 (*d*, J = 8.9 Hz)	C-1, C-2
4	163.3	C	-	-
7	171.6	C	-	-
1'	65.0	CH_2	4.57 (*d*, J = 6.7 Hz)	C-4, C-2', C-3'
2'	118.9	CH	5.48 (*t*, J = 6.7 Hz)	C-1', C-3', C-4', C-5'
3'	138.8	C	-	-
4'	25.8	CH_3	1.80 (*s*)	C-2', C-3', C-5'
5'	18.2	CH_3	1.75 (*s*)	C-2', C-3', C-4'

Table 24 Comparison of ^{1}H NMR spectral data between compounds **MNC4** and valencic acid (**R**, $CDCl_3$)

	MNC4	R
Position	δ_H (mult, J, Hz)	δ_H (mult, J, Hz)
1	-	-
2/6	8.04 (*d*, J = 8.9 Hz)	8.05 (*d*, J = 8.7 Hz)
3/5	6.94 (*d*, J = 8.9 Hz)	6.95 (*d*, J = 8.7 Hz)
4	-	-
7	-	-
1'	4.57 (*d*, J = 6.7 Hz)	4.59 (*d*, J = 7.1 Hz)
2'	5.48 (*t*, J = 6.7 Hz)	5.49 (*t*, J = 7.1 Hz)
3'	-	-
4'	1.80 (*s*)	1.81 (*s*)
5'	1.75 (*s*)	1.76 (*s*)

2.3.1.5 Compound MNC5



Compound **MNC5** was obtained as colorless oil. The UV spectrum showed absorption bands at λ_{max} 233, 291 and 306 nm, indicating the presence of a benzene chromophore. The IR spectrum exhibited absorption bands at 3384 and 1648 cm^{-1} for hydroxyl and carbonyl groups, respectively.

The ^{1}H NMR spectral data (**Table 25**), displayed characteristic signals of a 1,3,4-trisubstituted benzene [δ_H 7.42 (*d*, J = 1.8 Hz, H-2), 7.04 (*d*, J = 8.4 Hz, H-5) and 7.43 (*dd*, J = 8.4, 1.8 Hz, H-6)] and appearance of a singlet of an aldehydic group at δ_H 9.83 (1H, *s*, CHO). A singlet signal of a methoxyl group was evident at δ 3.98 (3H, *s*, 3-OMe). The location of a methoxyl group at C-3 was confirmed by HMBC correlation of OMe-3 (δ_H 3.98) with δ_C 148.5 (C-3). The presence of a carbonyl carbon at δ_C 190.8 in the ^{13}C NMR spectrum supported the IR data, whose position at C-1 was supported by HMBC correlations of H-2 (δ_H 7.43) with δ_C 148.5 (C-3), 151.7 (C-4), 127.5 (C-6) and 190.8 (C-7). The complete HMBC data were summarized in **Table 25**. Accordingly, the structure of **MNC5** was assigned as vanillin.

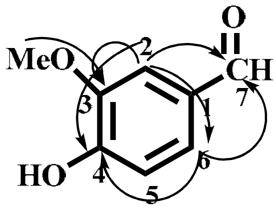
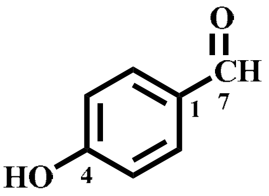


Figure 14 Selected HMBC correlations of **MNC5**

Table 25 ^{1}H, ^{13}C NMR and HMBC spectral data of **MNC5** ($CDCl_3$)

Position	δ_C		δ_H **(mult, *J*, Hz)**	**HMBC**
1	129.0	C	-	-
2	108.8	CH	7.42 (*d*, *J* = 1.8 Hz)	C-3, C-4, C-6, C-7
3	148.5	C	-	-
4	151.7	C	-	-
4-OH	-	-	6.19 (*s*)	-
5	114.4	CH	7.04 (*d*, *J* = 8.4 Hz)	C-3, C-4, C-1
6	127.5	CH	7.43 (*dd*, *J* = 8.4, 1.8 Hz)	C-4, C-2, C-7
7	190.8	CH	9.83 (*s*)	-
8	56.2	OCH_3	3.98 (*s*)	C-3

2.3.1.6 Compound MNC6



Compound **MNC6** was obtained as colorless oil. The UV spectrum showed absorption bands at λ_{max} 237, 293 and 306 nm, indicating the presence of a benzene chromophore. The IR spectrum exhibited absorption bands at 3367 and 1684 cm^{-1} for hydroxyl and carbonyl groups, respectively.

The ^{1}H NMR spectrum displayed characteristic signals of a 1,4-disubstituted benzene at δ 7.81 (2H, *d*, J = 8.7 Hz) and 6.86 (2H, *d*, J = 8.7 Hz) and appearance of a singlet of an aldehydic group at δ 9.88 (1H, *s*, CHO). The presence of a carbonyl carbon at δ_C 190.6 in the ^{13}C NMR spectrum was in agreement with the IR data. The complete HMBC data were summarized in **Table 26**. Accordingly, the structure of **MNC6** was assigned as 4-hydroxybenzaldehyde.

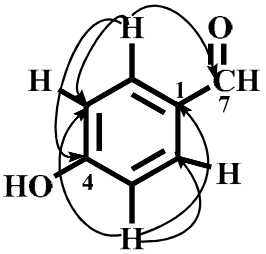
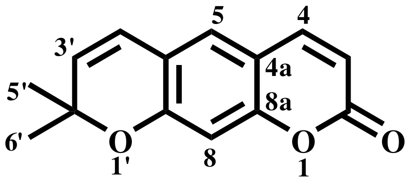


Figure 15 Selected HMBC correlations of **MNC6**

Table 26 ^{1}H, ^{13}C NMR and HMBC spectral data of **MNC6** ($CDCl_3$)

Position	δ_C		δ_H **(mult, *J*, Hz)**	**HMBC**
1	130.0	C	-	-
2/6	132.5	CH	7.81 (*d*, *J* = 8.7 Hz)	C-3, C-4, C-7
3/5	116.1	CH	6.86 (*d*, *J* = 8.7 Hz)	C-1, C-2
4	161.0	C	-	-
4-OH	-	-	-	-
7	190.6	C	9.88 (*s*)	-

2.3.1.7 Compound MNC7



Compound MNC7 was isolated as colorless crystals, m.p. 130-131°C. The UV spectrum showed absorption bands at λ_{max} 223, 265, 304 and 348 nm typical of a coumarin nucleus. The IR absorption indicated the presence of a conjugated carbonyl (1622 cm^{-1}) group.

In the ^1H NMR spectral data (**Table 27**), two pairs of AB-type doublets at δ 6.17 and 7.54 (each 1H, *d*, $J = 9.6$ Hz) and at δ 6.30 and 5.65 (each 1H, *d*, $J = 9.9$ Hz) were assigned to α- and β- protons of an α,β-unsaturated carbonyl system and two olefinic protons of the dimethylchromene ring, respectively. In addition two aromatic proton singlets at δ 7.01 and 6.66 were evident which were assigned as *para*-protons (H-5 and H-8), respectively of a 1,2,4,5-tetrasubstituted aromatic ring. A singlet signal of two methyl groups was displayed at δ_H 1.43 (6H) which was shown attached to an oxygenated carbon (δ_C 77.7).

The ^{13}C NMR spectral data **(Table 27)** exhibited thirteen resonances for fourteen carbons: six quaternary (δ 161.1, 156.8, 155.4, 118.5, 112.7 and 77.7), six methine (δ 143.4, 131.2, 124.8, 120.8, 112.9 and 104.3) and one singlet signal of two methyl carbons (δ 28.3). In the HMBC spectrum, one of the olefinic protons of the dimethylchromene ring at δ_H 6.30 (H-4') showed correlations with C-5 (δ_C 124.8), C-6 (δ_C 118.5) and C-7 (δ_C 156.8), whereas H-3' at δ_H 5.65 correlated with C-6 (δ_C 118.5), C-2' (δ_C 77.7) and C-5'/C-6' (δ_C 28.3). These data together with the downfield chemical shift of C-7 at δ_C 156.8 suggested that the dimethylchromene ring was fused to a coumarin skeleton at C-6 and C-7 with an ether linkage at C-7. From the spectral data and comparison with xanthyletin (Wu *et al.*, 1983), compound **MNC7** was assigned as xanthyletin.

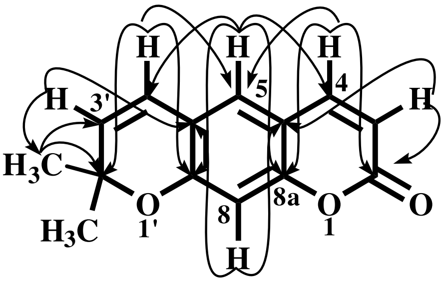


Figure 16 Selected HMBC correlations of **MNC7**

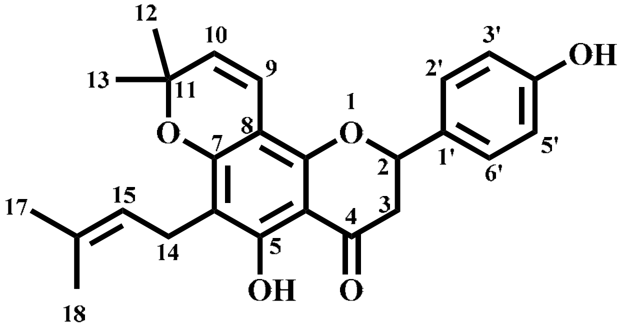
Table 27 ^{1}H, ^{13}C NMR and HMBC spectral data of **MNC7** ($CDCl_3$)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**
1	-	-	-	-
2	161.1	C	-	-
3	112.9	CH	6.17 (*d*, *J* = 9.6 Hz)	C-2, C-4a,
4	143.4	CH	7.54 (*d*, *J* = 9.6 Hz)	C-2, C-5, C-8a
4a	112.7	C	-	-
5	124.8	CH	7.01 (*s*)	C-4, C-7, C-8a, C-4'
6	118.5	C	-	-
7	156.8	C	-	-
8	104.3	CH	6.66 (*s*)	C-4a, C-6
8a	155.4	C	-	-
1'	-	-	-	-
2'	77.7	C	-	-
3'	131.2	CH	5.65 (*d*, *J* = 9.9 Hz)	C-6, C-2', C-5'/C-6'
4'	120.8	CH	6.30 (*d*, *J* = 9.9 Hz)	C-5, C-6, C-7, C-2'
5'/6'	28.3	CH_3	1.43 (*s*)	C-2', C-3'

Table 28 Comparison of ^{1}H NMR and ^{13}C NMR spectral data between compounds **MNC7** ($CDCl_3$) and xanthyletin (**R**, $CDCl_3$)

Position	**MNC7** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**	**δ_C, MNC7**	**δ_C, R**
1	-	-	-	-
2	-	-	161.1	161.1
3	6.17 (*d*, *J* = 9.6 Hz)	6.24 (*d*, *J* = 9.2 Hz)	112.9	112.9
4	7.54 (*d*, *J* = 9.6 Hz)	7.60 (*d*, *J* = 9.2 Hz)	143.4	143.4
4a	-	-	112.7	112.7
5	7.01 (*s*)	7.04 (*s*)	124.8	124.8
6	-	-	118.5	118.5
7	-	-	156.8	156.8
8	6.66 (*s*)	6.72 (*s*)	104.3	104.3
8a	-	-	155.4	155.4
1'	-	-	-	-
2'	-	-	77.7	77.7
3'	5.65 (*d*, *J* = 9.9 Hz)	5.71 (*d*, *J* = 9.6 Hz)	131.2	131.2
4'	6.30 (*d*, *J* = 9.9 Hz)	6.36 (*d*, *J* = 9.6 Hz)	120.8	120.8
5'/6'	1.43 (*s*)	1.43 (*s*)	28.3	28.3

2.3.1.8 Compound MNC8



Compound MNC8 was isolated as yellow oil, $[\alpha]^{27}_{D}$ -11.3° (*c* 0.2, MeOH). The UV spectrum showed absorption bands at λ_{max} 221, 226, 280 and 316 nm. The IR spectrum showed the stretching of hydroxyl (3418 cm^{-1}) and conjugated carbonyl group (1625 cm^{-1}).

The ^1H NMR spectrum **(Table 29)** displayed a singlet signal of a chelated proton 5-OH at δ 12.35 and two doublets of a *p*-disubstituted benzene at δ 7.33 and 6.88 (each 2H, *d*, *J* = 8.5 Hz) due to H-2'/H-6' and H-3'/H-5' respectively. The ^1H NMR spectrum displayed ABX system at δ5.33 (*dd*, *J* = 13.0, 3.0 Hz, H-2), 3.03 (*dd*, *J* = 17.0, 13.0 Hz, H-3_{ax}) and 2.78 (*dd*, *J* = 17.0, 3.0 Hz, H-3_{eq}). The prenyl unit was implied from distinctive signals of two equivalent methylene protons at δ 3.25 (*d*, *J* = 7.0 Hz, H-14), an olefinic proton at δ5.20 (*t*, *J* = 7.0 Hz, H-15) and two singlets of methyl protons at δ 1.67 (17-CH_3) and 1.78 (18-CH_3), whose location was assigned at C-6 by HMBC correlation of 5-OH and of H-15 to C-6. Moreover, a chromene ring was detected from the characteristic signals at δ5.46 (*d*, *J* = 10.0 Hz, H-10), 6.54 (*d*, *J* = 10.0 Hz H-9), 1.45 (12-CH_3) and 1.41 (13-CH_3). The HMBC correlations of H-9 to C-8a, C-7 and C-11 and of H-10 to C-8, confirmed the position of a 2,2-dimethylchromene ring at C-7 and C-8 position. The optical rotation of compound **MNC8** is levorotatory ($[\alpha]^{27}_{D}$ -11.3°, *c* 0.2, MeOH), similar to erythrisenegalone ($[\alpha]^{20}_{D}$ -9.3°, *c* 0.12, MeOH), (Khaomek *et al.*, 1985). From the spectral data and comparison with those of erythrisenegalone (Khaomek *et al.*, 1985), compound **MNC8** was assigned as erythrisenegalone.

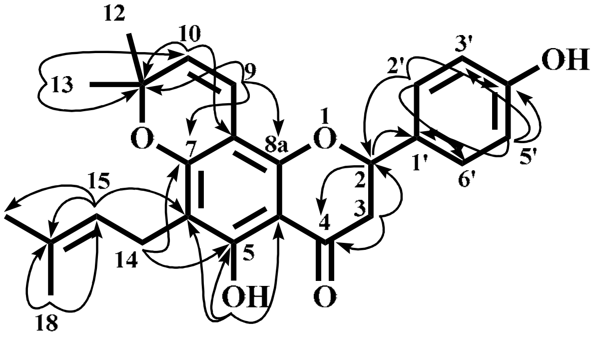


Figure 17 Selected HMBC correlations of **MNC8**

Table 29 ^{1}H, ^{13}C NMR and HMBC spectral data of **MNC8** (CDCl$_3$)

Position	δ_C		δ_H **(mult, *J*, Hz)**	**HMBC**
2_{ax}	78.9	CH	5.33 (*dd*, *J* = 13.0, 3.0 Hz)	C-4, C-2'/ C-6'
3_{ax}	43.3	CH_2	3.03 (*dd*, *J* = 17.0, 13.0 Hz)	C-2, C-1', C-5a
3_{eq}		CH_2	2.78 (*dd*, *J* = 17.0, 3.0 Hz)	C-2, C-1', C-5a
4	195.9	C	-	-
5	161.1	C	-	-
5-OH		-	12.35 (*s*)	C-5, C-6, C-5a
6	110.0	C	-	-
7	159.7	C	-	-
8	102.5	C	-	-
9	116.0	CH	6.54 (*d*, *J* = 10.0 Hz)	C-7, C-8a, C-11
10	126.1	CH	5.46 (*d*, *J* = 10.0 Hz)	C-8, C-9, C-12, C-13
11	78.0	C	-	-
12	28.5	CH_3	1.45 (*s*)	C-10, C-11, C-13
13	28.2	CH_3	1.41 (*s*)	C-10, C-11, C-12
14	20.9	CH_2	3.25 (*d*, *J* = 7.0 Hz)	C-5, C-7, C-16
15	122.4	CH	5.20 (*t*, *J* = 7.0 Hz)	C-6, C-17, C-18
16	131.5	C	-	-
17	25.7	CH_3	1.67 (*s*)	C-15, C-16, C-18
18	17.8	CH_3	1.78 (*s*)	C-15, C-16, C-17
5a	110.0	C	-	-

Table 29 (Continued)

Position	δ_C (C- type)		δ_H (mult, J, Hz)	HMBC
8a	160.0	C	-	-
1'	131.0	C	-	-
2'/6'	127.7	CH	7.33 (*d*, J = 8.5 Hz)	C-2, C-1', C-3', C-4'
3'/5'	115.6	CH	6.88 (*d*, J = 8.5 Hz)	C-1', C-2', C-4'
4'	155.9	C	-	
4'-OH	-	-	5.05 (*s*)	

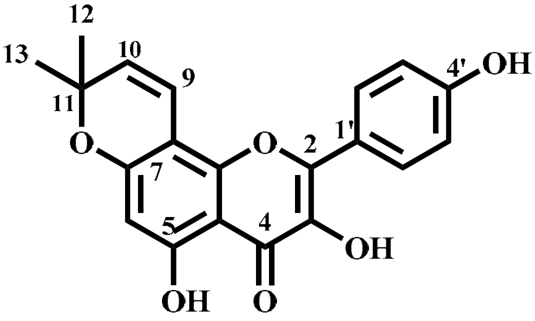
Table 30 Comparison of ^{1}H NMR spectral data between compounds **MNC8** ($CDCl_3$) and erythrisenegalone (**R**, $CDCl_3$)

Position	MNC8 δ_H (mult, J, Hz)	R δ_H (mult, J, Hz)	δ_C, MNC8	δ_C, R
2_{ax}	5.33 (*dd*, J = 13.0, 3.0 Hz)	5.32 (*dd*, J = 12.8, 3.0 Hz)	78.9	78.7
3_{ax}	3.03 (*dd*, J = 17.0, 13.0 Hz)	3.04 (*dd*, J = 17.0, 12.8 Hz)	43.3	43.2
3_{eq}	2.78 (*dd*, J = 17.0, 3.0 Hz)	2.78 (*dd*, J = 16.8, 3.2 Hz)	43.3	43.2
4	-	-	195.9	196.1
5	-	-	161.1	161.1
5-OH	12.35 (*s*)	-	-	-
6	-	-	110.0	109.7
7	-	-	159.7	159.8
8	-	-	102.5	101.7
9	6.54 (*d*, J = 10.0 Hz)	6.54 (*d*, J = 10.0 Hz)	116.0	116.0
10	5.46 (*d*, J = 10.0 Hz)	5.46 (*d*, J = 10.0 Hz)	126.1	126.1
11	-	-	78.0	77.9
12	1.45 (*s*)	1.45 (*s*)	28.5	28.5

Table 30 (Continued)

Position	**MNC8 δ_{H} (mult, *J*, Hz)**	**R δ_{H} (mult, *J*, Hz)**	**δ_{C}, MNC8**	**δ_{C}, R**
13	1.41 (*s*)	1.42 (s)	28.2	28.2
14	3.25 (*d*, *J* = 7.0 Hz)	3.25 (*d*, *J* = 7.2 Hz)	20.9	20.9
15	5.20 (*t*, *J* = 7.0 Hz)	5.21 (*t*, *J* = 7.4 Hz)	122.4	122.3
16	-	-	131.5	131.3
17	1.67 (*s*)	1.68 (s)	25.7	25.8
18	1.76 (*s*)	179 (s)	17.8	17.8
5a	-	-	110.0	102.5
8a	-	-	160.0	155.0
1'	-	-	131.0	130.8
2'/6'	7.33 (*d*, *J* = 8.5 Hz)	7.32 (*d*, *J* = 8.4 Hz)	127.7	127.7
3/'5'	6.88 (*d*, *J* = 8.5 Hz)	6.88 (*d*, *J* = 8.4 Hz)	115.6	115.6
4'	-	-	155.9	156.0
4'-OH	5.05 (*s*)	-	-	-

2.3.1.9 Compound MNC9



Compound MNC9 was isolated as a yellow needles, m.p. 253-254℃. The UV spectrum showed absorption bands at $\lambda_{\max}$ 239, 281, 332 and 382 nm. The IR spectrum showed the stretching of hydroxyl (3360 cm^{-1}) and conjugated carbonyl group (1620 cm^{-1}).

The ^1H NMR spectrum **(Table 31)** displayed signals with A_2B_2 pattern at δ8.05 and 6.92 (each 2H, *d*, *J* = 8.4 Hz) due to H-2'/H-6' and H-3'/H-5' respectively and one singlet signal of an aromatic proton at δ6.12 (H-6). The doublet signals of vinylic protons at δ5.54 (*d*, *J* = 10.2 Hz, H-10) and δ6.73 (*d*, *J* = 10.2 Hz, H-9) and a singlet signal of two methyl groups at δ1.42 (CH_3-12 and CH_3-13) were assigned for those of a 2,2-dimethylchromene ring. The correlations of H-9 (δ 6.73) to C-7 (δ 159.1), C-8 (δ 100.8), C-8a (δ 150.4), of H-10 (δ 5.54) to C-8 (δ 100.8), C-12 (δ 27.8) determined the positions of the chromene ring at C-7 and C-8. From the spectral data and comparison with citrusinol (Wu *et al.*, 1987), compound **MNC9** was assigned as citrusinol.

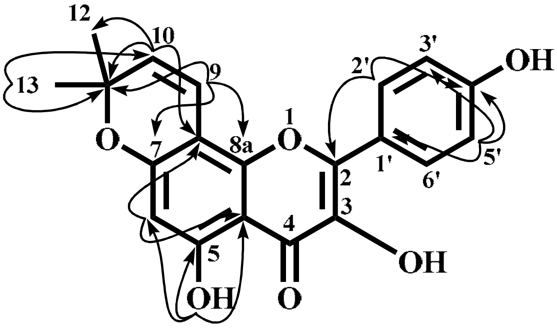


Figure 18 Selected HMBC correlations of **MNC9**

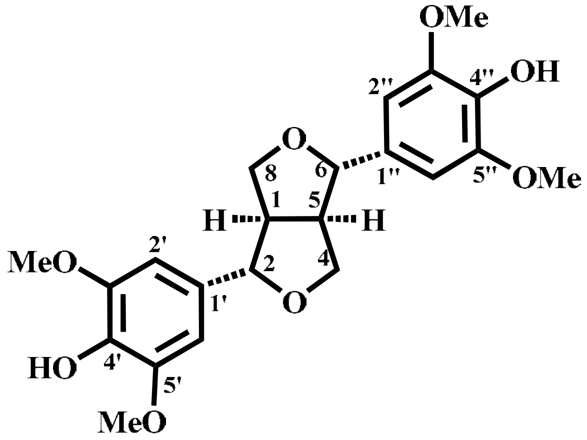
Table 31 ^{1}H, ^{13}C NMR and HMBC spectral data of **MNC9** ($CDCl_3$)

Position	δ_C		δ_H **(mult, *J*, Hz)**	**HMBC**
2	146.4	C	-	-
3	135.3	C	-	-
4	175.8	C	-	-
5	160.1	C	-	-
6	98.7	CH	6.12 (*s*)	C-8, C-5a
7	159.1	C	-	-
8	100.8	C	-	-
9	114.3	CH	6.73 (*d*, *J* = 10.2 Hz)	C-7, C-8a, C-11
10	126.9	CH	5.54 (*d*, *J* = 10.2 Hz)	C-8, C-9, C-12, C-13
11	77.7	C	-	-
12	27.8	CH_3	1.42 (*s*)	C-10, C-11
13	27.8	CH_3	1.42 (*s*)	C-10, C-11
5a	103.8	C	-	-
8a	150.4	C	-	-
1'	121.8	C	-	-
2'/6'	129.2	CH	8.05 (*d*, *J* = 8.4 Hz)	C-2, C-1', C-3', C-4'
3'/5'	115.4	CH	6.92 (*d*, *J* = 8.4 Hz)	C-1', C-2', C-4'
4'	158.7	C	-	
4'-OH	-	-	6.52 (*s*)	

Table 32 Comparison of ^{1}H NMR spectral data between compounds **MNC9** ($CDCl_3$) and citrusionol (**R**, $CDCl_3 + (CD_3)_2CO$)

Position	**MNC9** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**
2	-	-
3	-	-
4	-	-
5	-	-
6	6.12 (*s*)	6.18 (*s*)
7	-	-
8	-	-
9	6.73 (*d*, *J* = 10.2 Hz)	6.89 (*d*, *J* = 10.0 Hz)
10	5.54 (*d*, *J* = 10.2 Hz)	5.74 (*d*, *J* = 10.0 Hz)
11	-	-
12	1.42 (*s*)	1.50 (*s*)
13	1.42 (*s*)	1.50 (*s*)
5a	-	-
8a	-	-
1'	-	-
2'	8.05 (*d*, *J* = 8.4 Hz)	8.17 (*d*, *J* = 8.0 Hz)
3'	6.92 (*d*, *J* = 8.4 Hz)	7.03 (*d*, *J* = 8.0 Hz)
4'	-	-
4'-OH	6.52 (*s*)	-
5'	6.92 (*d*, *J* = 8.4 Hz)	7.03 (*d*, *J* = 8.0 Hz)
6'	8.05 (*d*, *J* = 8.4 Hz)	8.17 (*d*, *J* = 8.0 Hz)

2.3.1.10 Compound MNC10



Compound MNC10 was isolated as a colorless solid, m.p. 179-181 ℃, $[\alpha]^{27}_{D}$ +55.7°(c 0.17, $CHCl_3$), The UV spectrum showed absorption bands at λ_{max} 205, 237 and 281 nm. The IR spectrum showed the stretching of hydroxyl group (3435 cm^{-1}).

The ^1H NMR spectrum **(Table 33)** displayed the resonances of methine protons at δ 3.09 (2H, *m*, H-1/H-5), benzylic oxymethine protons at δ 4.73 (2H, *d*, J = 3.6 Hz, H-2/H-6), non-equivalent oxygenated methylene protons at δ 4.28 (2H, *dd*, J = 8.7, 6.9 Hz, H_{eq}-4/H_{eq}-8) and δ 3.91 (2H, *m*, H_{ax}-4/H_{ax}-8). The HMBC correlations of H-1/H-5 (δ 3.09) to C-1'/C-1''(δ 132.0), C-2/C-6 (δ 86.0) and C-4/C-8 (δ 71.7) as well as that of H-2'/H-2'' (δ 6.58) to C-2/C-6 (δ 86.0), C-1'/C-1''(δ 132.0) and C-4'/C-4'' (δ 134.2) indicated that C-2/C-6 of the furan ring were linked to benzene rings at C-1'/C-1''. In addition the spectral data exhibited the presence of a 1,3,4,5-tetrasubstituted benzene rings at δ 6.58, (4H, *s*, H-2'/H-2'', H-6'/H-6'') and a singlet of four methoxyl groups at C-3'/C-3''/C-5'/C-5'' at δ 3.95. The location of the methoxyl groups at δ 3.89 was assigned by their HMBC correlations to C-3'/C-3''/C-5'/C-5'' (δ 147.1) whereas the correlations of H-2'/H-2''(δ 102.6) to C-3'/C-3'' (δ 147.1) and C-4'/C-4'' (δ 134.2) suggested the position of OH at C-4'/C-4''. The stereochemistry at C-2/6 and C-1/5 was deduced from NOESY experiment, cross peaks were observed between H-1/H-5 and H-2'/H-2'', with the absence of cross peaks between H-1/H-5 and H-2/H-6. The optical rotation of compound **MNC10** is dextrorotatory ($[\alpha]^{27}_{D}$ +55.7°, c 0.17, $CHCl_3$), similar to (+)-syringaresinol $[\alpha]^{25}_{D}$

+42° (*c* 0.1, $CHCl_3$) (Das *et al.*, 1999). From the spectral data and comparison with those of (+)-syringaresinol (Das *et al.*, 1999) compound **MNC9** was assigned as (+)-syringaresinol.

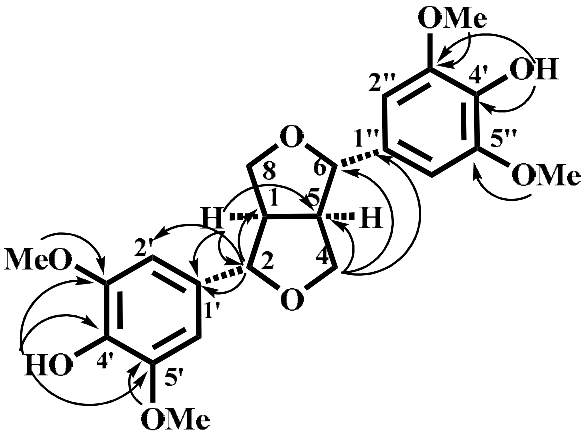


Figure 19 Selected HMBC correlations of **MNC10**

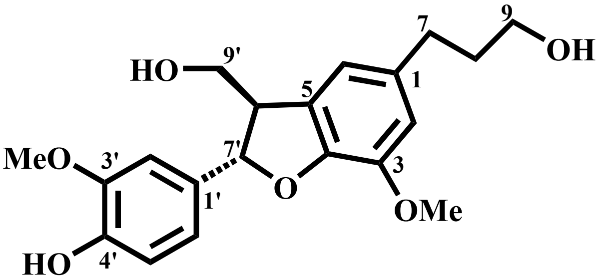
Table 33 ^1H, ^{13}C NMR and HMBC spectral data of **MNC10** ($CDCl_3$)

Position	δ_C		δ_H **(mult, *J*, Hz)**	**HMBC**
1/5	54.2	CH	3.09 (*m*)	C-2, C-6, C-1'
2/6	86.0	CH	4.73 (*d, J* = 3.6 Hz,)	C-1, C-8, C-1', C-2'
$4_{eq}/8_{eq}$	71.7	CH_2	4.28 (*dd, J* = 8.7, 6.9 Hz)	C-2, C-5, C-6
$4_{ax}/8_{ax}$	71.7	CH_2	3.91 (*m*)	C-1, C-6
1'/1"	132.0	C	-	-
2'/6'	102.6	CH	6.58 (*s*)	C-2, C-1', C-4'
3'/5'	147.1	C	-	-
4'/4"	134.2	C	-	-
2"/6"	102.6	CH	6.58 (*s*)	C-6, C-1", C-4"
3"/5"	147.1	C	-	-
3'/3"-OCH_3	56.3	2×OCH_3	3.89 (*s*)	C-3'/ C-3"
4'/4"-OH	-	-	5.61 (*s*)	C-4'/ C-4", C-5'/ C-5"
5'/5"-OCH_3	56.3	2×OCH_3	3.89 (*s*)	C-5'/ C-5"

Table 34 Comparison of ^{1}H NMR spectral data between compounds **MNC10** ($CDCl_3$) and (+)-syringaresinol (**R**, $CDCl_3$)

Position	**MNC10** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**
1/5	3.09 (*m*)	3.09-3.00 (*m*)
2/6	4.73 (*d, J* = 3.6 Hz)	4.68 (*d, J* = 4.0 Hz)
$4_{eq}/8_{eq}$	4.28 (*dd, J* = 8.7, 6.9 Hz)	4.23 (*dd, J* = 9.0, 7.0 Hz)
$4_{ax/}8_{ax}$	3.91 (*m*)	3.87 (*dd, J* = 9.0, 4.0 Hz)
1'/1"	-	-
2'/6'	6.58 (*s*)	6.52 (*s*)
3'/5'	-	-
4'/4"	-	-
2"/6"	6.58 (*s*)	6.52 (*s*)
3"/5"	-	-
3'/3"-OCH_3	3.89 (*s*)	3.90 (*s*)
4'/4"-OH	5.61 (*s*)	-
5'/5"-OCH_3	3.89 (*s*)	3.90 (*s*)

2.3.1.11 Compound MNC11



Compound MNC11 was isolated as colorless oil, $[\alpha]^{27}_{D}$ -11.3° (*c* 0.2, MeOH). The UV spectrum showed absorption bands at λ_{max} 208 and 282 nm. The IR spectrum showed the stretching of hydroxyl group (3392 cm^{-1}).

The ^{1}H NMR spectrum displayed the resonances of aromatic protons at δ 6.93 (*d*, *J* = 1.8 Hz, H-2'), 6.90 (*dd*, *J* = 8.1, 1.8 Hz, H-6') and 6.86 (*d*, *J* = 8.1 Hz, H-5') indicating the presence of a 1,3,4-trisubstituted benzene ring whereas a singlet signal of an aromatic proton was evident at δ 6.76 (2H, *s*, H-2/H-6). An oxygenated methine proton signal at δ 5.53 (1H, *d*, *J* = 7.5 Hz, H-7'), two oxygenated methylene signal at δ 3.68 (2H, *t*, *J* = 6.3 Hz, H-9) and δ 3.95 (2H, *m*, H-9'), a methine signal at δ 3.60 (1H, *m*, H-8'), two methylene signals at δ 1.88 (2H, *m*, H-8) and 2.67 (2H, *t*, *J* = 7.2 Hz, H-7) and two methoxyl signals at δ 3.85 (3H, *s*, 3′-OMe) and δ 3.87 (3H, *s*, 3-OMe) were observed.

The ^{13}C NMR spectrum showed 20 signals for 20 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested a presence of four oxygenated olefinic quaternary carbons at δ 144.2, 145.0, 146.6 and 146.7, three olefinic quaternary carbons at δ 127.8, 133.1 and 135.4, five aromatic carbons at δ 108.8, 112.5, 114.3, 116.0, and 119.4, an oxygenated methine carbon at δ 87.9, two oxygenated methylene carbons at δ 62.3 and 63.9, a methine carbon at δ 53.8, two methylene carbons at δ 32.0 and 34.6 and two methoxyl carbons at δ 55.9 and 56.0. Absence of NOESY cross peak between H-7' and H-8' indicated their *trans* relationship.

The ^{1}H and ^{13}C NMR spectral data of 7' and 8' positions agreed well with those reported by Shen *et al.*, 1997 together with similar levorotatory rotation ($[\alpha]^{25}_{D}$ -14.3°, *c* 0.2, MeOH), thus suggesting the same 7'*S* and 8'*R* configurations.

Compound **MNC11** was identified as dihydrodehydrodiconifenyl alcohol (Shen *et al.*, 1997).

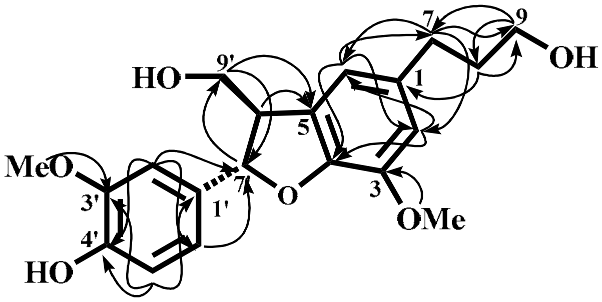


Figure 20 Selected HMBC correlations of **MNC11**

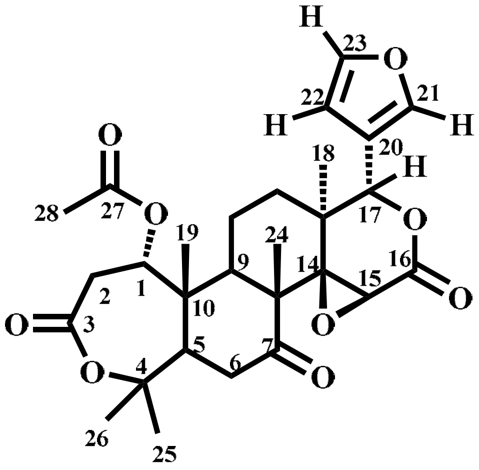
Table 35 ^{1}H, ^{13}C NMR and HMBC spectral data of **MNC11** ($CDCl_3$)

Position	δ_C		δ_H (mult, *J*, Hz)	HMBC
1	135.4	C	-	-
2	112.5	CH	6.67 (*s*)	C-1, C-4, C-6, C-7
3	144.2	C	-	-
4	146.6	C	-	-
5	127.8	C	-	-
6	116.0	CH	6.67 (*s*)	C-1, C-2, C-4, C-7
7	32.0	CH_2	2.67 (*t, J* = 7.2 Hz)	C-1, C-2, C-6, C-9
8	34.6	CH_2	1.88 (*m*)	C-1, C-7, C-9
9	62.3	CH_2	3.68 (*t, J* = 6.3 Hz)	C-6, C-7
1'	133.1	C	-	-
2'	108.8	CH	6.93 (*d, J* = 1.8 Hz)	C-1', C-4', C-6', C-7'
3'	146.7	C	-	-
4'	145.0	C	-	-
5'	114.3	CH	6.86 (*d, J* = 8.1 Hz)	C-1', C-3', C-4'
6'	119.4	CH	6.90 (*dd, J* = 8.1, 1.8 Hz)	C-1', C-2', C-4', C-7'
7'	87.9	CH	5.53 (*d, J* = 7.5 Hz)	C-1', C-2', C-6', C-9'
8'	53.8	CH	3.60 (*m*)	C-3, C-6, C-1', C-7'
9'	63.9	CH_2	3.95 (*m*)	C-5, C-7', C-8'
3-OCH_3	56.0	OCH_3	3.87 (*s*)	C-3
3-OCH_3	55.9	OCH_3	3.85 (*s*)	C-3'

Table 36 Comparison of ^{1}H NMR and ^{13}C NMR spectral data between compounds **MNC11** ($CDCl_3$) and dihydrodehydrodiconifenyl alcohol (**R**, $CDCl_3$)

Position	**MNC11** δ_H **(mult, *J*, Hz)**	**R** δ_H **(mult, *J*, Hz)**	δ_C**, MNC11**	δ_C**, R**
1	-	-	135.4	135.4
2	6.67 (*s*)	6.68 (*s*)	112.5	112.4
3	-	-	144.2	144.2
4	-	-	146.6	146.6
5	-	-	127.8	127.7
6	6.67 (*s*)	6.68 (*s*)	116.0	115.9
7	2.67 (*t, J* = 7.2 Hz)	2.68 (*t, J* = 7.5 Hz)	32.0	34.6
8	1.88 (*m*)	1.89 (*m*)	34.6	32.0
9	3.68 (*t, J* = 6.3 Hz)	3.70 (*t, J* = 6.3 Hz)	62.3	62.3
1'	-	-	133.1	133.1
2'	6.93 (*d, J* = 1.8 Hz)	6.94 (*s*)	108.8	108.8
3'	-	-	146.7	147.1
4'	-	-	145.0	145.6
5'	6.86 (*d, J* = 8.1 Hz)	6.90 (*d, J* = 8.0 Hz)	114.3	114.2
6'	6.90 (*dd, J* = 8.1, 1.8 Hz)	6.76 (*d, J* = 8.0)	119.4	119.4
7'	5.53 (*d, J* = 7.5 Hz)	5.55 (*d, J* = 7.5 Hz)	87.9	87.9
8'	3.60 (*m*)	3.63 (*m*)	53.8	53.8
9'	3.95 (*m*)	3.96 (*m*)	63.9	63.9
3-OCH_3	3.87 (*s*)	3.89 (*s*)	56.0	56.0
3-OCH_3	3.85 (*s*)	3.87 (*s*)	55.9	56.0

2.3.1.12 Compound MNC12



Compound MNC12 was obtained as white crystals, m.p. 189-190°C, $[\alpha]^{27}_{D}$ -79.3° (*c* 0.10, $CHCl_3$). The IR spectrum of compound **MNC12** indicated the presence of carbonyl absorption at 1730 cm^{-1} and *β*-substituted furan at 875 cm^{-1}.

The ^1H NMR spectrum (**Table 37**) suggested the presence of a *β*-substituted furan at *δ* 7.39 (1H, *br s*), 7.39 (1H, *br s*) and 6.31 (1H, *t, J* = 1.2 Hz). It was further established that compound **MNC12** was a limonoid with five tertiary C-methyl groups resonating as singlets at *δ* 1.54, 1.44, 1.31, 1.16 and 1.07 and a -OCOMe as a singlet at *δ* 1.99. The presence of an epoxy lactone moiety was revealed by the characteristic H-15 and H-17 singlet signals at *δ* 3.77 and 5.42 respectively. The presence of a system $-CH-CH_2-C=O$ in the molecule was inferred from an ABC pattern at *δ* 2.56 (1H, *dd, J* = 15.0, 3.6 Hz, H-5), 2.57 (1H, *dd, J* = 15.0, 3.6 Hz, H-6_b) and 2.71 (1H, *t*, *J* = 15.0 Hz, H-6_a). This result also revealed the presence of two fully substituted carbon atoms alpha to the methine carbon due to the absence of other coupling for these three protons in the ^1H NMR spectrum. Three mutually coupling protons at *δ* 3.07 (1H, *dd, J* = 15.6, 7.2 Hz, H-2_b), 3.20 (1H, *dd*, *J* = 15.6, 1.2 Hz, H-2_a), and 4.99 (1H, *d, J* = 7.2 Hz, H-1) were assigned to the moiety $O=C-CH_2-CH-O-$. These results were also supported by a HMBC experiment (**Figure 21**), (**Table 37**). Based on these data and the specific rotation of nomilin ($[\alpha]^{24}_{D}$ -122.87) (Bennett *et al.*, 2006, Zhang *et al.*, 2006), therefor the structure of **MNC12** was assigned as nomilin.

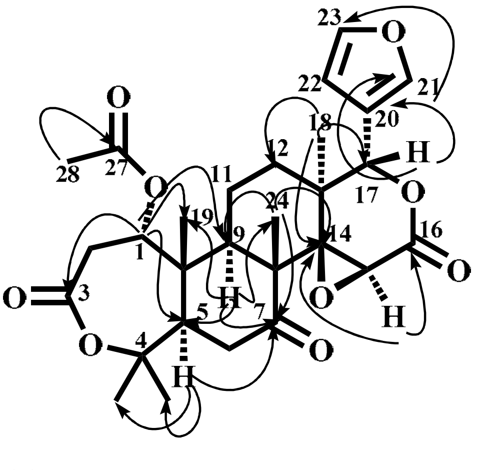


Figure 21 Selected HMBC correlations of **MNC12**

Table 37 ^{1}H, ^{13}C NMR and HMBC spectral data of **MNC12** ($CDCl_3$)

Position	δ_C		δ_H **(mult, *J*, Hz)**	**HMBC**
1	70.6	CH	4.99 (*d, J* = 7.2 Hz)	C-3, C-5, C-9, C-19, C-27
2	35.2	CH_{2a}	3.20 (*dd, J* = 15.6, 1.2 Hz)	C-1, C-3, C-10, C-27
		CH_{2b}	3.07 (*dd, J* = 15.6, 7.2 Hz)	
3	169.1	C	-	-
4	84.3	C	-	-
5	50.9	CH	2.56 (*dd, J* = 15.0, 3.6 Hz)	C-1, C-7, C-9, C-19, C-25, C-26
6	38.2	CH_{2a}	2.71 (*t, J* = 15.0 Hz)	C-4, C-10, C-7, C-8
		CH_{2b}	2.57 (*dd, J* = 15.0, 3.6 Hz)	
7	206.7	C	-	-
8	52.7	C	-	-
9	44.3	CH	2.45 (*dd, J* = 9.0, 4.2 Hz)	C-1, C-7, C-12, C-14, C-19, C-24
10	44.0	C	-	-
11	17.0	CH_2	1.77 (*m*)	C-8, C-9, C-10, C-13
12	32.1	CH_2	1.61 (*m*)	C-9, C-11, C-18, C-14, C-17
13	37.3	C	-	-
14	65.3	C	-	-

Table 37 (Continued)

Position	δ_C		δ_H (mult, J, Hz)	HMBC
15	53.2	CH	3.77 (*s*)	C-8, C-13, C-14, C-16
16	166.7	C	-	-
17	77.9	CH	5.42 (*s*)	C-12, C-14, C-18, C-21, C-22
18	20.7	CH_3	1.07 (*s*)	C-12, C-13, C-14, C-17
19	16.4	CH_3	1.31 (*s*)	C-1, C-5, C-9, C-10
20	119.6	CH	-	-
21	143.1	CH	7.39 (*br s*)	C-17, C-20, C-22, C-23
22	109.5	CH	6.31 (*t*, J = 1.2 Hz)	C-17, C-20, C-23
23	140.9	CH	7.39 (*br s*)	C-17, C-20, C-22
24	16.9	CH_3	1.16 (*s*)	C-7, C-8, C-9, C-14
25	33.3	CH_3	1.44 (*s*)	C-4, C-5, C-26
26	23.2	CH_3	1.54 (*s*)	C-4, C-5, C-25
27	169.1	C	-	-
28	20.7	CH_3	1.99 (*s*)	C-27

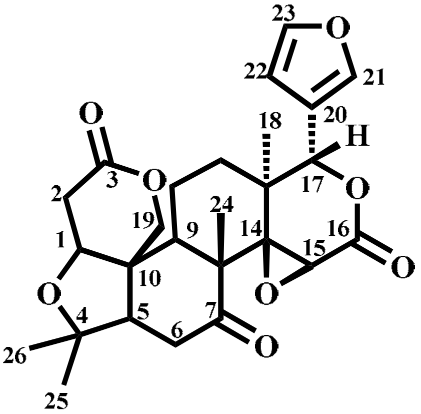
Table 38 Comparison of ^{13}C NMR spectral data between compounds **MNC12** ($CDCl_3$) and nomilin (**R**, $CDCl_3$)

Position	δ_C, **MNC12**	δ_C, **R**
1	70.6	70.8
2	35.2	35.3
3	169.1	169.2
4	84.3	84.3
5	50.9	51.1
6	38.2	38.9
7	206.7	206.9

Table 38 (Continued)

Position	**δ_C, MNC12**	**δ_C, R**
8	52.7	52.9
9	44.3	44.3
10	44.0	44.3
11	17.0	16.5
12	32.1	32.2
13	37.3	37.6
14	65.3	65.6
15	53.2	53.5
16	166.7	166.9
17	77.9	78.1
18	20.7	20.1
19	16.4	16.2
20	119.6	120.2
21	143.1	141.3
22	109.5	110.1
23	140.9	143.1
24	16.9	16.9
25	33.3	32.9
26	23.2	23.3
27	169.1	169.2
28	20.7	20.8

2.3.1.13 Compound MNC13



Compound MNC13 was obtained as white crystals, m.p. 285-286°C, $[\alpha]^{27}_{D}$ -139.5° (*c* 0.10, Me_2CO). The IR spectrum of compound **MNC13** indicated the presence of two carbonyl absorptions at 1730 and 1709 cm^{-1} and β-substituted furan at 883 cm^{-1}.

Compound **MNC13**, the second limonoid isolated, has spectroscopic properties similar to those of nomilin, compound **MNC12** (**Table 37**). Immediately recognizable are the β-substituted furan, H-17 (δ 5.50) and H-15 (δ 4.04) of an epoxy lactone, and four tertiary methyls (δ 1.08, 1.17, 1.18 and 1.29). Furthermore, the ^{1}H NMR spectrum also showed signal of a system -O-CH-CH_2-C=O at δ 2.72 (1H *dd, J* = 16.8, 1.5, H-2$_a$), 2.96 (1H *dd, J* = 16.8, 3.3, H-2$_b$) and 4.07 (1H *d, J* = 3.3, H-1). The signal of non-equivalent oxymethylene protons were observed at δ 4.81 and 4.49 (1H each, *d*, *J* = 13.2 Hz, H-19). The HMBC correlations of H-19 at δ 4.81 and 4.49 to the carbons at δ 79.2 (C-1), 60.4 (C-5), and 45.9 (C-10) together with HMBC correlations of H-1 at δ 4.07 to the carbons at δ 170.1 (C-3), 60.4 (C-5), 48.1(C-9) and 65.6 (C-19) suggested an ester bridge from C-2 to C-19. The ^{13}C NMR spectrum of limonin (see **Table 39**) was in accord with this assignment. This result was also supported by a HMBC experiment (**Figure 22**). Based on these data, the structure of **MNC13** was assigned as limonin (Breksa *et al.*, 1979) ($[\alpha]^{23}_{D}$ -124.7°, *c* 0.12, Me_2CO).

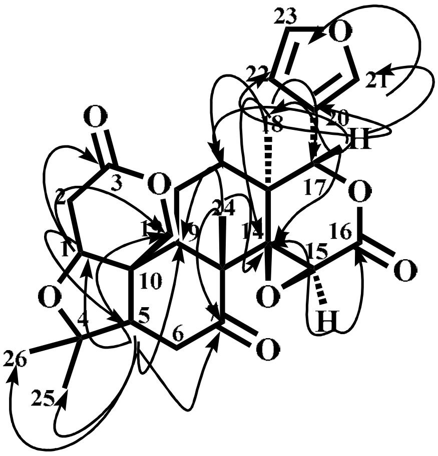


Figure 22 Selected HMBC correlations of **MNC13**

Table 39 ^{1}H, ^{13}C NMR and HMBC spectral data of **MNC13** (CDCl$_3$)

Position	**δ$_C$**		**δ$_H$ (mult, *J*, Hz)**	**HMBC**
1	79.2	CH	4.07 (*d, J* = 3.3 Hz)	C-3, C-5, C-9, C-19
2	35.7	CH$_{2a}$	2.72 (*dd, J* = 16.8, 1.5 Hz)	C-1, C-3, C-10
		CH$_{2b}$	2.96 (*dd, J* = 16.8, 3.3 Hz)	
3	170.1	C	-	-
4	80.5	C	-	-
5	60.4	CH	2.27 (*dd, J* = 14.4, 3.3 Hz)	C-1, C-7, C-9, C-19, C-25, C-26
6	36.4	CH$_{2a}$	2.90 (*t, J* = 14.4 Hz)	C-4, C-10, C-7, C-8
		CH$_{2b}$	2.46 (*dd, J* = 14.4, 3.3 Hz)	
7	206.7	C	-	-
8	51.4	C	-	-
9	48.1	CH	2.57 (*dd, J* = 12.0, 3.0 Hz)	C-1, C-7, C-12, C-14, C-19, C-24
10	45.9	C	-	-
11	18.8	CH$_2$	1.80 (*m*)	C-8, C-9, C-10, C-13
12	30.8	CH$_2$	1.67 (*m*)	C-9, C-11, C-18, C-14, C-17
13	38.1	C	-	-
14	65.9	C	-	-

Table 39 (Continued)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**
15	53.9	CH	4.04 (*s*)	C-8, C-13, C-14, C-16
16	167.3	C	-	-
17	78.1	CH	5.50 (*s*)	C-12, C-14, C-18, C-21, C-22
18	20.6	CH_3	1.17 (*s*)	C-12, C-13, C-14, C-17
19	65.6	CH_{2a}	4.81 (*d, J* = 13.2 Hz)	C-1, C-3, C-5, C-9, C-10
	65.6	CH_{2b}	4.49 (*d, J* = 13.2 Hz)	
20	125.4	C	-	-
21	143.4	CH	7.42 (*m*)	C-17, C-20, C-22, C-23
22	109.3	CH	6.35 (*d, J* = 0.9 Hz)	C-17, C-20, C-22, C-23
23	141.3	CH	7.42 (*m*)	C-17, C-20, C-22, C-23
24	17.6	CH_3	1.08 (*s*)	C-7, C-8, C-9, C-14
25	21.3	CH_3	1.29 (*s*)	C-4, C-5, C-26
26	30.1	CH_3	1.18 (*s*)	C-4, C-5, C-25

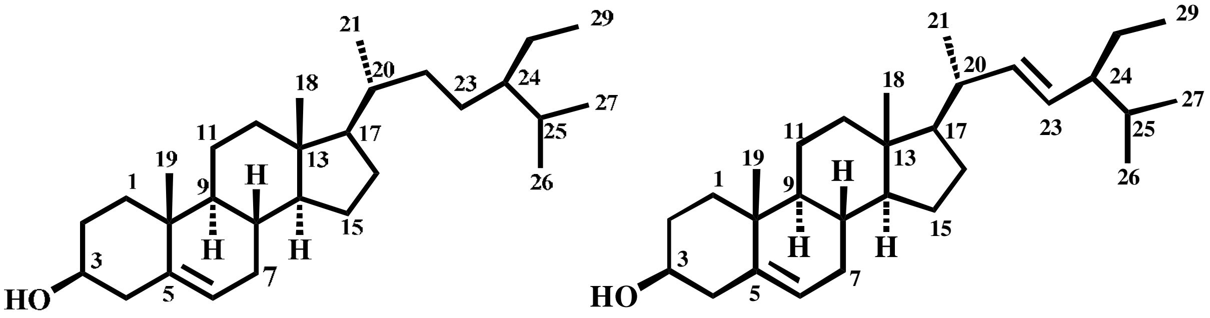
Table 40 Comparison of ^{1}H NMR and ^{13}C NMR spectral data between compounds **MNC13** ($CDCl_3$) and limonin (**R**, $CDCl_3$)

Position	**MNC13** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**	**δ_C, MNC13**	**δ_C, R**
1	4.07 (*d, J* = 3.3 Hz)	4.03 (*m*)	79.2	79.2
2	2.72 (*dd, J* = 16.8, 1.5 Hz)	2.67 (*dd, J* = 16.8, 2.0 Hz)	35.7	35.7
2	2.96 (*dd, J* = 16.8, 3.3 Hz)	2.98 (*dd, J* = 16.8, 4.0 Hz)		
3	-	-	170.1	169.0
4	-	-	80.5	80.3
5	2.27 (*dd, J* = 14.4, 3.3 Hz)	2.22 (*dd, J* = 15.8, 3.4 Hz)	60.4	60.7
6	2.90 (*t, J* = 14.4 Hz)	2.85 (*t, J* = 14.4 Hz)	36.4	36.4

Table 40 (Continued)

Position	**MNC13** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**	**δ_C, MNC13**	**δ_C, R**
6	2.46 (*dd, J* = 14.4, 3.3 Hz)	2.46 (*dd, J* = 14.4, 3.2 Hz)		
7	-	-	206.7	206.0
8	-	-	51.4	51.4
9	2.57 (*dd, J* = 12.0, 3.0 Hz)	2.55 (*dd, J* = 12.2, 3.0 Hz)	48.1	48.2
10	-	-	45.9	46.0
11	1.80 (*m*)	1.72-1.95 (*m*)	18.8	19.0
12	1.67 (*m*)	1.46-1.58 (*m*)	30.8	30.9
13	-	-	38.1	38.0
14	-	-	65.9	65.7
15	4.04 (*s*)	4.05 (*s*)	53.9	53.9
16	-	-	167.3	166.5
17	5.50 (*s*)	5.47 (*s*)	78.1	77.8
18	1.17 (*s*)	1.18 (*s*)	20.6	20.7
19	4.81 (*d, J* = 13.2 Hz)	4.76 (*d, J* = 13.0 Hz)	65.6	65.4
19	4.49 (*d, J* = 13.2 Hz)	4.46 (*d, J* = 13.0 Hz)		
20	-	-	125.4	120.4
21	7.42 (*m*)	7.40 (*m*)	143.4	143.3
22	6.35 (*d, J* = 0.9 Hz)	6.34 (*m*)	109.3	109.7
23	7.42 (*m*)	7.41 (*m*)	141.3	141.2
24	1.08 (*s*)	1.08 (*s*)	17.6	17.7
25	1.29 (*s*)	1.29 (*s*)	30.1	30.2
26	1.18 (*s*)	1.18 (*s*)	21.3	21.4

2.3.1.14 Compounds MNC14 and MNC15



The mixture of **MNC14** and **MNC15** was isolated as a white solid. The ^{1}H NMR spectra showed an oxymethine proton at δ 3.57-3.47 (*m*) and three olefinic protons at δ 5.35 (*d*, J = 5.1 Hz), 5.16 (*dd*, J = 8.4, 15.1 Hz) and 5.01 (*dd*, J = 8.4, 15.1 Hz). The ^{1}H NMR spectral data of this compound corresponded to a previous reported data (Thongdeeying 2005). Thus, the mixture was identified as β-sitosterol (**MNC14**) and stigmasterol (**MNC15**).

CHAPTER 4
CONCLUSION

Seven known compounds; two curcuminoids: curcumin (**CC1**) and demethoxycurcumin (**CC2**), one gingerdione: 1-dehydrogingerdione (**CC3**), together with four sesquiterpenes: germacrone (**CC4**), (+)-germacrone-4,5-epoxide (**CC5**), zederone (**CC6**) and comosone II **(CC7**) were isolated from the rhizomes of *Curcuma zedoaria*. Their structures were elucidated by spectroscopic methods. Compound **CC2** (5.13 g) was a major component.

Fifteen known compounds; three acridone alkaloids: citrusinine-I **(MNC1**), *N*-methylataphyllinine **(MNC2)** and citracridone I **(MNC3)**, three benzene derivative: valencic acid **(MNC4)**, vanillin **(MNC5)** and 4-hydroxybenzaldehyde, **(MNC6)**, a coumarin: xanthyletin **(MNC7)**, two flavonoids: erythrisenegalone **(MNC8)** and citrusinol **(MNC9)**, two lignans: (+)-syringaresinol **(MNC10)** and dihydrodehydrodiconifenyl alcohol **(MNC11)**, two limonoids: nomilin **(MNC12)** and limonin **(MNC13)** and two steroids: a mixture of *β*-sitosterol (**MNC14**) and stigmasterol (**MNC15**) were isolated from the stems of *Citrus medica*. Their structures were elucidated by spectroscopic methods. A mixture of **MNC14** and **MNC15** (178.0 g) was a major component.

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APPENDIX

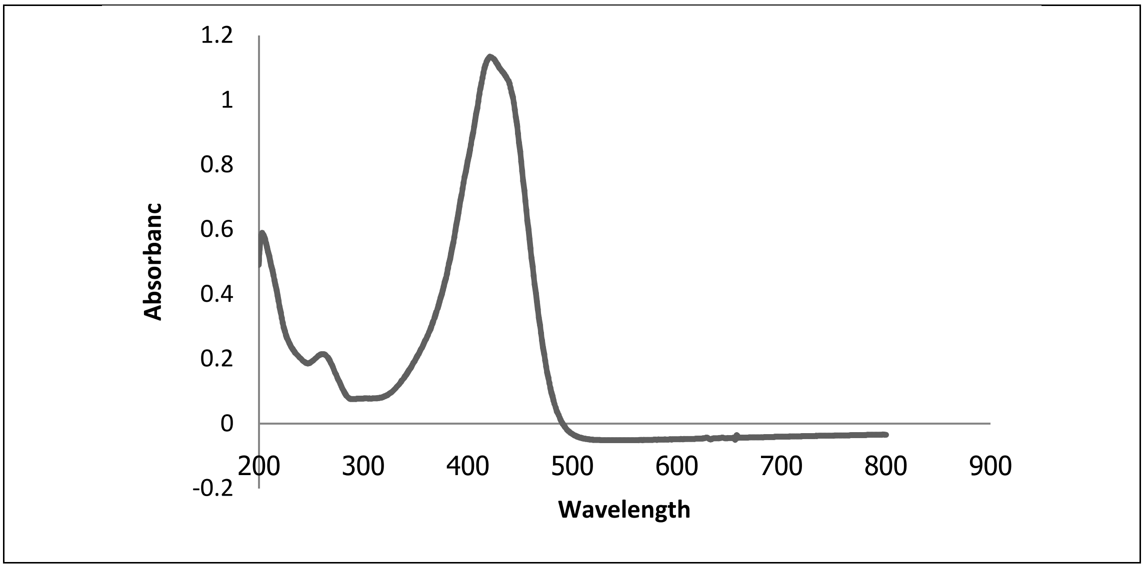

Figure 23 UV (MeOH) spectrum of compound **CC1**

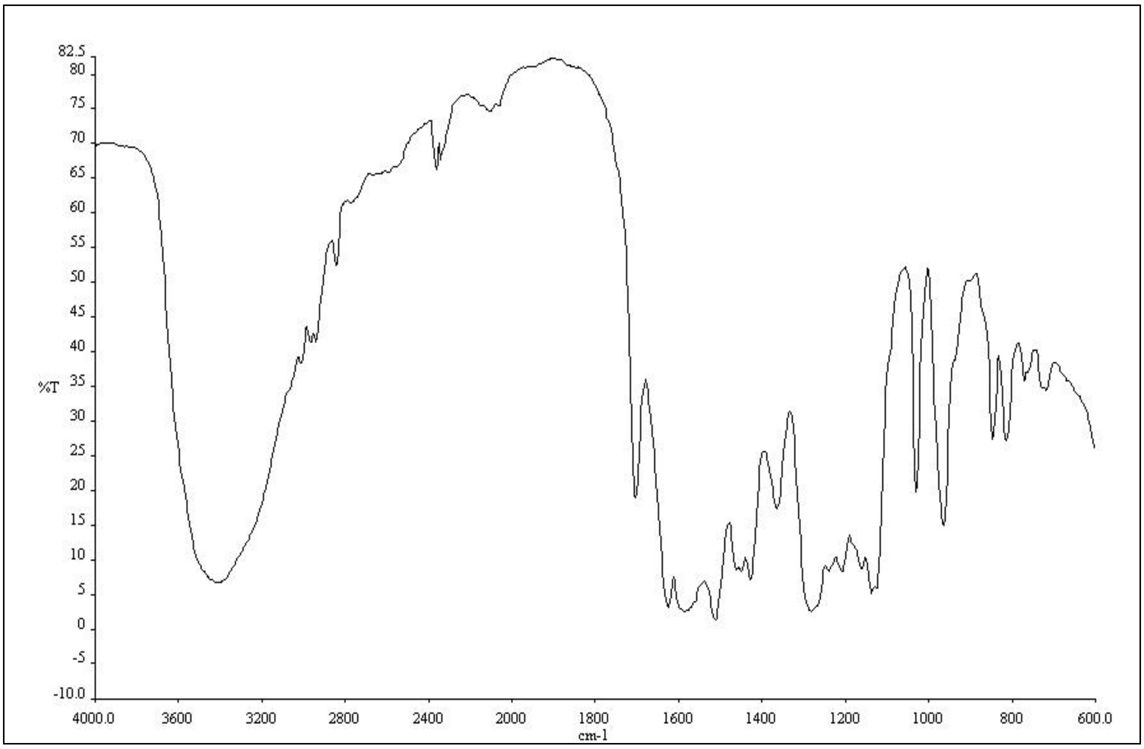

Figure 24 IR (KBr) spectrum of compound **CC1**

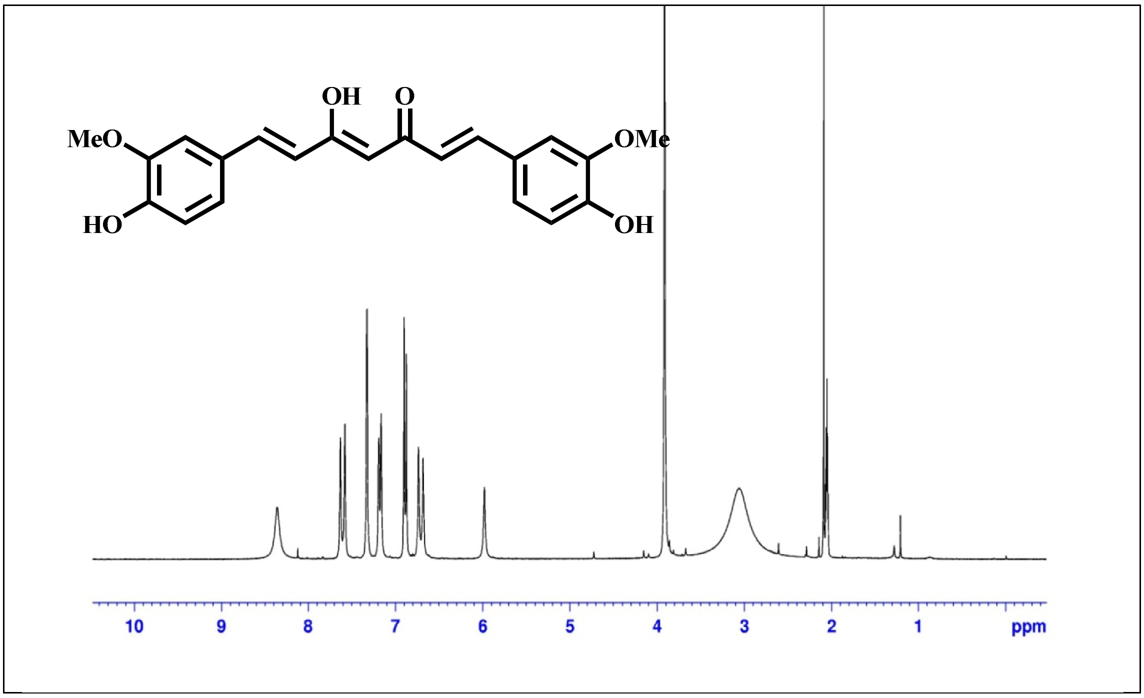

Figure 25 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **CC1**

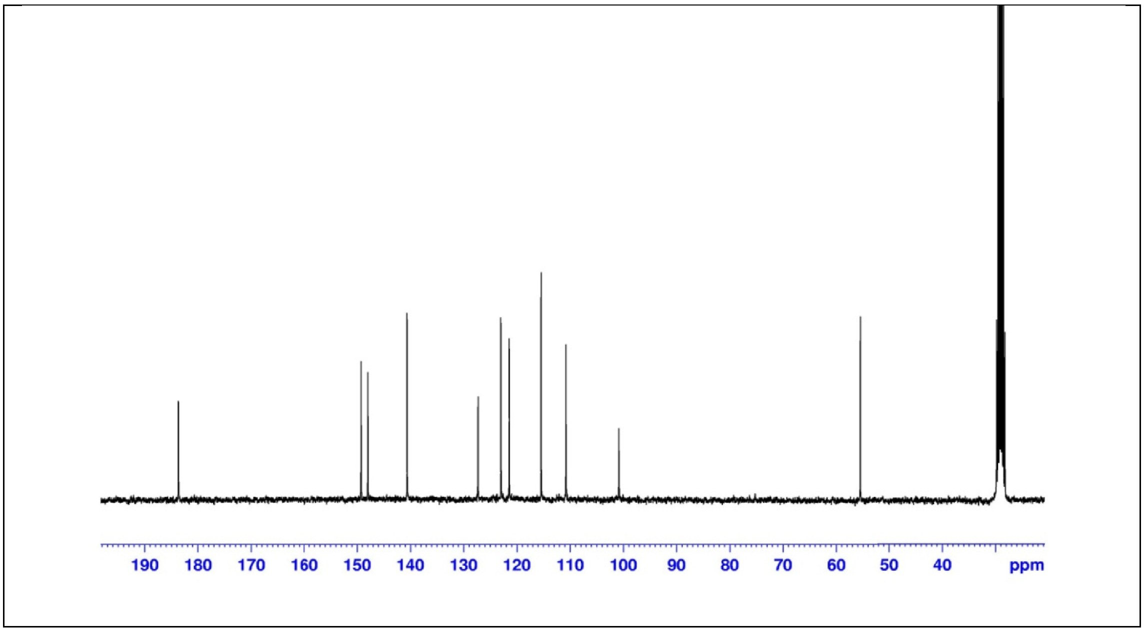

Figure 26 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **CC1**

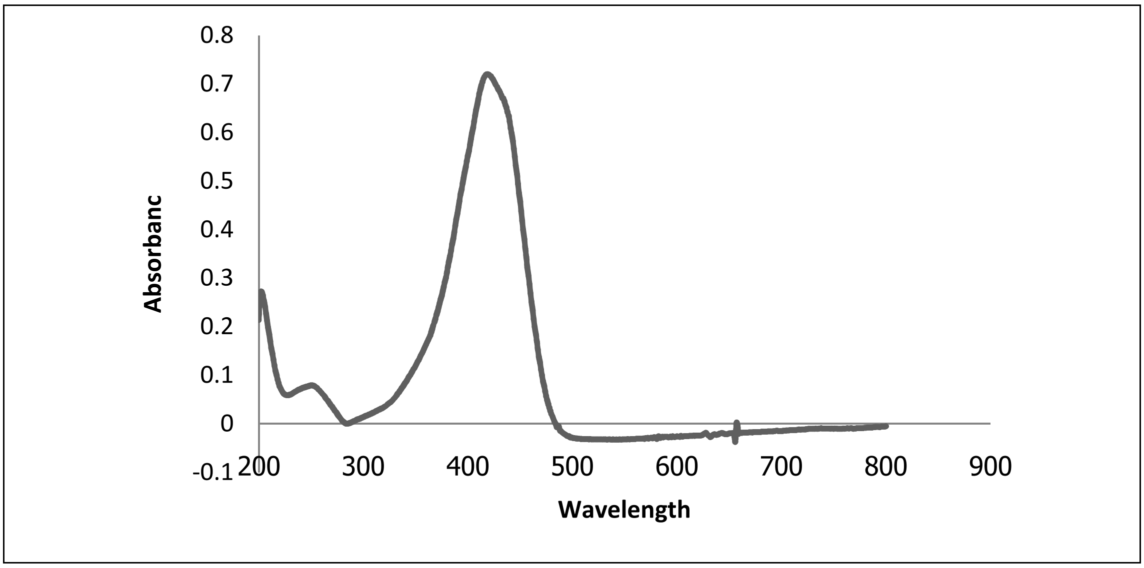

Figure 27 UV (MeOH) spectrum of compound **CC2**

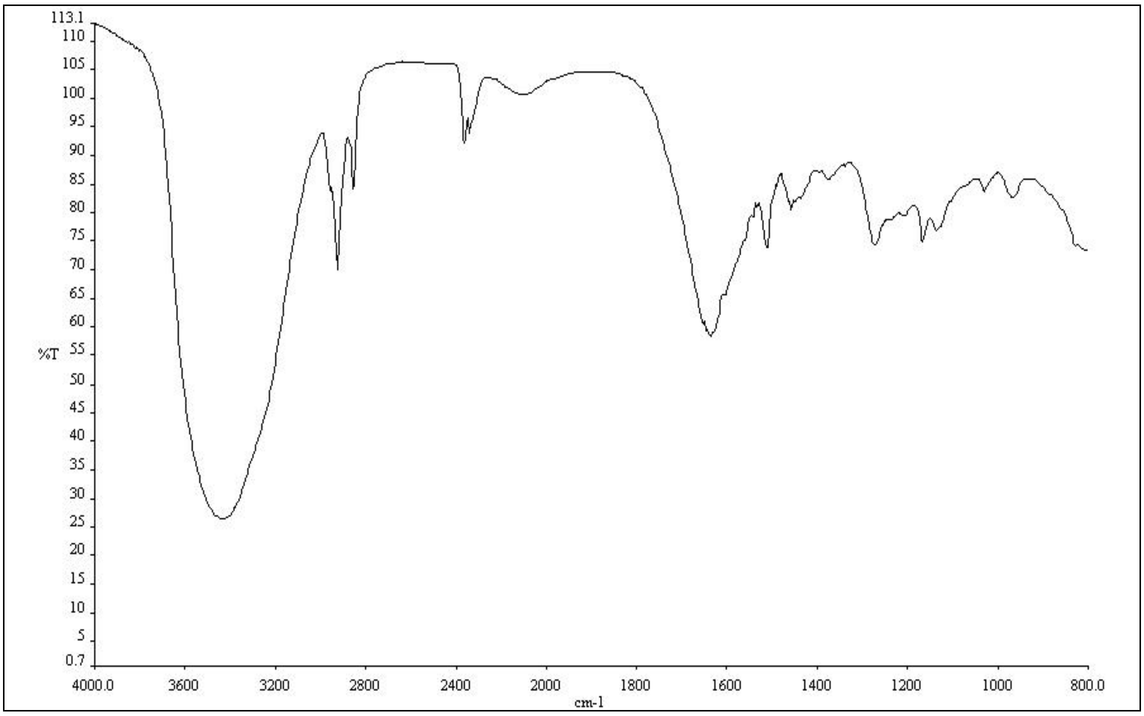

Figure 28 IR (KBr) spectrum of compound **CC2**

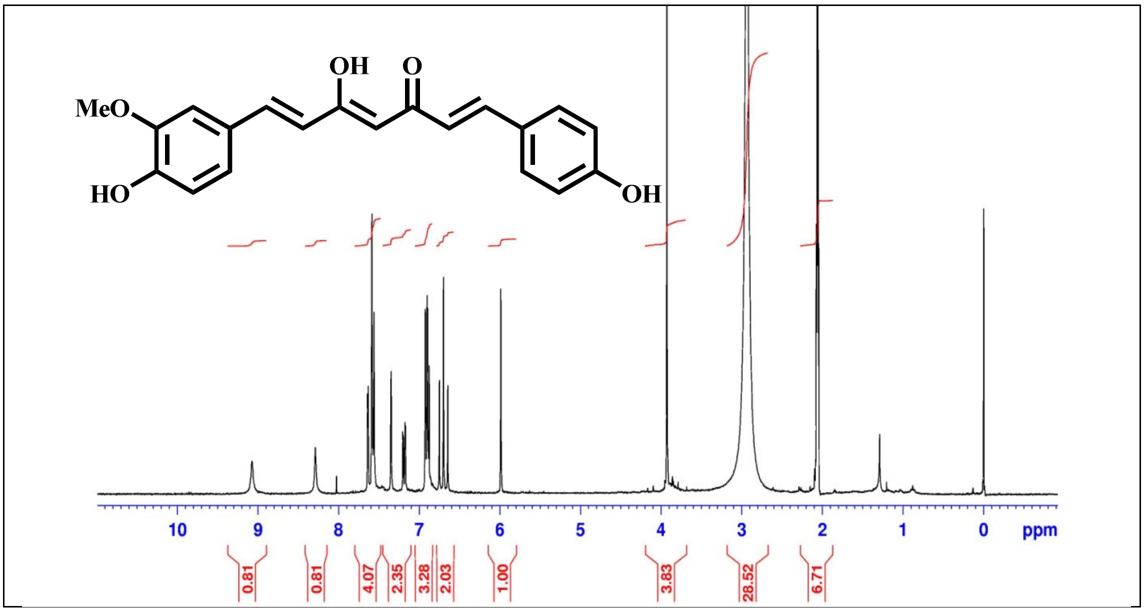

Figure 29 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **CC2**

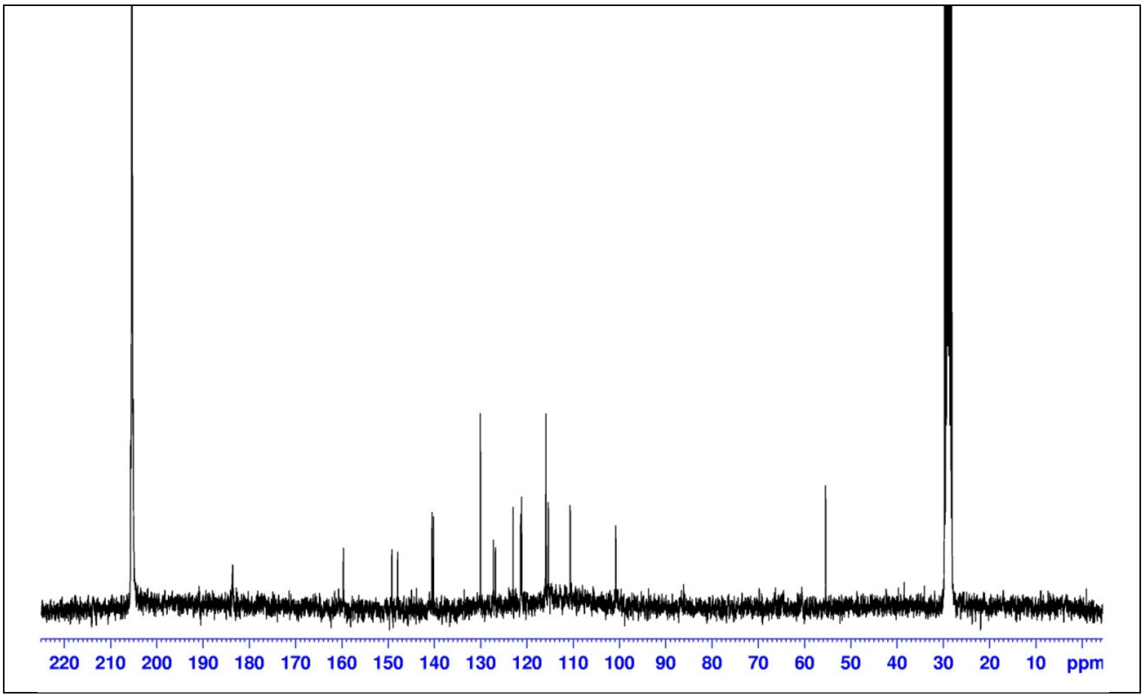

Figure 30 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **CC2**

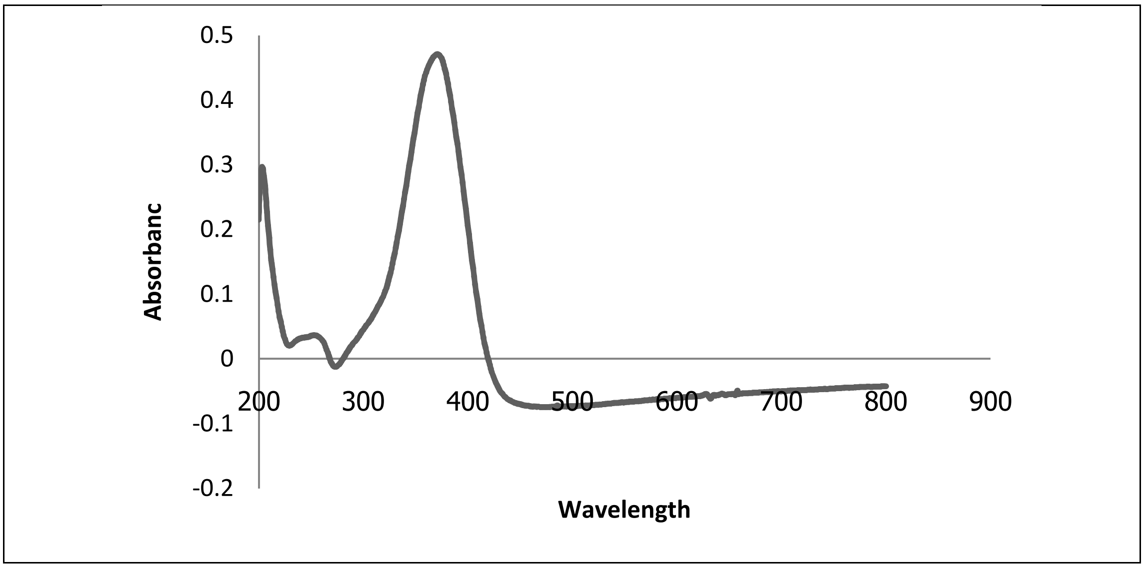

Figure 31 UV (MeOH) spectrum of compound **CC3**

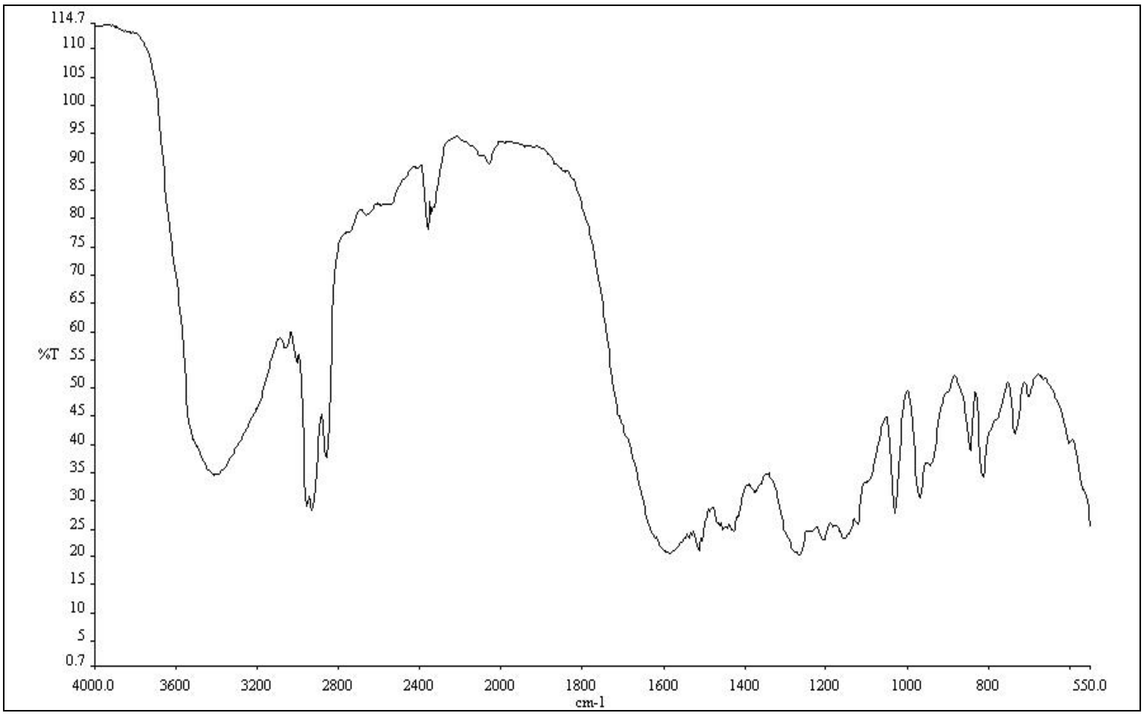

Figure 32 IR (neat) spectrum of compound **CC3**

Figure 33 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CC3**

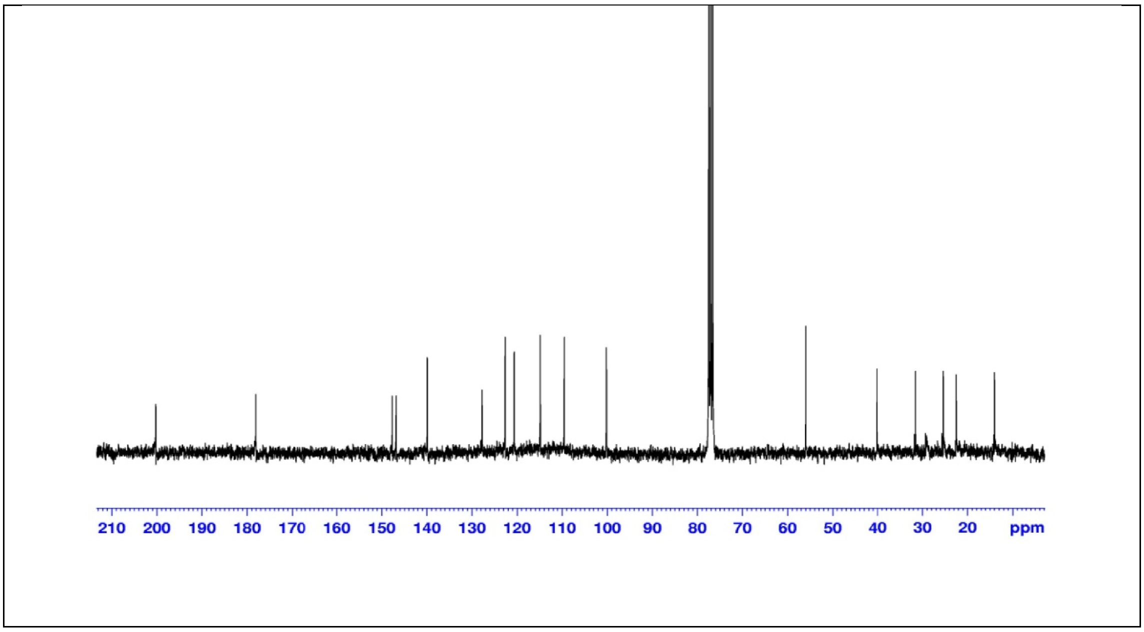

Figure 34 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CC3**

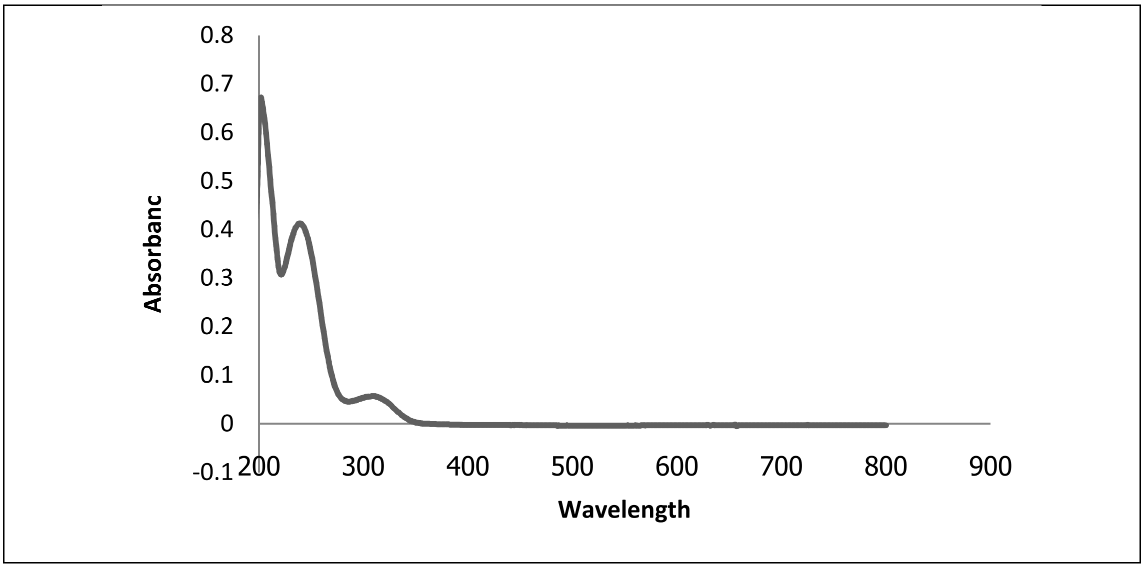

Figure 35 UV (MeOH) spectrum of compound **CC4**

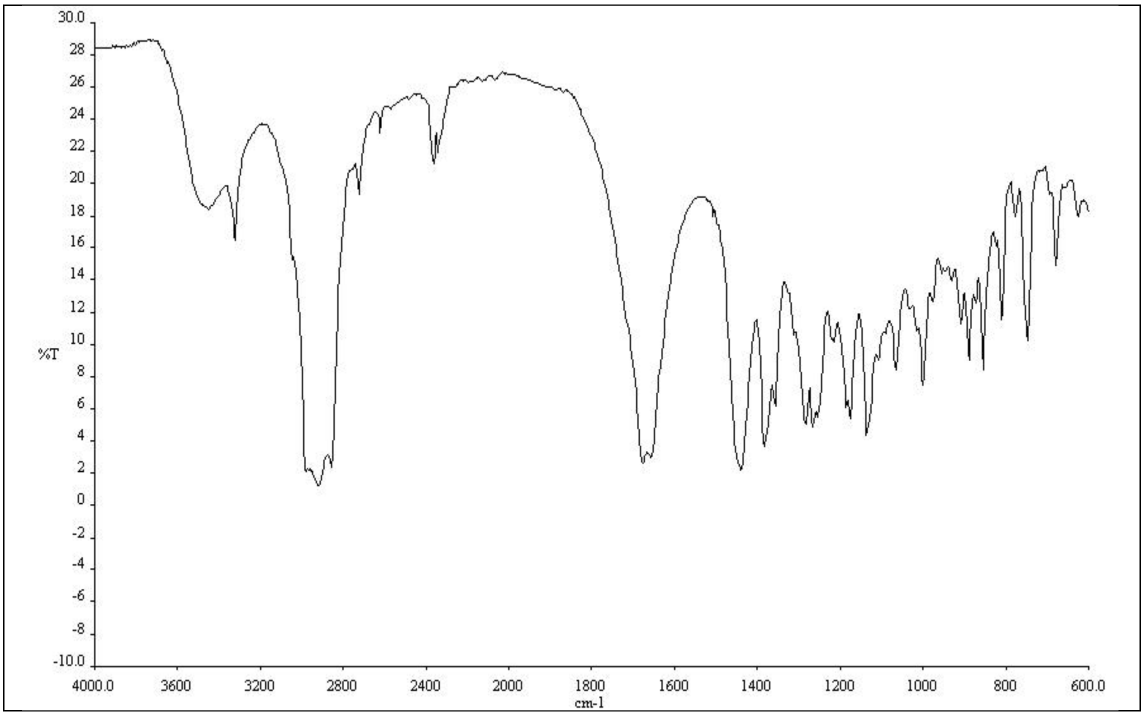

Figure 36 IR (neat) spectrum of compound **CC4**

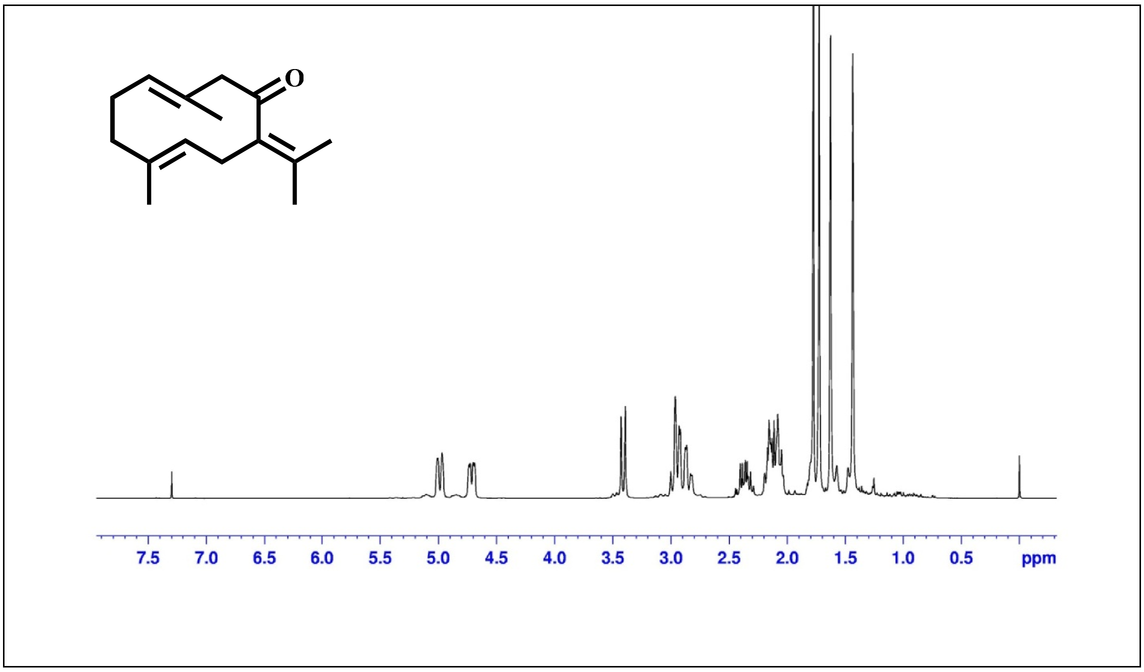

Figure 37 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CC4**

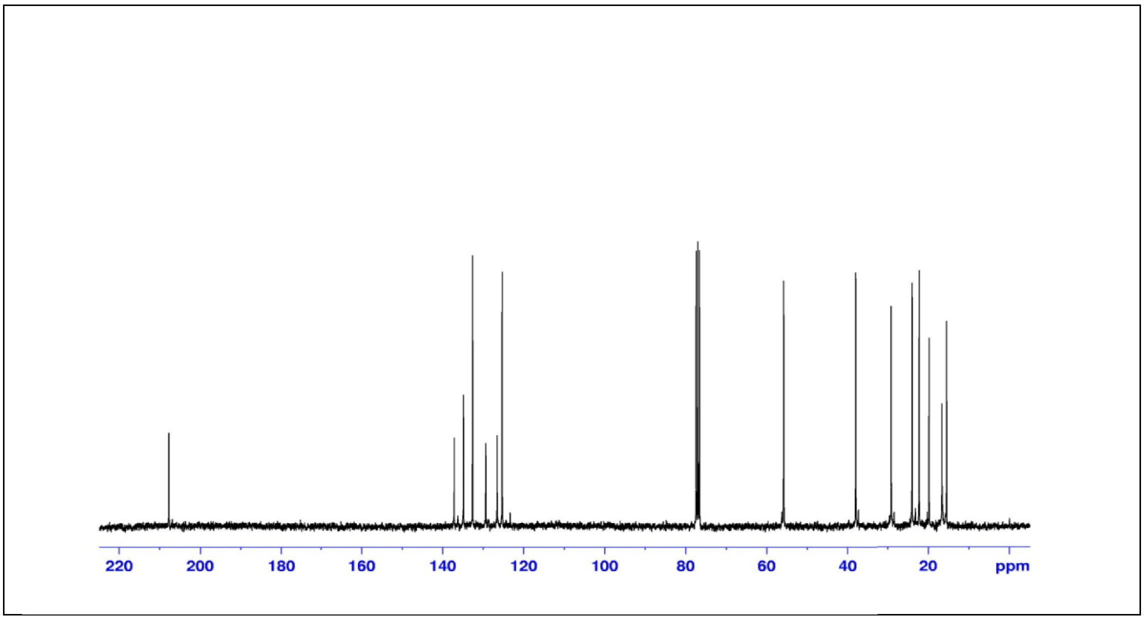

Figure 38 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CC4**

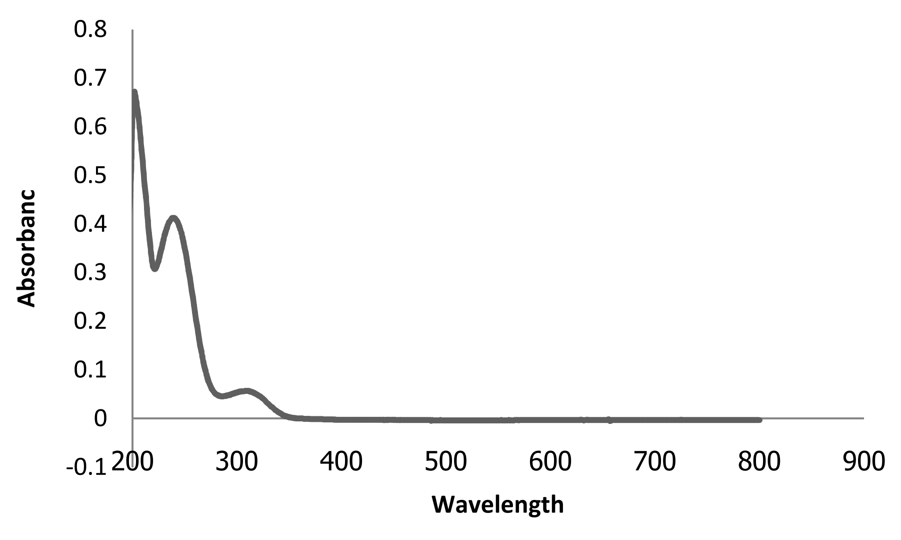

Figure 39 UV (MeOH) spectrum of compound **CC5**

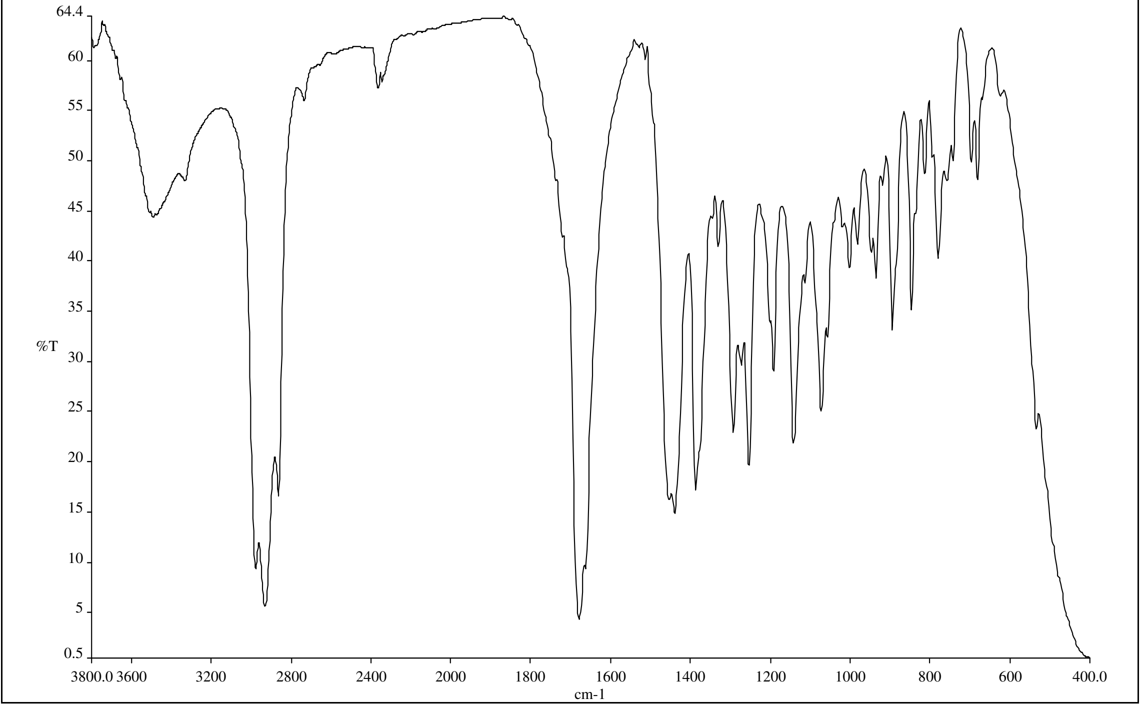

Figure 40 IR (neat) spectrum of compound **CC5**

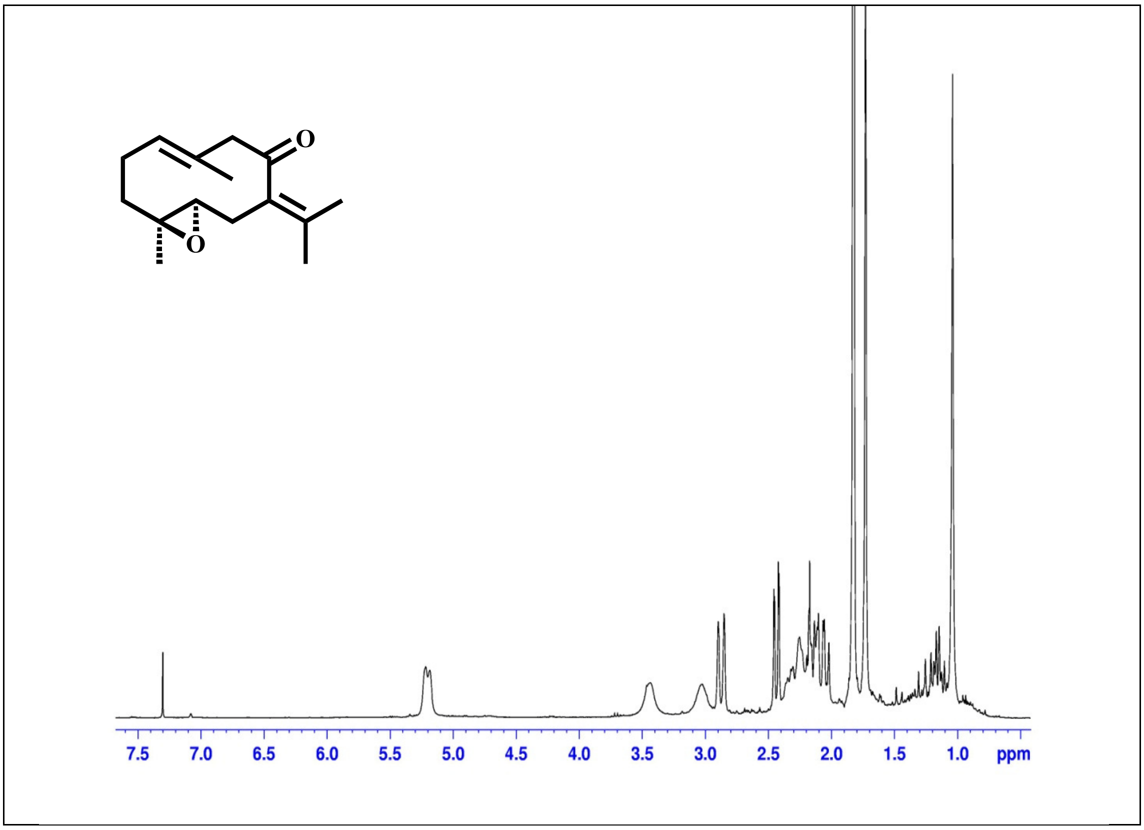

Figure 41 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **CC5**

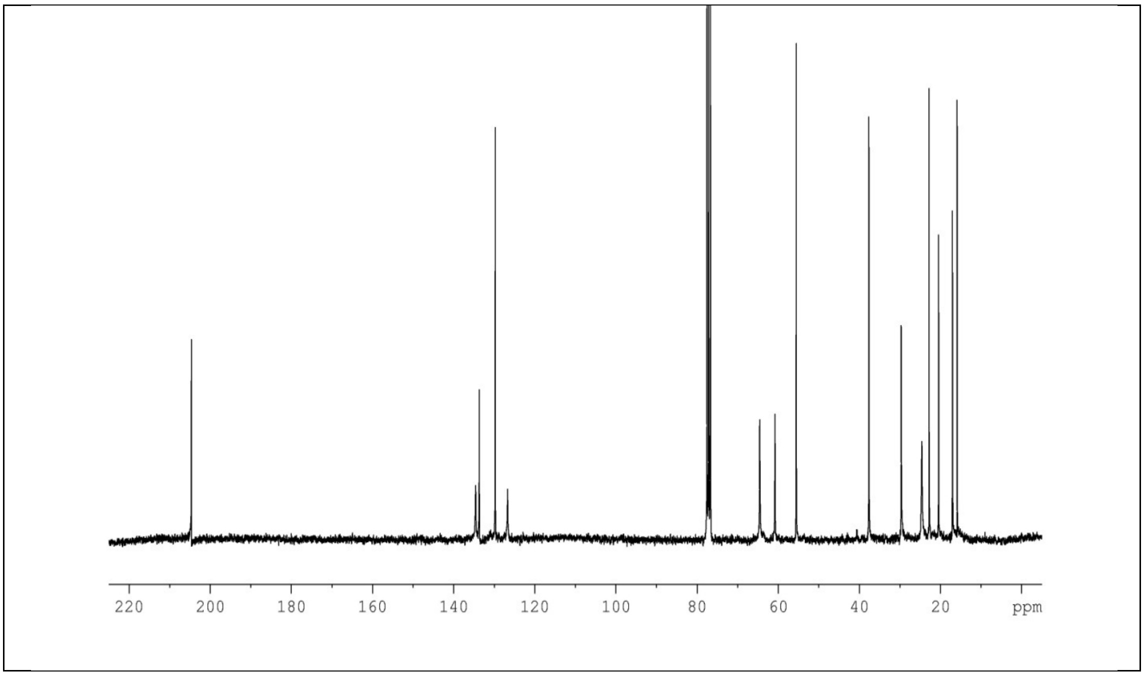

Figure 42 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **CC5**

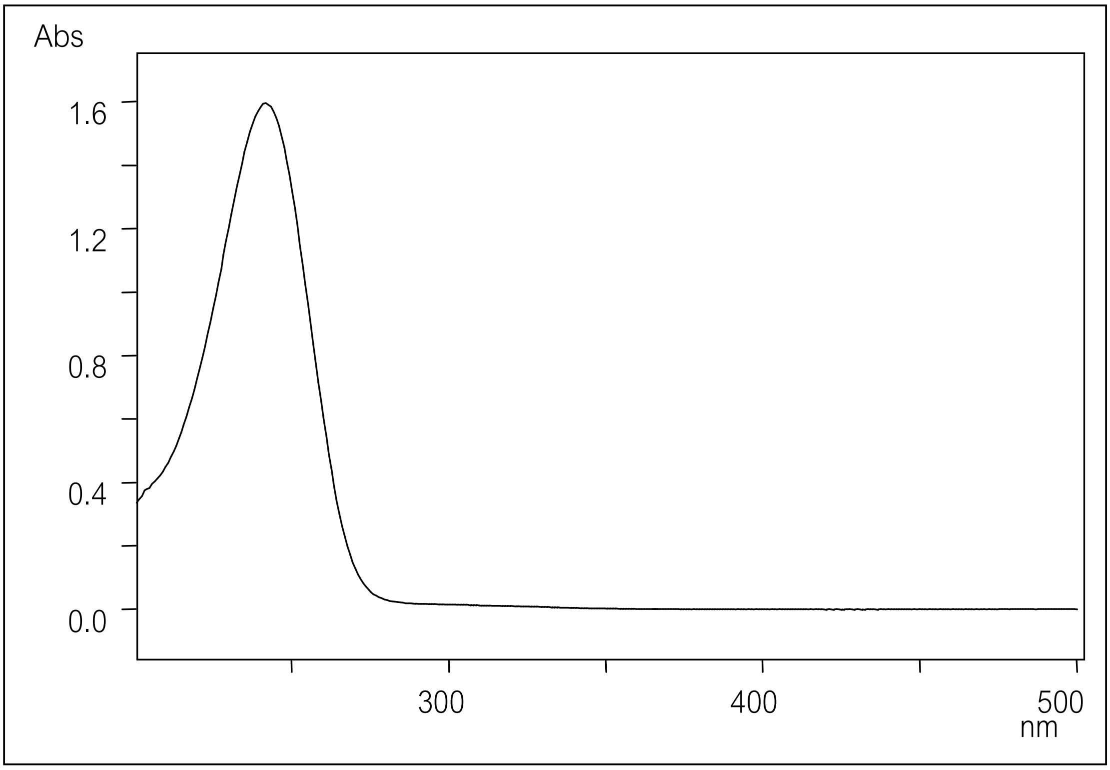

Figure 43 UV (MeOH) spectrum of compound **CC6**

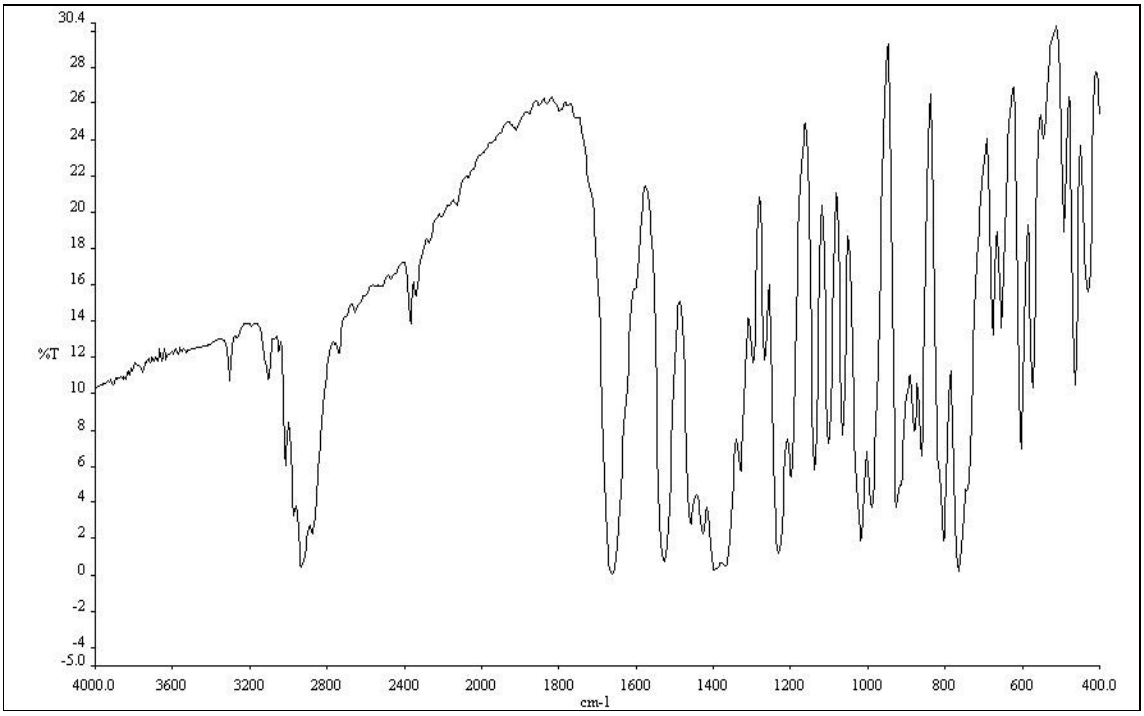

Figure 44 IR (KBr) spectrum of compound **CC6**

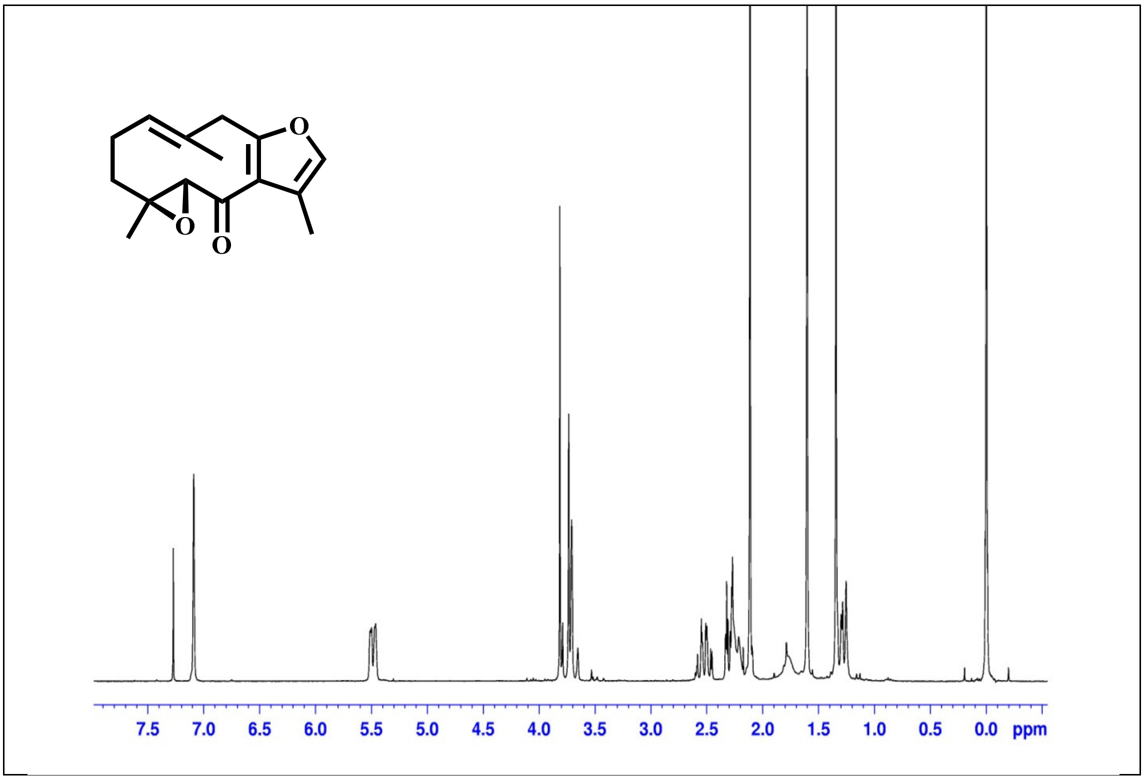

Figure 45 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CC6**

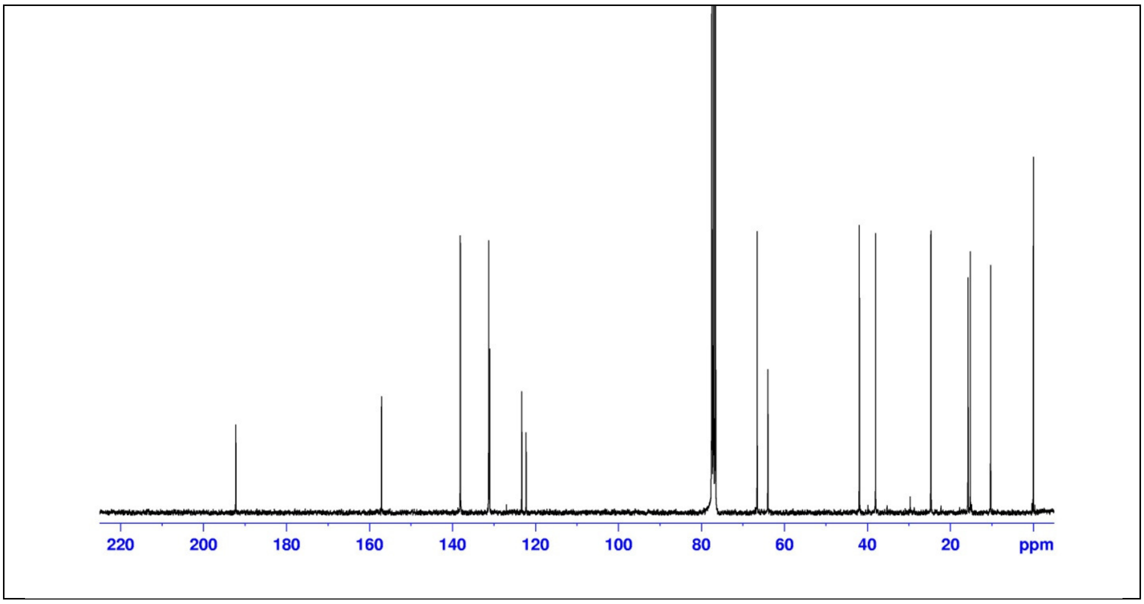

Figure 46 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CC6**

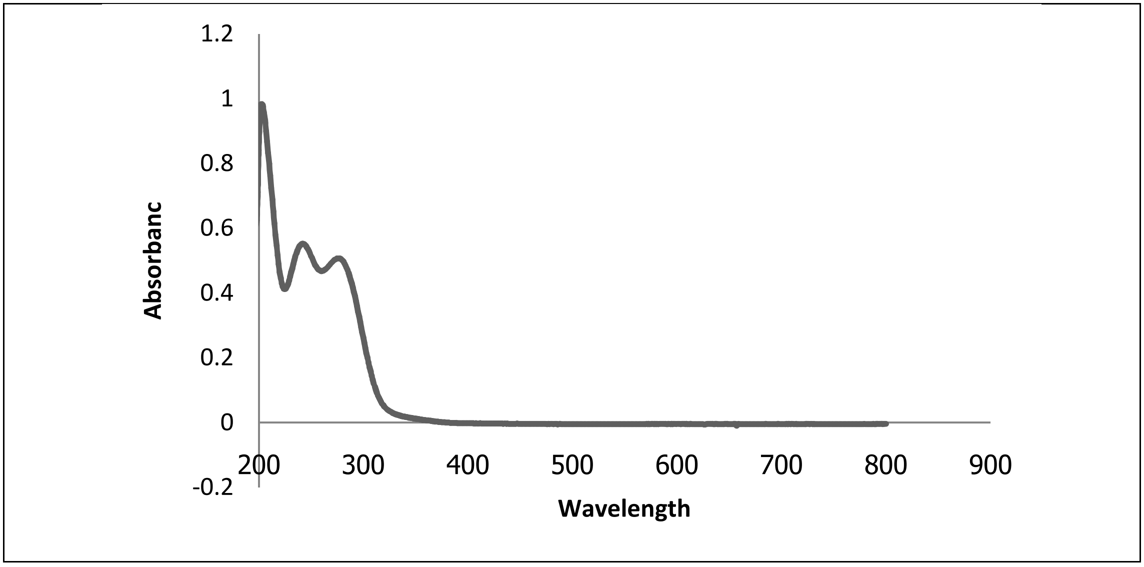

Figure 47 UV (MeOH) spectrum of compound **CC7**

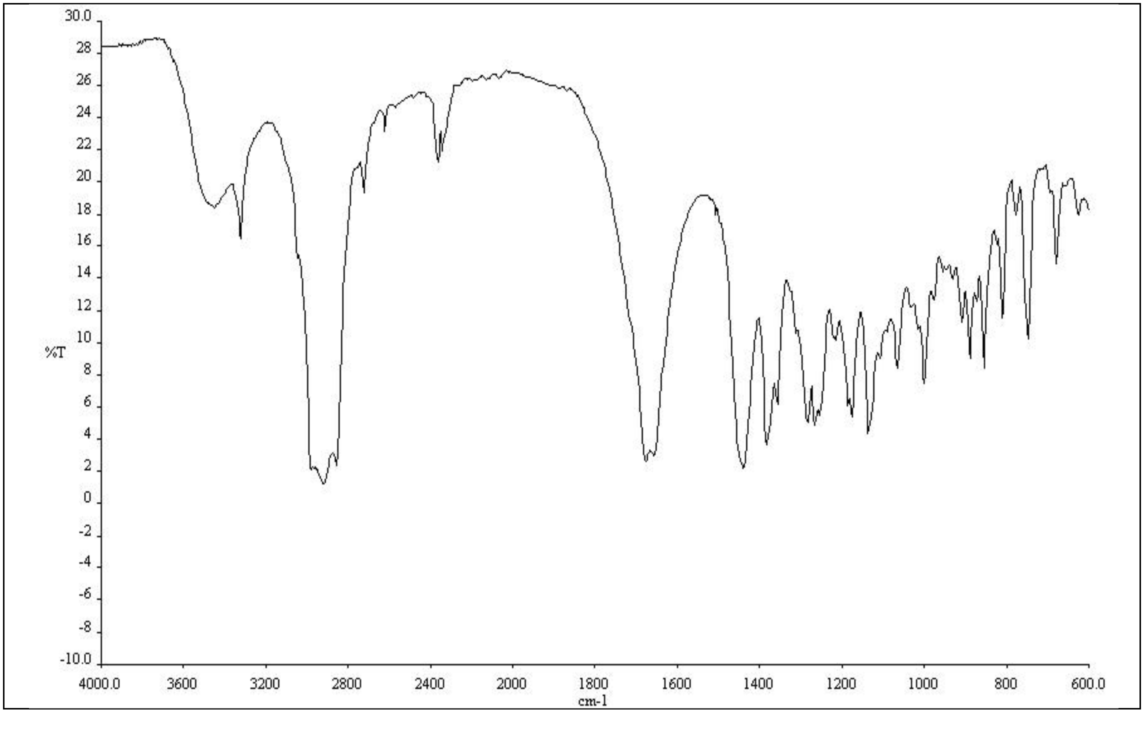

Figure 48 IR (neat) spectrum of compound **CC7**

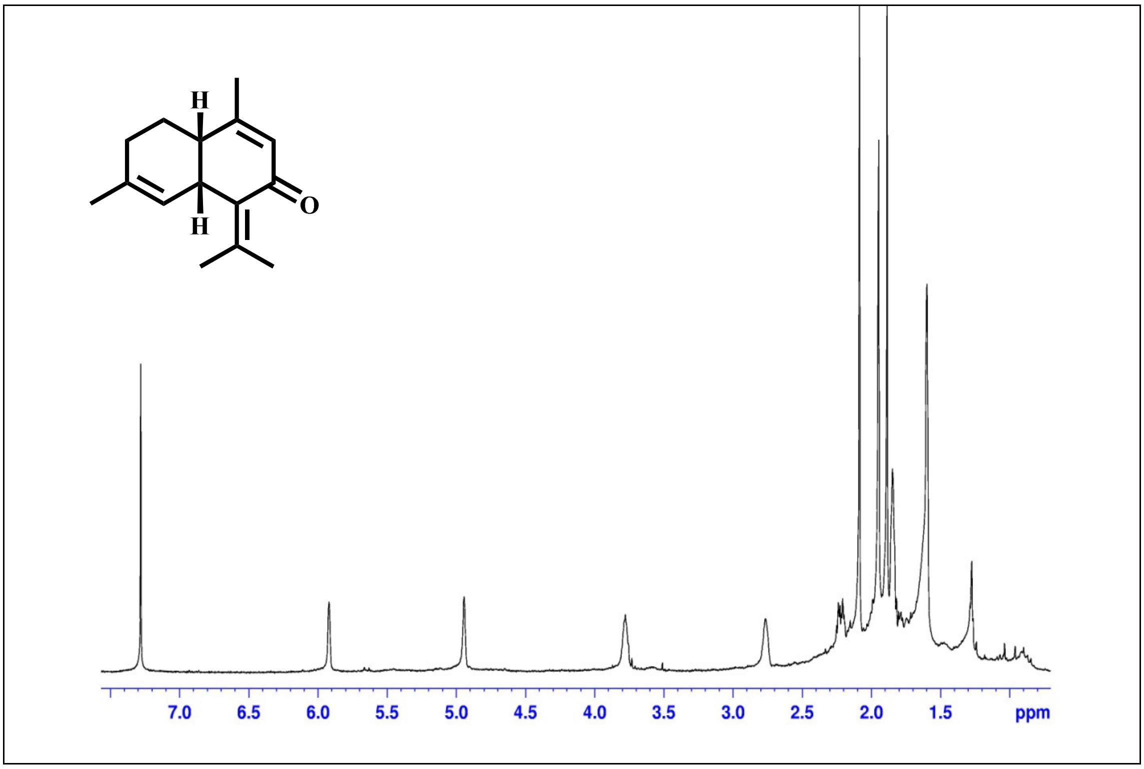

Figure 49 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **CC7**

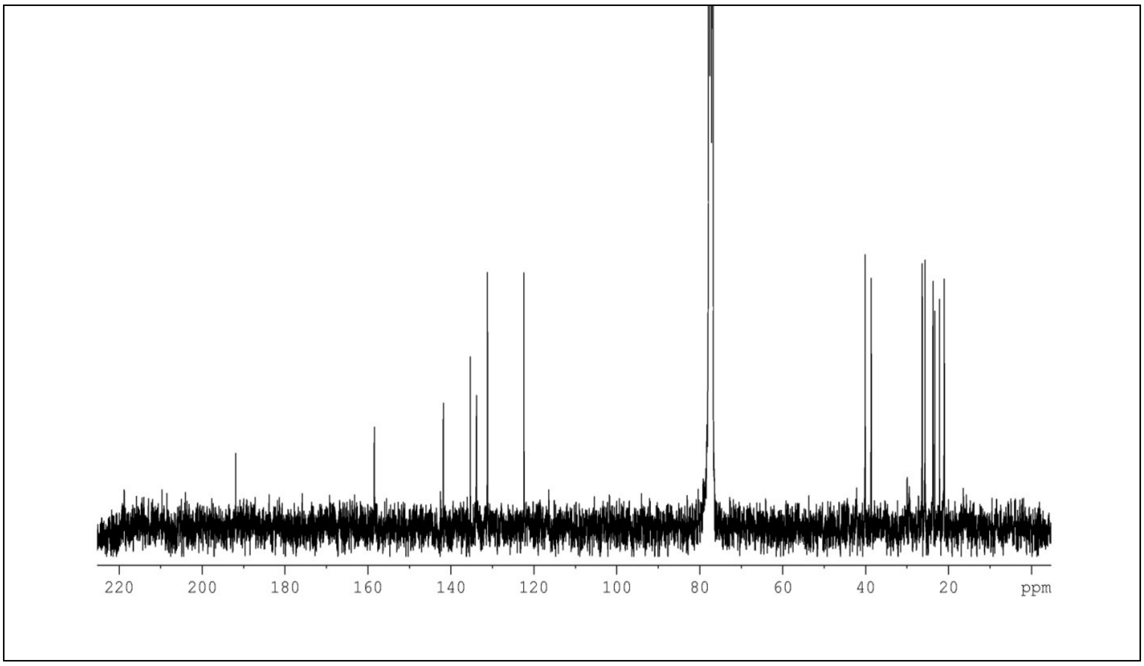

Figure 50 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **CC7**

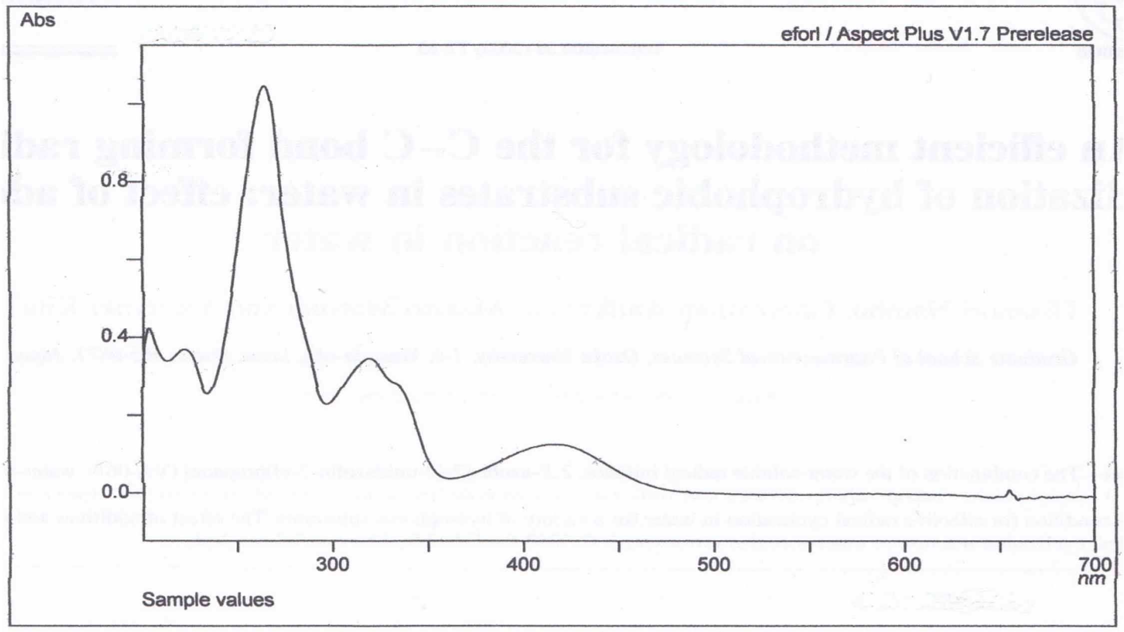

Figure 51 UV (MeOH) spectrum of compound **MNC1**

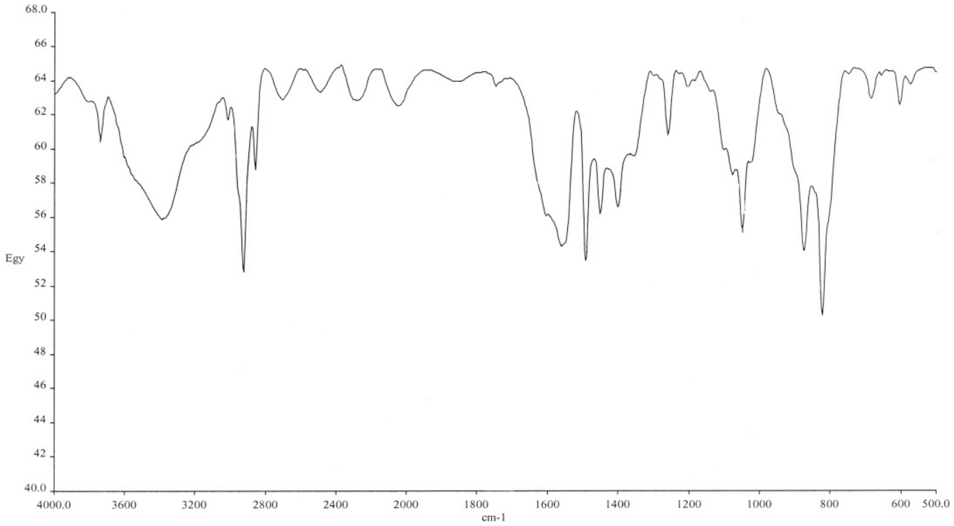

Figure 52 IR (neat) spectrum of compound **MNC1**

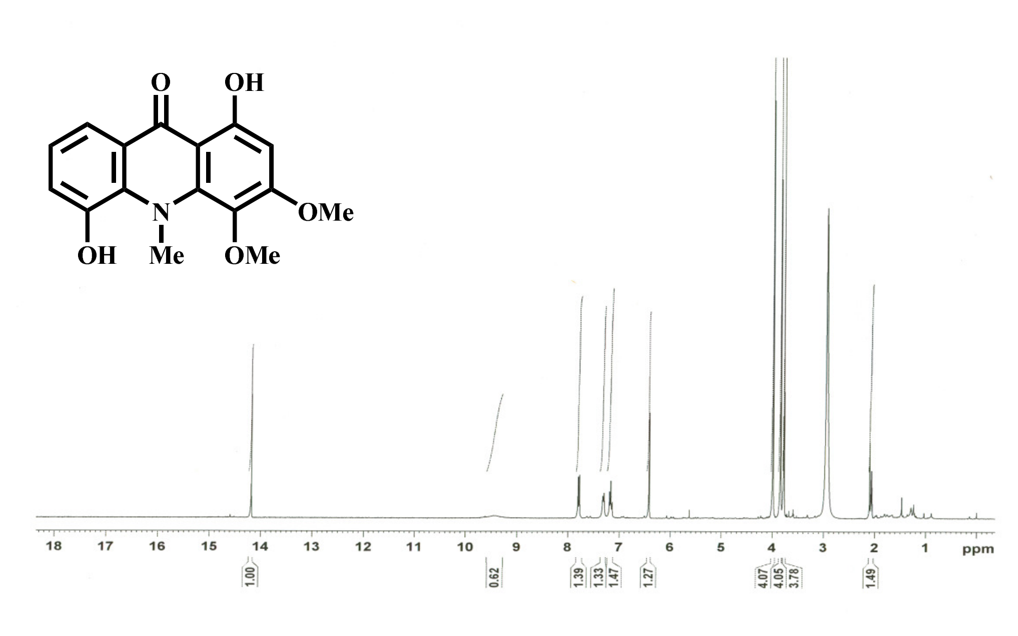

Figure 53 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **MNC1**

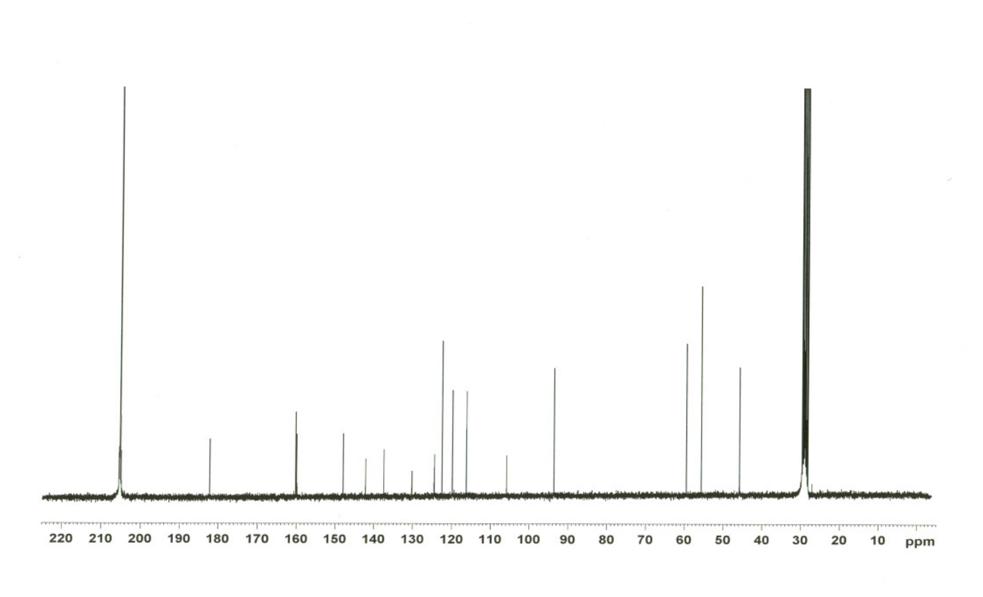

Figure 54 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **MNC1**

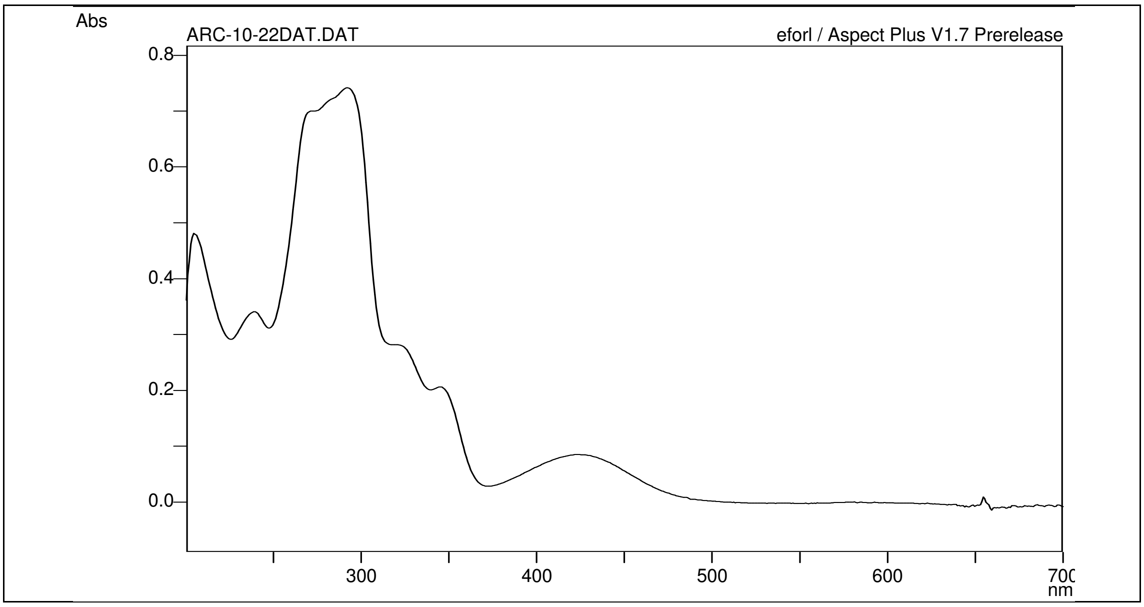

Figure 55 UV (MeOH) spectrum of compound **MNC2**

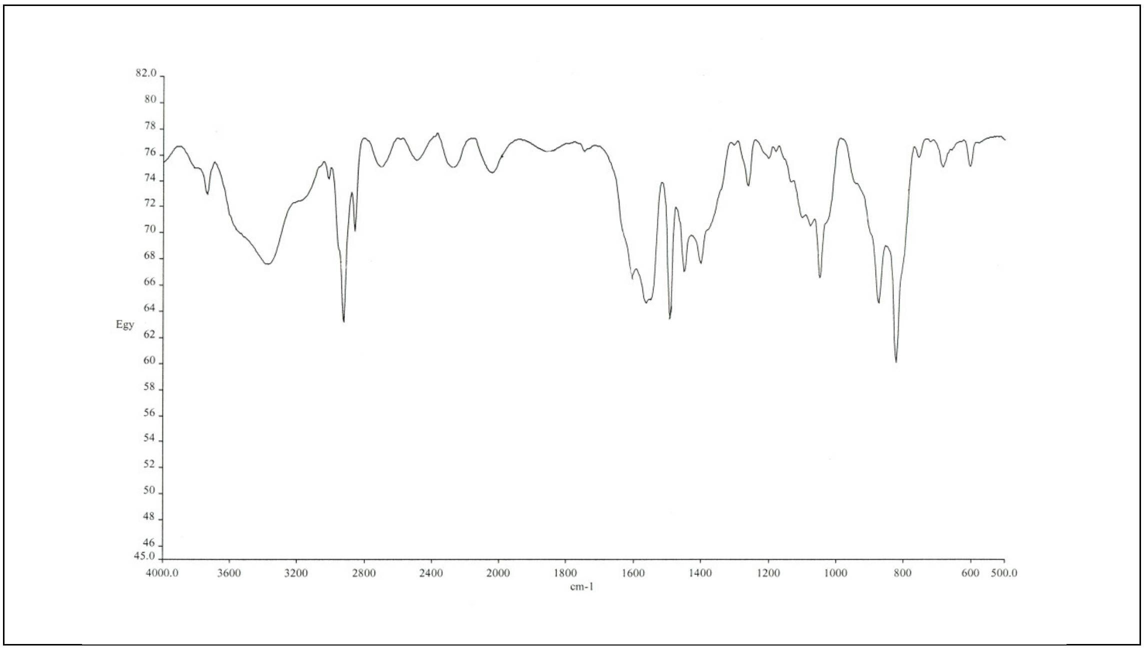

Figure 56 IR (neat) spectrum of compound **MNC2**

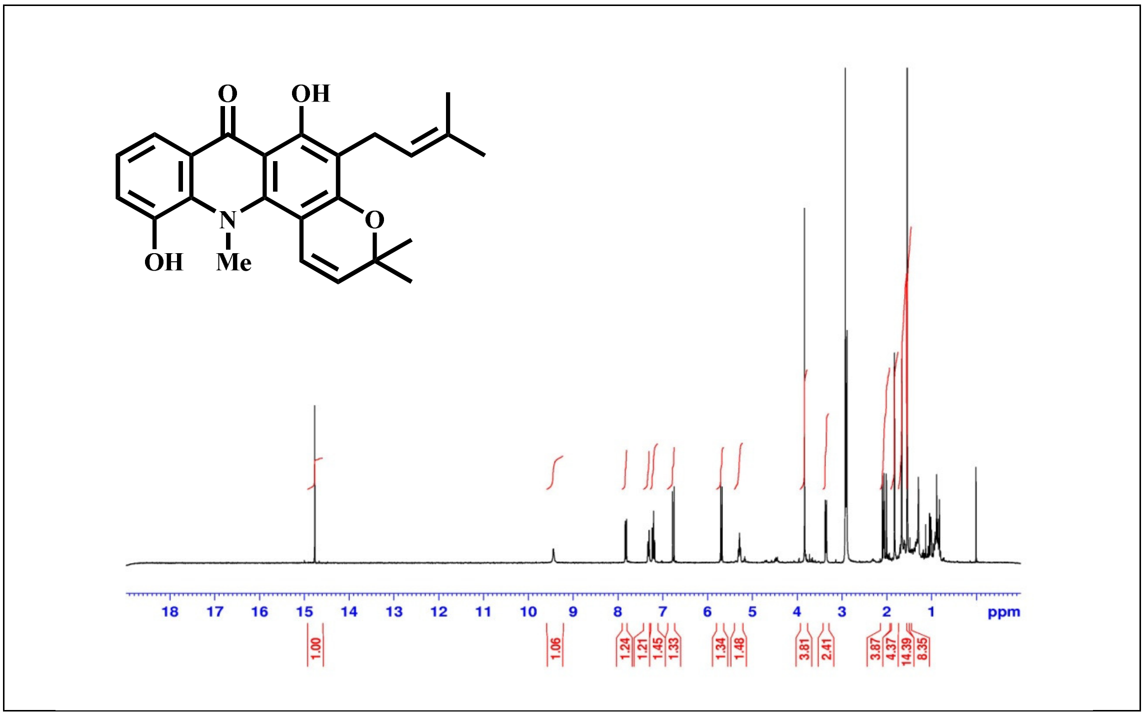

Figure 57 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **MNC2**

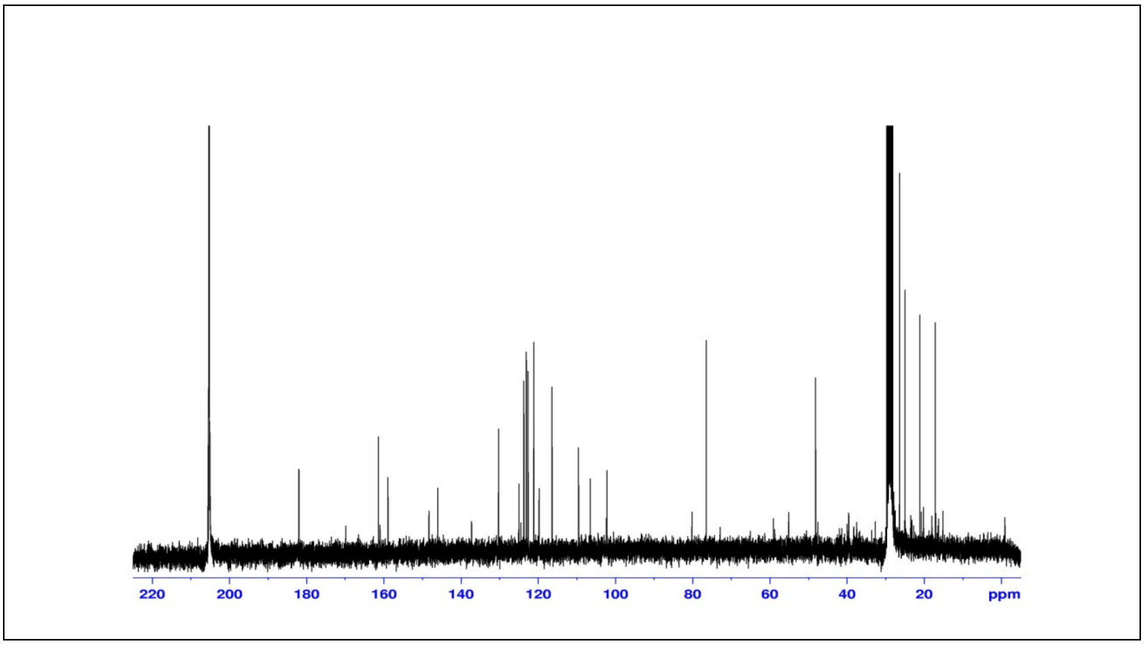

Figure 58 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **MNC2**

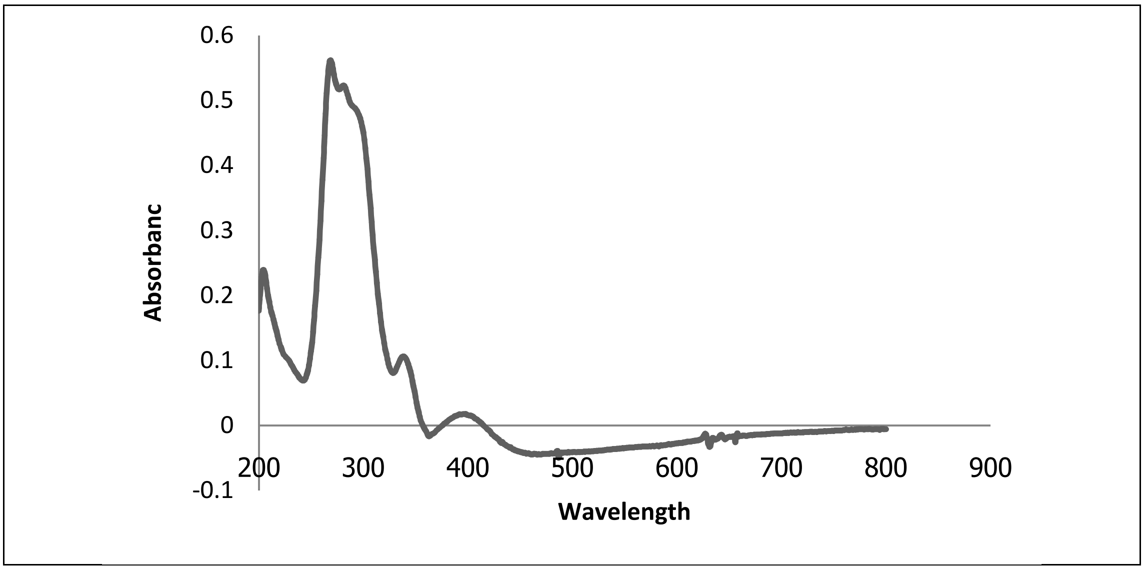

Figure 59 UV (MeOH) spectrum of compound **MNC3**

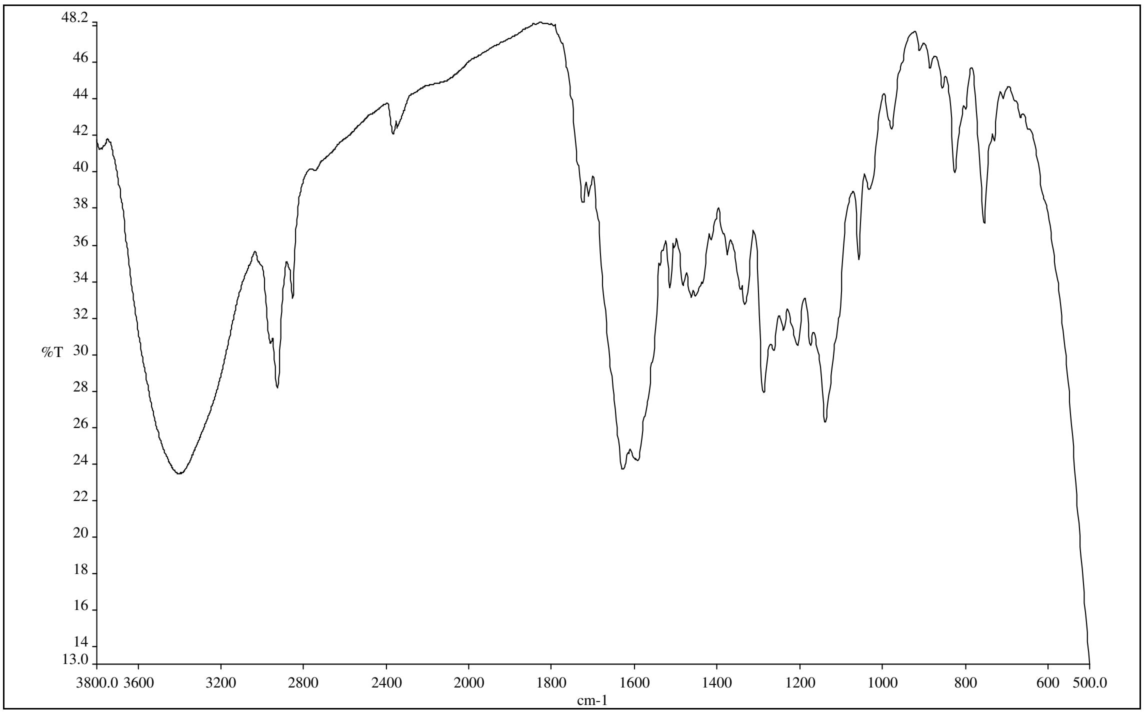

Figure 60 IR (neat) spectrum of compound **MNC3**

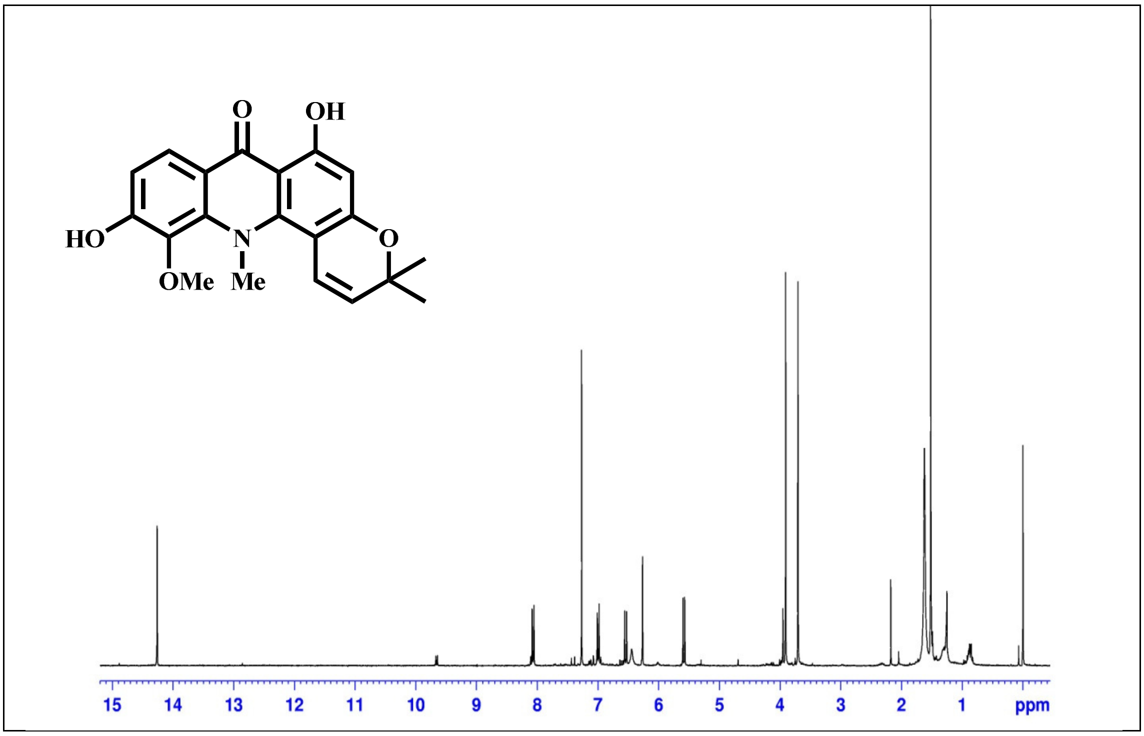

Figure 61 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **MNC3**

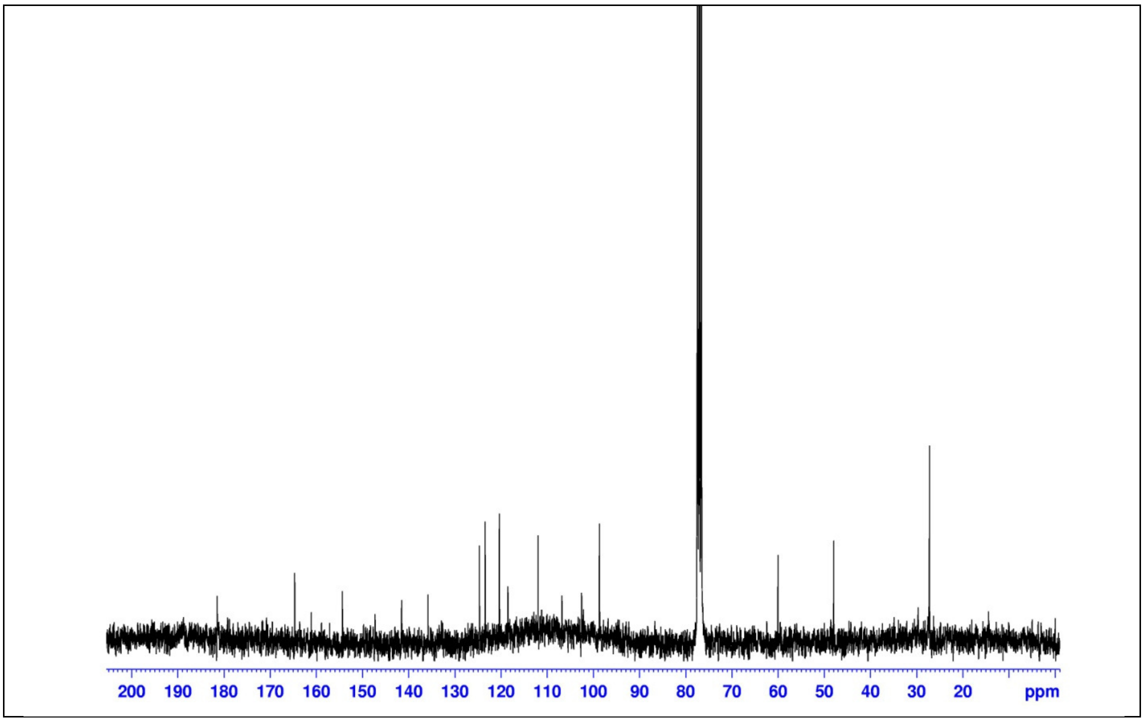

Figure 62 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **MNC3**

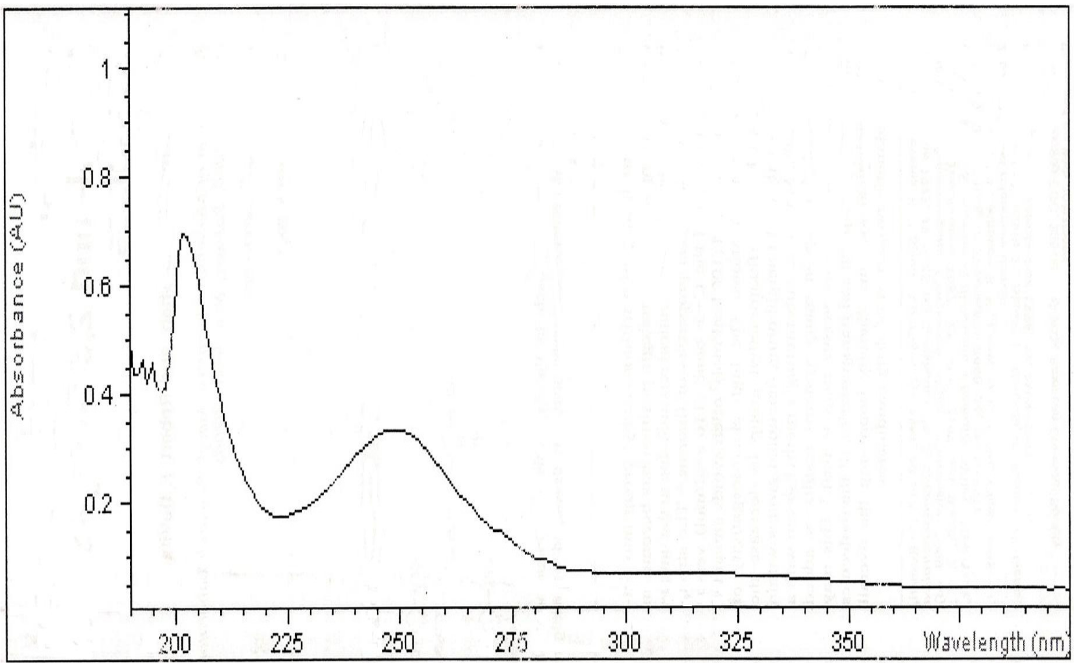

Figure 63 UV (MeOH) spectrum of compound **MNC4**

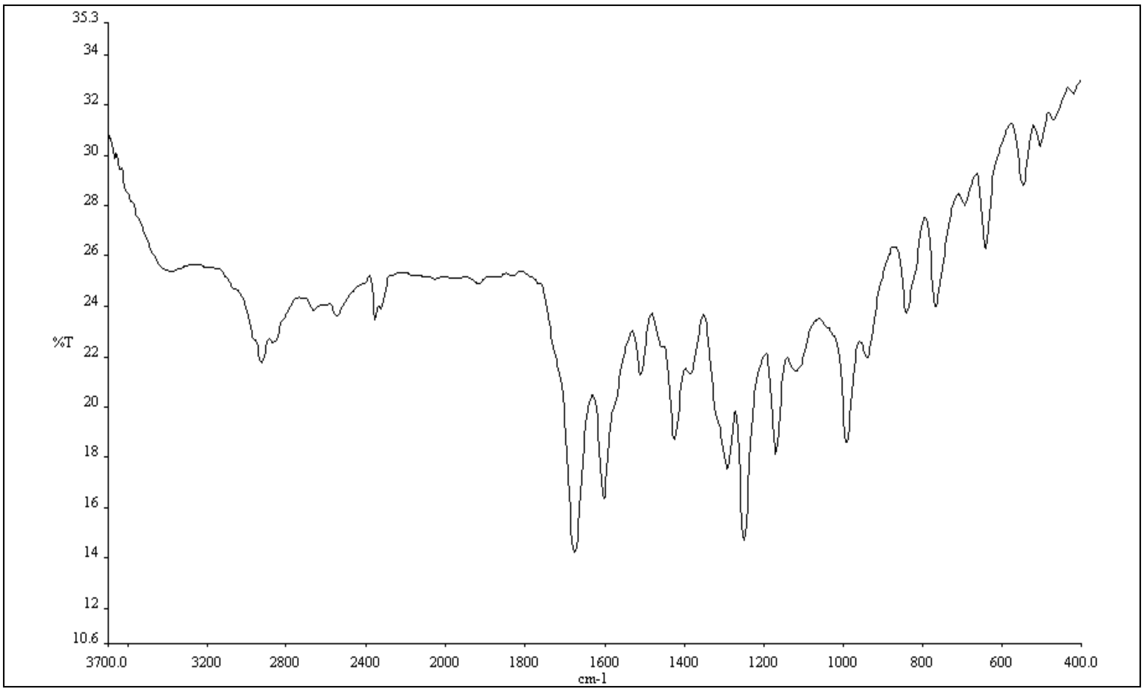

Figure 64 IR (neat) spectrum of compound **MNC4**

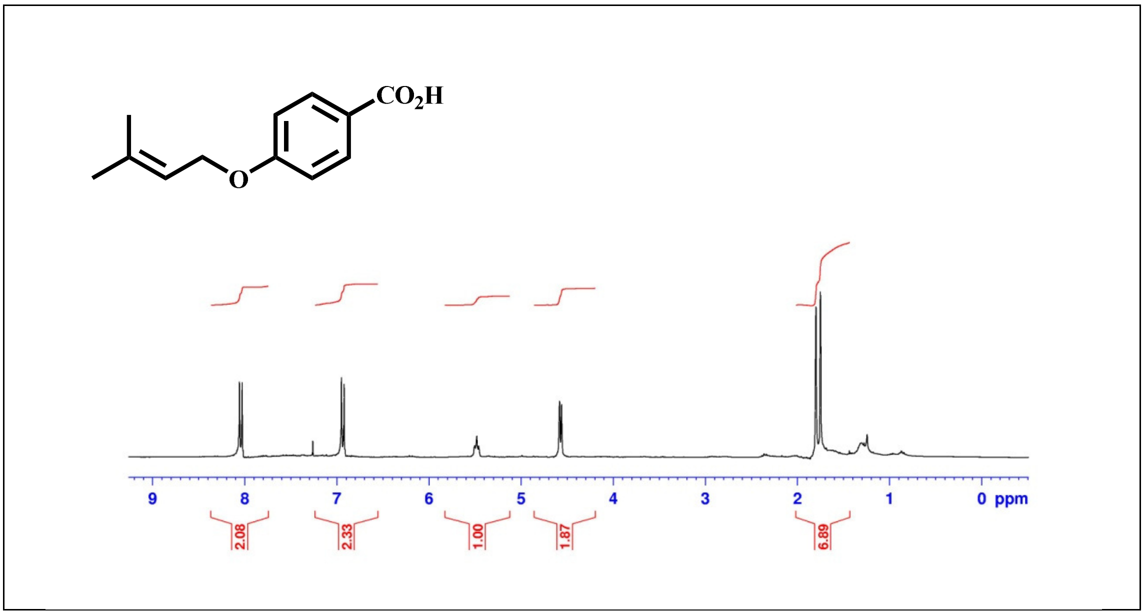

Figure 65 ^{1}H NMR (300 MHz) (CDCl$_3$) of compound **MNC4**

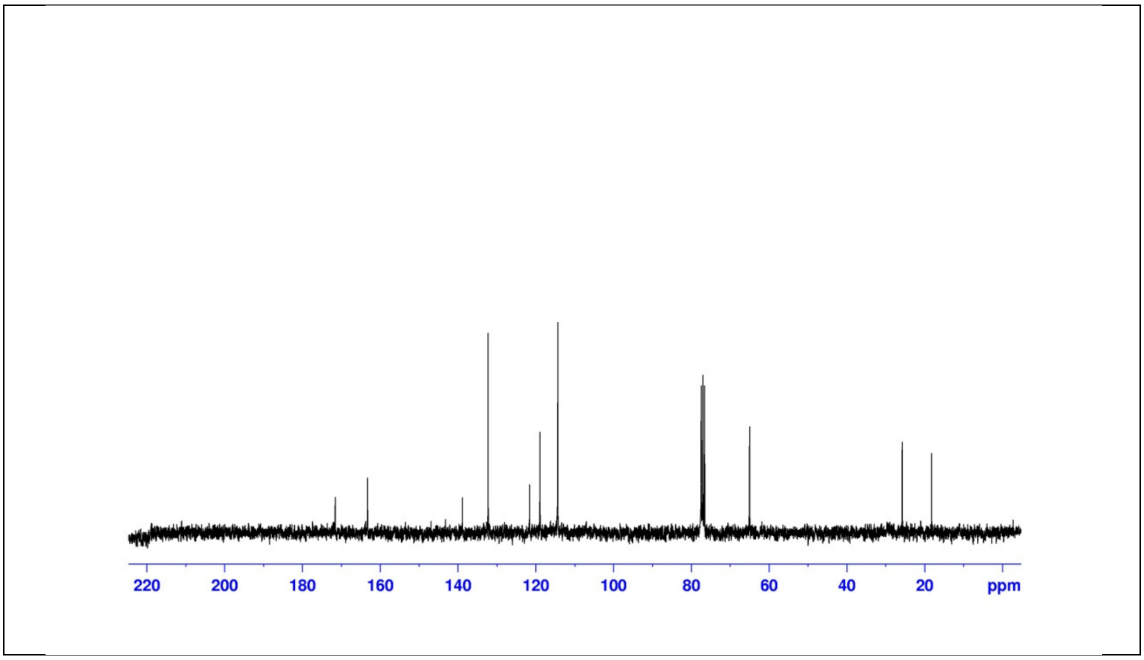

Figure 66 ^{13}C NMR (75 MHz) (CDCl$_3$) of compound **MNC4**

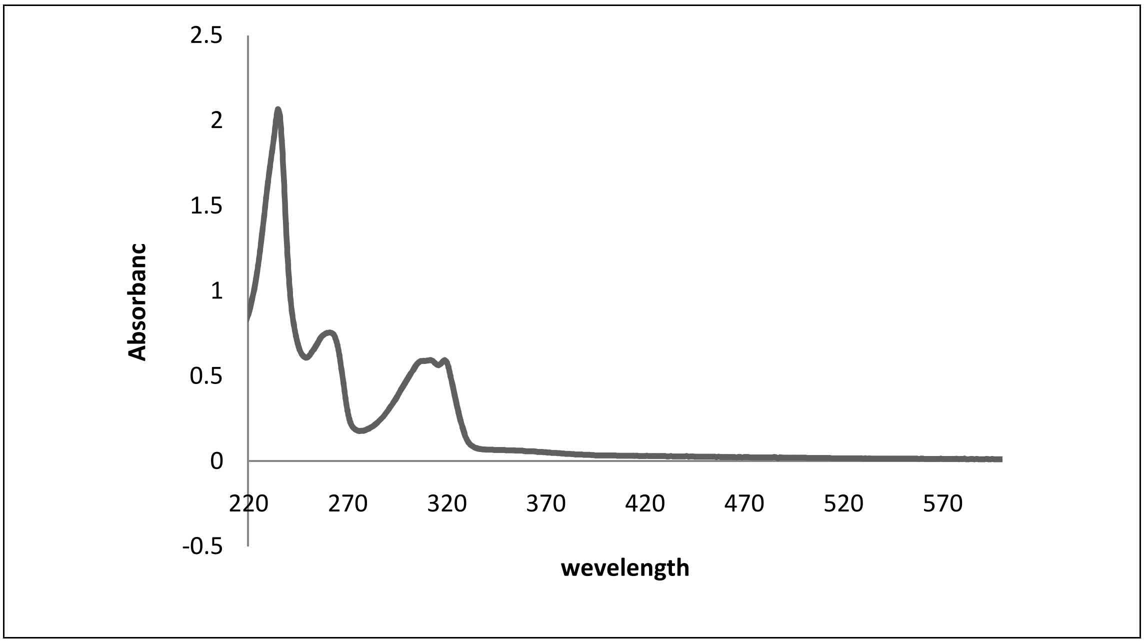

Figure 67 UV (MeOH) spectrum of compound **MNC5**

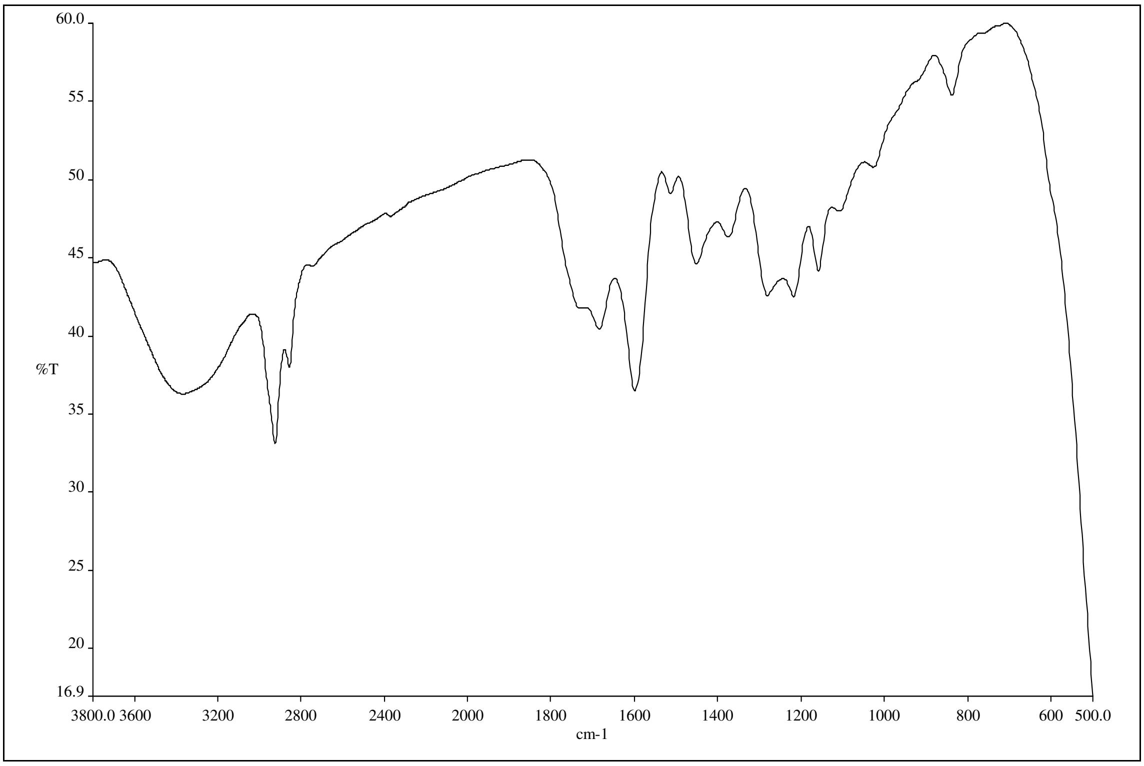

Figure 68 IR (neat) spectrum of compound **MNC5**

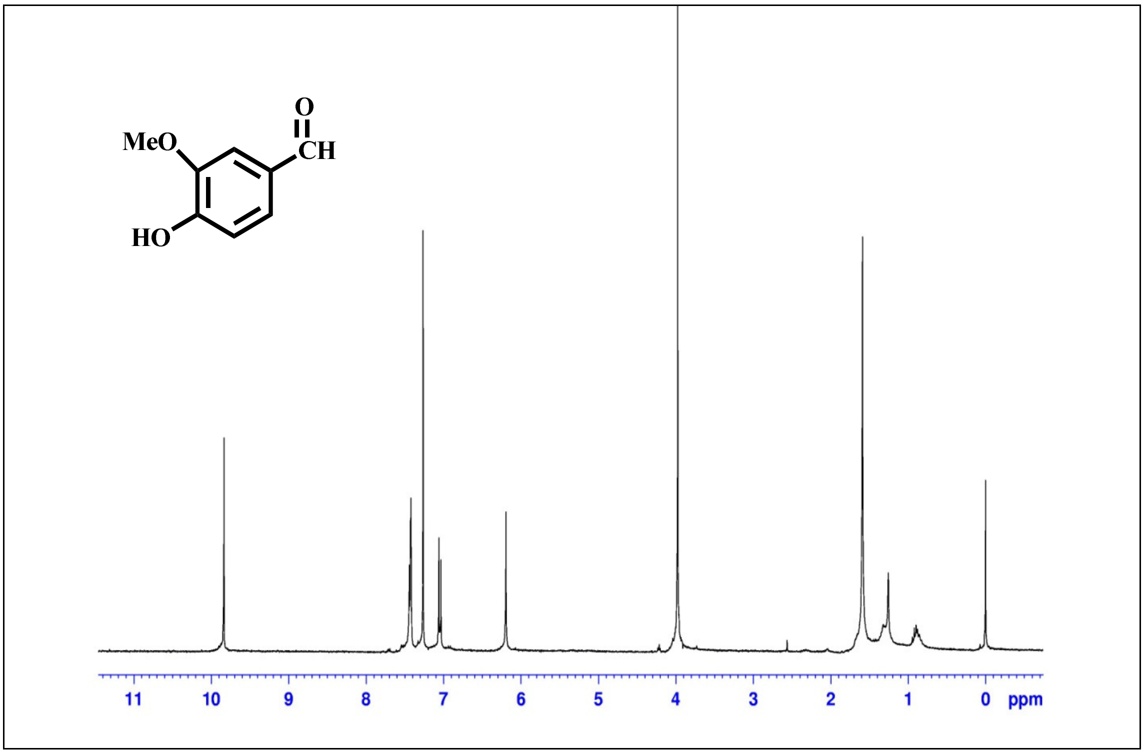

Figure 69 ^{1}H NMR (300 MHz) (CDCl$_3$) of compound **MNC5**

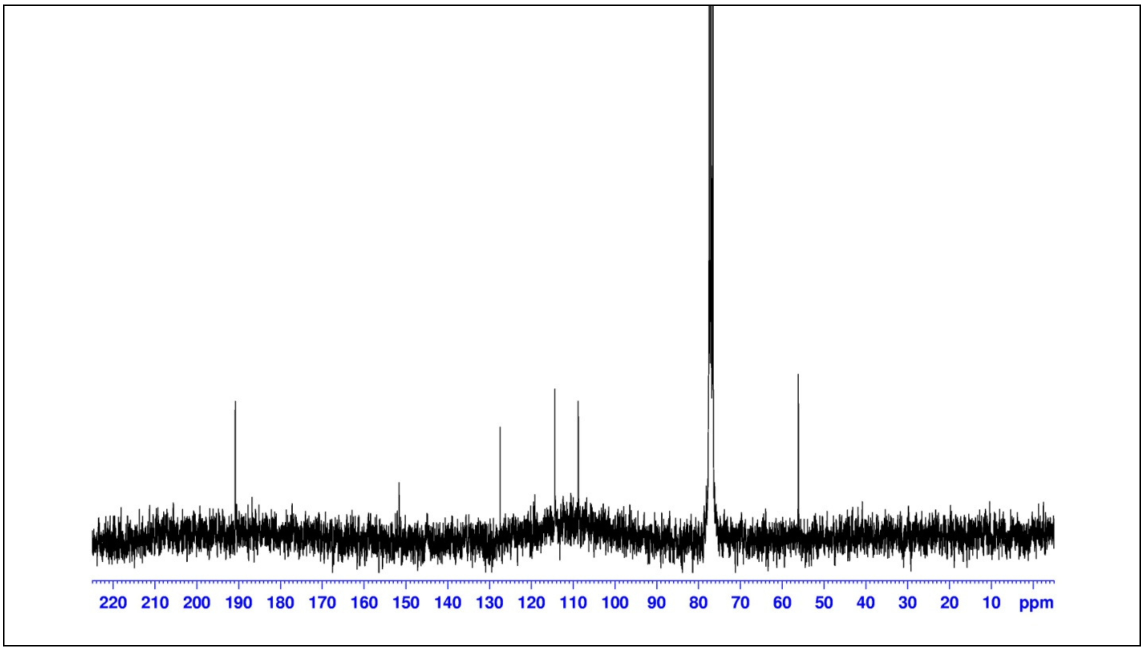

Figure 70 ^{13}C NMR (75 MHz) (CDCl$_3$) of compound **MNC5**

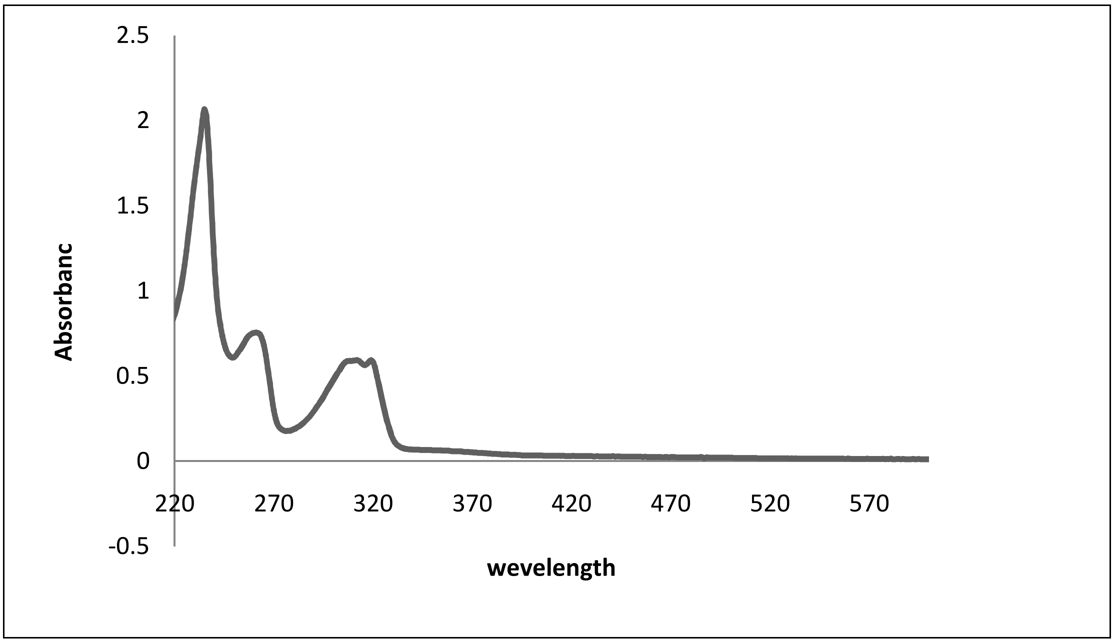

Figure 71 UV (MeOH) spectrum of compound **MNC6**

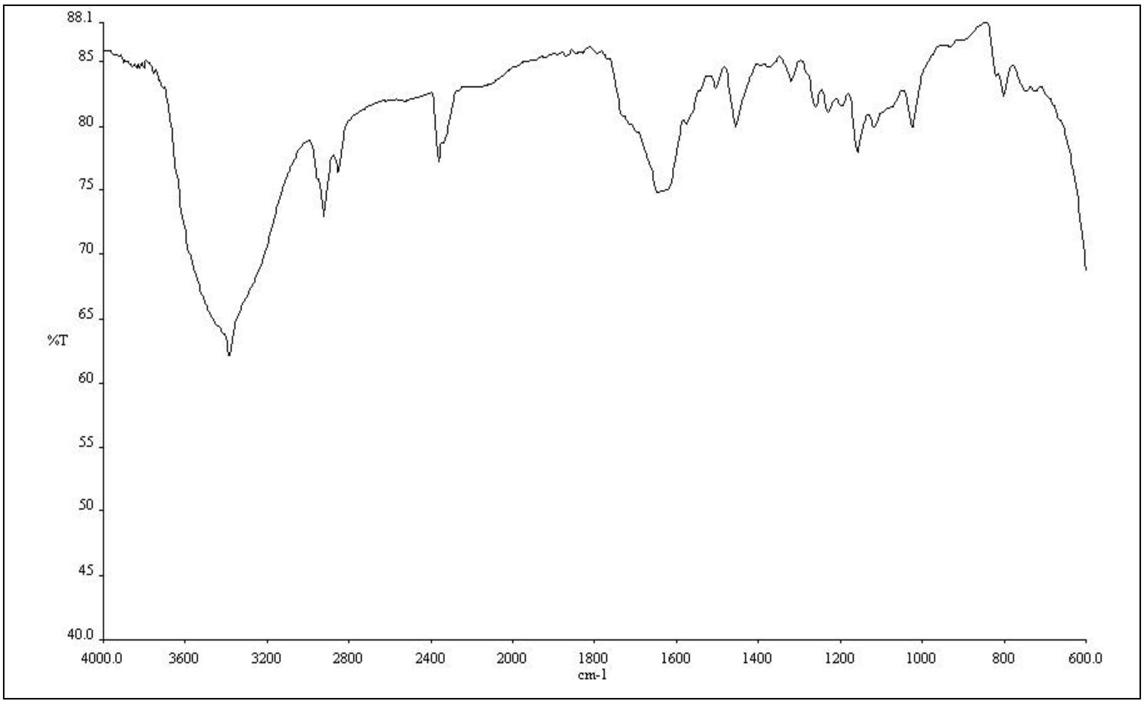

Figure 72 IR (neat) spectrum of compound **MNC6**

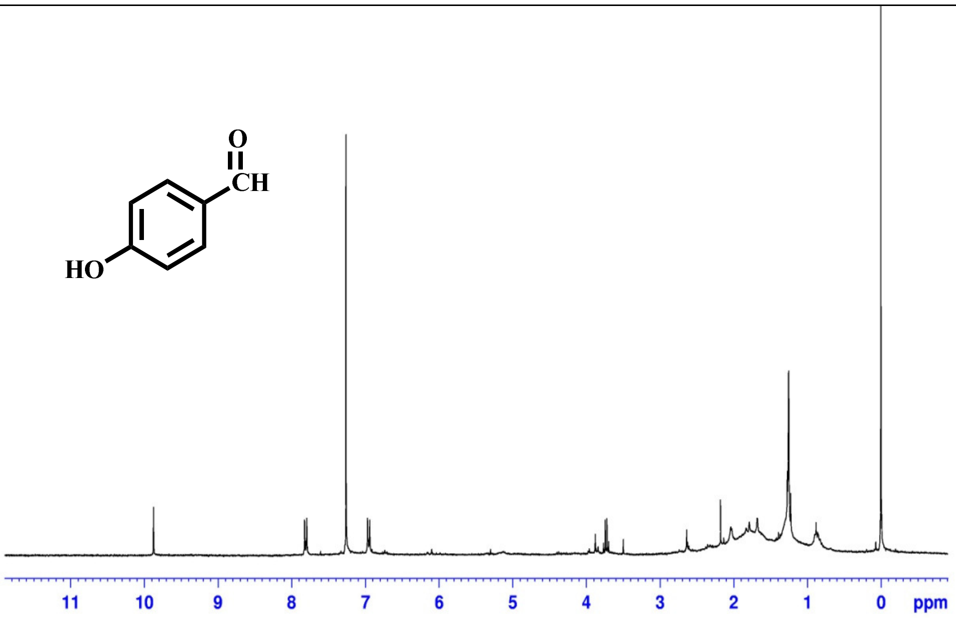

Figure 73 ^{1}H NMR (300 MHz) ($CDCl_3$) of compound **MNC6**

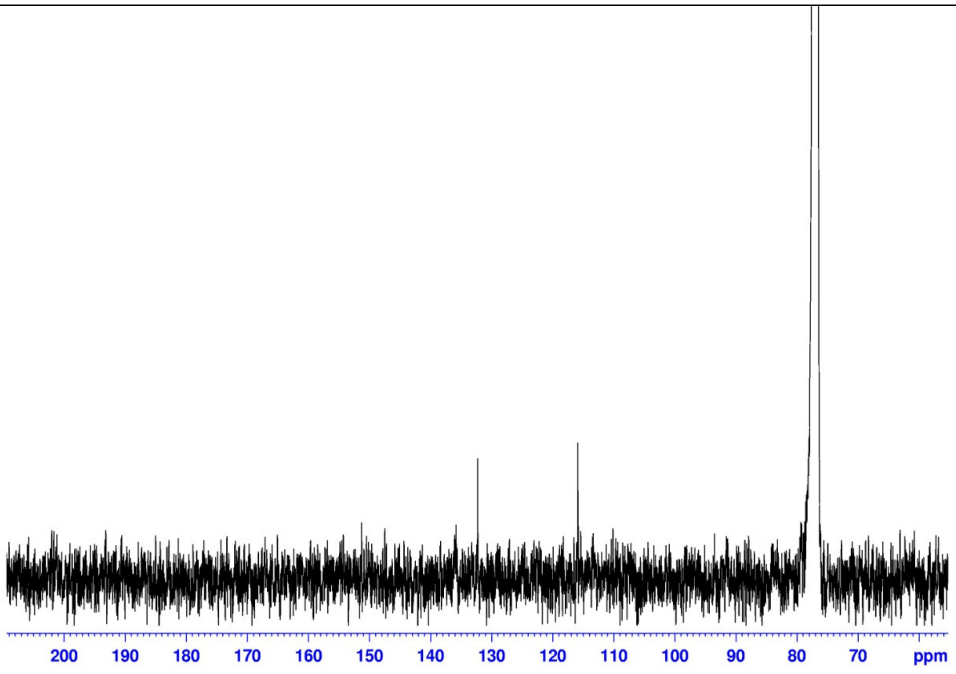

Figure 74 ^{13}C NMR (75 MHz) ($CDCl_3$) of compound **MNC6**

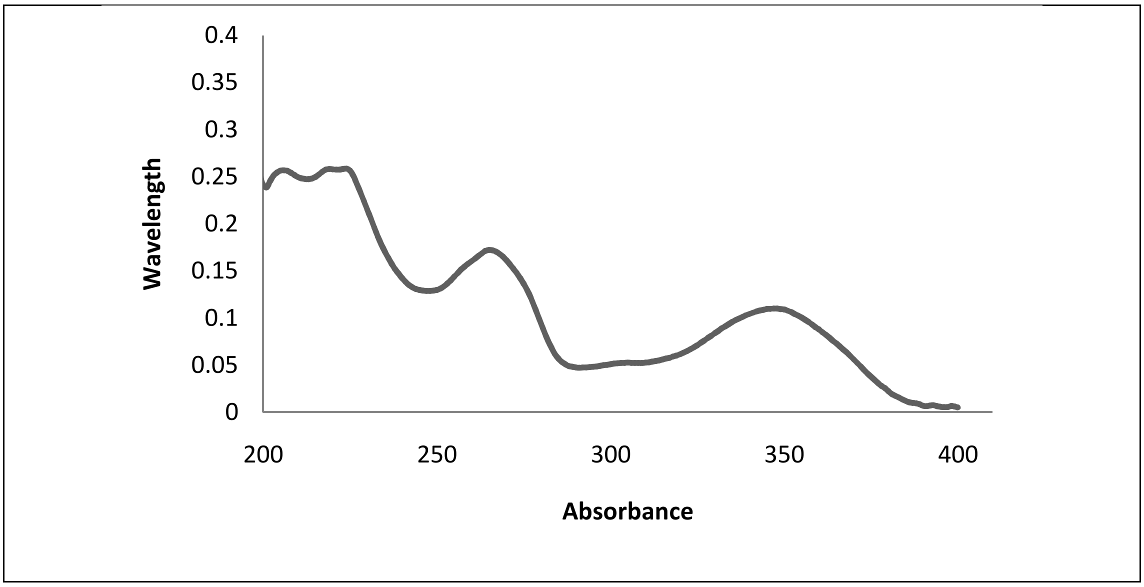

Figure 75 UV (MeOH) spectrum of compound **MNC7**

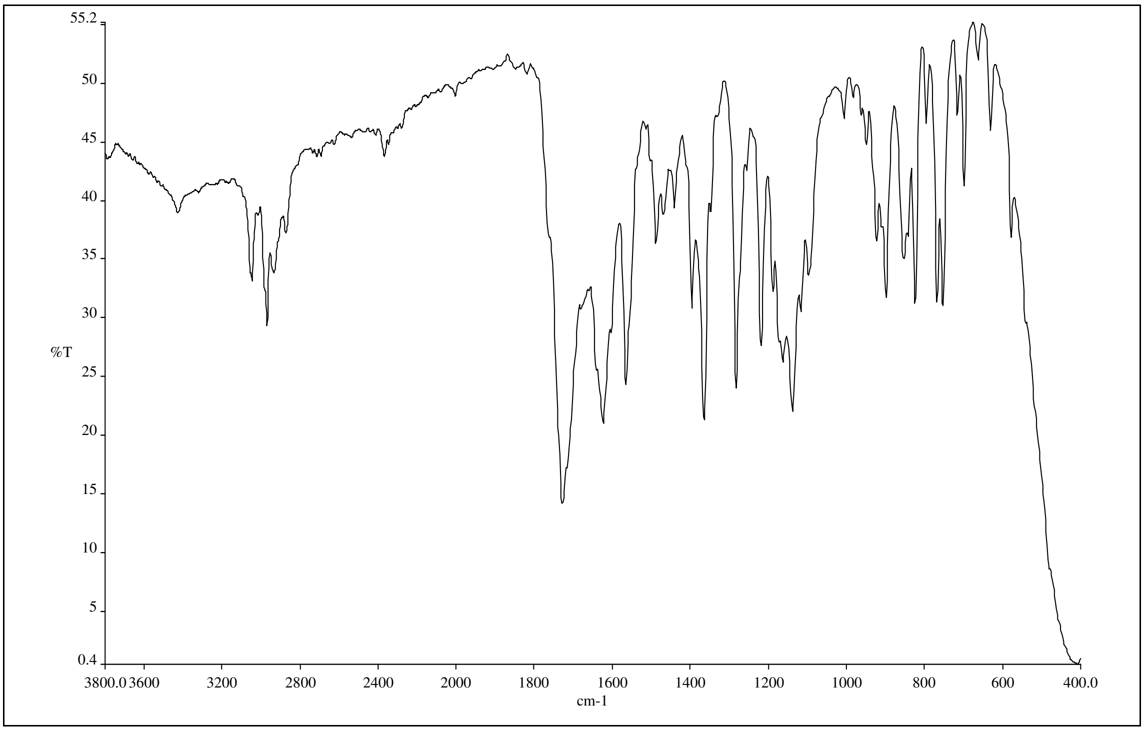

Figure 76 IR (neat) spectrum of compound **MNC7**

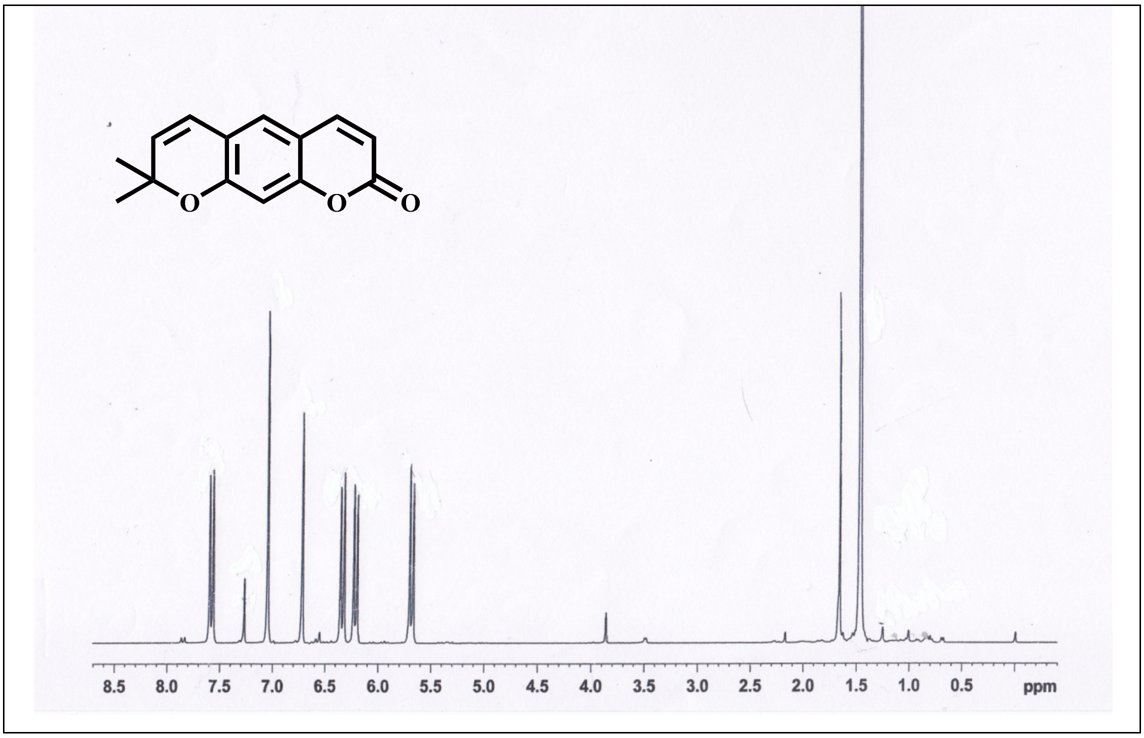

Figure 77 ^{1}H NMR (300 MHz) ($CDCl_3$) of compound **MNC7**

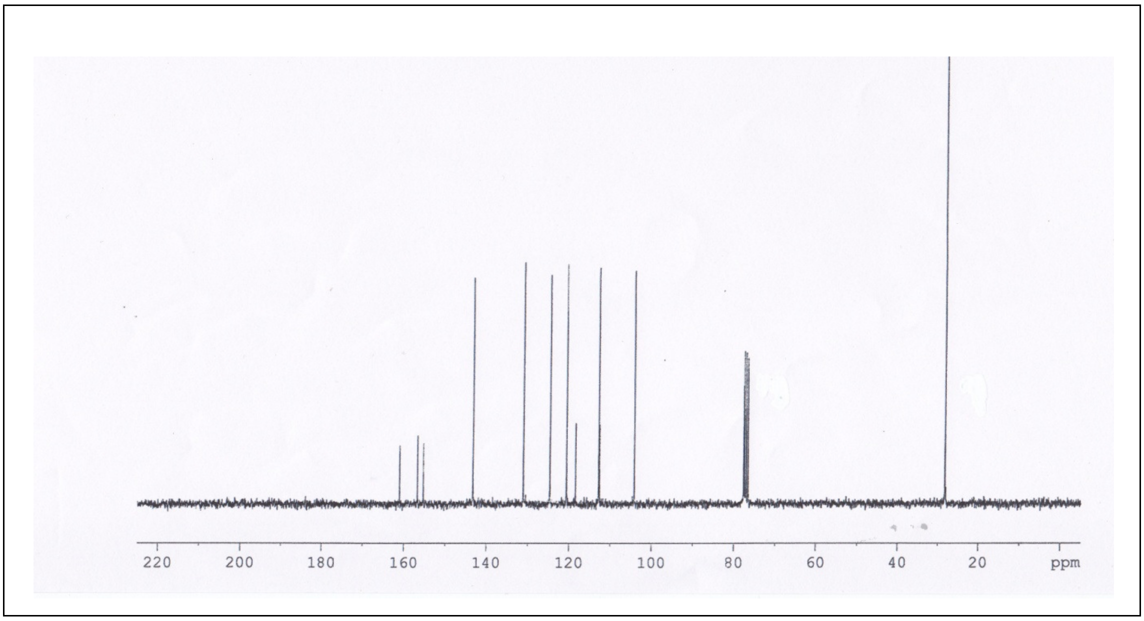

Figure 78 ^{13}C NMR (75 MHz) ($CDCl_3$) of compound **MNC7**

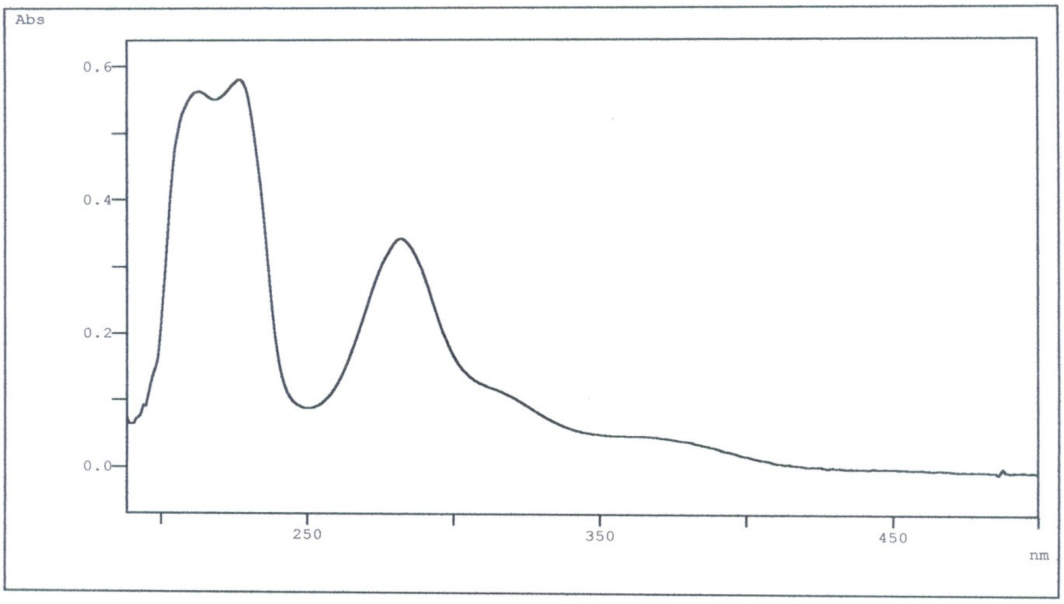

Figure 79 UV (MeOH) spectrum of compound **MNC8**

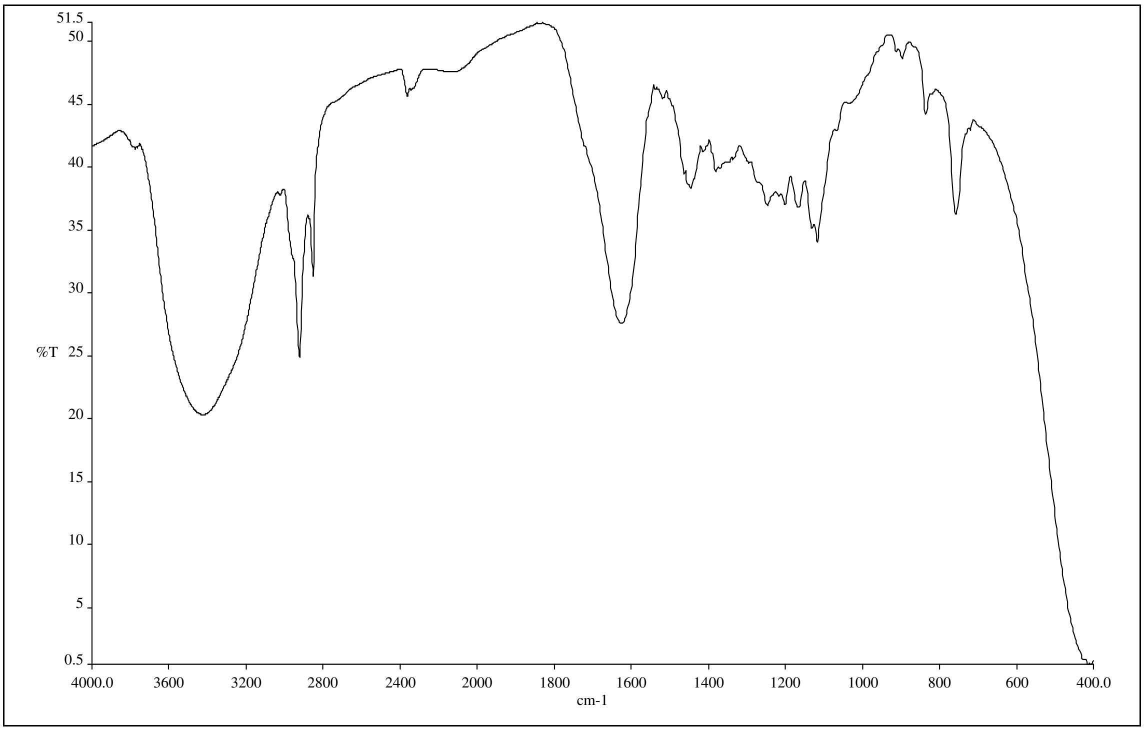

Figure 80 IR (neat) spectrum of compound **MNC8**

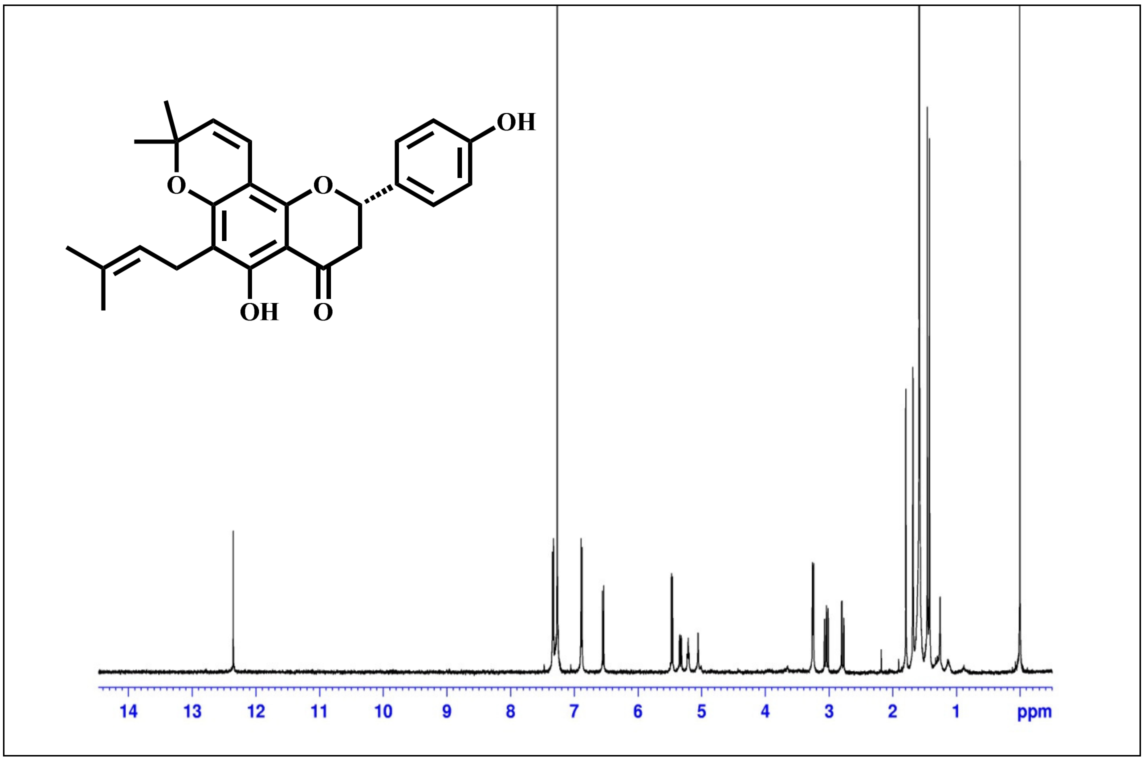

Figure 81 ^{1}H NMR (300 MHz) ($CDCl_3$) of compound **MNC8**

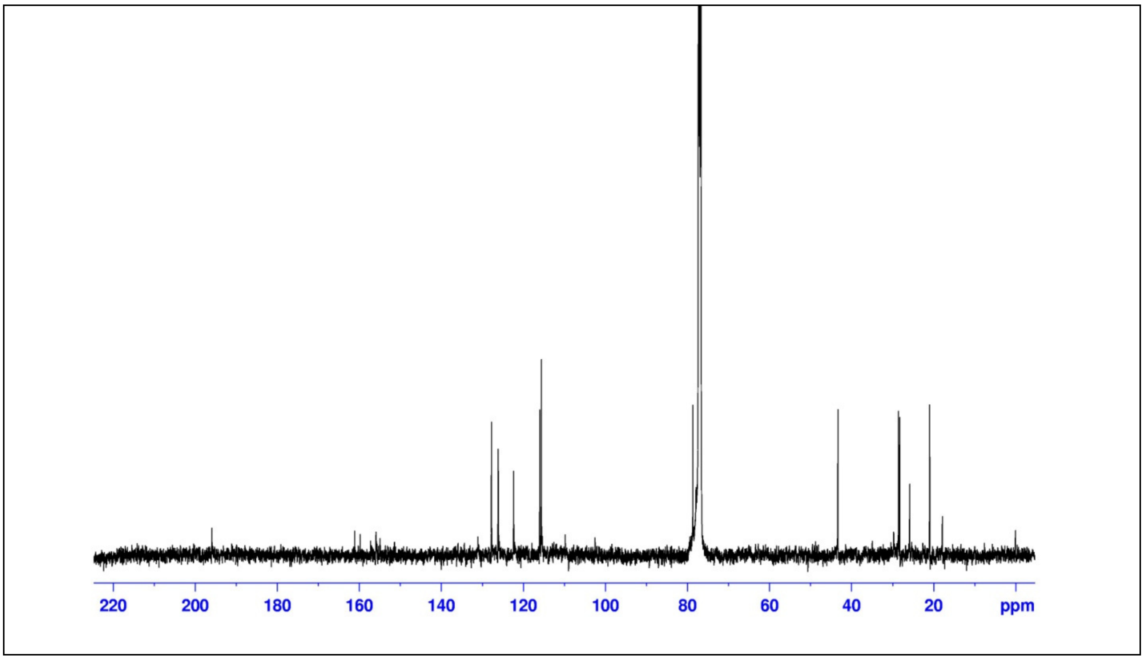

Figure 82 ^{13}C NMR (75 MHz) ($CDCl_3$) of compound **MNC8**

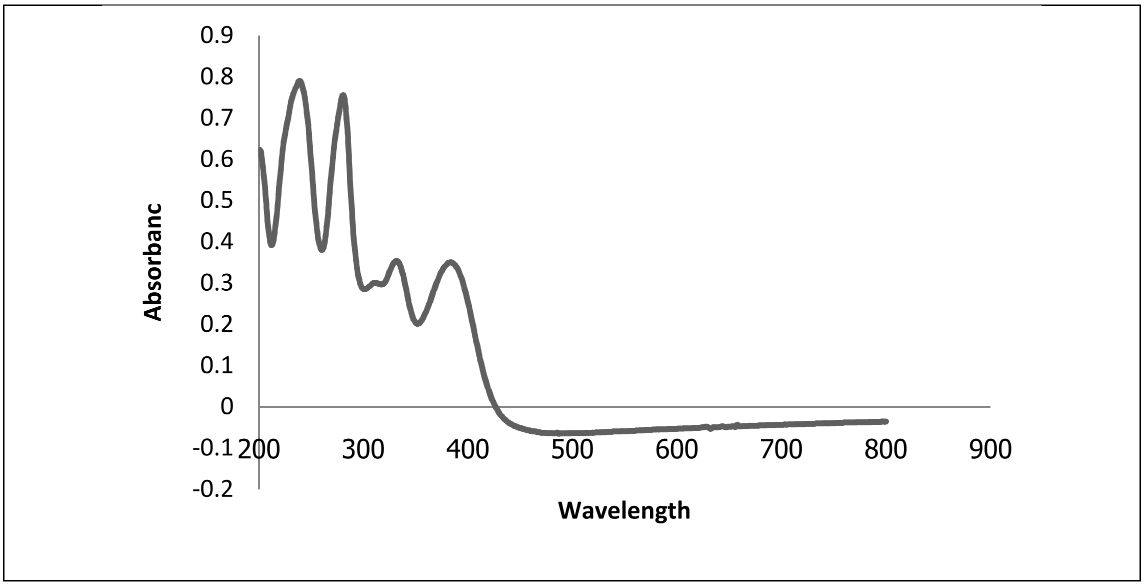

Figure 83 UV (MeOH) spectrum of compound **MNC9**

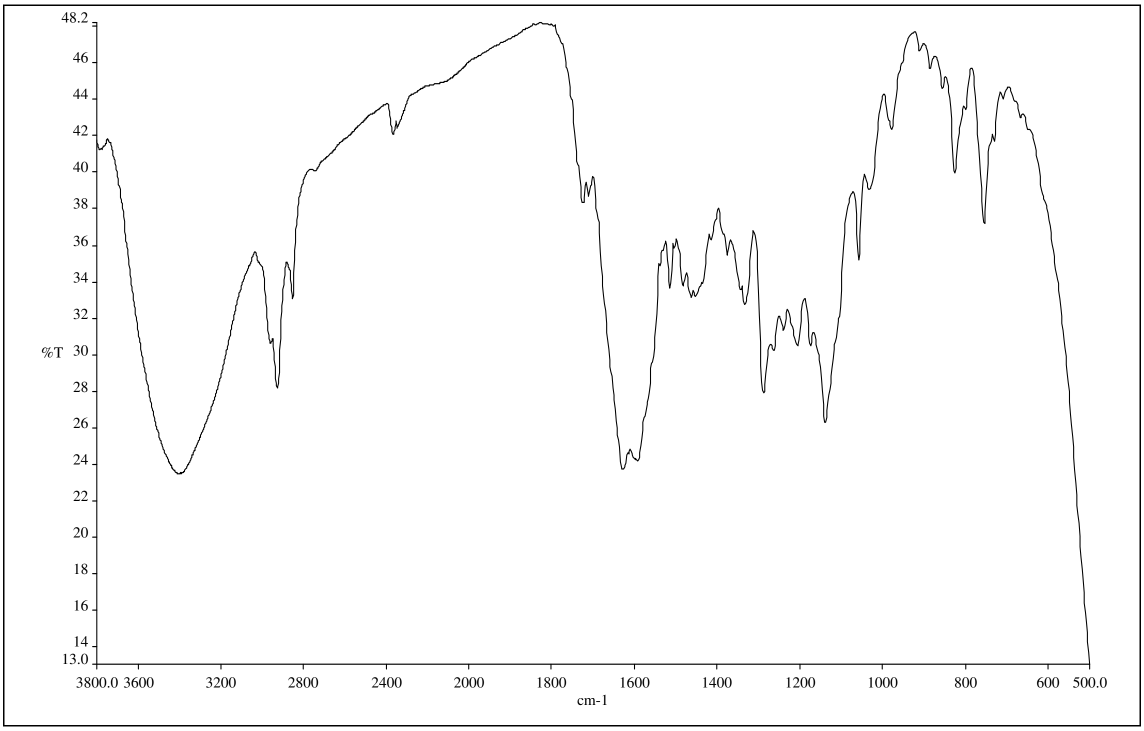

Figure 84 IR (neat) spectrum of compound **MNC9**

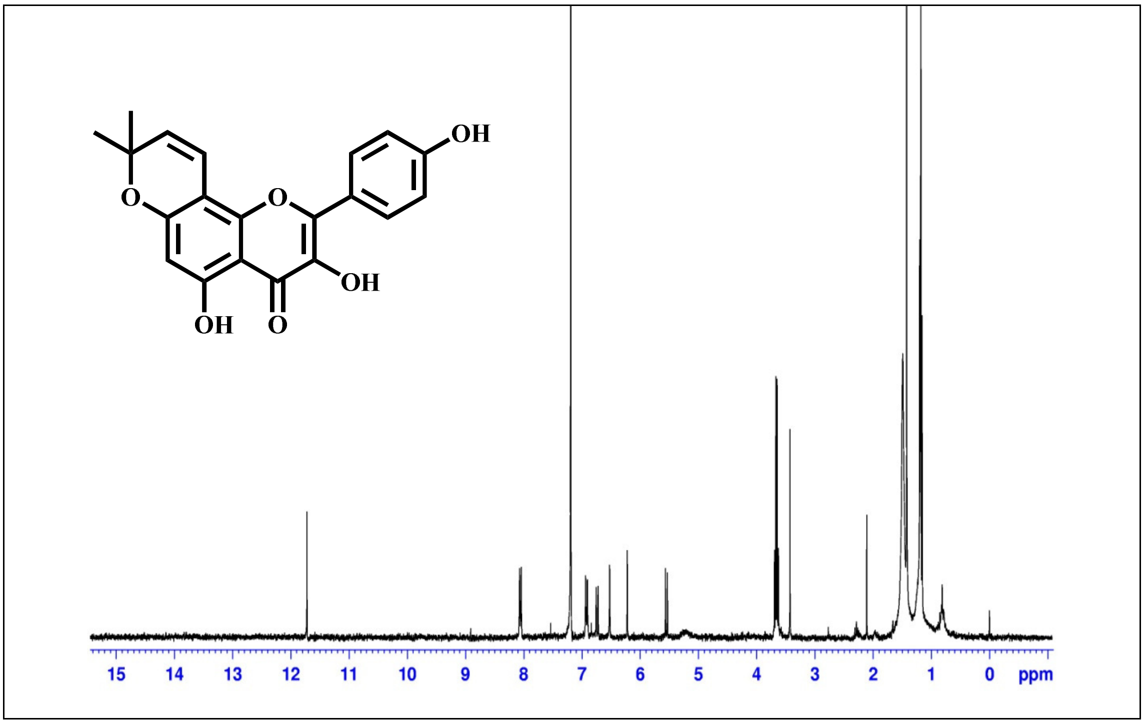

Figure 85 ^{1}H NMR (500 MHz) ($CDCl_3$) of compound **MNC9**

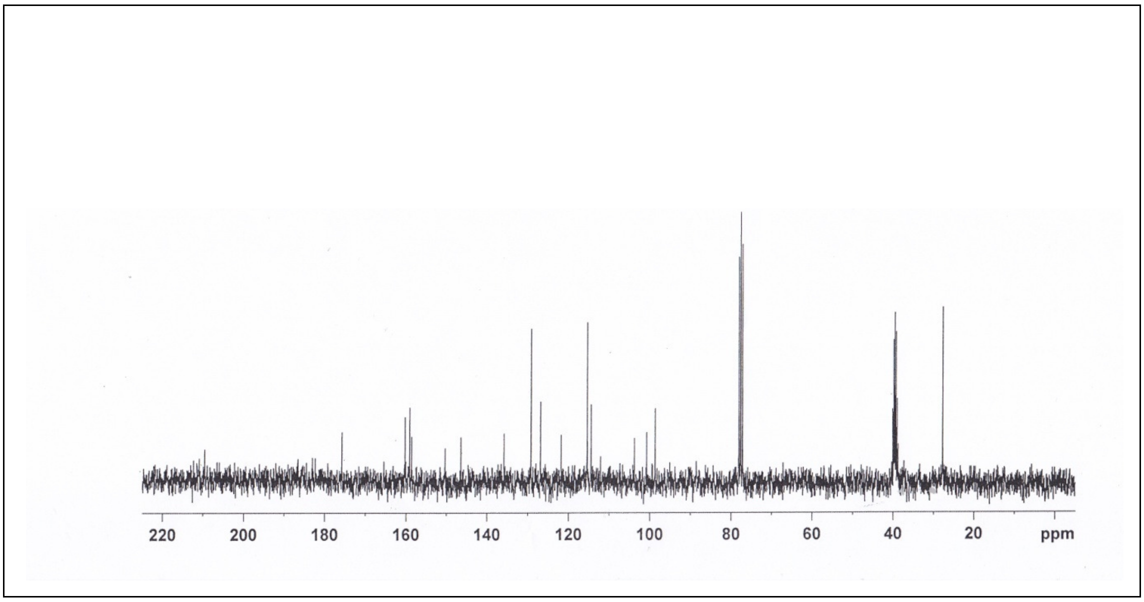

Figure 86 ^{13}C NMR (125 MHz) ($CDCl_3$) of compound **MNC9**

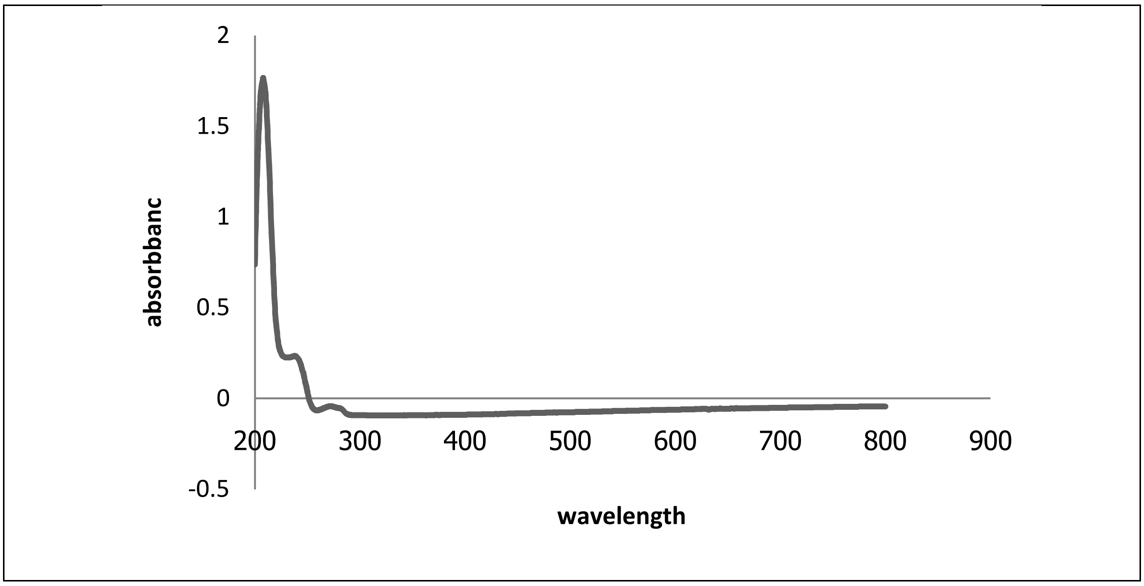

Figure 87 UV (MeOH) spectrum of compound **MNC10**

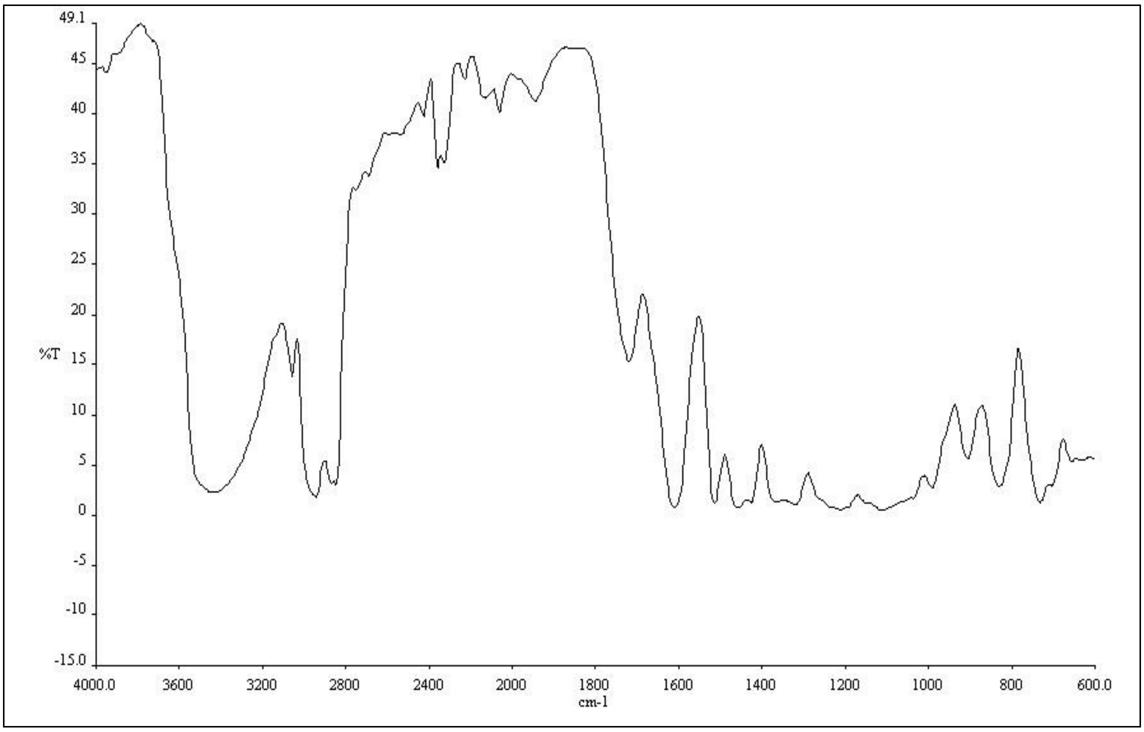

Figure 88 IR (neat) spectrum of compound **MNC10**

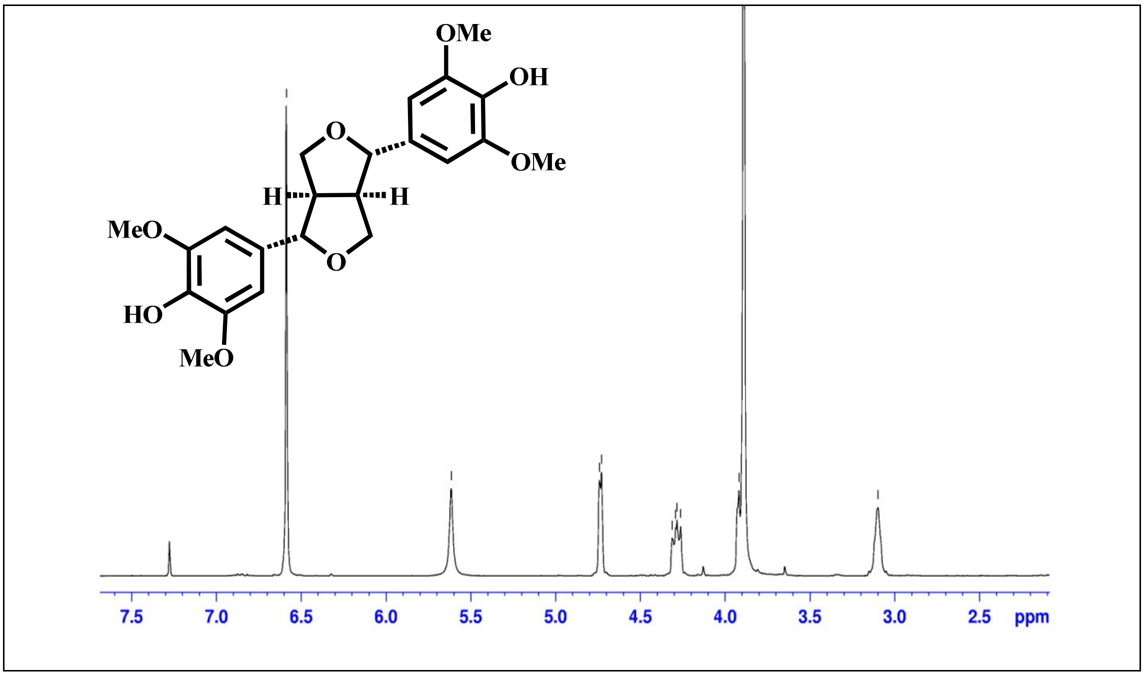

Figure 89 ^{1}H NMR (300 MHz) ($CDCl_3$) of compound **MNC10**

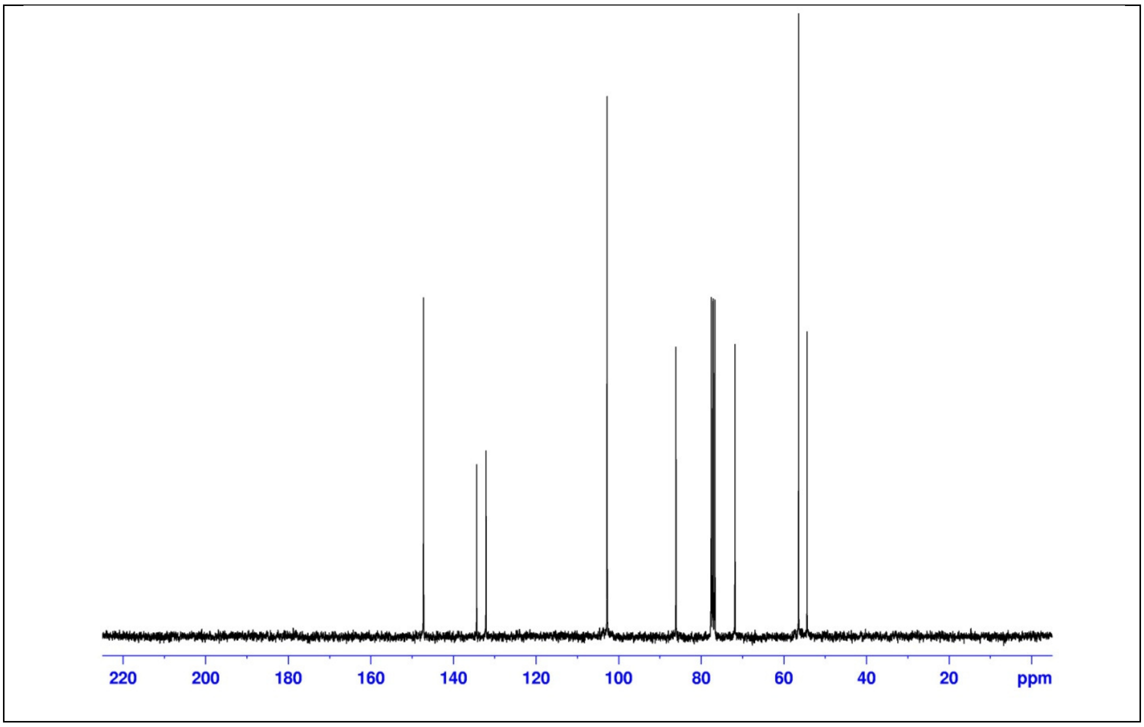

Figure 90 ^{13}C NMR (75 MHz) ($CDCl_3$) of compound **MNC10**

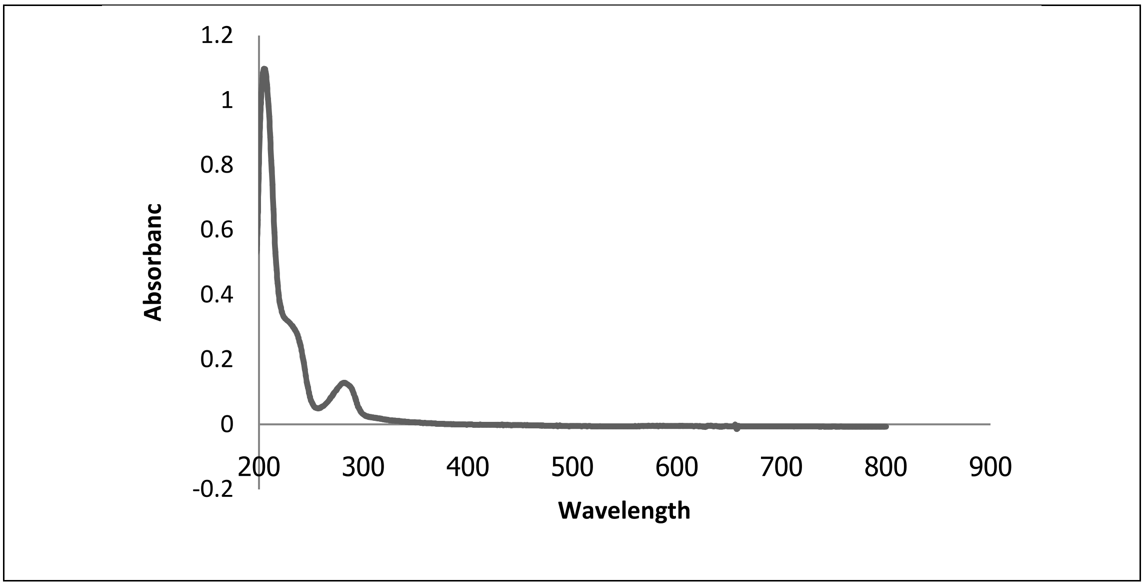

Figure 91 UV (MeOH) spectrum of compound **MNC11**

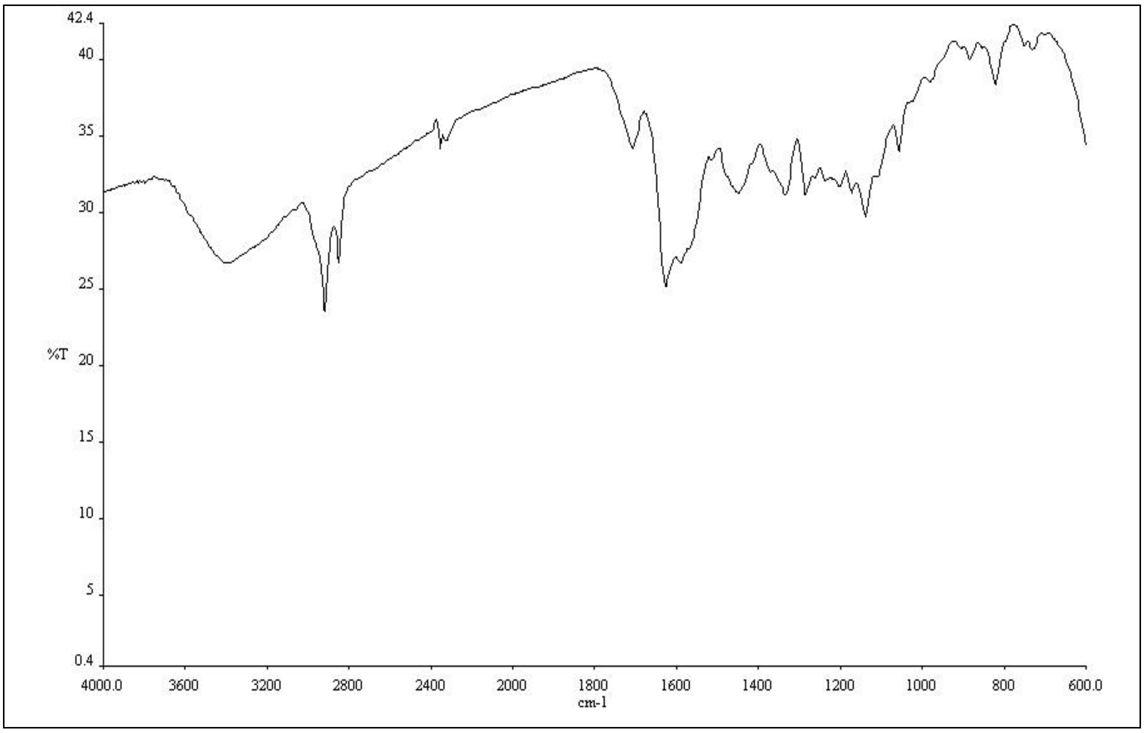

Figure 92 IR (neat) spectrum of compound **MNC11**

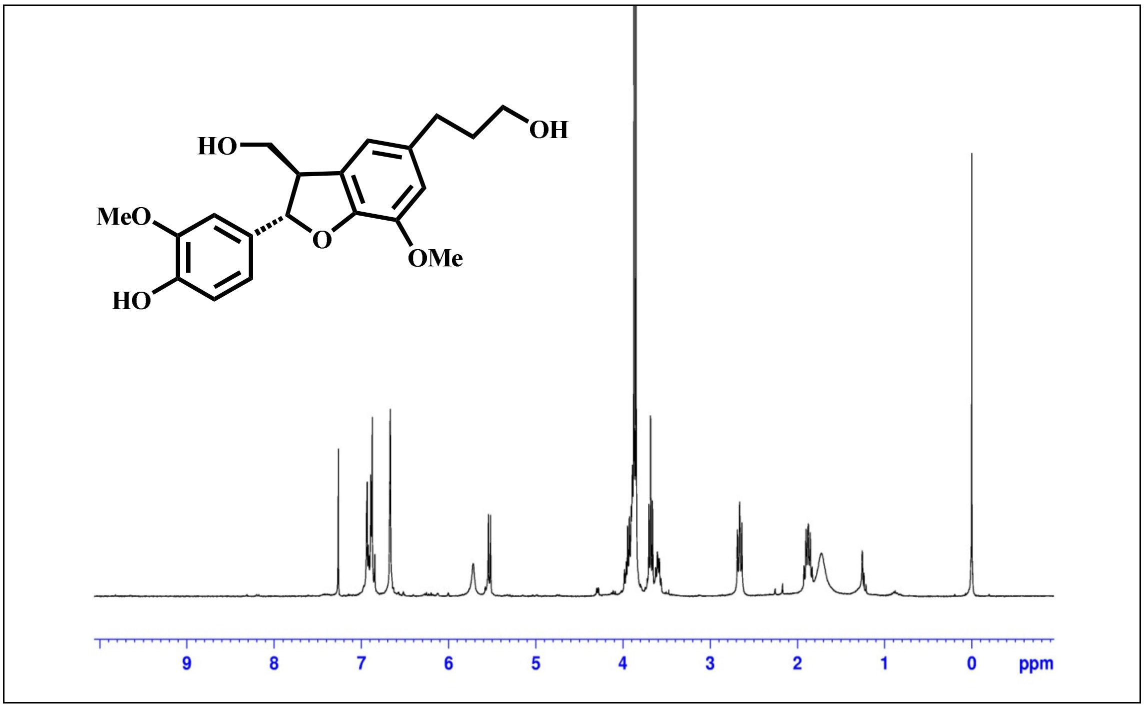

Figure 93 ^{1}H NMR (300 MHz) (CDCl$_3$) of compound **MNC11**

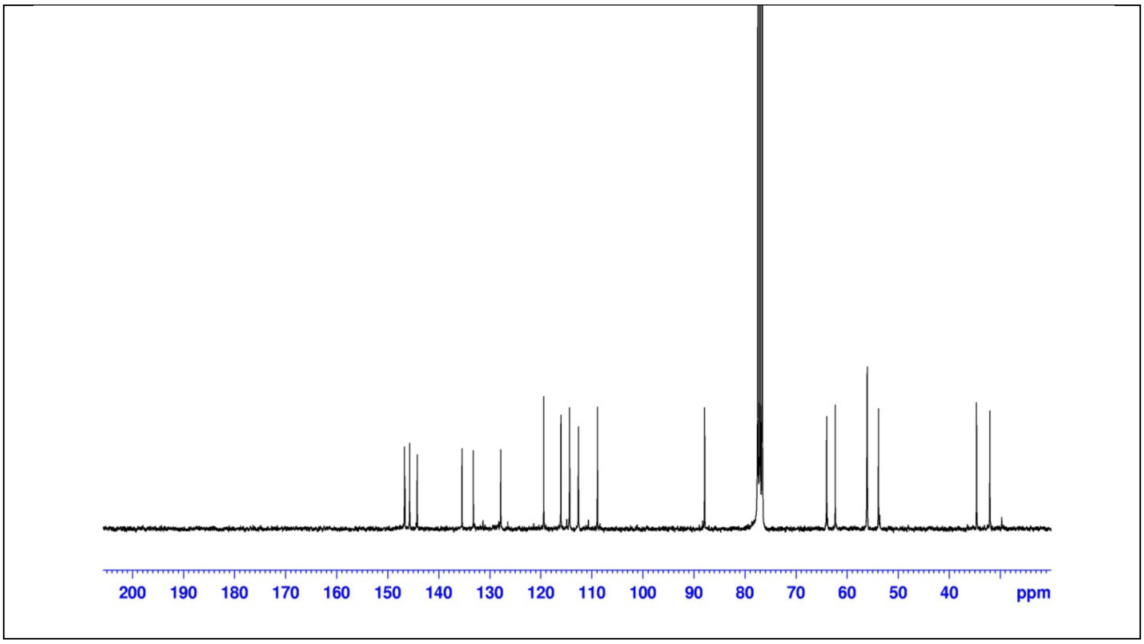

Figure 94 ^{13}C NMR (75 MHz) (CDCl$_3$) of compound **MNC11**

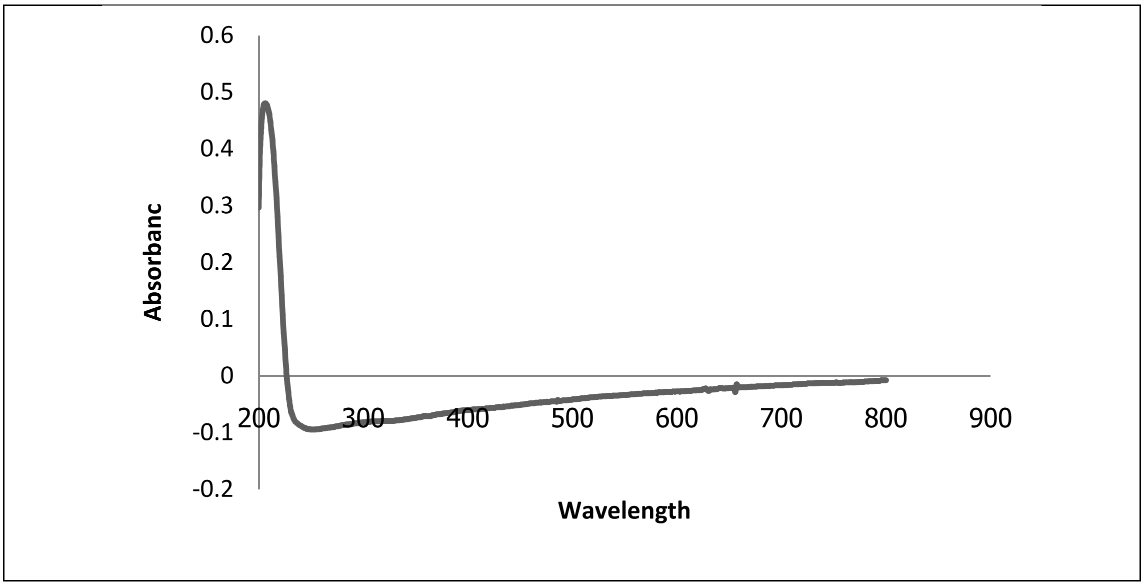

Figure 95 UV (MeOH) spectrum of compound **MNC12**

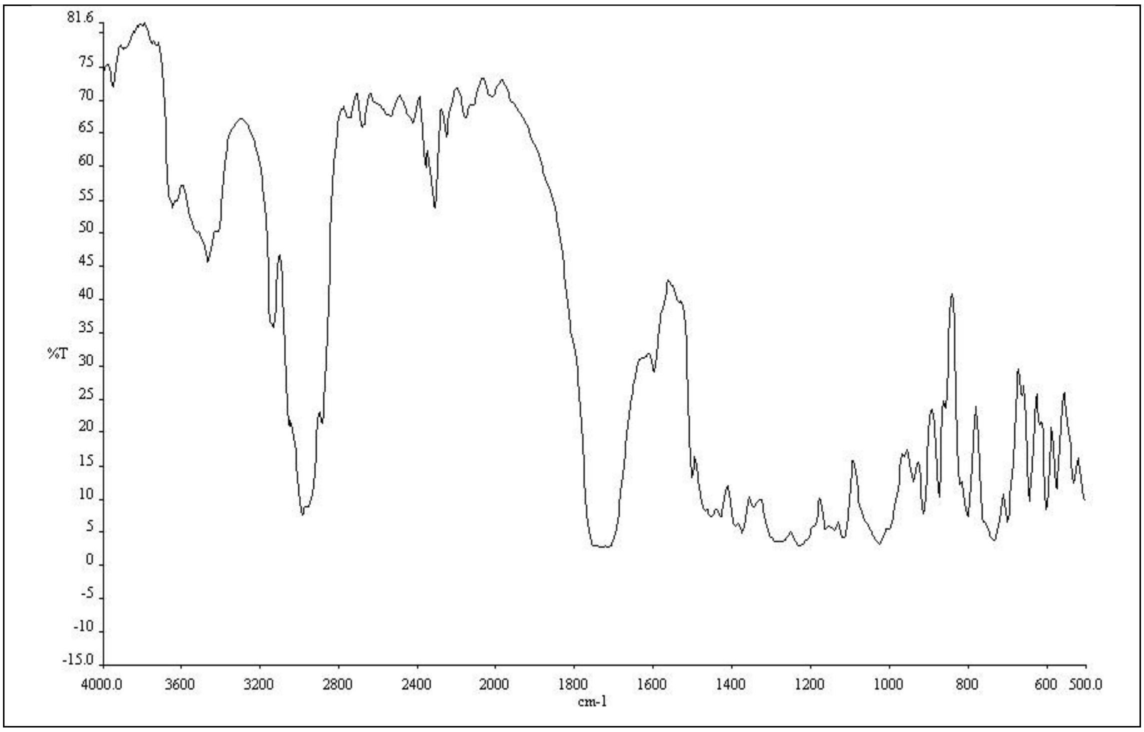

Figure 96 IR (KBr) spectrum of compound **MNC12**

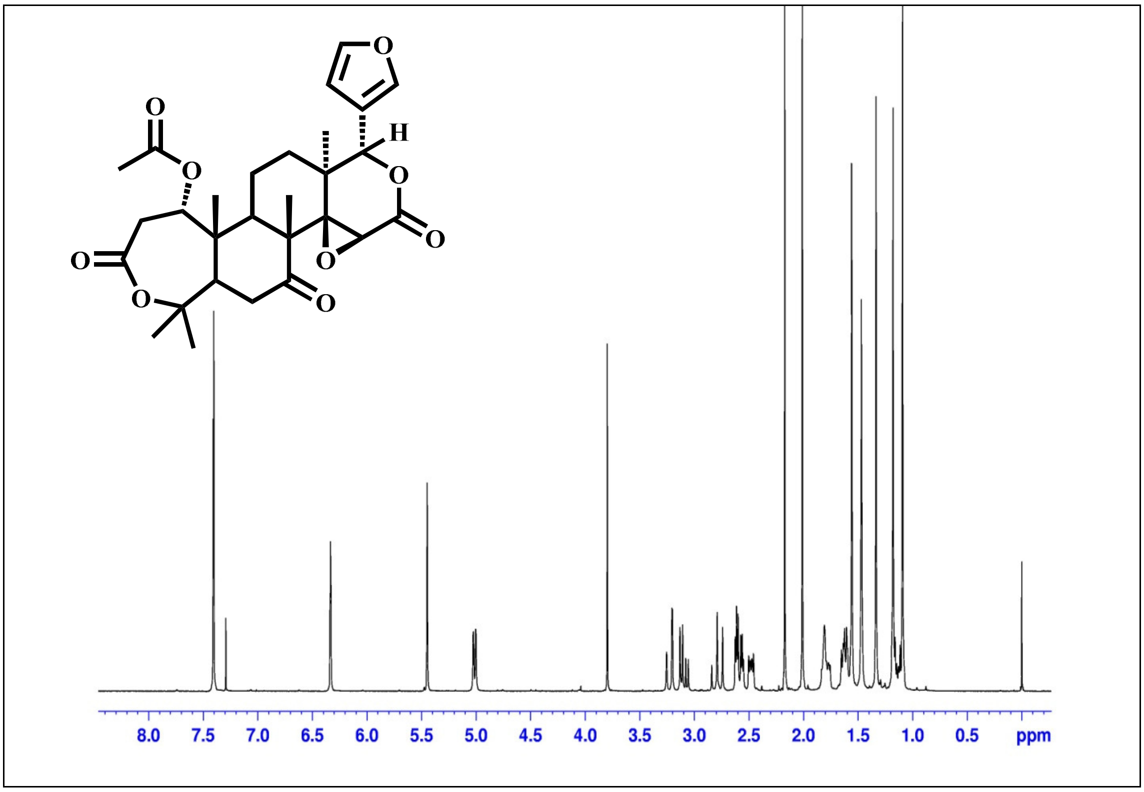

Figure 97 ^{1}H NMR (300 MHz) ($CDCl_3$) of compound **MNC12**

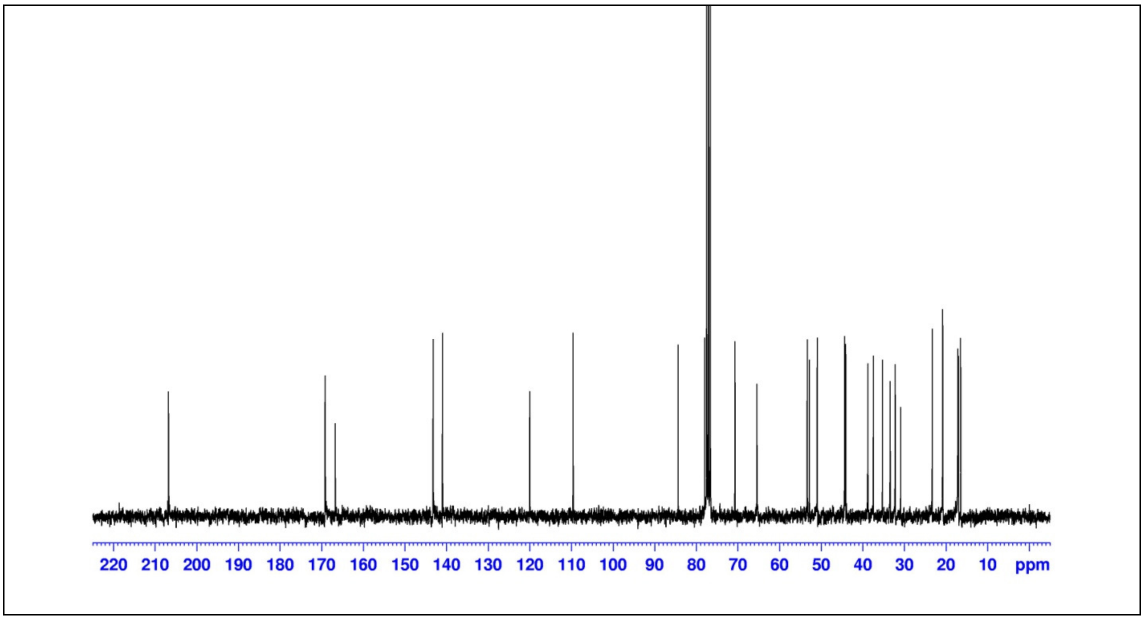

Figure 98 ^{13}C NMR (75 MHz) ($CDCl_3$) of compound **MNC12**

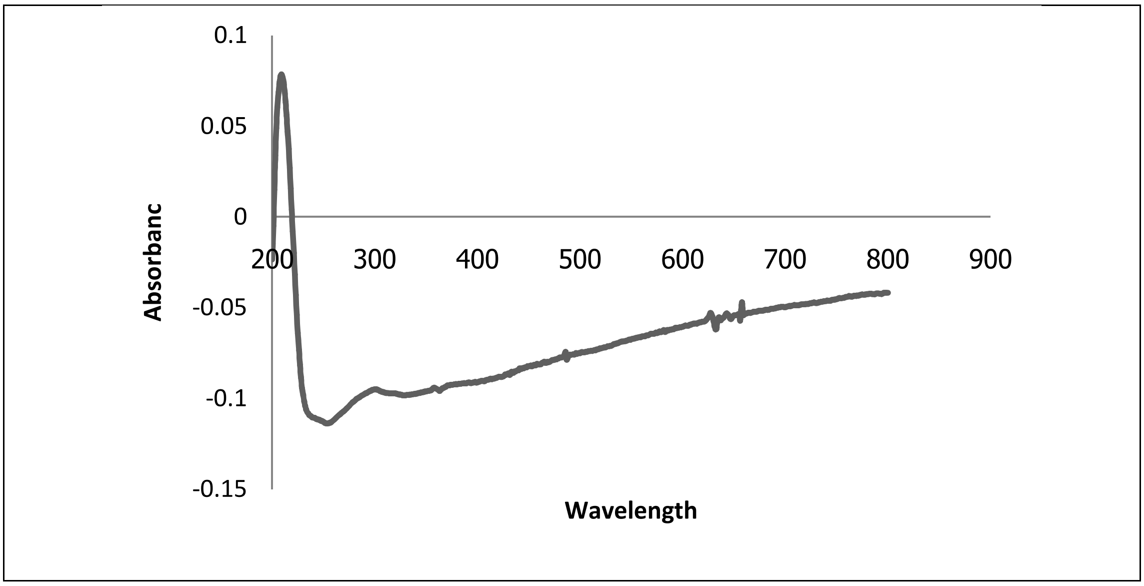

Figure 99 UV (MeOH) spectrum of compound **MNC13**

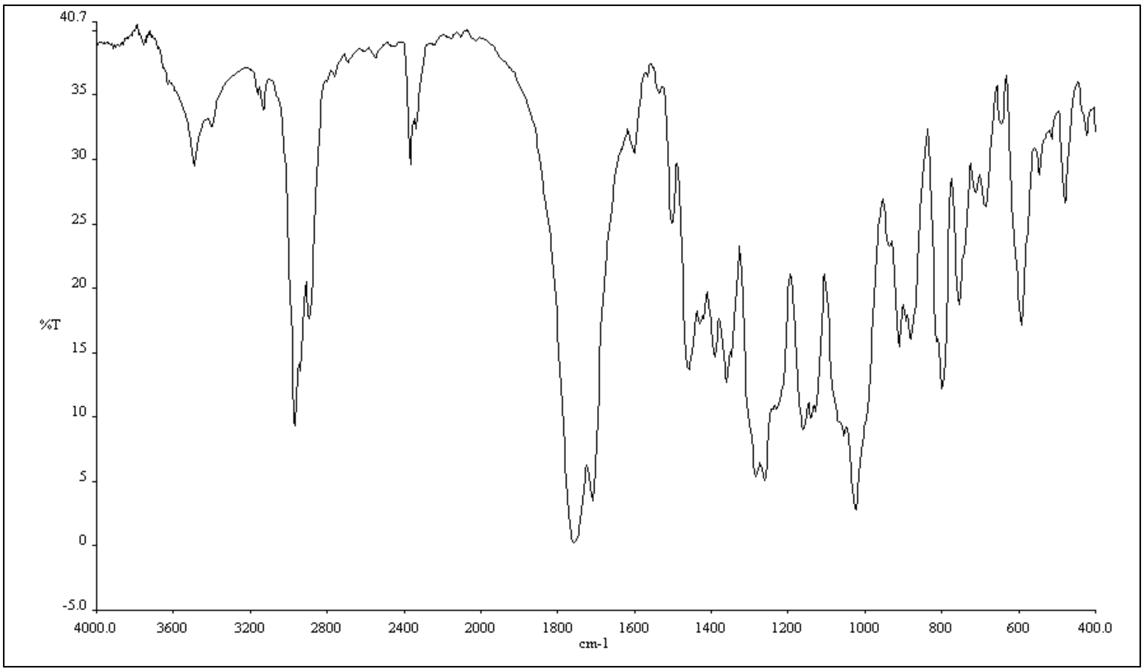

Figure 100 IR (KBr) spectrum of compound **MNC13**

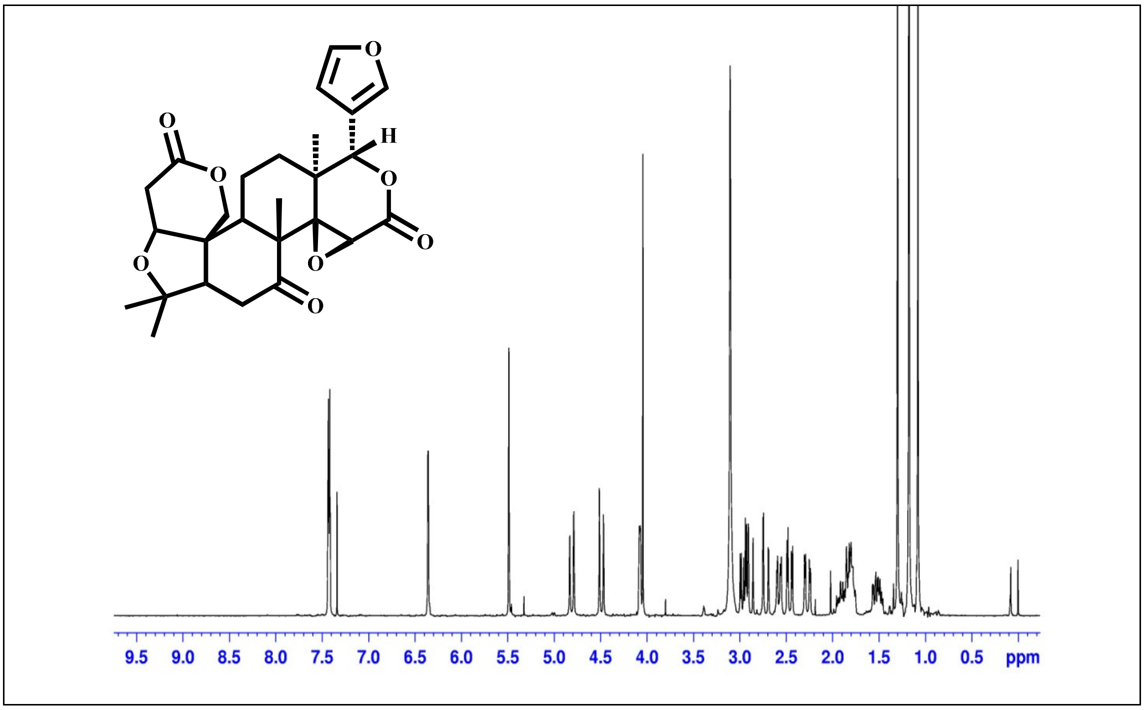

Figure 101 ^{1}H NMR (300 MHz) (CDCl$_3$) of compound **MNC13**

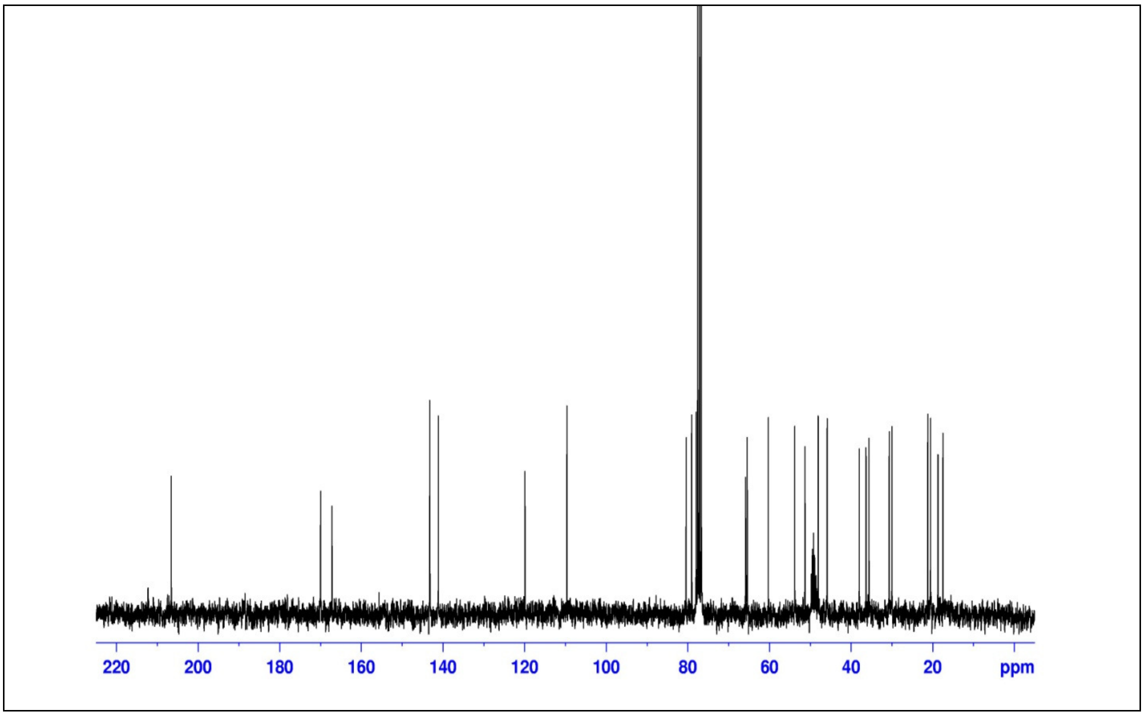

Figure 102 ^{13}C NMR (75 MHz) (CDCl$_3$) of compound **MNC13**

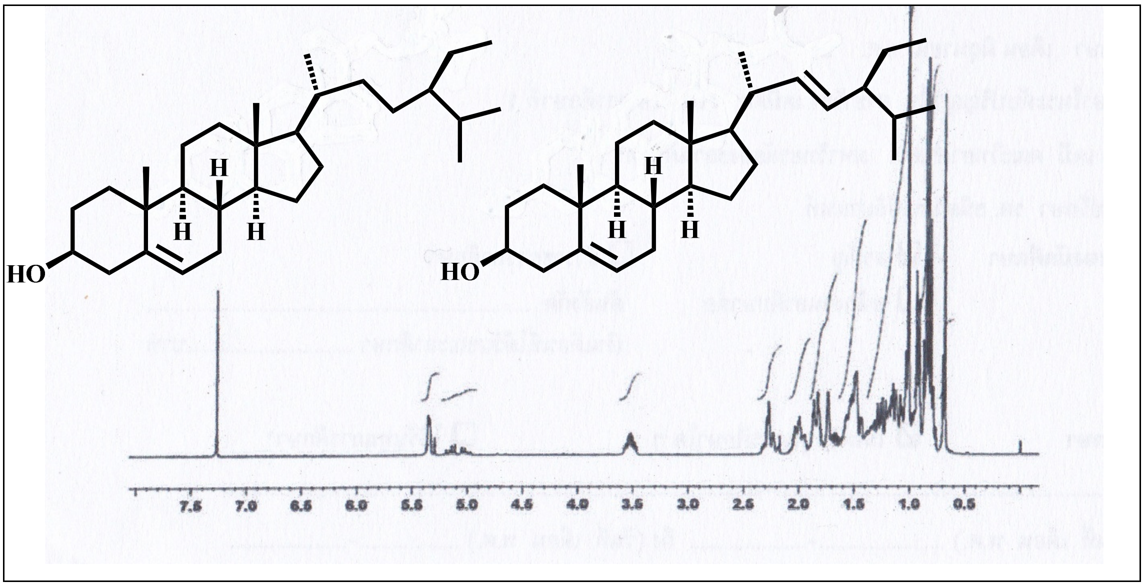

Figure 103 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compounds **MNC14+MNC15**

VITAE

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The Center for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education.

List of Publication and Proceedings

Pomkeua, S., Ponglimanont, C. and Karalai, C. 2010. Chemical constituents from the rhizome of *Curcuma zedoaria* (Christm.) Rosc. 16th National Graduate Research Conference, Maejo University, Chiang Mai, Thailand, March 11-12, 2010, pp. 17. (Poster presentation)