Chemical Constituents from the Leaves and Stems of *Citrus aurantifolia* Swingle

Tareeyah Malee

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Chemical Studies
Prince of Songkla University
2011

Thesis Title	Chemical Constituents from the Leaves and Stems of *Citrus aurantifolia* Swingle
Author	Miss Tareeyah Malee
Major Program	Chemical Studies

Major Advisor :

……………..……………………………..

(Assoc. Prof. Dr. Wilawan Mahabusarakam)

Co-advisor :

……………………………………

(Dr. Suda Chakthong)

Examining Committee :

…………..……….………….Chairperson

(Dr. Asadhawut Hiranrat)

…………..……….…………………………….

(Assoc. Prof. Dr. Wilawan Mahabusarakam)

…………..……….…………………………….

(Dr. Suda Chakthong)

…………..……….…………………………….

(Assoc. Prof. Dr. Chatchanok Karalai)

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.

…..…..……………………………..

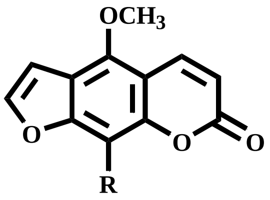
(Prof. Dr. Amornrat Phongdara)

Dean of Graduate School

ชื่อวิทยานิพนธ์ องค์ประกอบทางเคมีจากใบและลำต้นมะนาว
ผู้เขียน นางสาวตารีย๊ะ มะลี
สาขาวิชา เคมีศึกษา
ปีการศึกษา 2554

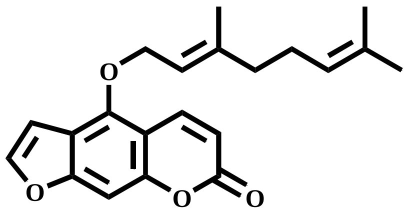
บทคัดย่อ

การศึกษาองค์ประกอบทางเคมีของส่วนสกัดหยาบไดคลอโรมีเทนและส่วนสกัดหยาบ อะซีโตนของใบมะนาว แยกได้สารกลุ่ม coumarins 5 สาร คือ bergapten (**TM1**), isopimpinellin (**TM2**), citropten (**TM3**), bergamottin (**TM5**) และ umbeliferone **(TM7)** กลุ่ม flavones 1 สาร คือ 5,7,4'-trihydroxy-3-methoxyflavone (**TM6**) และ กลุ่ม benzene derivatives 1 สาร คือ 4-hydroxybenzaldehyde (**TM4**) ส่วนสกัดหยาบอะซีโตนของลำต้นมะนาวแยกได้สารประกอบกลุ่ม flavones 3 สาร คือ atalantoflavone (**TM9**), chrysin (**TM12**) และ citrusinol (**TM13**) กลุ่ม acridone alkaloids 2 สาร คือ 5-hydroxynoracronycine (**TM10**) และ citracridone-III (**TM11**) กลุ่ม limonoids 2 สาร คือ limonin (**TM8**) และ limonexic acid (**TM15**) และกลุ่ม lignins 1 สาร คือ syringaresinol (**TM14**) โครงสร้างของสารประกอบเหล่านี้วิเคราะห์โดยใช้ข้อมูลทางสเปกโทรสโกปีและเปรียบเทียบกับสารที่มีรายงานการวิจัยแล้ว

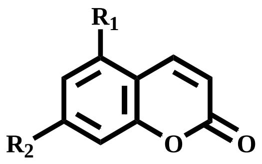


TM1 : R = H

TM2 : R = OCH_3

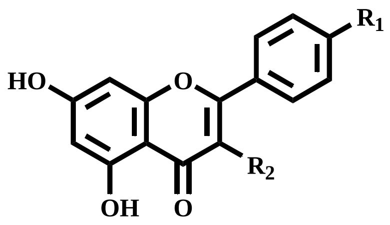


TM5



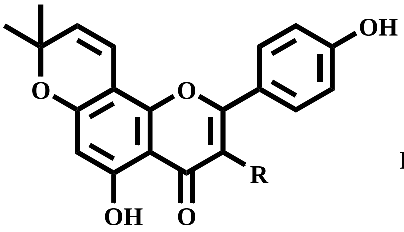
TM3 : R_1 = OCH_3, R_2 = OCH_3

TM7 : R_1 = H, R_2 = OH



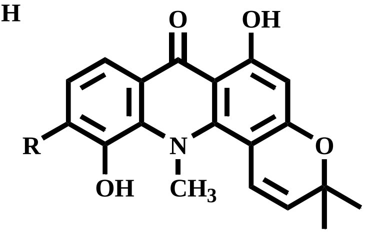
TM6 : R_1 = OH, R_2 = OCH_3

TM12 : R_1 = H, R_2 = H



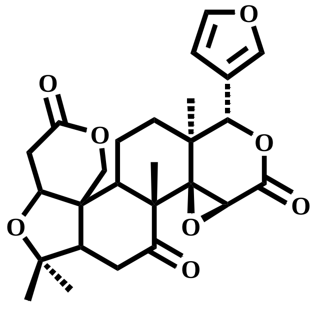
TM9 : R = H

TM13 : R = OH

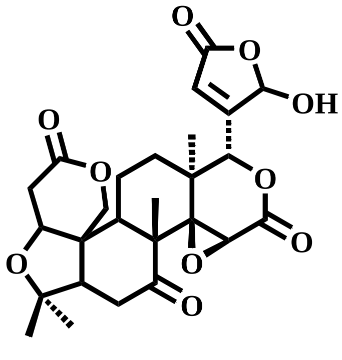


TM10 : R = H

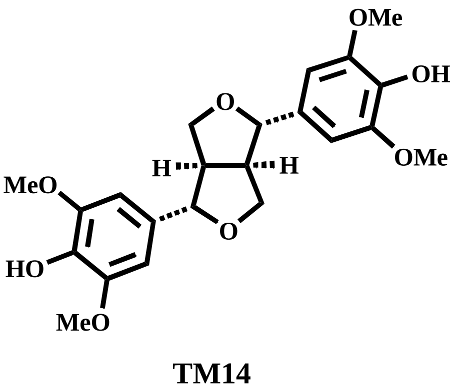
TM11 : R = OH



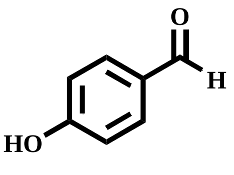
TM8



TM15



TM14

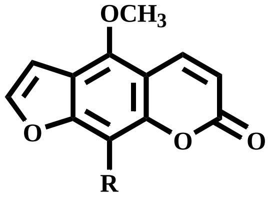


TM4

Thesis Title	Chemical Constituents from the Leaves and Stems of *Citrus aurantifolia* Swingle
Author	Miss Tareeyah Malee
Major Program	Chemical Studies
Academic Year	2011

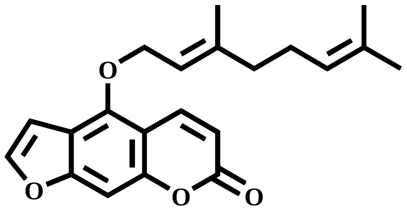
ABSTRACT

Investigation of the dichloromethane and acetone extracts of the leaves of *Citrus aurantifolia* Swingle, yielded five coumarins: bergapten (**TM1**), isopimpinellin (**TM2**), citropten (**TM3**), bergamottin (**TM5**) and umbeliferone **(TM7)**, one benzene derivative: 4-hydroxybenzaldehyde (**TM4**) and one flavone: 5,7,4'-trihydroxy-3-methoxy flavone (**TM6**). Investigation of the acetone extract of the stems of *C. aurantifolia* yielded three flavones: atalantoflavone (**TM9**), chrysin (**TM12**) and citrusinol (**TM13**), two acridone alkaloids: 5-hydroxynoracronycine (**TM10**) and citracridone-III (**TM11**), two limonoids: limonin (**TM8**) and limonexic acid (**TM15**), and one lignin: syringaresinol (**TM14**). Their structures were elucidated by spectroscopic methods and comparison with those reported in the literatures.

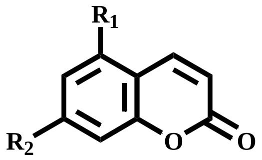


TM1 : R = H

TM2 : R = OCH_3

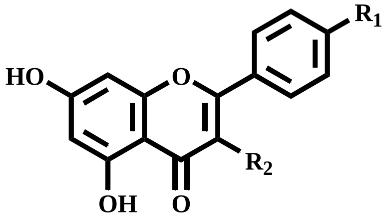


TM5



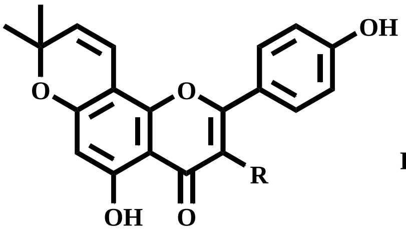
TM3 : R_1 = OCH_3, R_2 = OCH_3

TM7 : R_1 = H, R_2 = OH



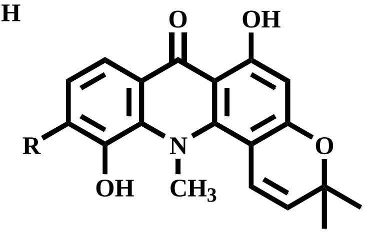
TM6 : R_1 = OH, R_2 = OCH_3

TM12 : R_1 = H, R_2 = H



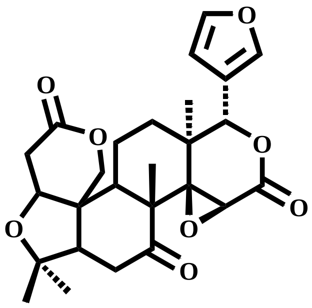
TM9 : R = H

TM13 : R = OH

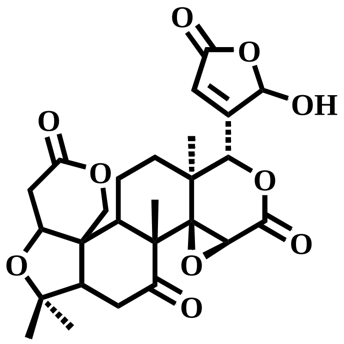


TM10 : R = H

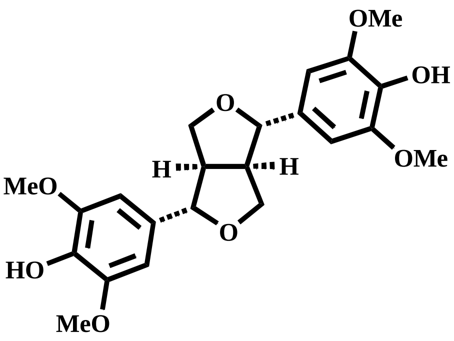
TM11 : R = OH



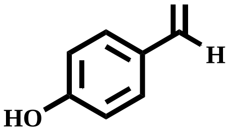
TM8



TM15



TM14



TM4

ACKNOWLEDGEMENT

I wish to express my deepest and sincere gratitude to supervisor, Associate Professor Doctor Wilawan Mahabusarakam, for her valuable instructions, constant guidance, useful suggestions, appreciation, sincere advice and kindness. This was a great motivator for me and will remain to be deep-rooted in my heart. My sincere thanks are expressed to co-advisor, Doctor Suda Chakthong, for her valuable advice and kindness. I would like to express my appreciation to the staffs of the Department of Chemistry, Faculty of Science, Prince of Songkla University for making this thesis possible.

This research was supported by a scholarship from the Center for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Educaion. I would like to acknowledge the Faculty of Science Research Fund and the Graduate School, Prince of Songkla University for partial financial support.

Tareeyah Malee

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

The purpose of this research is to investigate the chemical constituents from the leaves and stems of *Citrus aurantifolia* Swingle. They are part of the basic researches on the Thai medicinal plants. Seven compound and eight compounds have been isolated from the leaves and stems of *Citrus aurantifolia* Swingle, respectively.

CONTENTS

CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF ILLUSTRATIONS

LIST OF ILLUSTRATIONS (Continued)

LIST OF ILLUSTRATIONS (Continued)

LIST OF SCHEMES

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
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
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

INTRODUCTION

1.1 Introduction

Citrus aurantifolia Swingle, 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.

Figure 1 *Citrus aurantifolia* Swingle

According to Smitinand (2001), Seventeen species of genus *Citrus* are found in Thailand

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

1.2 Review of Literatures

The chemical constituents which were isolated from the *Citrus* plants were summarized in **Table 1** Information obtained from Scifinder Scholar will be presented and classified in to group: Acridone alkaloids, Benzene derivatives, Coumarins, Flavonoids and Limonoids.

1.3 The Biological Activities of *Citrus* genus

The chemical constituents isolated from the *Citrus* genus were summarized in **Table 1** (The literature survey from Scifinder Scholar database). Some of acridone alkaloids from the stem bark of C. *maxima* f. Buntan showed cytotoxicity. For example, 5-hydroxynoracronycine alcohol, glycocitrine-I, 5-hydroxynoracronycine, citrusinine-I and citracridone-III were found to display cytotoxic activity against two tumor cells (HepG2 and KB) with inhibitory concentration, IC_{50} 15.1 μM (Teng *et al.*, 2010). Some of the compounds which were isolated from the root bark of *C. maxima* showed interesting activities such as nordentatin inhibited the growth of *Bacillus substilis, Staphyllococus aureus and Micrococus luteus* at $IC_{100} \leq 10$ μg/ml. Xanthyletin inhibited the growth of *Klebsiella pneumonia, Pseudomonas aeruginosa* and *Salmonella typhi* at $IC_{100} \leq 100$ μg/ml and *Bordetella bronchiseptica at* $IC_{100} \leq 50$ μg/ml. *Bordetella bronchiseptica* was distinctly inhibited by scopoletin and glycocitrine-I *at* $IC_{100} \leq$

100 μg/ml (Wu *et al.*, 1988). Polymethoxyflavones from the fruit peels of C. *reticulata* Blanco cv. Ponkan, such as isosinensetin showed antiproliferative activity against breast cancer cell line (MCF-7) with IC_{50} 15.1 μM and ovarian cancer cell line (HO8910) with IC_{50} 15.1 μM (Du *et al.*, 2010).

Table 1 Compounds isolated from the plant of *Citrus* genus

A. Acridones   **D**. Flavonoids
B. Benzene derivatives   **E**. Limonoids
C. Coumarins

Scientific name (Investigated part)	**Compounds / Structures**	**Bibliography**
C. aurantifolia Swingle peel	Psoralene, **C1** Bergapten, **C2** Isopimpinellin, **C3** Imperatorin, **C4** Myricetin, **D1**	Nagwa *et al*., 2010
Leave	Isobergapten, **C5** Angelicin, **C6** Kaempferol, **D2**	Nagwa *et al*., 2010
C. hytrix **DC** fruit	Bergamottin, C7 Oxypeucedanin, C8	Murakami *et al*., 1999
C. Japonica **Thunb** seed	Limonin, **E1** Limonyl acetate, **E2** Ichagensin, **E3** Calamin, **E4** Limonin-17-β-*D*-glucopyranoside, **E5**	Hasegawa *et al*., 1998

Table 1 (continued)

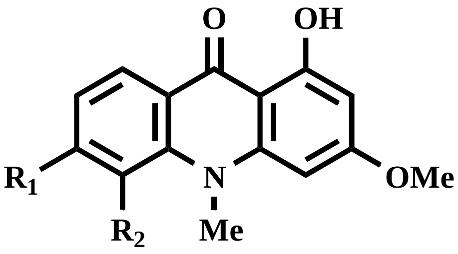
Scientific name (Investigated part)	**Compounds / Structures**	**Bibliography**
C. maxima *Stem bark*	Grandisine-I, **A1** Natsucitrine-II, **A2** Citrusinine-I, **A3** Glycocitrine-I, **A4** 5-Hydroxynoracronycine alcohol, **A5** 5-Hydroxynoracronycine, **A6** Citracridone-III, **A7**	Teng *et al*., 2005
***C. medica* L. var. *sarcodactylis*.**		
fruit	3-(3-Methoxy-4-hydroxyphenyl)-acrylic acid, **B1** Vanillic acid, **B2** 3,4-Dihydroxy-benzoic acid**, B3** 7-Hydroxy-5-methoxycoumarin, **C9** Umbelliferone, **C10** 6,7-Dimethoxycoumarin, **C11**	Yin *et al*., 2004
	Limettin, **C12** Scopoletin, **C13** 3,5,6-Trihydroxy-3',4',7-trimethoxy flavone, **D3** 3,5,6-Trihydroxy-4',7-dimethoxy flavone, **D4**	He *et al*., 1985
***C. mitis* Blanco** fruit	Citromitin, **D5** 5-Demethylcitromitin, **D6**	Sastry *et al*., 1931

Table 1 (continued)

Scientific name **(Investigated part)**	**Compounds / Structures**	**Bibliography**
C. nobilis Root bark	Citropone A, **A8** 5-Hydroxynoracronycine, **A9** Citracridone-I, **A10** Crenyllatin, **C14** Xanthyletin, **C15** Suberenol, **C16** Suberosin, **C17** Xanthoxyletin, **C18** Nordentatin, **C19**	Wu *et al*., 1987
C. nobilis Seed	Citrobilin, **E6** Limonin, **E7** Limonexic acid, **E8** Obacunone, **E9** Nomilin, **E10** Deacetyl nomilin, **E11**	Bui *et al*., 2004
***C. reticulate* Blanco** fruit	Isosinensetin, **D7** Sinensetin, **D8** Tetramethyl-oisoscutellarein, **D9** Nobiletin, **D10**	Du *et al*., 2010

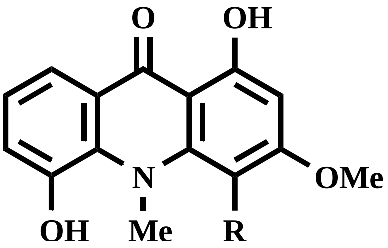
Structures of compounds from *Citrus* genus

A. Acridone alkaloids



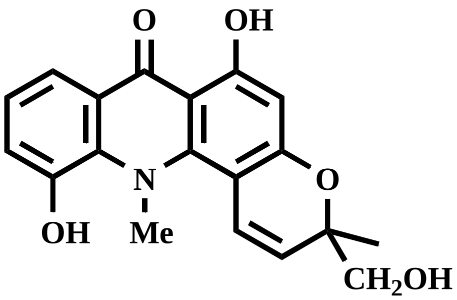
A1 : Grandisine-I R_1 = OMe R_2 = OH

A2 : Natsucitrine-II R_1 = OH R_2 = OMe

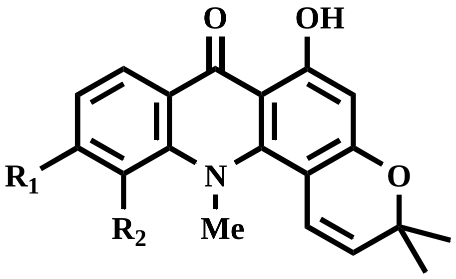


A3 : Citrusinine-I R = OMe

A4 : Glycocitrine-I R = prenyl

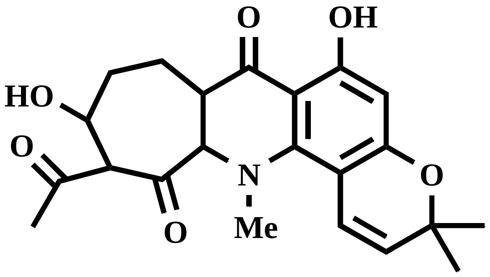


A5 : 5-Hydroxynoracronycine alcohol

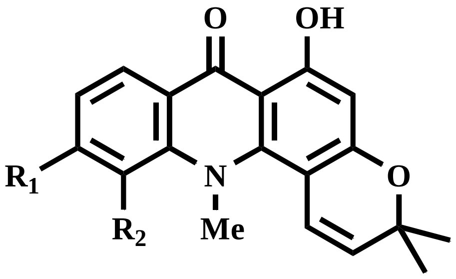


A6 : 5-Hydroxynoracronycine R_1 = OMe R_2 = OH

A7 : Citracridone-III R_1 = OH R_2 = OMe



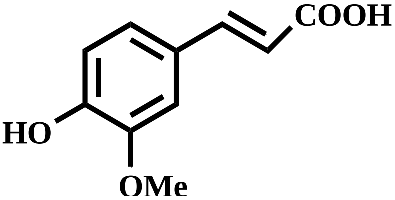
A8 : Citropone A



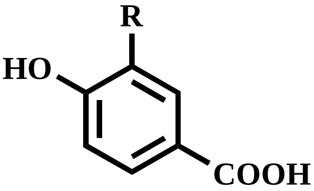
A9 : 5-Hydroxynoracronycine R_1 = H R_2 = OH

A10 : Citracridone-I R_1 = OH R_2 = OMe

B. Benzene derivatives



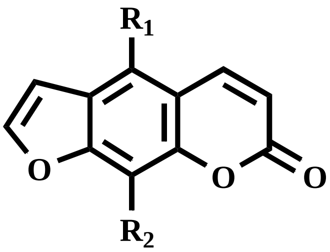
B1 : 3-(3-Methoxy-4-hydroxyphenyl)-acrylic acid

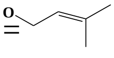


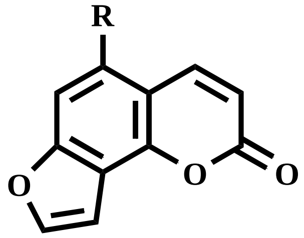
B2 : Vanillic acid R = OMe

B3 : 3,4-Dihydroxybenzoic acid R = OH

C. Coumarins

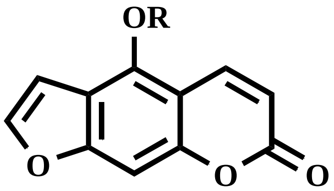


C1 : Psoralene	R_1 = H	R_2 = H
C2 : Bergapten	R_1 = H	R_2 = OMe
C3 : Isopimpinellin	R_1 = OMe	R_2 = OMe
C4 : Imperatorin	R_1 = 	R_2 = H

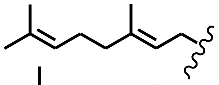


C5 : Isobergapten : R = OMe

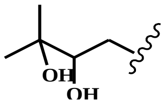
C6 : Angelicin : R = H

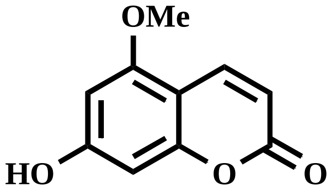


C7 : Bergamottin R =

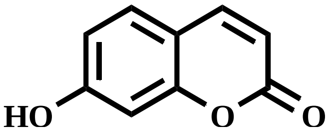


C8 : Oxypeucedanin R =

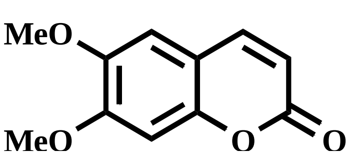




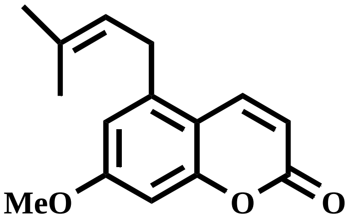
C9 : 7-Hydroxy-5-methoxycoumarin



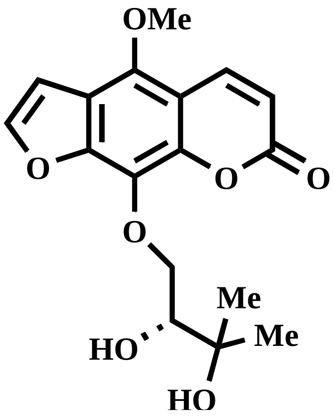
C10 : Umbelliferone



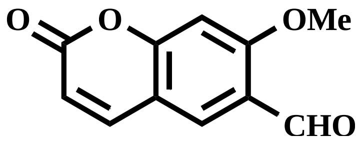
C11 : 6,7-Dimethoxycoumarin



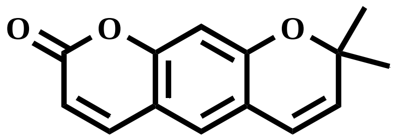
C12 : Limettin



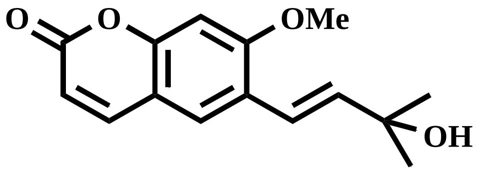
C13 : Scopoletin



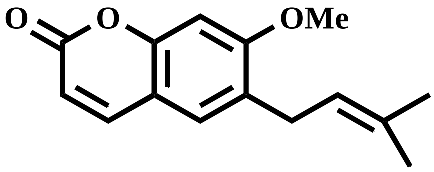
C14 : Crenyllatin



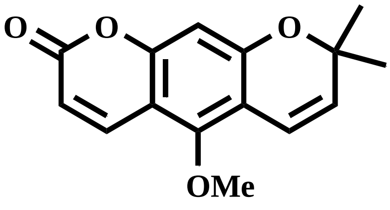
C15 : Xanthyletin



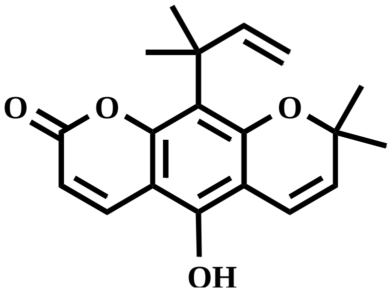
C16 : Suberenol



C17 : Suberosin

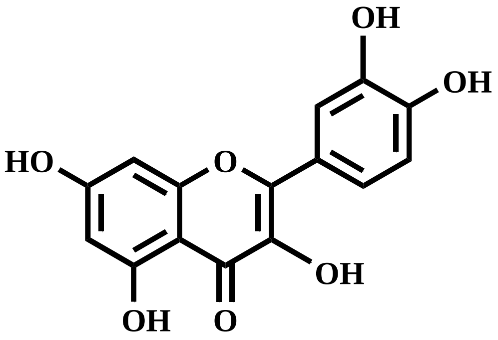


C18 : Xanthoxyletin

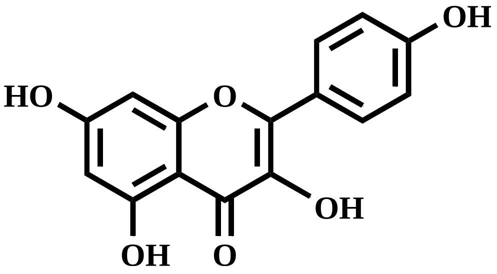


C19 : Nordentatin

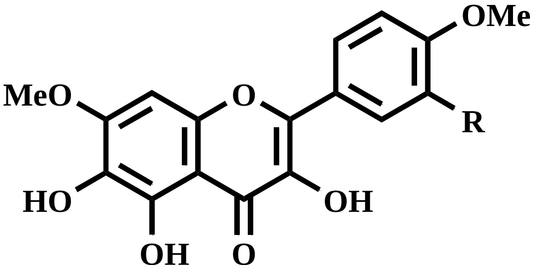
D. Flavonoids



D1 : Myricetin

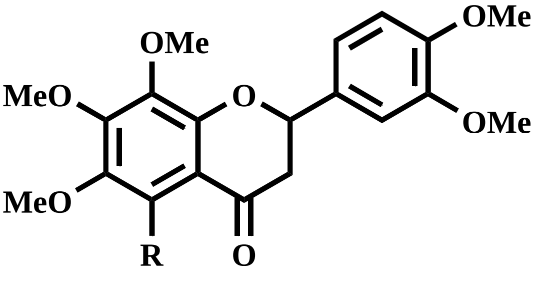


D2 : Kaempferol



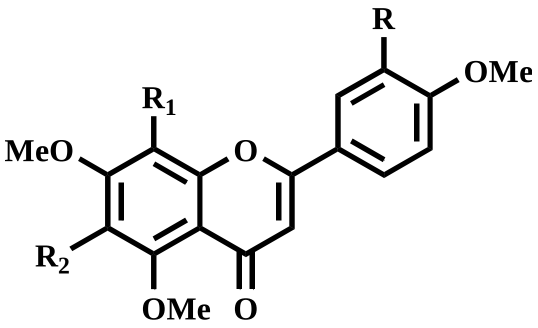
D3 : 3,5,6-Trihydroxy-3',4',7-trimethoxyflavone : R = Me

D4 : 3,5,6-Trihydroxy-4',7-dimethoxyflavone : R = H



D5 : Citromitin : R = OMe

D6 : 5-Demethylcitromitin : R = OH



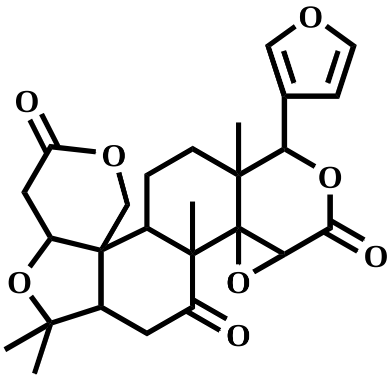
D7 : Isosinensetin : $R = H$ $R_1 = OMe$ $R_2 = H$

D8 : Sinensetin : $R = OMe$ $R_1 = OMe$ $R_2 = H$

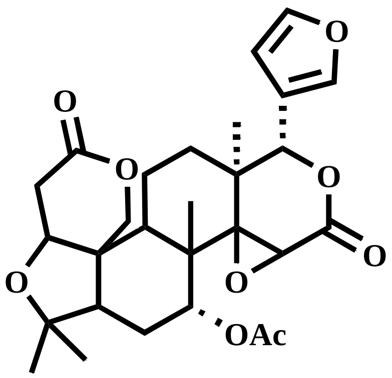
D9 : Tetramethyl-oisoscutellarein : $R = OMe$ $R_1 = H$ $R_2 = OMe$

D10 : Nobiletin : $R = OMe$ $R_1 = OMe$ $R_2 = OMe$

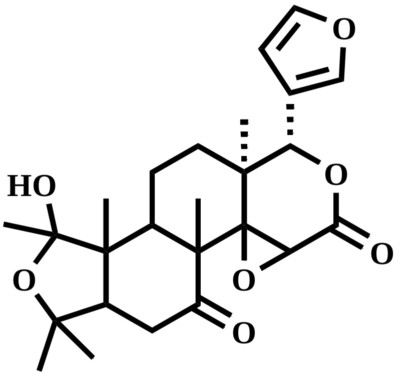
E. Limonoids



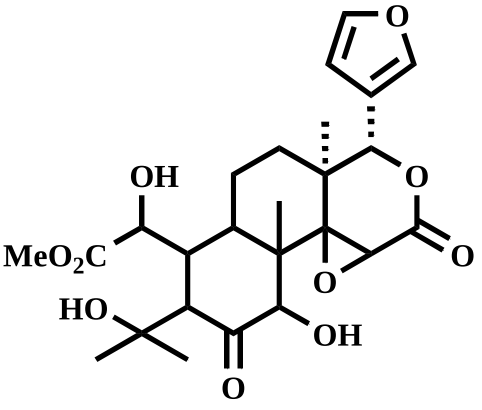
E1 : Limonin



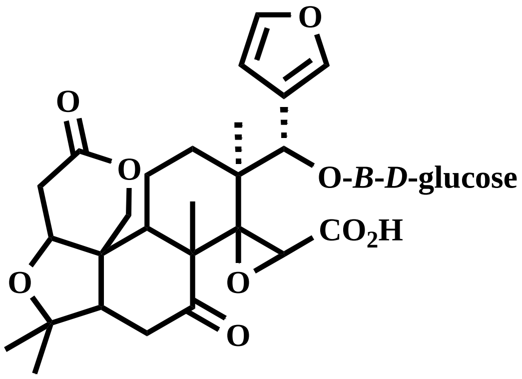
E2 : Limonyl acetate

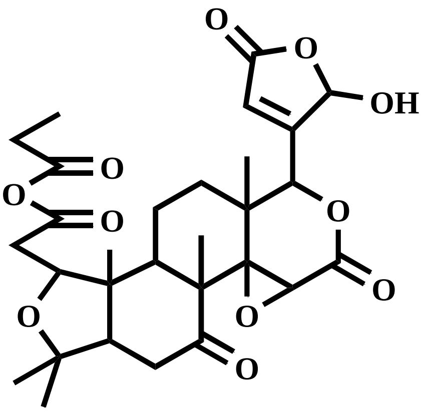


E3 : Ichangensin



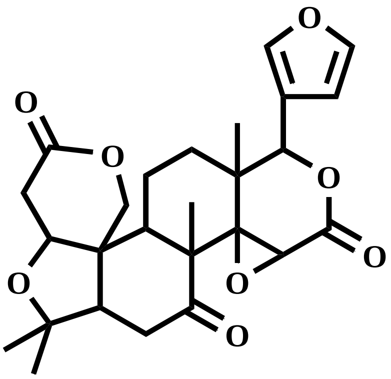
E4 : Calamin

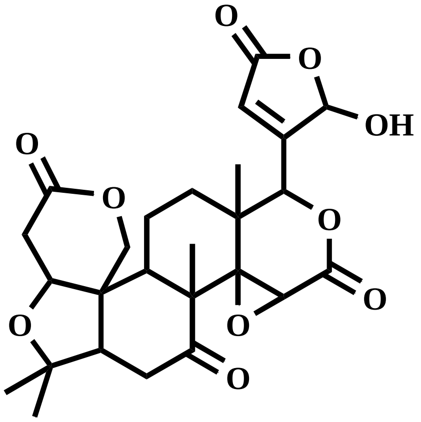




E5 : Limonin-17-*β*-*D*-glucopyranoside

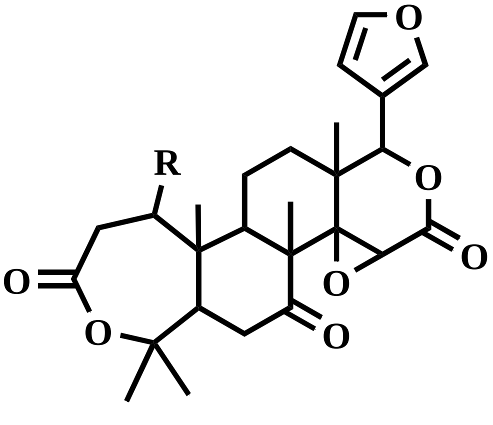
E6 : Citrobilin

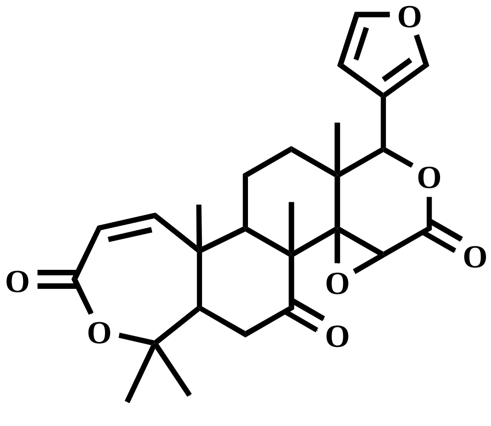




E7 : Limonin

E8 : Limonexic acid





E9 : Obacunone

E10 : Nomilin : R = OAc

E11 : Deacetyl nomilin : R = OH

1.4 Objective

This research work is to investigate the chemical constituents from the leaves and stems of *Citrus aurantifolia* Swingle. It involved isolation, purification and structure elucidation.

CHAPTER 2
EXPERIMENTAL

2.1 Instruments and Chemicals

Column chromatography was performed by using silica gel 60(230-400 Mesh ASTM, Merck) or silica gel 100 (70-230 Mesh ASTM, Merck) or Sephadex^{TM} LH-20 (Amersham Biosciences, Sweden). For thin-layer chromatography (TLC), aluminium sheets of silica gel 60 F_{254} (20×20 cm, layer thickness 0.2 mm, Merck) were used for analytical purpose and the compounds were visualized under ultraviolet light. Solvent for extraction and chromatography were distilled at their boiling ranges prior to use. Melting point was recorded in $^{\circ}$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. Infrared spectra (IR) were obtained on a Perkin-Elmer 783 FTS165 FT-IR spectrophotometer and were recorded in wave number (cm^{-1}). ^{1}H and ^{13}C- Nuclear resonance spectra were recorded on FT-NMR Bruker Ultra Shield™ 300 and 500 MHz spectrometer at Department of Chemistry, Faculty of Science, Prince of Songkla university. Spectra were recorded in $CDCl_3$, Acetone-d_6 and DMSO-d_6 as δ value in ppm down field from TMS (internal standard δ 0.00) and coupling constant (J) are expressed in hertz.

2.2 Plant material

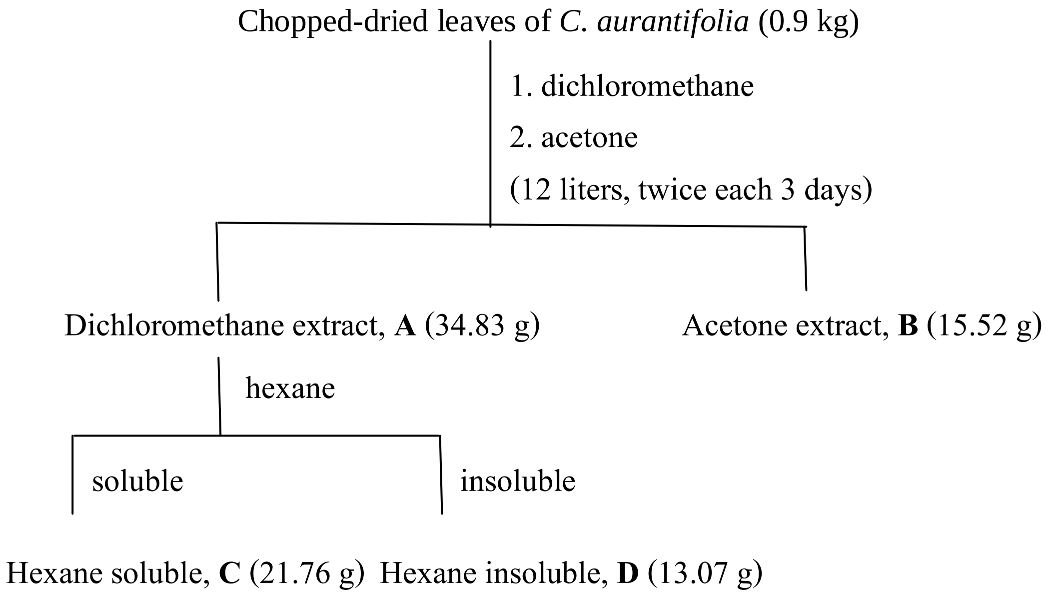
The leaves and stems of *C. aurantifolia* (Rutaceae) were collected from Amphur Panare, Pattani Province in the southern part of Thailand, on May, 2009 and were air-dried at room temperature, with no direct sunlight.

2.3 Extraction and isolation

2.3.1 Purification and isolation of the leaves of *C. aurantifolia*

2.3.1.1 Purification of dichloromethane extract

Chopped-dried leaves of *C. aurantifolia* (0.9 kg) was immersed in dichloromethane and acetone at room temperature (12 liters, twice each 3 days). After removal of solvents, a green-brown gum of crude dichloromethane (34.83 g) and the dark-brown gum of crude acetone (15.52 g) were obtained. The dichloromethane extract was further dissolved in hexane to remove wax. The process of extraction was shown in **Scheme 1**.

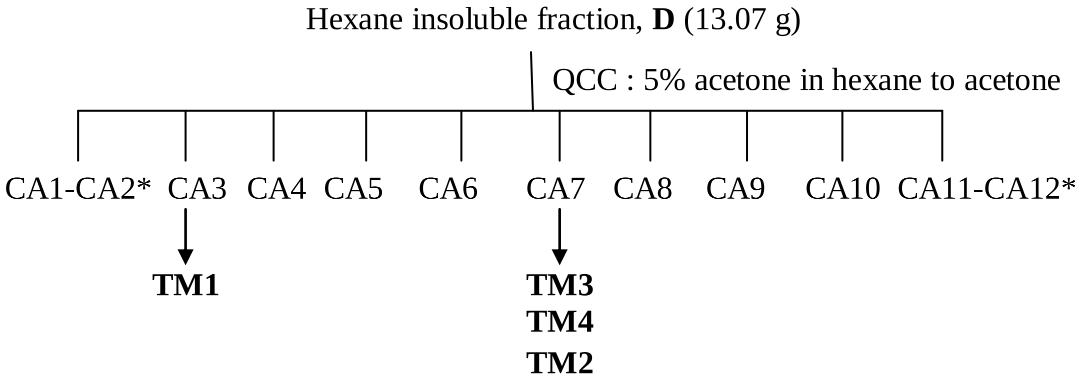


Scheme 1 Extraction of the crude extracts from the leaves of *C. aurantifolia.*

The hexane insoluble fraction, D (13.07 g), was separated by quick column chromatography over silica gel 60H using gradient solvent of 5% acetone in hexane to acetone. Fractions with the similar characteristic on TLC were combined to afford 12 fractions (CA1-CA12). Further purification of each fraction gave four compounds (**Scheme 2).**

Table 2 Physical characteristic and weights of fraction obtained from QCC of the hexane insoluble fraction (**D**)

Fraction	Weight (g)	Physical characteristic
CA1	1.116	yellow gel
CA2	1.036	orange gel
CA3	1.373	brown viscous liquid
CA4	1.308	brown viscous liquid
CA5	0.309	brown viscous liquid
CA6	1.081	brown viscous liquid
CA7	1.535	brown viscous liquid
CA8	1.347	brown viscous liquid
CA9	1.329	brown viscous liquid
CA10	0.704	brown viscous liquid
CA11	1.334	brown solid
CA12	0.532	brown solid



*no further investigation

Scheme 2 Isolation of **TM1-TM4** from hexane insoluble fraction, **D**

Fraction CA3 yielded a white solid of **TM1** (3.0 mg).

Fraction CA7 (1.535 g) was further separated by CC and eluted with hexane : dichloromethane: acetone (8:1:1) to afford subfractions CA7.1-CA7.12. Subfraction CA7.7 (83.40 mg) was further separated by CC eluting with hexane:

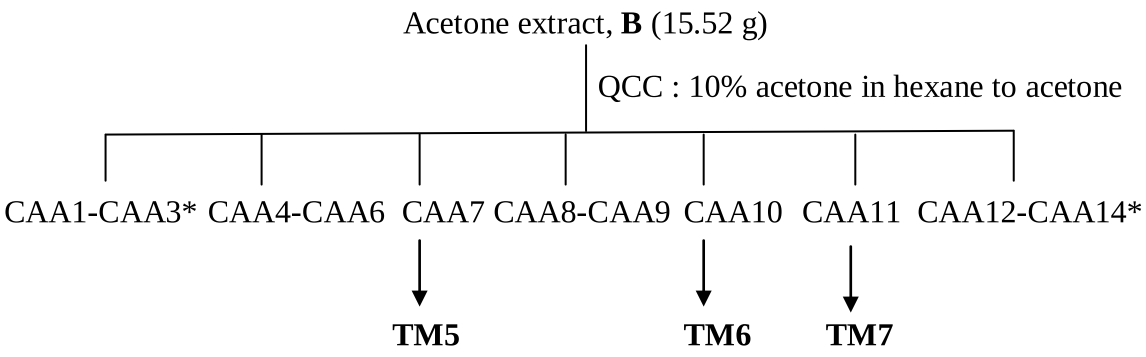
dichloromethane: acetone (7.5:1:1.5) to afford a white solid of **TM2** (3.6 mg). Subfraction CA7.10 (345.0 mg) was further separated by CC and eluted with 5% acetone in hexane to give a white solid of **TM3** (2.9 mg). Subfraction CA7.11 (89.80 mg) was further separated by CC and eluted with 5% acetone in hexane to give a white solid of **TM4** (0.9 mg).

2.3.1.2 Purification of acetone extract

The acetone extract, **B** (15.52 g) was separated by quick column chromatography over silica gel 60H using gradient solvent of 10% acetone in hexane to acetone as eluents. Fractions with the similar characteristic on TLC were combined to afford 14 fractions (CAA1-CAA14) (**Table 3**). Further purification of each fraction gave three compounds (**Scheme 3**).

Table 3 Physical characteristic and weights of fraction obtained from QCC of the acetone extract (**B**)

Fraction	Weight (g)	Physical characteristic
CAA1	1.884	yellow gel
CAA2	1.343	orange gel
CAA3	1.086	yellow-brown viscous
CAA4	0.969	liquid
CAA5	1.811	brown viscous liquid
CAA6	1.263	brown viscous liquid
CAA7	0.837	brown viscous liquid
CAA8	1.327	brown viscous liquid
CAA9	0.535	brown viscous liquid
CAA10	0.816	brown viscous liquid
CAA11	0.832	brown viscous liquid
CAA12	0.535	brown solid
CAA13	2.311	brown solid
CAA14	1.005	brown solid



*no further investigation

Scheme 3 Isolation of **TM5-TM7** from acetone extract (**B)** of the leaves of *C. aurantifolia*.

Fraction CAA7 (0.837 g) was further separated by CC and eluted with 15% acetone in hexane to afford a white solid of **TM5** (0.9 mg).

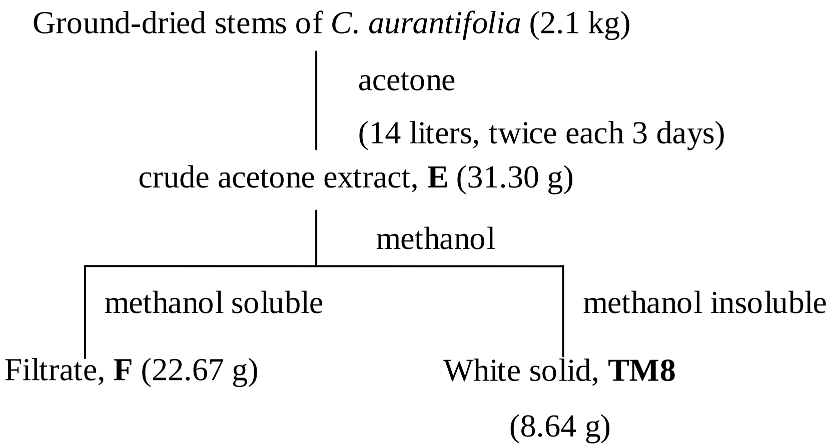
Fraction CAA10 yielded a yellow solid of **TM6** (14.3 mg).

Fraction CAA11 yielded a white solid of **TM7** (2.8 mg).

2.3.2 Extraction and isolation of the stems of *C. aurantifolia*

2.3.2.1 Purification of acetone extract

Ground-dried stems of *C. aurantifolia* (2.1 kg) was immersed in acetone at room temperature (14 liters, twice each 3 days). After removal of solvent, the dark-brown viscous acetone extract, **E** (31.30 g) was obtained. The crude extract was further dissolved in methanol to give white solid, **TM8** (8.64 g) and methanol soluble fraction, **F** (22.67 g). The process of extraction was shown in **Scheme 4**.



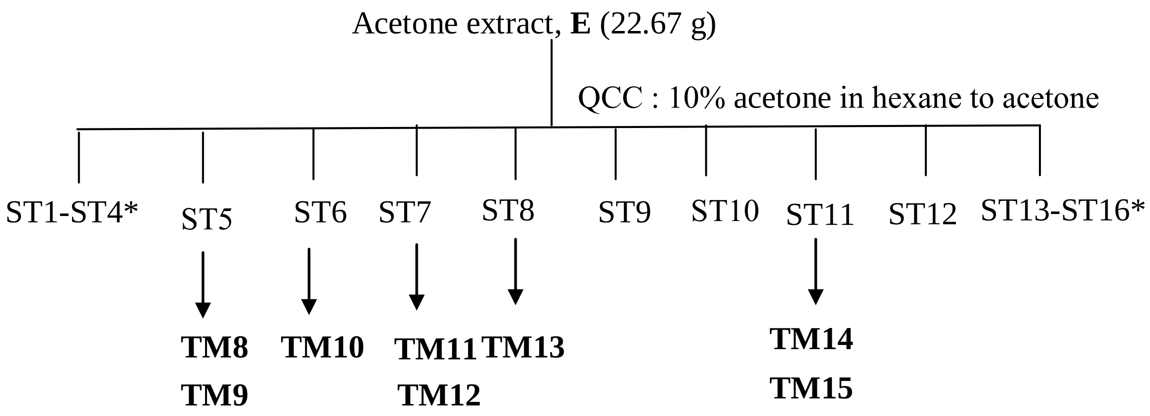
Scheme 4 Extraction of the crude extracts from the stems of *C. aurantifolia*.

2.3.2.1 Purification of acetone extract

The methanol soluble of acetone extract, **F** (22.67 g) was separated by quick column chromatography over silica gel 60H using gradient solvent of 10% acetone in hexane to acetone. Fractions with the similar characteristic on TLC were combined to afford 16 fraction (ST1-ST16) (**Table 4**). Further purification of each fraction gave seven compounds (**Scheme 5**).

Table 4 Physical characteristic and weights of fraction obtained from QCC of the methanol soluble fraction (**F**)

Fraction	**Weight (g)**	**Physical characteristic**
ST1	0.553	yellow gel
ST2	1.224	yellow gel
ST3	1.007	yellow-brown viscous liquid
ST4	0.934	brown viscous liquid
ST5	1.112	brown viscous liquid
ST6	0.884	brown viscous liquid
ST7	0.782	brown viscous liquid
ST8	1.243	brown viscous liquid
ST9	0.952	brown viscous liquid
ST10	0.712	brown viscous liquid
ST11	1.778	brown viscous liquid
ST12	1.114	brown solid
ST13	0.541	brown solid
ST14	1.375	brown solid
ST15	2.442	brown solid
ST16	2.228	brown solid



*no further investigation

Scheme 5 Isolation of **TM8**-**TM15** from acetone extract **(E)**.

Fraction ST5 yielded a white solid of **TM8** (16.4 mg) and a yellow solid of **TM9** (25.3 mg).

Fraction ST6 (0.884g) was further separated by CC and eluted with hexane : dichloromethane: acetone (8:1:1) to afford subfractions ST6.1-ST6.14. Subfraction ST6.12 (96.40 mg) was further separated by CC eluting with hexane: dichloromethane: acetone (7.5:1:1.5) to yield a yellow solid of **TM10** (7.7 mg).

Fraction ST7 (0.782 g) was further separated by CC over Sephadex™ LH-20 and eluted with 50% methanol-dichloromethane to yield a yellow solid of **TM11** (0.9 mg) and **TM12** (2.7 mg).

Fraction ST8 (1.243 g) was further separated by CC over Sephadex™ LH-20 and eluted with 50% methanol-dichloromethane to give a yellow solid of **TM13** (2.4 mg).

Fraction ST11 (1.778 g) was further separated by CC over Sephadex™ LH-20 and eluted with 50% methanol-dichloromethane to afford subfractions ST11.1-ST11.6. Subfraction ST11.1 was further separated by CC over Sephadex™ LH-20 and eluted with 50% methanol-dichloromethane to a yellow solid of **TM14** (2.6 mg). and a white solid of **TM15** (2.4 mg).

TM1: Bergapten

m.p. 190-193 ^{o}C

UV λ_{max} nm (MeOH) (log ε): 222 (4.00), 249 (3.89), 259 (3.85), 267 (3.86), 311 (3.79) nm

IR (KBr) ν (cm^{-1}): 1620 (C=O stretching)

For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 5**

TM2: Isopimpinellin

m.p. 150-152 ^{o}C

UV λ_{max} nm (MeOH) (log ε): 235 (3.89), 253 (3.85), 263 (3.86), 325 (3.79)

IR (KBr) ν (cm^{-1}): 1705, 1690 (C=O stretching)

For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 6**

TM3: Citropten

m.p. 224-226 ^{o}C

UV λ_{max} nm (MeOH) (log ε): 216 (3.21), 258 (3.27),324 (3.23)

IR (KBr) ν (cm^{-1}): 1611 (C=O stretching)

For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 7**

TM4: 4-Hydroxybenzaldehyde

m.p. 224-226 ^{o}C

UV λ_{max} nm (MeOH) (log ε): 257 (3.68), 275 (3.04) nm

IR (neat) ν (cm^{-1}): 3209 (O-H stretching), 1674 (C=O stretching)

For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 8**

TM5: Bergamottin

m.p. 194-196 °C

UV λ_{max} nm (MeOH) (log ε): 217 (3.45), 246 (3.43), 288 (3.33), 319 (3.23) nm

IR (KBr) ν (cm^{-1}): 1632 (C=O stretching)

For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 9**

TM6: 5,7,4'-Trihydroxy-3-methoxyflavone

m.p. 123-125 °C

UV λ_{max} nm (MeOH) (log ε): 235 (3.55), 286 (3.50), 330 (3.43), 387 (3.04) nm

IR (neat) ν (cm^{-1}): 3368 (O-H stretching), 1655 (C=O stretching)

For ^{1}H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 10**

TM7: Umbelliferone

m.p. 224-226 °C

UV λ_{max} nm (MeOH) (log ε): 216 (3.67), 324 (3.55) nm

IR (neat) ν (cm^{-1}): 3411 (O-H stretching), 1720 (C=O stretching)

For ^{1}H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 11**

TM8: Limonin

m.p. 285-286 °C

IR (neat) ν (cm^{-1}): 1730, 1709 (C=O stretching)

For ^{1}H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 12**

TM9: Atalantoflavone

m.p. 285-287 ^{o}C

UV λ_{max} nm (MeOH) (log ε): 239 (3.57), 281 (3.44), 332 (3.41), 382 (3.35) nm

IR (neat) ν (cm^{-1}): 3360 (O-H stretching), 1620 (C=O stretching)

For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 13**

TM10: 5-Hydroxynoracronycine

m.p. 261-263^{o}C

UV λ_{max} nm (MeOH) (log ε): 222 (3.33), 279 (3.20), 321 (3.18), 388 (3.09) nm

IR (neat) ν (cm^{-1}): 3446 (O-H stretching), 1723 (C=O stretching)

For ^{1}H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 14**

TM11: Citracridone-III

m.p. 274-276 ^{o}C

UV λ_{max} nm (MeOH) (log ε): 225 (3.87), 281 (3.77), 343 (3.74), 386 (3.53) nm

IR (neat) ν (cm^{-1}): 3368 (O-H stretching), 1655 (C=O stretching)

For ^{1}H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 15**

TM12: Chrysin

m.p. 280-282 ^{o}C

UV λ_{max} nm (MeOH) (log ε): 231 (3.42), 287 (3.37), 340 (3.25) and 366 (3.21) nm

IR (neat) ν (cm^{-1}): 3550 (O-H stretching), 1620 (C=O stretching)

For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 16**

TM13: Citrusinol

m.p. 253-254 °C

UV λ_{max} nm (MeOH) (log ε): 222 (3.25), 241 (3.20), 248 (3.14), 267 (3.09), 331 (3.02) nm

IR (neat) ν (cm^{-1}): 3550 (O-H stretching), 1620 (C=O stretching)

For ^{1}H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 17**

TM14: Syringaresinol

m.p. 261-263 °C

UV λ_{max} nm (MeOH) (log ε): 205 (3.44), 237 (3.35), 281 (3.21) nm

IR (neat) ν (cm^{-1}): 3435 (O-H stretching), 1620 (C=O stretching)

For ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 18**

TM15: Limonexic acid

m.p. 285-286 °C

IR (neat) ν (cm^{-1}): 3233 (O-H stretching), 1743 (C=O stretching)

For ^{1}H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 19**

CHAPTER 3

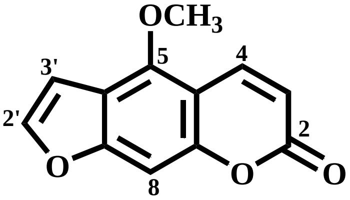
RESULTS AND DISCUSSION

3.1 Structure elucidation of compounds from the leaves and the stems of *C. aurantifolia*

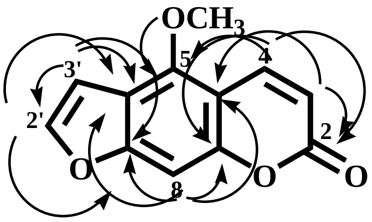
The investigation of chemical constituents in the dichloromethane and acetone extract of the leaves of *C. aurantifolia* Swingle resulted in the isolation of 7 compounds. They were identified as bergapten (**TM1**), isopimpinellin (**TM2**), citropten (**TM3**), 4-hydroxybenzaldehyde (**TM4**), bergamottin (**TM5**), 5,7,4'ttri hydroxy-3-methoxyflavone (**TM6**), umbeliferone **(TM7)**. While investigation of chemical constituents in the acetone extract of the stems resulted in the isolation of 8 compounds. They were identified as limonin (**TM8**), atalantoflavone (**TM9**), 5-hydroxynoracronycine (**TM10**), citracridone-III (**TM11**), chrysin (**TM12**), citrusinol (**TM13**), syringaresinol (**TM14**) and limonexic acid (**TM15**).

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

TM1 : Bergapten



TM1 was obtained as a white solid. The UV spectrum showed maximum absorption bands at 222, 249, 259, 267 and 311 nm. The IR absorption showed the absorption band of C=O stretching at 1620 cm^{-1}. The ^{1}H NMR spectrum **(Table 5)** showed an AB-type doublet of the α- and β-olefinic protons of coumarin system at δ 6.29 and δ 8.17 (J = 9.9 Hz), a siglet of aromatic proton H-8 at δ 7.15, and singlet of methoxyl group at δ 4.29 (5-OMe). The resonances at δ 7.04 (*d*) and δ 7.61 (*d*) with a coupling constant of 2.4 Hz were assigned for the olefinic protons H-3' and H-2' of furan ring. The HMBC correlations of α-olefinic proton, methoxyl proton and olefinic proton H-3' to C-5 (δ 149.7) indicated that the methoxyl group located at C-5 not C-8 position. The correlations of H-2' to C-6 (δ 116.2), C-7 (δ 158.4)) indicated that a furan ring was fused to coumarin at C-6 and C-7 position. **TM1** then was identified as 5-methoxy-2*H*-furo[3,2-*g*]chromen-2-one. The assignment and spectroscopic data were in agreement to those of Bergapten (Patil *et al.*, 2010).



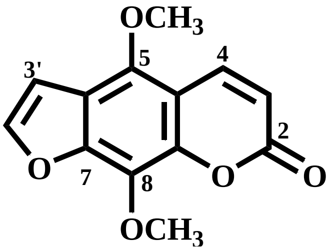
Major HMBC correlations of **TM1**

Table 5 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM1** (Bergapten)

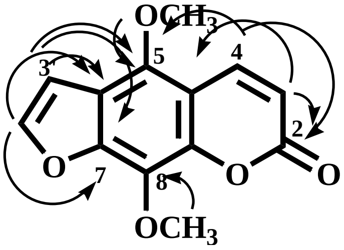
Position	δ_H **(multiplicity)**	δ_C **(C-Type)**	**HMBC**
2	-	161.4 (C=O)	-
3	6.29 (*d*, *J* = 9.9)	112.6 (CH)	C-2, C-4a
4	8.17 (*d*, *J* = 9.9)	139.7 (CH)	C-2, C-8a, C-5
4a		106.4 (C)	
5	-	149.7 (C)	-
6	-	116.2 (C)	-
7	-	158.4 (C)	-
8	7.15 (*s*)	98.4 (CH)	C-4a, C-6, C-7, C-8a
8a	-	156.5 (C)	-
2'	7.61 (*d*, *J* = 2.4)	144.8 (CH)	C-6, C-7, C-3'
3'	7.04 (*d*, *J* = 2.4)	105.3 (CH)	C-6, C-7, C-2'
5-OCH$_3$	4.29 (*s*)	60.1 (CH$_3$)	C-5

in CDCl$_3$

TM2 : Isopimpinellin



TM2 was obtained as a white solid. m.p. 150-152. ^{o}C. The UV spectrum showed maximum absorption bands at 235, 253, 263 and 325 nm. The IR absorption bands showed the absorption bands of C=O stretching at 1705 cm^{-1} and aromatic 1690 cm^{-1}. Its ^{1}H and ^{13}C NMR spectrum **(Table 6)** were similar to those of **TM1** with the presence of methoxyl signal (δ 4.18) instead of aromatic proton signal (δ 7.15). The ^{1}H NMR spectrum showed an AB-type doublet of the α - and β - olefinic protons of coumarin system at δ 8.14 and δ 6.30 (*d*, *J* = 9.9 Hz), and singlet of two methoxyl groups at δ 4.18 (5-OMe and 8-OMe). The resonances at δ 7.01 (*d*) and δ 7.64 (*d*) with a coupling constant of 2.4 Hz were assigned for the olefinic protons H-3' and H-2' of furan ring. The HMBC correlations of α -olefinic proton, methoxyl proton and olefinic proton H-3' to C-5 (δ 155.2) indicated that the methoxy group located at C-5. The correlations of H-2' to C-6 (δ 122.8), C-7 (δ 159.1) indicated that a furan ring was fused to coumarin at C-6 and C-7 position. **TM2** then was identified as 4,9-dimethoxy-2*H*-furo[3,2-*g*]chromen-2-one. It was known as Isopimpinellin (Patil *et al.*, 2010).



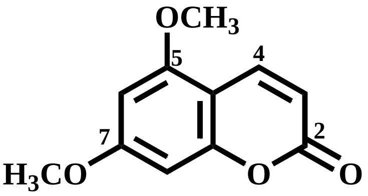
Major HMBC correlations of **TM2**

Table 6 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM2** (Isopimpinellin)

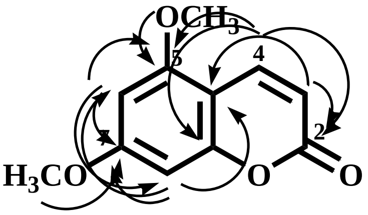
Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
2	-	168.3 (C=O)	-
3	6.30 (*d*, *J* = 9.9)	112.6 (CH)	C-2, C-4a
4	8.14 (*d*, *J* = 9.9)	141.4 (CH)	C-2, C-8a, C-5
4a		112.2 (C)	
5	-	155.2 (C)	-
6	-	122.8 (C)	-
7	-	159.1 (C)	-
8	-	138.8 (C)	-
8a	-	144.9 (C)	-
2'	7.64 (*d*, *J* = 2.4)	146.8 (CH)	C-6, C-7, C-3'
3'	7.01 (*d*, *J* = 2.4)	104.5 (CH)	C-6, C-7, C-2'
5-OCH_3	4.18 (*s*)	60.4 (CH_3)	C-5
8-OCH_3	4.18 (*s*)	61.2 (CH_3)	C-8

in $CDCl_3$

TM3 : Citropten



TM3 was obtained as a white solid. m.p. 224-226 °C. The UV spectrum showed maximum absorption bands at 216, 258 and 324 nm. The IR absorption bands showed the absorption bands of C=O stretching at 1611 cm^{-1}. The ^{1}H NMR spectrum **(Table 7)** showed the characteristic signal of α- and β- olefinic protons of coumarin at δ 7.97 and δ 6.16 (*d*, *J* = 9.9 Hz), *meta*-aromatic protons with *J* = 2.1 Hz at δ 6.42 (H-8) and δ 6.28 (H-6), and two methoxyl groups at δ 3.89 (5-OMe) and δ 3.85 (7-OMe). The HMBC correlations of α-olefinic proton and 5-OMe to C-5 (δ 163.7), and that of H-6 (δ 6.28), H-8 (δ 6.42) to C-7 (δ 156.9) confirmed that the methoxyl group were located at the C-5 and C-7, respectively. **TM3** then was identified as 5,7-dimethoxy-2*H*-chromen-2-one which was known as Citropten (Kawaii *et al.*, 1999).



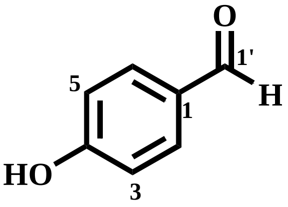
Major HMBC correlations of **TM3**

Table 7 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM3** (Citropten)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
2	-	161.3 (C=O)	-
3	6.16 (*d*, *J* = 9.6)	111.1 (CH)	C-2, C-4a
4	7.97 (*d*, *J* = 9.6)	138.7 (CH)	C-2, C-8a, C-5
4a	-	104.1 (C)	-
5	-	163.7 (C)	-
6	6.28 (*d*, *J* = 2.1)	94.9 (CH)	C-4a, C-5, C-7, C-8
7	-	156.9 (C)	-
8	6.42 (*d*, *J* = 2.1)	92.9 (CH)	C-4a, C-6, C-7
8a	-	156.9 (C)	-
5-OCH_3	3.89 (*s*)	55.9(CH_3)	C-5
7-OCH_3	3.85 (*s*)	55.8 (CH_3)	C-7

in $CDCl_3$

TM4 : 4-Hydroxybenzaldehyde



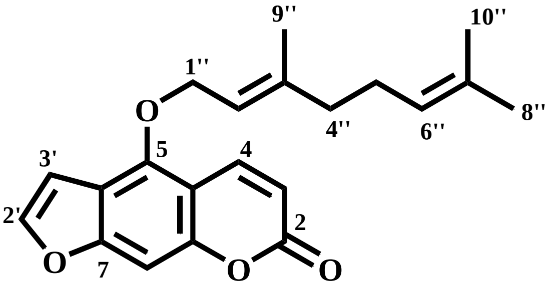
TM4 was obtained as a white solid. Its ^{1}H NMR spectrum **(Table 8)** showed characteristic signal of a *para*-disubstituted benzene at δ 7.81(*d*, J = 8.4 Hz, H-2/H-6), δ 6.96 (*d*, J = 8.4 Hz, H-3/H-5). One of substituent was assigned for a formyl group which its proton resonated at δ 9.88 (*s*, CHO) and its cabonyl carbon resonated at δ190.6. Another substituent was suggested to be a hydroxyl group from the resonance of an oxy-carbon signal at δ 161.0. It was then identified as 4-hydroxybenzaldehyde. Its spectroscopic data were in agreement to the previously reported data (Jang *et al*., 2004).

Table 8 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM4** (4-Hydroxybenzaldehyde)

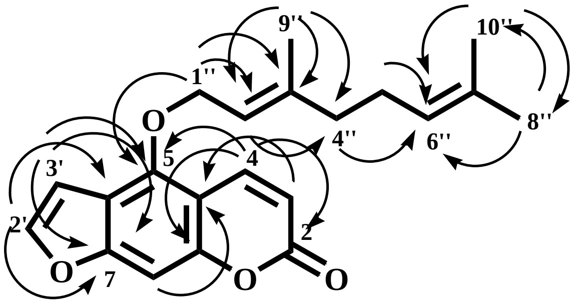
Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
1	-	130.0 (C)	-
2/6	7.81 (*d*, J = 8.4)	132.5 (CH)	C-3, C-4, C-7
3/5	6.96 (*d*, J = 8.4)	116.1 (CH)	C-1, C-2
4	-	161.0 (C)	-
1'	9.88 (*s*)	190.6 (C)	-

in $CDCl_3$

TM5 : Bergamottin



TM5 was obtained as a white solid. The UV spectrum showed maximum absorption bands at 217, 246, 288 and 319 nm. The IR absorption showed the absorption band of C=O stretching at 1632 cm^{-1}. The ^{1}H NMR spectrum **(Table 9)** showed an AB-type doublet of α- and β-olefinic protons of coumarin at δ 6.26 and δ 8.16 (J = 9.8 Hz) and a siglet of aromatic proton H-8 at δ 7.10. The presence of furan ring was proposed from the doublet olefinic protons with J = 2.4 Hz, at δ 7.50 (H-2') and δ 6.96 (H-3') together with a low field carbon signal at δ 144.8 (C-2'). The correlations of H-2' to C-6 (δ 114.2) and C-7 (δ 158.1) indicated that the furan ring was fused at C-6 and C-7 of coumarin. The presence of *O*-geranyl group was shown by the characterisric signal of oxy-methylene proton H-1" at δ 4.95 (*d*, J = 6.9 Hz), methine proton H-2" at δ 5.54 (*t*, J = 6.7 Hz) and H-6" at δ 5.06 (*br s*), methylene proton H-1" at δ 4.95 (*d*, J = 6.9 Hz), H-4" and H-5" at δ 2.10 (*s*) and methyl proton at δ 1.68 (H-8"), 1.60 (H-9") and 1.69 (H-10"). The side chain was placed at C-5 and *peri*- to β-proton according to the HMBC correlations of H-3', H-1" and H-4 to C-5 (δ 148.9). **TM5** then was identified as 4-(3,7-dimethylocta-2,6-dienoxy)furo[3,2-g]chromen-7-one which corresponded to Bergamottin (Kawaii *et al.*, 1999).



Major HMBC correlations of **TM5**

Table 9 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM5** (Bergamottin)

Position	δ_H (multiplicity)	δ_C (C-Type)	HMBC
2	-	161.3 (C=O)	-
3	6.26 (*d*, *J* = 9.8)	112.5 (CH)	C-2, C-4a
4	8.16 (*d*, *J* = 9.8)	139.6 (CH)	C-2, C-8a, C-5
4a		107.5 (C)	
5	-	148.9 (C)	-
6	-	114.2 (C)	-
7	-	158.1 (C)	-
8	7.10 (*s*)	94.2 (CH)	C-4a, C-6, C-7, C8a
8a	-	152.7 (C)	-
2'	7.50 (*d*, *J* = 2.4)	144.8 (CH)	C-6, C-7
3'	6.96 (*d*, *J* = 2.4)	105.1 (CH)	C-6, C-7
1"	4.95 (*d*, *J* = 6.9)	69.7 (CH_2)	C-5, C-2", C-3"
2"	5.54 (*t*, *J* = 6.7)	118.9 (CH)	C-4", C-9"
3"	-	143.0 (C)	-
4"	2.10 (*br s*)	39.5 (CH_2)	C-2", C-3", C-5"

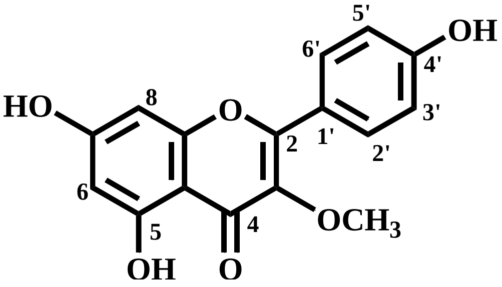
in $CDCl_3$

Table 9 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM5** (Bergamottin) (continued)

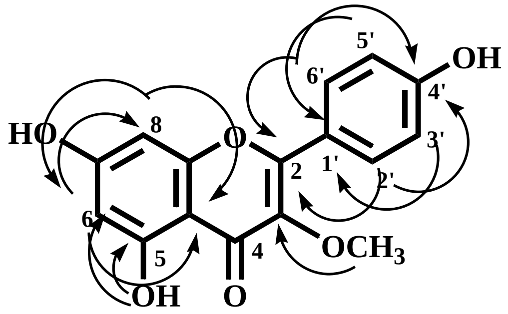
Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
5"	2.10 (*br s*)	26.2 (CH_2)	C-4", C-6", C-7"
6"	5.06 (*br s*)	123.5 (CH)	-
7"	-	132.0 (C)	-
8"	1.68 (*s*)	17.7 (CH_3)	C-6", C-7", C-10"
9"	1.60 (*s*)	25.7 (CH_3)	C-2", C-3", C-5"
10"	1.69 (*s*)	16.7 (CH_3)	C-6", C-7", C-8"

in $CDCl_3$

TM6 : 5,7,4'-Trihydroxy-3-methoxyflavone



TM6 was obtained as a yellow solid. The UV spectrum showed absorption bands at λ_{max} 235, 286, 330 and 387 nm. The IR spectrum showed the stretching of hydroxyl (3368 cm^{-1}) and conjugated carbonyl group (1655 cm^{-1}). The ^{1}H NMR spectrum **(Table 10)** showed the resonance of a hydrogen bonded hydroxyl group at δ 13.00 (5-OH), a methoxyl group at δ 3.92 (3-OCH$_3$) and two hydroxyl group at δ 9.80 (7-OH) and 9.68 (4'-OH). The spectrum further showed signal of *meta*-proton at δ 6.50 and 6.55. The higher field one was assigned for H-6 according to the HMBC correlation of the chelated hydroxyl 5-OH to C-6 (δ 105.4). The *ortho* coupling pattern in aromatic region at δ 7.55 (*d*, J = 9.0 Hz) and δ 6.96 (*d*, J = 9.0 Hz,) corresponded to H-2'/H-6' and H-3'/H-5', respectively. Attached to the *para* position of the B ring is a hydroxyl group resonating at δ 9.68 confirmed by HMBC correlations of H-2'/H-6' to C-2 (δ 160.8). The methoxyl group was at olefinic carbon (C-3) not aromatic carbon due to HMBC correlation to the high field oxygenated quaternary carbon (δ 130.4, C-3). It was proposed as 5,7,4'-Trihydroxy-3-methoxyflavone. Its spectroscopic data was in agreement to those of previously reported data (Oesterle *et al.*, 1917).



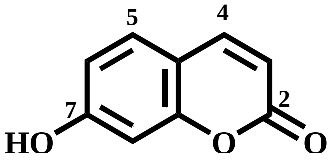
Major HMBC correlations of **TM6**

Table 10 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM6** (5,7,4'-Trihydroxy-3-methoxyflavone)

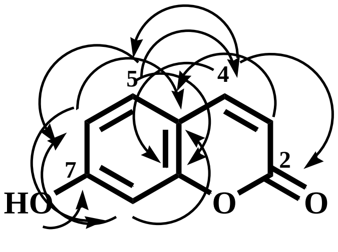
Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
2	-	160.8 (C)	-
3	-	130.4 (C)	-
4	-	197.8 (C=O)	-
4a	-	131.6 (C)	-
5	-	153.2 (C)	-
6	6.50 (*d*, *J* = 2.1)	105.4 (CH)	C-7
7	-	164.7 (C)	-
8	6.55 (*d*, *J* = 2.1)	94.0 (CH)	C-4a, C-5, C-8a
8a	-	156.4 (C)	-
1'	-	121.8 (C)	-
2',6'	7.55 (*d*, *J* = 9.0)	128.3 (CH)	C-2, C-6'
3',5'	6.96 (*d*, *J* = 9.0)	116.2 (CH)	C-1'
4'	-	155.6 (C)	-
5-OH	13.00 (*s*)	-	C-6, C-5, C-4a
7-OH	9.80 (*s*)	-	
4'-OH	9.68 (*s*)	-	-
3-OCH_3	3.92 (*s*)	60.1 (CH_3)	

in $CDCl_3$ + DMSO-*d*$_6$

TM7 : Umbelliferone



TM7 was obtained as a white solid. m.p. 224-226 °C. The UV spectrum showed maximum absorption bands at 216 and 324 nm. The IR absorption showed the absorption bands of hydroxyl at 3411 cm^{-1} C=O stretching at 1720 cm^{-1}. The ^{1}H NMR spectral data **(Table 11)** indicated that it was a coumarin from AB-type doublet of α - and β - olefinic protons at δ 6.18 and δ 7.26 (J = 9.6 Hz). The trisubstituted benzene ring was proposed from an ABX signal of aromatic protons H-5, H-6 and H-8 at δ 7.32 (*d*, J = 8.4 Hz), δ 6.82 (*dd*, J = 8.4, 2.1 Hz) and δ 6.79 (*d*, J = 2.1 Hz), respectively. The HMBC correlation of H-4 (δ 7.62) to C-5 (δ 128.8) and H-5 (δ 7.32) to C-4 (δ 143.8) confirmed the aromatic proton H-5 *peri* to olefinic proton H-4. A hydroxyl group resonated at δ 9.81 was assigned for 7-OH due to the HMBC correlations of H-5 and 7-OH to C-7 (δ 161.4). **TM7** then was identified as 7-Hydroxychromen-2-one which corresponded to umbelliferone (Khalil *et al.*, 2003).



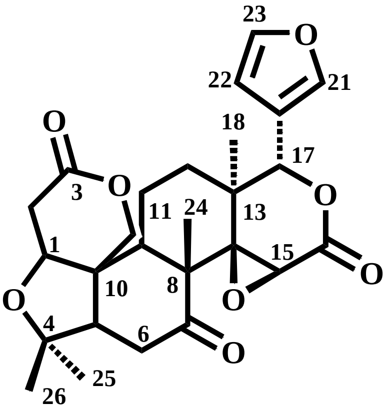
Major HMBC correlations of **TM7**

Table 11 ^{1}H,^{13}C NMR and HMBC spectral data of **TM7** (Umbelliferone)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
2	-	161.6 (C=O)	-
3	6.18 (*d*, *J* = 9.6)	113.4 (CH)	C-2, C-4a
4	7.26 (*d*, *J* = 9.6)	143.8 (CH)	C-2, C-8a, C-5
4a	-	111.6 (C)	-
5	7.32 (*d*, *J* = 8.4)	128.8 (CH)	C-4, C-8a, C-7
6	6.82 (*dd*, *J* = 8.4, 2.1)	111.8 (CH)	C-8, C-4a
7	-	161.4 (C)	-
8	6.79 (*d*, *J* = 2.1)	103.1 (CH)	C-6, C-8a
8a	-	155.9 (C)	-
7-OH	9.81 (*s*)	-	C-6, C-7, C-8

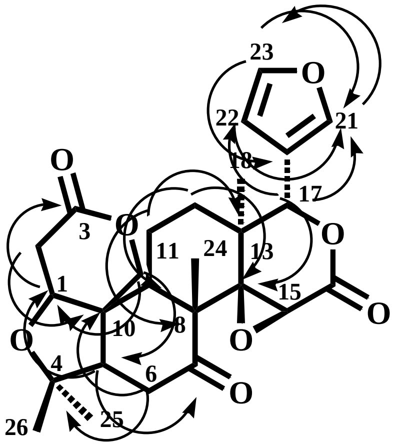
in $CDCl_3$+ DMSO-d_6

TM8 : Limonin



TM8 was obtained as a white solid, m.p. 285-286°C, $[\alpha]^{27}_{D}$ -132.5° (*c* 0.10, Me_2CO). The IR spectrum of **TM8** showed stretching of carbonyl at 1730 and 1709 cm^{-1}. The ^1H NMR spectrum **(Table 12)** suggested the presence of substituted furan from a singlet of H-21 at δ 7.42 (*s*) and doublets of H-23 at δ 7.45 and H-22 at δ 6.36 associated with a coupling constant value of 1.5 Hz. It was further established that **TM8** was a limonoid with four of singlet methyl groups at δ 1.25 (H-25), 1.17 (H-18), 1.16 (H-26) and at δ 1.08 (H-24). The presence of an epoxy lactone moiety of limonoid was revealed by the signals of carbonyl carbon at δ 171.7 (C-16), oxy carbon at δ 83.8 (C-17) and epoxy carbon at δ 58.6 (C-15) and δ 70.7 (C-14) together with the characteristic H-15 and H-17 singlet signal at δ 4.05 and 5.48, respectively. The HMBC correlation of H-17 to C-21 (δ 147.9) and C-22 (δ 114.5) suggested the attachment of furan ring at C-17. Moreover, lactone moiety was indicated by the signals of carbonyl carbon at δ 174.3 (C-3) and oxy carbon at δ 70.0 (C-19) along with signals of methylene protons at δ 2.30 and 2.70 (2 x *dd*, *J* = 15.0, 3.0 Hz, H-2α, H-2β), oxy-methylene protons at δ 4.50 and 4.82 (2 x *d*, *J* = 12.0 Hz, H-19α, H-19β) and oxy methine proton at δ 4.09 (*br s*, H-1). The presence of system –$(CH_3)_2$-C-CH-CH_2-C=O in the molecules was inferred from an ABC pattern at δ 2.73 (*dd*, *J* = 12.0, 3.0 Hz, H-6α), 3.16 (*dd*, *J* = 12.0, 3.0 Hz, H-6β) and δ 2.44 (*dd*, *J* = 12.0, 3.0 Hz, H-5α) as well as two methyl singlets at δ 1.25 (H-25) and δ 1.16 (H-26), The ^3J

correlations of H-5 to oxy-methine carbon C-1 (δ 84.9), H-6 to and quaternary carbon C-10 (δ 50.6), H-26 to C-5 (δ 64.8) and H-19 to C-5 (δ 64.8) implied that –(CH$_3$)$_2$-C-CH-CH$_2$-C=O was linked to the lactone ring by C-5 to C-10. Methylene proton resonated as multiplet at δ 1.87, 1.78 were assigned for H-11*α,* H-11*β* which was coupled by methylene proton H-12*α,* H-12*β* (δ 1.51, δ 1.82, *m*,) and methine proton H-9 (δ 2.58, *dd*, J = 9.0, 3.0 Hz). The ^3J correlation of H-11 to C-10 (δ 50.6), C-8 (δ 55.9) and C-13 (δ 44.5) together with ^3J correlation of H-12 to C-14 (δ 70.7) confirmed the location of four quaternary carbons. The carbon signals of quaternary carbon, methine carbon, methylene carbon and methyl carbon were in agreement with the assigned structure. **TM8** then was identified as 7,16-dioxo-7,16-dideoxylimondiol which was known as Limonin (Khalil *et al.*, 2003) ([α]$^{23}_{D}$ -124.7°, *c* 0.12, Me$_2$CO).



Major HMBC correlations of **TM8**

Table 12 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM8** (Limonin)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
1	4.09 (*br s*)	84.9 (CH)	C-3
2	2.30 (*dd*, *J* = 15.0, 3.0)	41.1 (CH_2)	C-1, C-9
	2.70 (*dd*, *J* = 15.0, 3.0)		C-3
3	-	174.3 (C=O)	-
4	-	82.7 (C)	-
5	2.44 (*dd*, *J* = 12.0, 3.0)	64.8 (CH)	C-1, C-7, C-10
6	2.73 (*dd*, *J* = 12.0, 3.0)	42.7 (CH_2)	C-4, C-7
	3.16 (*dd*, *J* = 12.0, 3.0)		C-5, C-10
7	-	211.4 (C=O)	-
8	-	55.9 (C)	-
9	2.58 (*dd*, *J* = 9.0, 3.0)	52.6 (CH)	C-11
10	-	50.6 (C)	-
11	1.87 (*m*)	23.6 (CH_2)	C-10, C-13
	1.78 (*m*)		C-8
12	1.51 (*m*)	35.2 (CH_2)	C-11, C-12, C-14
	1.82 (*m*)		C-11, C-17
13	-	44.5 (C)	-
14	-	70.7 (C)	-
15	4.05 (*s*)	58.6 (CH)	C-14, C-16
16	-	171.7 (C)	-
17	5.48 (*s*)	83.8 (CH)	C-12, C-18, C-21, C-22
18	1.17 (*s*)	22.3 (CH_3)	C-12, C-14, C-13, C-17
19	4.50 (*d*, *J* = 12.0)	70.0 (CH_2)	C-1, C-5, C-10
	4.82 (*d*, *J* = 12.0)		C-1, C-5, C-3

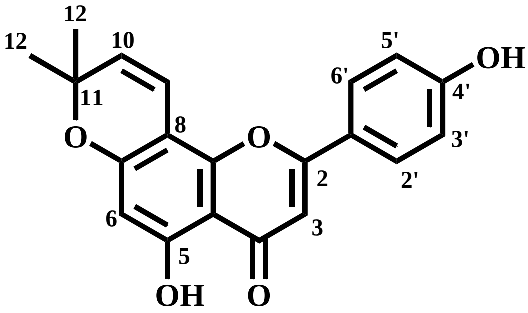
in $CDCl_3$+ DMSO-d_6

Table 12 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM8** (limonin) (continued)

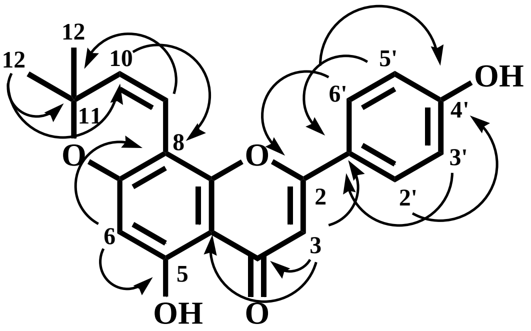
Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
20	-	124.8 (C)	-
21	7.42 (*s*)	147.9 (CH)	C-20, C-22, C-23
22	6.36 (*d*, *J* = 1.5)	114.5 (CH)	C-20, C-21, C-23
23	7.45(*d*, *J* = 1.5)	145.8 (CH)	C-20, C-21
24	1.08 (*s*)	22.3 (CH_3)	C-9, C-14, C-7
25	1.25 (*s*)	34.8 (CH_3)	C-26
26	1.16 (*s*)	25.2 (CH_3)	C-25, C-4, C-5

in $CDCl_3$+ DMSO-d_6

TM9 : Atalantoflavone



TM9 was obtained as a yellow solid. The UV spectrum showed absorption bands at λ_{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 ^{1}H NMR spectrum **(Table 13)** showed the presence of a *para*-substituted B ring on the basis of the characteristic aromatic AA'BB' spin system with chemical shift at δ 7.79 (H-2'/ H-6') and δ 7.16 (H-3'/H-5') with a coupling constant of J = 8.7 Hz. Attached to the *para* position of the B ring is a hydroxyl group resonating at δ 9.91. Moreover, the ^{1}H NMR spectrum revealed a deshielded singlet at δ 12.99 attributed to C-5 hydroxyl chelated to the C-4 carbonyl group. Furthermore, a singlet at δ 6.57 and δ 6.25 were assigned to H-3 and H-6, respectively. Both were identified by the use of an HMBC experiment : H-3 by the detection to C-1' (δ 126.6) of ring B and to C-4 (δ 187.2), and H-6 by a cross-peak of 5-OH to the corresponding C-6 (δ 104.7). The remaining signals in the ^{1}H NMR at δ 1.52 (*s*, 6H, H-12), δ 5.67 (*d*, J = 9.9 Hz, H-10) and δ 6.82 (*d*, J = 9.9 Hz, H-9) are typical for a 2,2-dimethylchromene ring which was suggested to be attached to C-7 and C-8 (δ 108.1) of the A ring by the HMBC correlation of H-10 and H-6 to C-8. **TM9** then was identified as 5-hydroxy-2-(4-hydroxyphenyl)-8,8-dimethy-pyrano[2,3-*f*]chromen-4-one. The assignment and spectroscopic data were in agreement to those of Atalantoflavone (Bacher *et al.*, 2010).



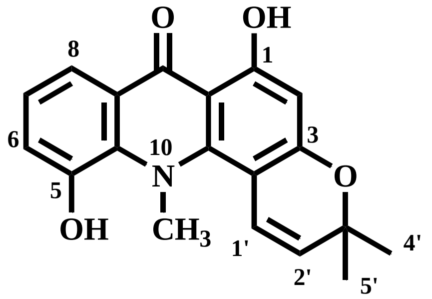
Major HMBC correlations of **TM9**

Table 13 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM9** (Atalantoflavone)

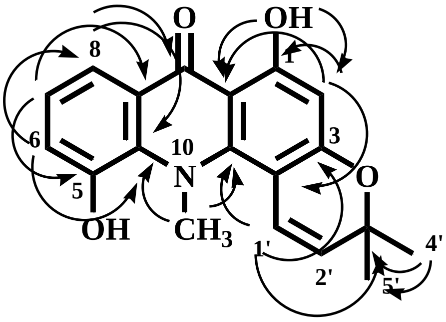
Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
2	-	168.8 (C=O)	-
3	6.57 (*s*)	106.6 (CH)	C-2, C-4, C-4a, C-1'
4	-	187.1 (C)	-
4a	-	109.8 (C)	-
5	-	166.2 (C)	-
6	6.25 (*s*)	104.6 (CH)	C-5, C-8
7	-	168.9 (C)	-
8	-	108.1 (C)	-
8a	-	156.5 (C)	-
9	6.82 (*d*, *J* = 9.9)	119.5 (CH)	C-8, C-11
10	5.67 (*d*, *J* = 9.9)	134.2 (CH)	C-8, C-11
11	-	82.7 (C)	-
12	1.52 (*s*)	32.8 (CH_3)	C-10, C-11, C-12
1'	-	126.5 (C)	-
2',6'	7.79 (*d*, *J* = 8.7)	132.7 (CH)	C-2', C-4'
3',5'	7.16 (*d*, *J* = 8.7)	121.0 (CH)	C-1', C-4'
4'	-	166.0 (C)	-
5-OH	12.99 (*s*)	-	-
4'-OH	9.91 (*s*)	-	-

in $CDCl_3$+DMSO-d_6

TM10 : 5-Hydroxynoracronycine



TM10 was obtained as a yellow solid. m.p. 261-263°C. The UV spectrum showed maximum absorption bands at 222, 279, 321 and 388 nm. The IR absorption showed the absorption bands of hydroxyl at 3446 cm^{-1} and C=O stretching at 1723 cm^{-1}. The ^1H NMR spectrum **(Table 14)** acquired in $CDCl_3$ and DMSO showed the characteristic signal of a chelated hydroxyl proton (1-OH) and *N*-methyl proton of acridone skeleton at δ 14.43 and δ 3.81, respectively. A singlet at δ 6.13 (H-2) was assigned for H-2 according to HMBC correlations of H-2 to C-1 (δ 169.1) and C-9a (δ 111.7). The remaining resonances appearing as an ABM system at δ 7.14 (*t*, J = 7.8 Hz), δ 7.26 (*d*, J = 7.8 Hz) and δ 7.75 (*d*, J = 7.8 Hz) were assigned for H-7, H-6 and H-8, respectively. Proton H-8 was confirmed at *peri* position to a carbonyl group by HMBCcorrelations of H-8 to carbonyl carbon C-9 (δ 186.6). Consequently, a hydroxyl proton that resonated at δ 9.98 was placed at C-5. The presence of 2,2-dimethylchromene ring was suggested from the resonance of methyl proton at δ 1.51 (H-4'/H-5', 6H) and *cis*-olefenic proton (J = 9.8 Hz) at δ 6.68 (*d*, H-1') and δ 5.56 (H-2'). It was placed at C-3 and C-4 according to HMBC correlations of H-2' to C-4 (δ 101.8), and H-1' to C-3' (δ 81.4) and C-4a (δ 148.8). **TM10** then was identified as 1,5,-diihydroxy-10,3',3'-trimethyl-10,3'-dihydro-3*H*-pyrano[2,3-*c*]acridin-9-one which was 5-Hydroxynoracronycine (Teng *et al.*, 2007).



Major HMBC correlations of **TM10**

Table 14 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM10** (5-Hydroxynoracronycine)

Position	***δ*****$_H$ (multiplicity)**	***δ*****$_C$ (C-Type)**	**HMBC**
1	-	169.1 (C)	-
2	6.13 (*s*)	102.2 (CH)	C-1, C-4, C-9a
3	-	165.9 (C)	-
4	-	107.0 (C)	-
4a	-	148.8 (C)	-
5	-	152.5 (C)	-
6	7.26 (*d*, *J* = 7.8)	124.9 (CH)	C-5, C-10a, C-8
7	7.14 (*t*, *J* = 7.8)	128.1 (CH)	C-5, C-8a
8	7.75 (*d*, *J* = 7.8)	120.7 (CH)	C-9, C10a
8a	-	129.5 (C)	-
9	-	186.6 (C=O)	-
9a	-	111.7 (C)	-
10a	-	141.8 (C)	-
1'	6.68 (*d*, *J* = 9.8)	125.8 (CH)	C-3, C-4a,C-9a, C-3'

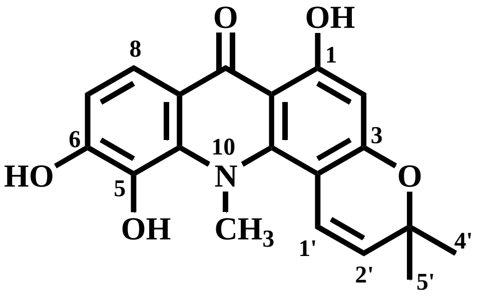
in $CDCl_3$ + DMSO-*d$_6$*

Table 14 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM10** (5-Hydroxynoracronycine) (continued)

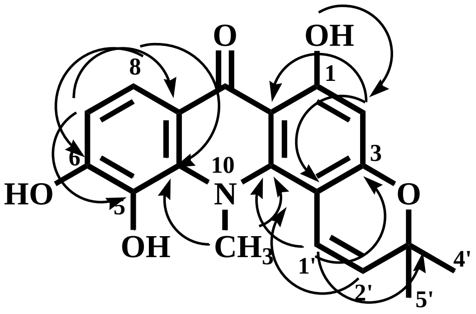
Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
2' 3' 4',5' 1-OH 5-OH *N*-CH$_3$	5.56 (*d*, *J* = 9.8) - 1.51 (*s*, 6H) 14.43 (*s*) 9.98 (*s*) 3.81 (*s*)	128.4 (CH) 81.4 (C) 31.8 (CH$_3$) - - 53.4 (CH$_3$)	C-4, C-3' - C-2', C-3' C-1, C-2, C-9a - C-4a, C-10a

in CDCl$_3$ + DMSO-d_6

TM11 : Citracridone-III



TM11 was obtained as a yellow solid. The UV spectrum showed absorption bands at λ_{max} 225, 281, 343 and 386 nm. The IR spectrum showed the stretching of hydroxyl (3368 cm^{-1}) and conjugated carbonyl group (1655 cm^{-1}). The ^{1}H NMR spectrum **(Table 15)** showed the characteristic signal of a chelated hydroxyl proton (1-OH) and *N*-methyl proton of acridone skeleton at δ 14.54 and δ 3.81, respectively. A singlet at δ 6.12 (H-2) was assigned for H-2 according to HMBC correlations to C-1(δ 158.8) and C-9a (δ 107.2). A doublet (*J* =9.0 Hz) of *ortho*-coupled aromatic proton H-7 (δ 6.96) and H-8 (δ 7.85) indicated the presence of a *tetra*-substituted benzene ring. Proton H-8 was confirmed at *peri* to a carbonyl group by HMBC correlations to carbonyl carbon C-9 (δ 176.8). Furthermore H-8 showed correlation to oxy-carbon resonated at δ 145.2 where as H-7 correlated to oxy-carbon resonated at δ 129.3 (C-5), suggested that δ 145.2 and δ 129.3 belonged to C-6 and C-5, respectively and the substituents at C-6 and C-5 were hydroxyl groups. A hydroxyl group that resonated at δ 9.64 could belong to 6-OH or 5-OH. The presence of 2,2-dimethylchromene ring was suggested from the resonance of methyl proton at δ 1.51 (H-4'/H-5', 6H) and *cis*-olefenic proton (*J* = 9.0 Hz) at δ 6.70 (*d*, H-1') and δ 5.53 (H-2'). It was placed at C-3 and C-4 according to HMBC correlations of H-2' to C-4 (δ 101.8), and H-1' to C-3' (δ 97.5) and C-4a (δ 143.1). **TM11** then was identified as 1,5,6-trihydroxy-10,3',3'-trimethyl-3,12-dihydro-3*H*-pyrano[2,3-*c*]acridin-9-one which was known as Citracridone-III (Teng *et al.*, 2007).



Major HMBC correlations of **TM11**

Table 15 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM11** (Citracridone-III)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
1	-	158.8 (C)	-
2	6.19 (*s*)	97.5 (CH)	C-1, C-2, C-3, C-4, C-9a
3	-	156.1 (C)	-
4	-	101.8 (C)	-
4a	-	143.0 (C)	-
5	-	129.3 (C)	-
6	-	145.2 (C)	-
7	6.97 (*d*, *J* = 9.0)	118.8 (CH)	C-5, C-6, C-8a
8	7.85 (*d*, *J* = 9.9)	111.8 (CH)	C-6, C-9, C10a
8a	-	113.1 (C)	-
9	-	176.8 (C=O)	-
9a	-	107.2 (C)	-
10a	-	132.9 (C)	C-3, C-14a, C-3'
1'	6.70 (*d*, *J* = 9.0)	116.4 (CH)	

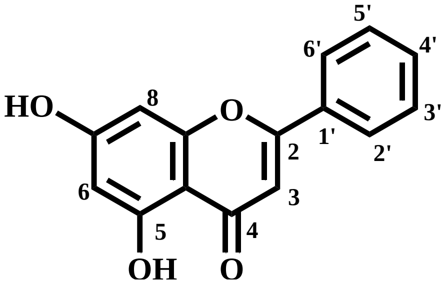
in $CDCl_3$ + DMSO-d_6

Table 15 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM11** (Citracridone-III) (continued)

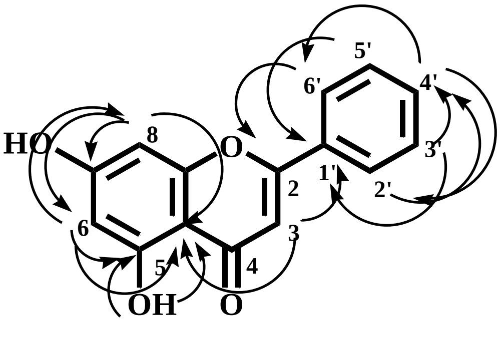
Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
2'	5.53 (*d*, *J* = 9.0)	113.1 (CH)	C-4, C-3'
3'	-	97.5 (C)	-
4',5'	1.51 (*s*)	26.7 (CH_3)	C-3'
1-OH	14.54 (*s*)	-	C-1, C-2, C-9a
5-OH	9.64 (*s*)	-	-
6-OH	-	-	-
N-CH_3	3.81 (*s*)	43.5 (CH_3)	C-4a, C-10a

in $CDCl_3$ + DMSO-d_6

TM12 : Chrysin



TM12 was obtained as a yellow solid. The UV spectrum showed absorption bands at λ_{max} 231, 287, 340 and 366 nm. The IR spectrum showed the stretching of hydroxyl (3550 cm^{-1}) and conjugated carbonyl group (1620 cm^{-1}). The ^{1}H NMR spectrum **(Table 16)** showed the resonance of a flavone of a hydrogenbonded hydroxyl proton at δ 12.66 (5-OH). The appearance of *meta*-coupled signals was resonanceed at δ 6.29 (H-6) and δ 6.43 (H-8) with coupling constant of 2.1 Hz. The spectrum further showed signals of equivalent aromatic proton H-2'/H-6' at δ 7.84 (2H, *dd*, *J* = 8.1, 2.1 Hz) , aromatic proton H-3'/H-5' at δ 7.49 (*m*) and H-4' at δ 7.48-7.46 (*m*). The ^{1}H NMR exhibited the resonance of a hydroxyl group at δ 10.01 (*br s*, 7-OH). The HMBC correlations of H-6 to C-7 (δ 162.1), C-8 (δ 94.3) and C-4a (δ 105.5) confirmed the position of H-6 and correlation of H-2'/H-6' and H-3'/H-5' to C-4' (δ 131.6) and H-4' to C-2'/C-6' confirmed the position of H-4'. Therefore, **TM12** was assigned to be 5,7-dihydroxyflavone which was known as Chrysin (Chen *et al.*, 2003).



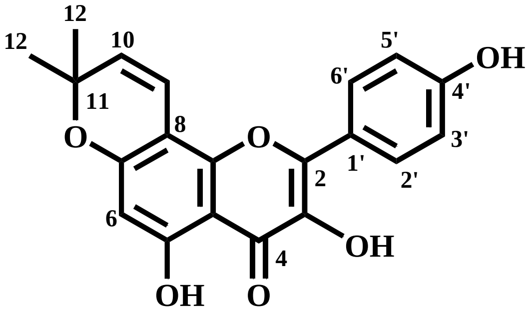
Major HMBC correlations of **TM12**

Table 16 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM12** (Chrysin)

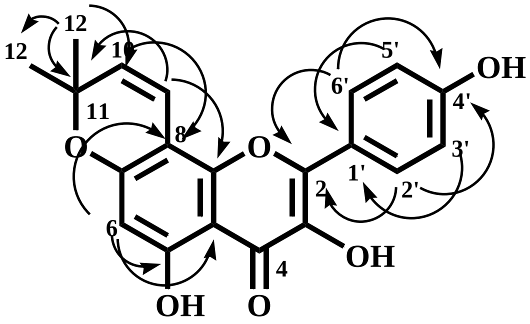
Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
2	-	163.6 (C)	-
3	6.58 (*s*)	105.5 (CH)	C-2, C-4a, C-1'
4	-	182.4 (C=O)	-
4a	-	105.5 (C)	-
5	-	164.4 (C)	-
6	6.29 (*d*, *J* = 2.1)	99.7 (CH)	C-74a, C-5, C-7, C-8
7	-	162.1 (C)	-
8	6.43 (*d*, *J* = 2.1)	94.3 (CH)	C-6, C-7, C-8a
8a	-	158.0 (C)	-
1'	-	126.2 (C)	-
2',6'	7.84 (*dd*, *J* = 8.1, 2.1)	126.6 (CH)	C-2, C-4'
3',5'	7.49 (*m*)	128.6 (CH)	C-4', C-1'
4'	7.48-7.46 (*m*)	131.6 (CH)	C-2'
5-OH	12.66 (*s*)	-	-
7-OH	10.01 (*s*)	-	-

in $CDCl_3$ + DMSO-d_6

TM13 : Citrusinol



TM13 was obtained as a yellow solid. m.p. 253-254°C. The UV spectrum showed absorption bands at λ_{max} 222, 241, 248, 267 and 331 nm. The IR spectrum showed the stretching of hydroxyl (3550 cm^{-1}) and conjugated carbonyl group (1620 cm^{-1}). The ^{1}H NMR spectrum **(Table 17)** acquired in $CDCl_3$ and DMSO-d_6 showed the presence of a *para*-substituted B ring (δ 8.22, H-2'/H-6'; δ 7.06 (H-3'/H-5', J = 9.0 Hz), a hydroxyl group (δ 9.14, 4-OH), a chelated hydroxyl group (δ 12.30, 5-OH), a singlet aromatic proton (δ 6.25, H-6) and 2,2-dimethylchromene ring (δ 1.49, *s*, H-12; δ 6.63, *d*, J = 9.9 Hz, H-9; δ 5.79, *d*, J = 9.9 Hz, H-10) as for **TM9**. The absence of a characteristic singlet of flavones proton H-3 (δ 6.57) together with the carbon signal of C-3 was moved to lower field (δ 136.0) than the relevant C-3 of **TM9** (δ 106.7) was in agreement with the flavonol structure. The HMBC correlation of H-6 and H-10 to C-8 (δ 103.8), and H-9 to C-7 (δ 159.5) confirmed the attachment of chromene ring to C-7 and C-8 of the A ring. **TM9** then was identified as 4*H*,8*H*-benzo[1,2-*b*:3,4-*b'*]dipyran-4-one,3,5-dihydroxy-2-(4-hydroxyphenyl)-8,8-dimethyl-pyrano[2,3-*f*]chromen-4-one. The assignment and spectroscopic data were in agreement to those of Citrusinol (Shang *et al.*, 2007).



Major HMBC correlations of **TM13**

Table 17 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM13** (Citrusinol)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
2	-	146.3 (C)	-
3	-	136.0 (C)	-
4	-	175.6 (C=O)	-
4a	-	101.2 (C)	-
5	-	160.85 (C)	-
6	6.25 (*s*)	98.8 (CH)	C-4a, C-5, C-8
7	-	159.5 (C)	-
8	-	103.8 (C)	-
8a	-	150.9 (C)	-
9	6.63 (*d*, *J* = 9.9)	114.5 (CH)	C-7, C-8a, C-11
10	5.79 (*d*, *J* = 9.9)	127.5 (CH)	C-8, C-11
11	-	78.1 (C)	-
12	1.49 (*s*)	28.4 (CH_3)	C-10, C-11, C-12

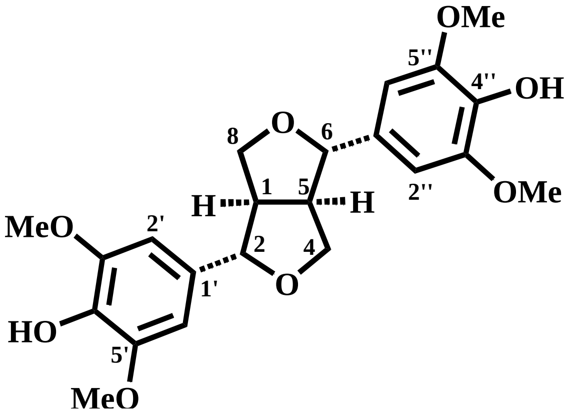
in Acetone-d_6

Table 17 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM13** (Citrusinol) (continued)

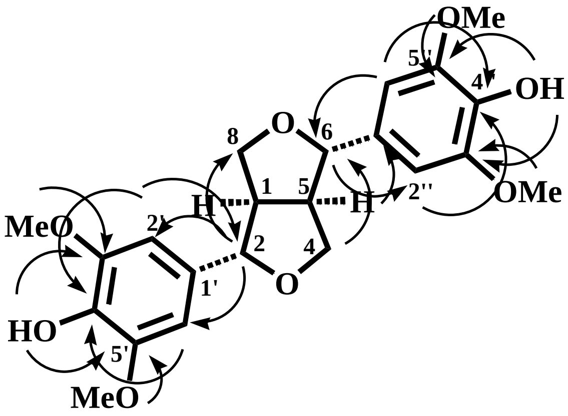
Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
1'	-	122.5 (C)	-
2',6'	8.22 (*d*, *J* = 9.0)	129.6 (CH)	C-2, C-2', C-4'
3',5'	7.06 (*d*, *J* = 9.0)	115.6 (CH)	C-1', C-3', C-4'
4'	-	159.3 (C)	-
3-OH	-	-	-
5-OH	12.30 (*s*)	-	-
4'-OH	9.14 (*s*)	-	-

in Acetone-d_6

TM14 : Syringaresinol



TM14 was obtained as a yellow solid. The UV spectrum showed absorption bands at λ_{max} 205, 237 and 281 nm. The IR spectrum showed the stretching of hydroxyl group at 3435 cm^{-1}. The ^{1}H and ^{13}C NMR spectra **(Table 18)** showed characteristic signal due to the symmetrical dibenzylfurofuran type lignin structure. The ^{1}H NMR spectrum showed the resonances of methine proton H-1/H-5 (δ 3.12), benzylic oxymethine proton H-2/H-6 (δ 4.75) and oxygenated methylene protons H$_e$-4/H$_e$-8 (δ 4.30). The HMBC correlations of H-1/H-5 to C-1'/C-1" (δ 132.1), C-2/C-6 (δ 86.1) and C-4/C-8 (δ 71.8), as well as that of H-2'/H-2" to C-2/C-6 (δ 86.1), C-1'/C-1" (δ 132.1), C-6'/C-6" (δ 102.8) and C-4'/C-4" (δ 134.3) indicated that the furan ring and benzene ring were linked to each other C-2/C-6 and C-1'/C-1". The phenyl ring were suggested to be 1,2,3,5-tetrasubstituted benzene ring due the resonance of *meta*-proton at 6.60 (H-2'/H-2", H-6'/H-6"). The methoxyl proton resonated at δ 3.91 (3'/3"-OCH$_3$, 5'/5"-OCH$_3$) and hydroxyl proton resonated at δ 5.53 (4'/4"-OH) were assigned for 3'/3", 5'/5"-OCH$_3$ and 4'/4"-OH. The HMBC correlations of 3'/3"-OCH$_3$ and 5'/5"-OCH$_3$ (δ 3.91) to C-3'/C-3"(δ 56.4) and C-5'/C-5" (δ 56.4), respectively. The HMBC correlations of 4'/4"-OH (δ 5.53), H-2'/H-2"and H-6'/H-6" (δ 6.60) to C-4'/C-4" (δ 134.3) confirmed the position of methoxyl group at C-3'/C-3" and C-5'/C-5" and hydroxyl group at C-4'/C-4", respectively. It was identical to 4,4'-(tetrahydro-1*H*,3*H*-foro[3,4-*c*]furan-1,4-diyl)bis-2,6-dimethoxyphenol **TM14** was assigned to be Syringaresinol (Lin-gen *et al.*,1982).



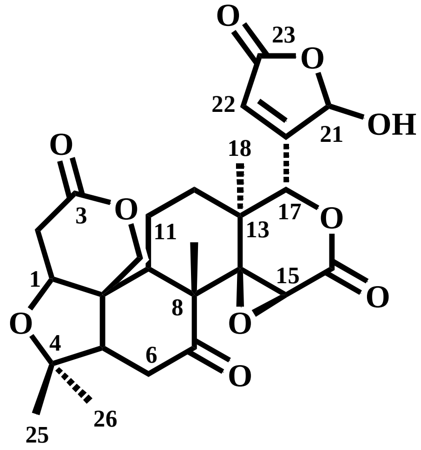
Major HMBC correlations of **TM14**

Table 18 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM14** (Syringaresinol)

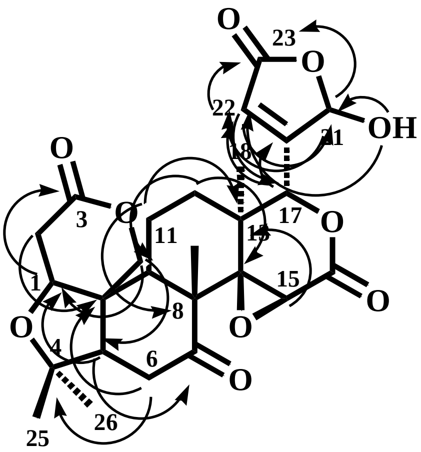
Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
1/5	3.12 (*m*)	54.4 (CH)	C-2, C-5, C-6, C-8, C-1"
2/6	4.75 (*d*, *J* = 4.2)	86.1 (CH)	C-1, C-8, C-1", C-2"
$4_e/8_e$	3.94 (*m*)	71.8 (CH_2)	C-1, C-6
$4_a/8_a$	4.30 (*m*)	71.8 (CH_2)	C-1, C-2, C-5, C-6
1'/1"	-	132.1 (C)	-
2'/2"	6.60 (*s*)	102.7 (CH)	C-6, C-1', C-6', C-4'
3'/3"	-	147.2 (C)	-
4'/4"	-	134.3 (C)	-
5'/5"	-	147.2 (CH)	C-6, C-1', C-6', C-3', C-4'
6'/6"	6.60 (*s*)	102.8 (CH)	C-6, C-1', C-2', C-4', C-5'
3'/3", 5'/5"-OCH_3	3.91 (*s*)	56.4 (4×OCH_3)	C-3'/3", 5'/5"
4'/4"-OH	5.53 (*br s*)	-	C-4'/4"

in $CDCl_3$

TM15 : Limonexic acid



TM15 was obtained as white solid, the second limonoid isolated, has spectroscopic properties similar to those of limonin, **TM8**. The IR spectrum indicated the presence of carbonyl absorption at 1730 cm^{-1} and *β*-substituted furan at 875 cm^{-1}. The ^1H NMR spectrum **(Table 19)** suggested the presence of proton H-17 (*δ* 5.33) and H-15 (*δ* 3.85) of epoxy lactone, and four tertiary methyls (*δ* 1.04, 1.10, 1.15 and 1.32). Furthermore, the ^1H NMR spectrum showed signal of a system -O-CH-CH_2-C=O at *δ* 2.26 (*dd*, *J* = 14.8, 3.2, H-2_a), 2.60 (*dd*, *J* = 14.8, 3.2, H-2_b) and 4.12 (*s*, H-1). The signal of non-equivalent oxymethylene protons were observed at *δ* 4.85 and 4.43 (1H each, *d*, *J* = 13.0 Hz, H-19). The ^1H NMR spectrum suggested the presence of a *β*-substituted furan at *δ* 5.98 (1H, *br s*, H-21), *δ* 6.26 (1H, *br s*, H-22) and *δ* 8.06 (1H, *br s*, 21-OH). The absence of signal at *δ* 8.06 when addition of a drop of D_2O confirmed that it was hydroxyl signal. The HMBC correlations of H-22 (*δ* 6.26) to the carbons at *δ* 78.9 (C-17), *δ* 98.8 (C-21) and *δ* 169.0 (C-23) and H-21 (*δ* 5.98) to the carbons at *δ* 122.7 (C-22), *δ* 169.0 (C-23) together with HMBC correlations of 21-OH at *δ* 8.06 to the carbons at *δ* 98.8 (C-21) and 165.4 (C-20) confirmed the position of H-22, H-21 and 21-OH, respectively. Based on these data, **TM15** was assigned as Limonexic acid (Khalil *et al.*, 2003).



Major HMBC correlations of **TM15**

Table 19 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM15** (Limonexic acid)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
1	4.12 (*br s*)	80.0 (CH)	C-3
2	2.26 (*dd*, *J* = 14.8, 3.2)	36.0 (CH_2)	C-1, C-9
	2.60 (*dd*, *J* = 14.8, 3.2)		C-3
3	-	169.3 (C=O)	-
4	-	80.0 (C)	-
5	2.48 (*m*)	50.3 (CH)	C-1, C-7, C-10
6	2.79 (*m*)	36.3 (CH_2)	C-4, C-7
	3.02 (*t*, *J* = 15.0)		C-5, C-10
7	-	206.3 (C=O)	-
8	-	51.4 (C)	-
9	2.51 (*dd*, *J* = 10.0, 2.0)	48.2 (CH)	C-11
10	-	45.8 (C)	-
11	1.84 (*m*)	18.2 (CH_2)	C-10, C-13
	1.99 (*m*)		C-8

in $CDCl_3$+DMSO-d_6

Table 19 ^{1}H, ^{13}C NMR and HMBC spectral data of **TM15** (Limonexic acid) (continued)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
12	1.32 (*m*) 1.72 (*m*)	29.4 (CH_2)	C-11, C-12, C-14 C-11, C-17
13	-	37.1 (C)	-
14	-	65.3 (C)	-
15	3.85 (*s*)	53.2 (CH)	C-14, C-16
16	-	165.5 (C)	-
17	5.33 (*s*)	78.9 (CH)	C-12, C-18, C-20, C-22
18	1.10 (*s*)	20.9 (CH_3)	C-12, C-14, C-13, C-17
19	4.43 (*d*, *J* = 13.0) 4.85 (*d*, *J* = 13.0)	65.3 (CH_2)	C-1, C-5, C-10 C-1, C-5, C-3
20	-	165.4 (C)	-
21	5.98 (*br s*)	98.8 (CH)	C-22, C-23
22	6.26 (*br s*)	122.7 (CH)	C-17, C-21, C-23
23	-	169.0 (C=O)	-
24	1.15 (*s*)	20.9 (CH_3)	C-9, C-14, C-7
25	1.32 (*s*)	29.6 (CH_3)	C-26
26	1.04 (*s*)	21.1 (CH_3)	C-25, C-4, C-5
21-OH	8.06 (*br s*)	-	C-20, C-21

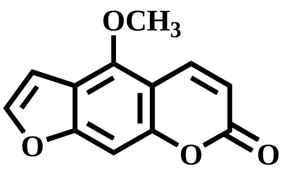
in $CDCl_3$+DMSO-d_6

CHAPTER 4

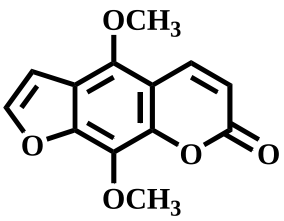
CONCLUSION

Investigation of the chemical constituents of the dichloromethane and acetone extracts of the leaves and acetone extract of the stems of *C. aurantifolia* Swingle, led to the isolation of six types of compounds; five coumarins: bergapten (**TM1**), isopimpinellin (**TM2**), citropten (**TM3**), bergamottin (**TM5**) and umbeliferone **(TM7)**, one benzene derivative : 4-hydroxybenzaldehyde (**TM4**), four flavones: 5,7,4'-trihydroxy-3-methoxyflavone (**TM6**), atalantoflavone (**TM9**), chrysin (**TM12**)and citrusinol (**TM13**),two acridone alkaloids: 5- hydroxynoracronycine (**TM10**) and citracridone-III (**TM11**), two limonoids: limonin (**TM8**) and limonexic acid (**TM15**) and one lignin: syringaresinol (**TM14**).

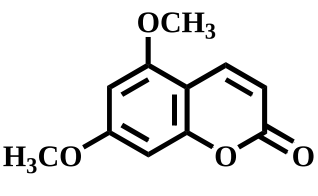
Coumarins



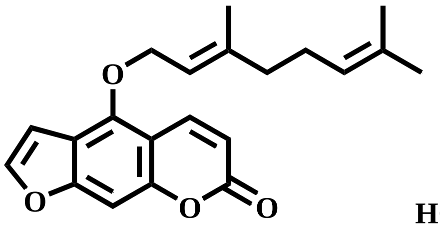
TM1, Bergapten



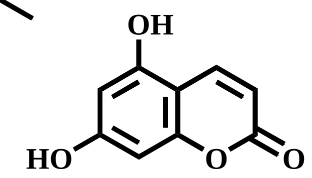
TM2, Isopimpinellin



TM3, Citropten

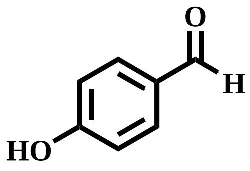


TM5, Bergamottin



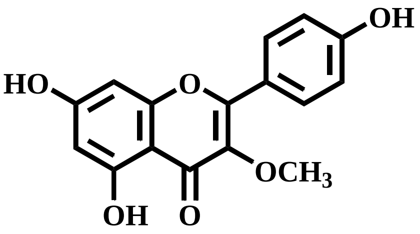
TM7, Umbeliferone

Benzene derivatives

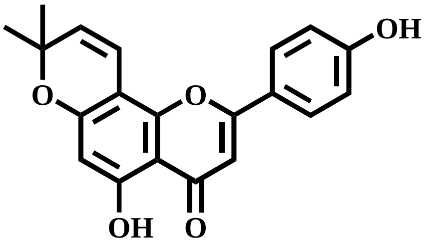


TM4, 4-Hydroxybenzaldehyde

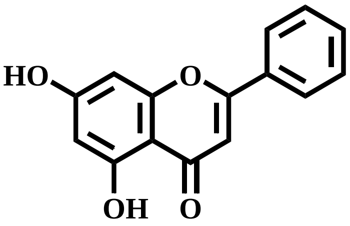
Flavonoids



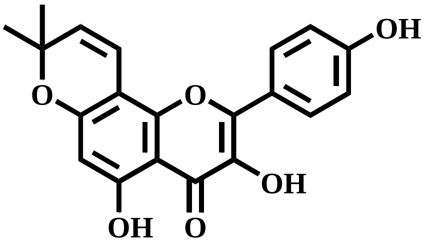
TM6, 5,7,4'-Trihydroxy-3-methoxyflavone



TM9, Atalantoflavone

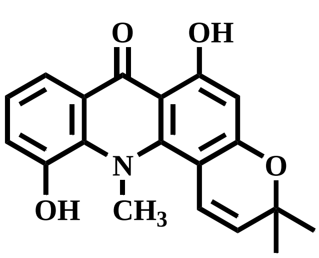


TM12, Chrysin

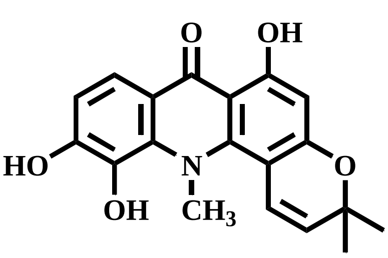


TM13, Citrusinol

Acridone alkaloids

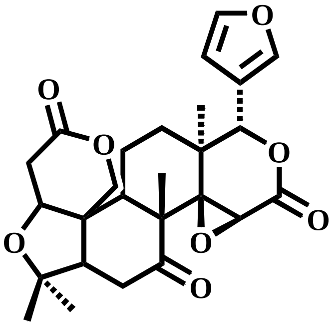


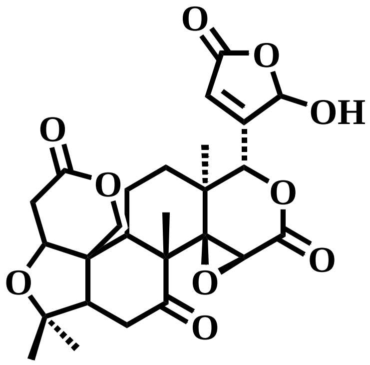
TM10, 5- Hydroxynoracronycine



TM11, Citracridone-III

Limonoids

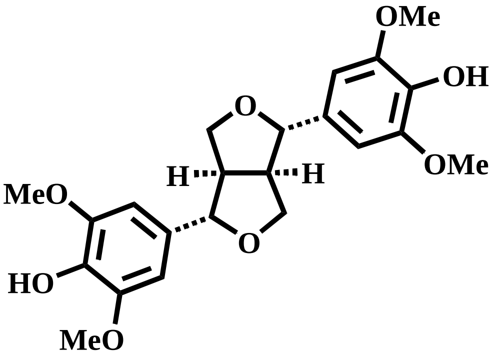




TM8, Limonin

TM15, Limonexic acid

Lignins



TM14, Syringaresinol

REFERENCES

Abdel-Fattah, M. E.; Taha, K. E.; Abdel Aziz, M. H.; Missalem, A. A.; El-Khrisy, E. A. M. 2003. "Chemical constituents of *Citrus Limonia* and *Foeniculum vulgare*", *Indian Journal of Heterocyclic Chemistry,* 13(1), 45-48.

Bacher, M.; Brader, G.;Greger, H.;Hofer, O. 2010. "Complete ^{1}H and ^{13}C NMR data assignment of new constituents from *Severinia buxifolia*", *Magn. Reson.*

. *Chem,* 48, 83-88.

Bennett, R. D.; Hasegawa, S. 1981. "Limonoids of calamondin seed", *Tetrahedron,* 37, 17-24.

Breksa, A. P., III; Dragull, K.; Wong, R. Y. 2008. "Isolation and identification of the first C-17 limonin epimer, epilimonin", *J. Agric. Food Chem.*, 56, 5595-5598.

Bui, K. A.; Duong, A. T.; Tran, V. S.; Seip, S. 2004. "Isolation and structure elucidation of a new limonoid from Vietnamese *Citrus nobilis* seeds", *Tap. Chi. Hoa. Hoc.,* 42(4), 520-523.

Chen, N., Ariyaprayoon, J. 2003. "Three new carbazole alkaloids isolated from *Murrya siamensis*". *Journal of Natural Products*, 53(2), 946-952.

Das, B.; Venkataiah, B.; Kashinatham, A. 1999. "(+)-Syringaresinol from *Parthenium hysterophorus*", *Fitoterapia*, 70, 101-102.

Du, Q.; Chen, H. 2010. "The methoxyflavones in *Citrus reticulate* Blanco cv. Ponkan and their antiproliferatic activity against cancer cells", *Food Chemistry,* 119, 567-572.

Girennavar, B.2007. "Grapefruit-drug interaction: isolation, synthesis, and Biological activity of furocoumarins and their variation Due to pre and post–harvest factors", *Horticulture*, 70, 1-149.

Hasegawa, Z.; Chaohuan, C.; Yulin, W. 1998. "Limonoids in Miyamashikimi (*Skimmia japonica* Thunb)", *J. Japan. Soc. Hort. Sci*, 67(6), 835-838.

He, H.; Ling, L. 1985. "Chemical studies on a Chinese traditional drug fingered citron (*Citrus medica* L.Var. *Sarcodactylis* (Noot.) Swingle)", *Yaoxue Xuebao,* 20(6), 433-435.

Ito, C.; Mizono, T.; Matsuoka, M.; Kimura, Y.; Sato, K.; Kajiura, I.; Omura, M.; Ju-Ichi, M.; Furukawa, H. 1988. "A new flavonoid and other components from Citrus Plants", *Chem. Pharm. Bull.,* 36(9), 3292-3295.

Jang, Y.; Wang, X. B; Kong, L. Y. 2004. "Benzene derivatives from *Citrus medica* L.Var. *sarcodactylis*". 62, 2502-2004.

Kawaii, O. S.; Choi, Z. S.; Choi, U. S.; Kim, H. G.; Kim, C. K.; Lee, R. K. 1999. "Identification of coumarins from the fruits of *Citrus hystrix* DC". *Arch. Pharm. Res*., 29, 845-848.

Khalil, F.; Lou, F. 2003. "Studies on the constituents of *Citrus medica* L.Var. *sarcodactylis*". Zhongguo Yaoxue *Zazhi (Beijing, China),* 39(1), 20-21.

Lin-gen, Y. Y.; Li, C. H.; Shen, Y. C.; Wu, T. S. 1982. "Anti-inflammatory principle from the stemand root barks of *Cristrus medica*". *Chemical & Pharmaceutical Bulletin,* 58(1), 65-66.

Murakami, A.; Nakamura, Y.; Koshimitsu, K.; Oshigashi, H. 1999. "Identification fo coumarins from the fruits of *Citrus hytrix* DC". J. Agric. Food Chem.,47, 333-339.

Nagwa, S. C.; Wu, P. L.; Wu, T. S. 2010. "Two coumarins from the root bark of *Citrus aurantifolia* Swingle". *Phytochemistry,* 44(1), 179-181.

Oesterle, M.; Shibuya, H.; Kitano, E.; Yanagi, K.; Kitagawa, I. 1917. "Flavonoidsof the stems bark of *Oroxylum indicum*". *Chem. Pharm. Bull.,* 32(5), 2059-2062.

Patil, P.; Ichino, C.; Ishiyama, A.; Sekiguchi, H.; Namatame, M.; Ruangrungsi, N.; Saifah, E.; Kiyohara, H.; Otoguro, K.; Omura, S.; Yamada, H. 2010. "In vitro antimalarial activity of prenylated flavonoids from *Erythrina fusca*", *J. Nat. Med*., 62, 217-220.

Ponce, M, A.; Scervino, J, M.; Rosa, E.; Ocampo, J, A.; Godeas, A, M. 2004. "Flavonoids from shoots and roots of *Trifolium repens* (white clover) grown in presence or absence of the arbuscular mycorrhizal fungus Glomus intraradices", *Phytochemistry*., 65, 1925-1930.

Sastry, A.; Kongkathip, N.; Kongkathip, B.; Phonnakhu, S.; Daly, J. W.; Spande, T. F.; Nimit, Y.; Rochanaruangrai, S. 1931. Coumarins and carbazoles from *Citrus miltis* Blanco exhibited antimycobacterial and antifungal activities. *Planta Medica*, 69(2), 155-157.

Shang, T. S.; Huang, S. C.; Lai, J. S.; Teng, C. M.; Ko, F. N.; Kuoh, C. -S. 2007. Chemical and antiplatelet aggregative investigation of the leaves of *Clausena excavata*. *Phytochemistry*, 32(2), 449-451.

Sultana, S.; Ali, M.; Ansari, S. H.; Bagri, P. 2008. “New 4′-substituted flavones from the fruit peels of *Citrus limon* (L.) Burm.f”, *J. Asian. Nat Products Research,* 10(12), 1123–1127.

Smitinand, T. 2001. “Thai Plant Names (Botanical Name-Vernacular Names)., 2nd ed”, Funny Publishing Limited Partnership, Bangkok, Thailand, p 159-160, 131-133.

Teng, W. Y.; Huang, Y. L.; Shen, C. C.; Huang, R. L.; Chung, R. S.; Chen, C-C. 2010. “Cytotoxic acridone alkaloids from the stem bark of *Citrus maxima*”, *J. Chinese Chemical Society (Taipei, Taiwan),* 52(6), 1253-1255.

Teng, Y. H.; Peh, T. H.; Ee, G. C. L.; Rahmani, M.; Sukari, M. A.; Ali, A. M.; Muse, R. 2007. “A new cytotoxic carbazole alkaloid from *Clausena excavata*”. *Natural Product Research*, 21(9), 810-813.

Wu, T. S.; Kuoh, C. S.; Furukawa, H. 1987. Acridone alkaloids VI. The constituents of *Citrus depressa*. “Isolation and structure elucidation of new acridone alkaloids from *Citrus* genus”, *Chem. Pharm. Bull.,* 31(3), 895-900.

Wu, T. S.; Furukawa, H. 1988. “Acridone alkaloids VII. Constituents of *Citrus sinensis* osbeck var *brasiliensis* Tanaka. Isolation and characterization of three new acridone alkaloids, and new coumarin”, *Chem. Pharm. Bull.,* 31(3), 901-906.

Wu, T. S. 1987. “A 2,2-dimethylpyranoflavonol from *Citrus nobilis*”, *Phytochemistry,* 26(11), 3094-3095.

Yin, F.; Lou, F. 2004. “Studies on the constituents of *Citrus medica* L.Var. *sarcodactylis* (Noot.) Swingle”, *Zhongguo Tianran Yaowu,* 2(3), 149-151.

Yin, F.; Lou, F. 2004. “Studies on the constituents of *Citrus medica* L.Var. *sarcodactylis*”, Zhongguo Yaoxue *Zazhi (Beijing, China),* 39(1), 20-21.

APPENDIX

1. ^{1}H NMR and ^{13}C NMR spectrum of compounds TM1-TM15

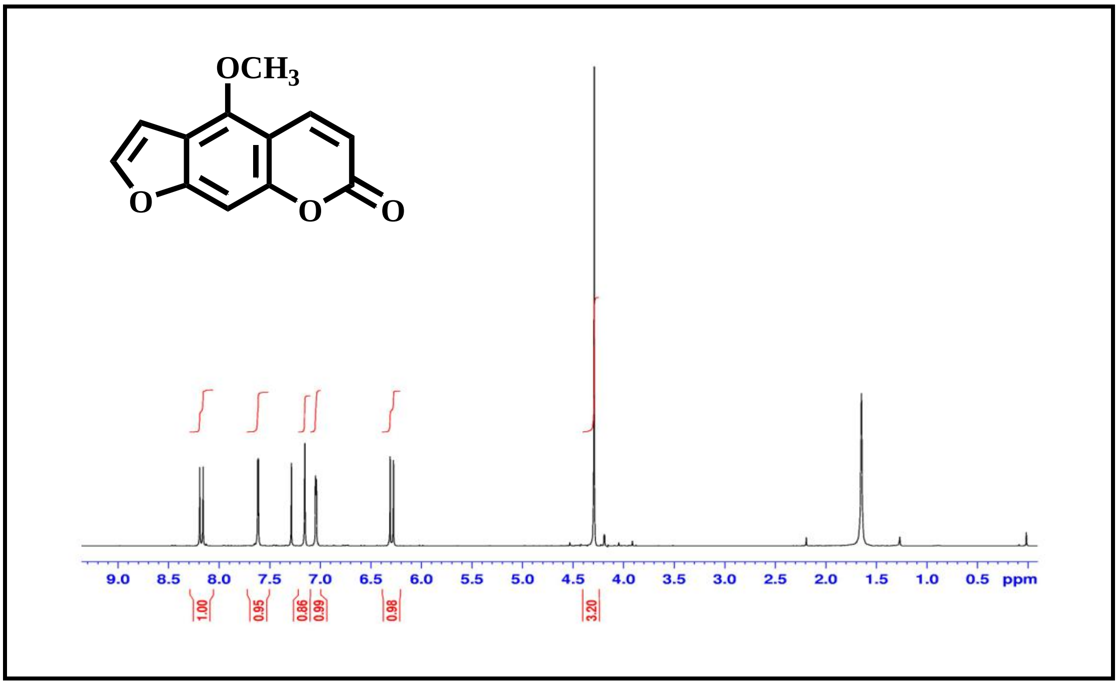


Figure A-1 ^{1}H NMR spectrum (in CDCl$_3$) of **TM1** (Bergapten)

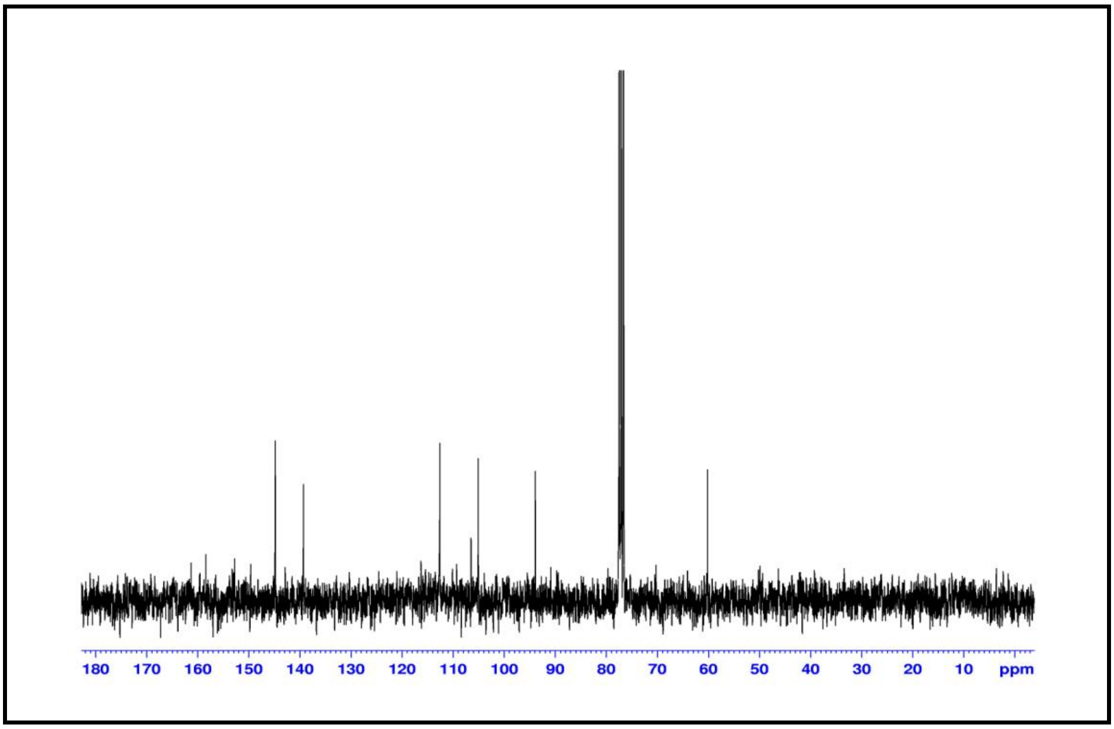


Figure A-2 ^{13}C NMR spectrum (in CDCl$_3$) of **TM1** (Bergapten)

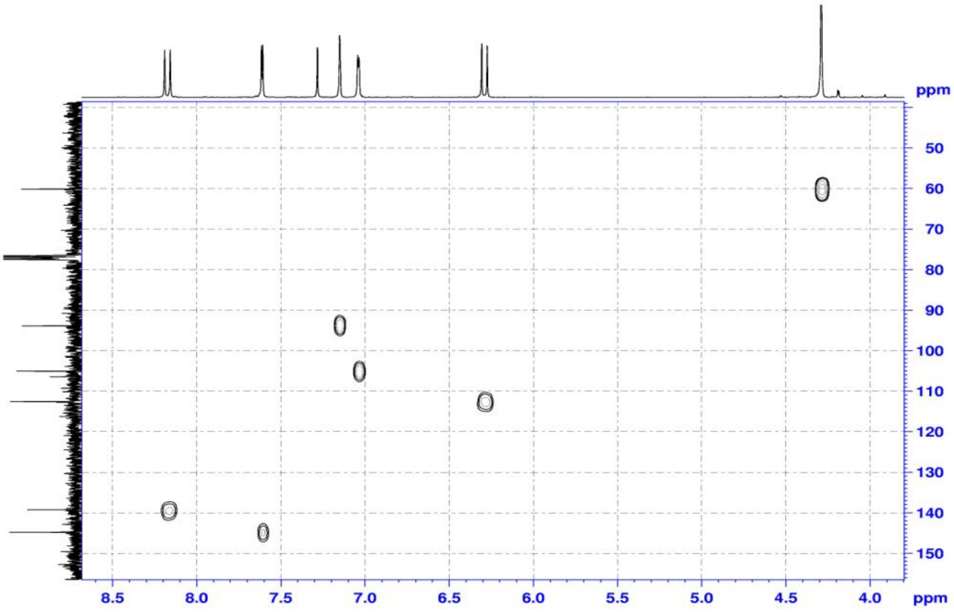


Figure A-3 HMQC spectrum (in $CDCl_3$) of **TM1** (Bergapten)

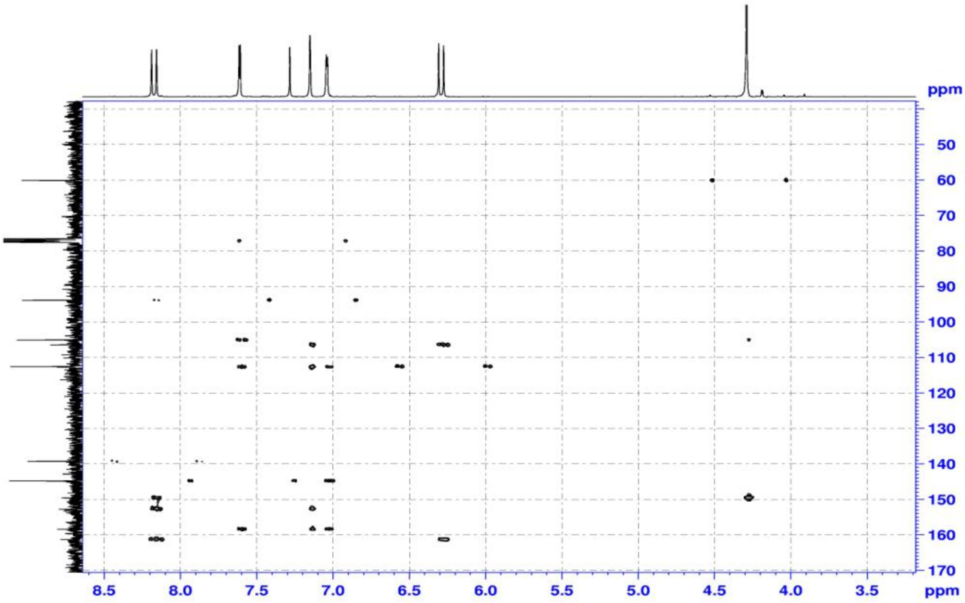


Figure A-4 HMBC spectrum (in $CDCl_3$) of **TM1** (Bergapten)

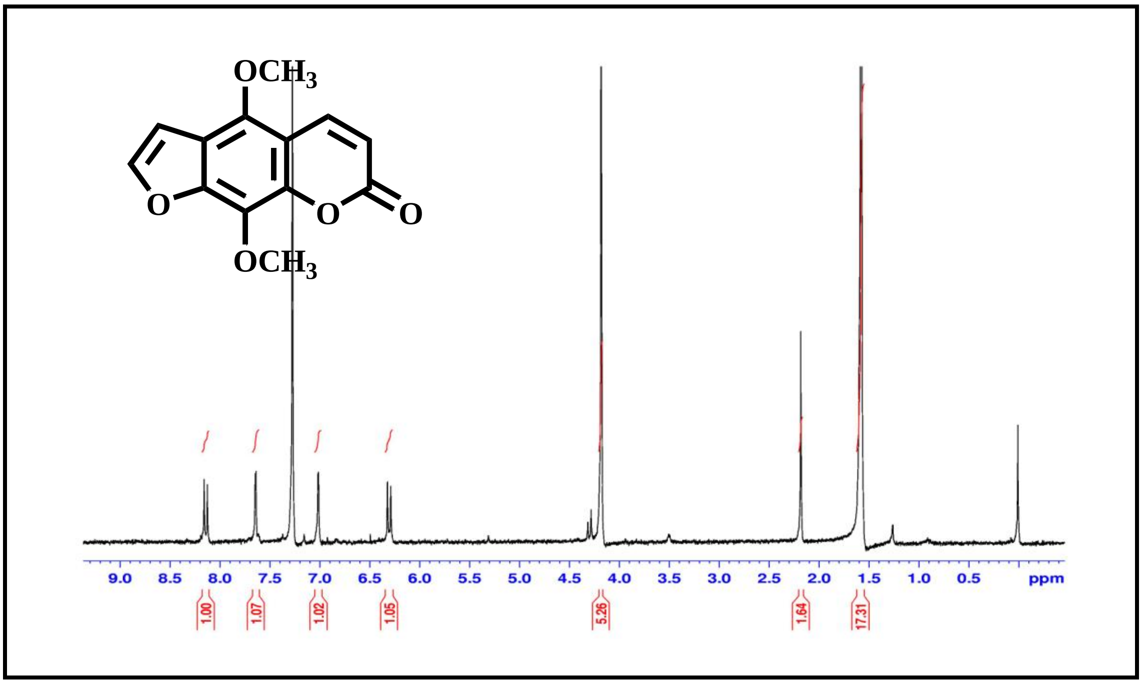


Figure A-5 ^{1}H NMR spectrum (in $CDCl_3$) of **TM2** (Isopimpinellin)

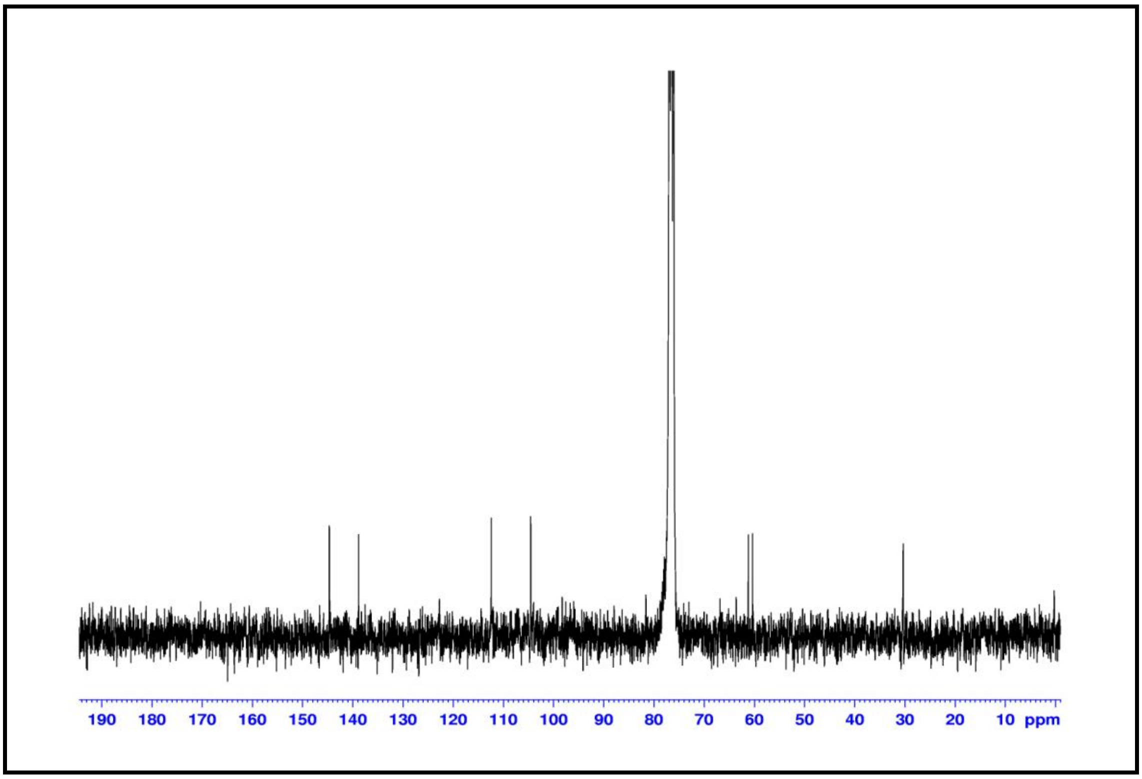


Figure A-6 ^{13}C NMR spectrum (in $CDCl_3$) of **TM2** (Isopimpinellin)

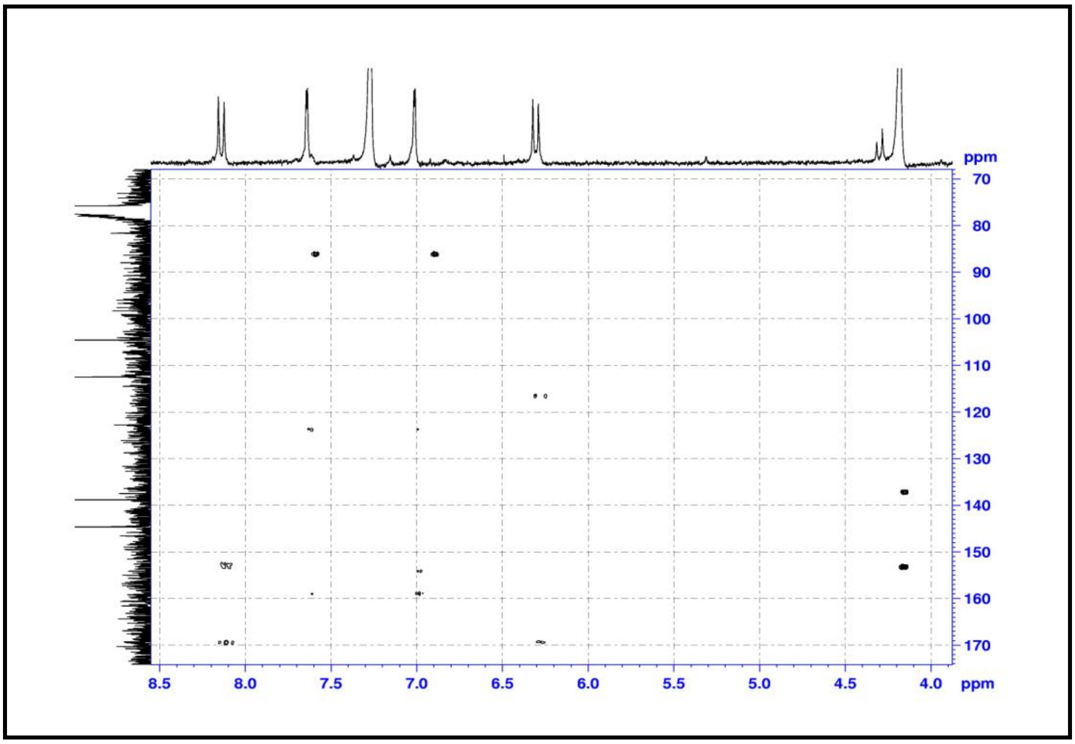


Figure A-7 HMBC spectrum (in $CDCl_3$) of **TM2** (Isopimpinellin)

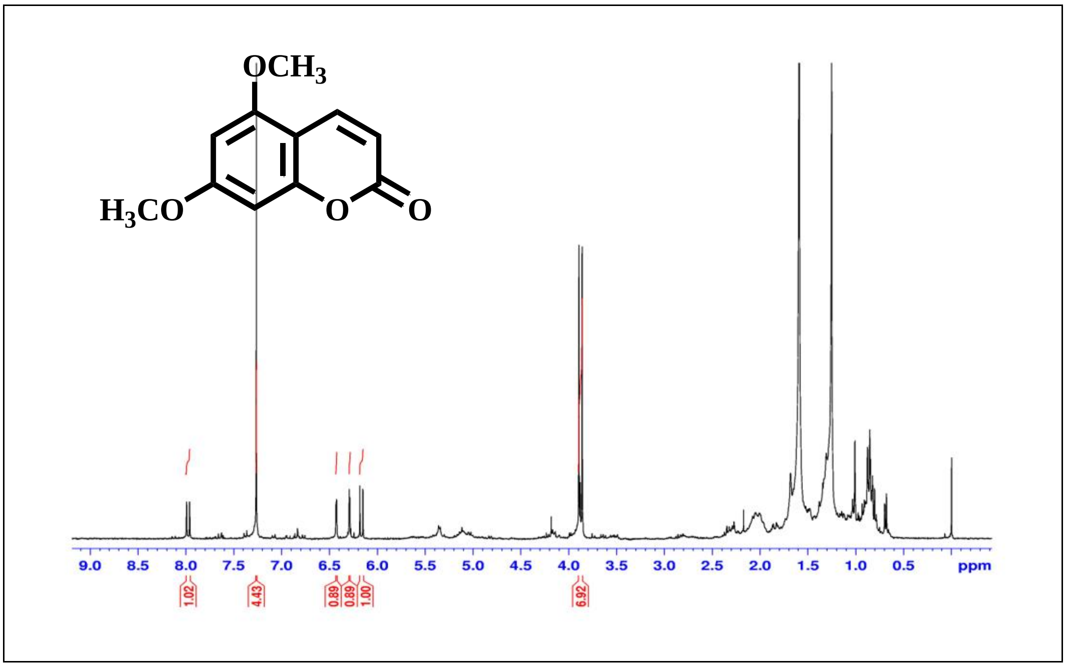


Figure A-8 ^1H NMR spectrum (in $CDCl_3$) of **TM3** (Citropten)

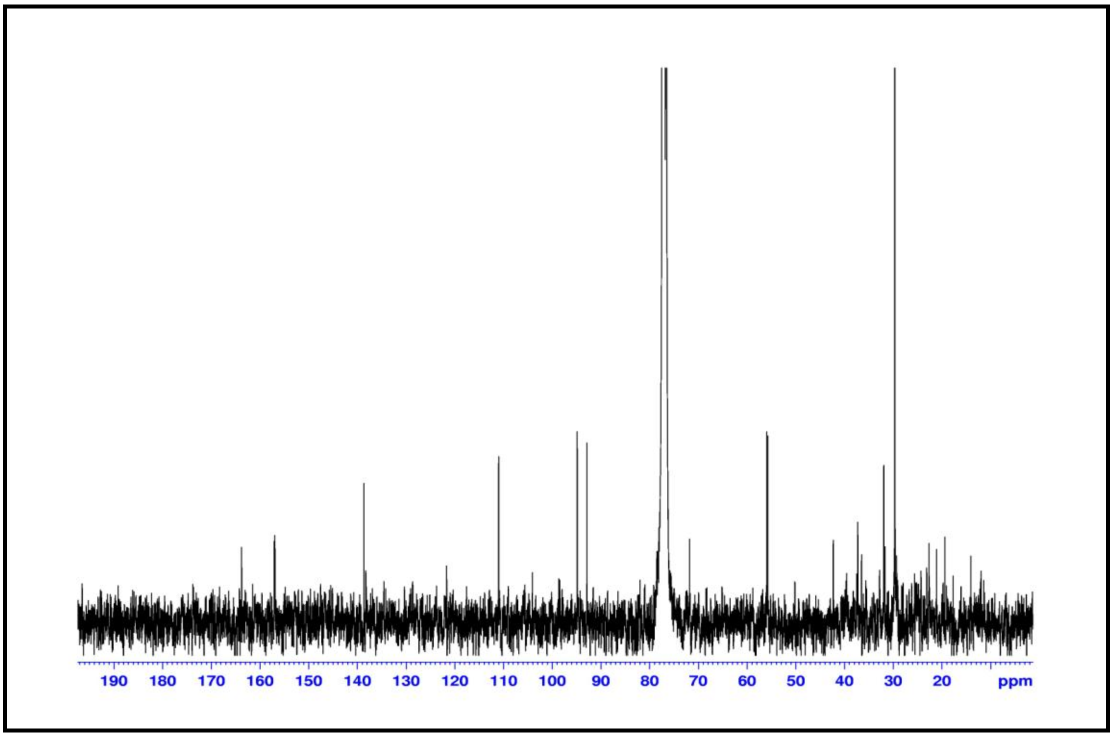


Figure A-9 ^{13}C NMR spectrum (in $CDCl_3$) of **TM3** (Citropten)

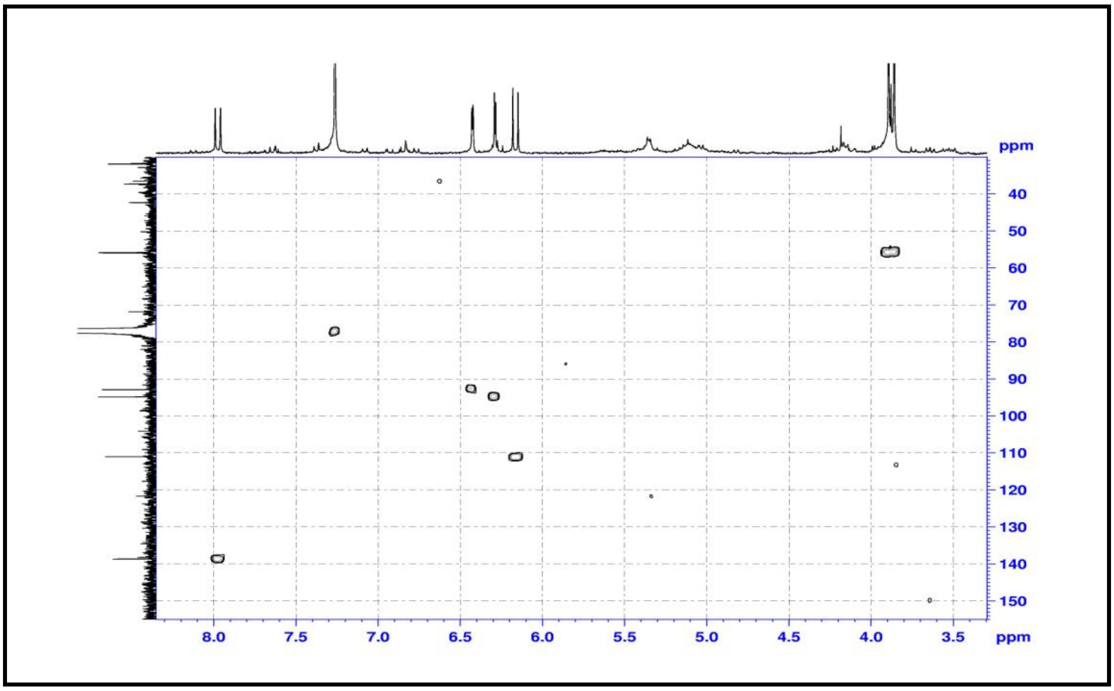


Figure A-10 HMQC spectrum (in $CDCl_3$) of **TM3** (Citropten)

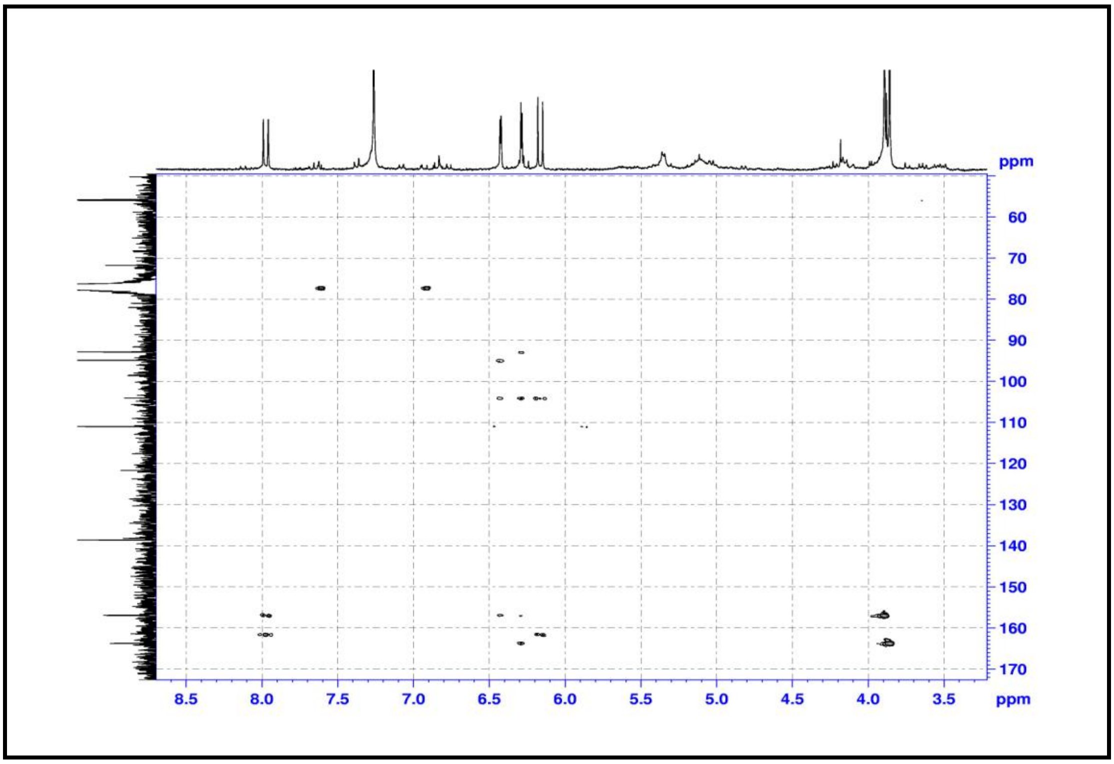


Figure A-11 HMBC spectrum (in $CDCl_3$) of **TM3** (Citropten)

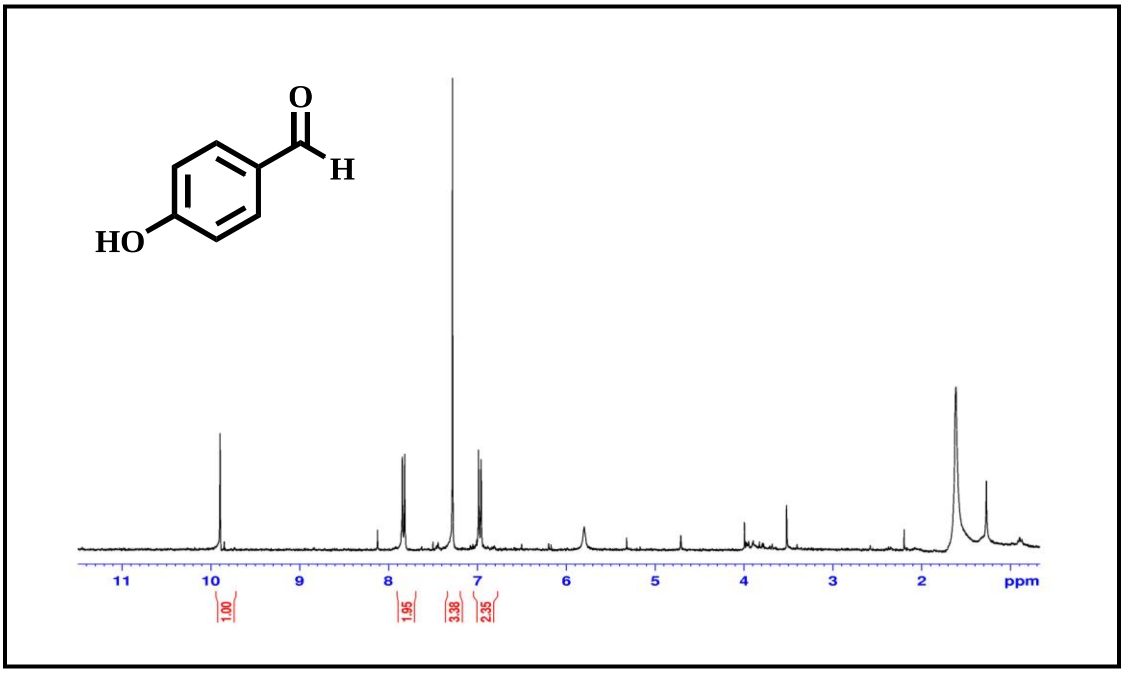


Figure A-12 ^{1}H NMR spectrum (in $CDCl_3$) of **TM4** (4-Hydroxybenzaldehyde)

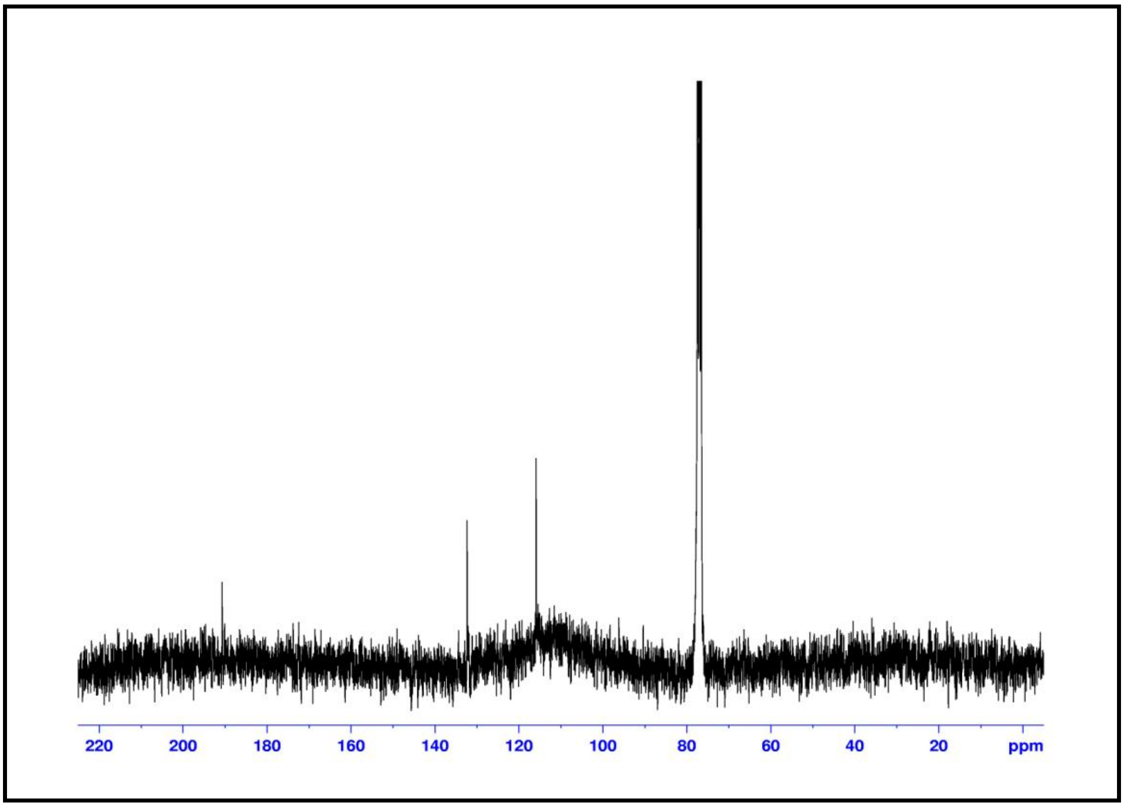


Figure A-13 ^{13}C NMR spectrum (in $CDCl_3$) of **TM4** (4-Hydroxybenzaldehyde)

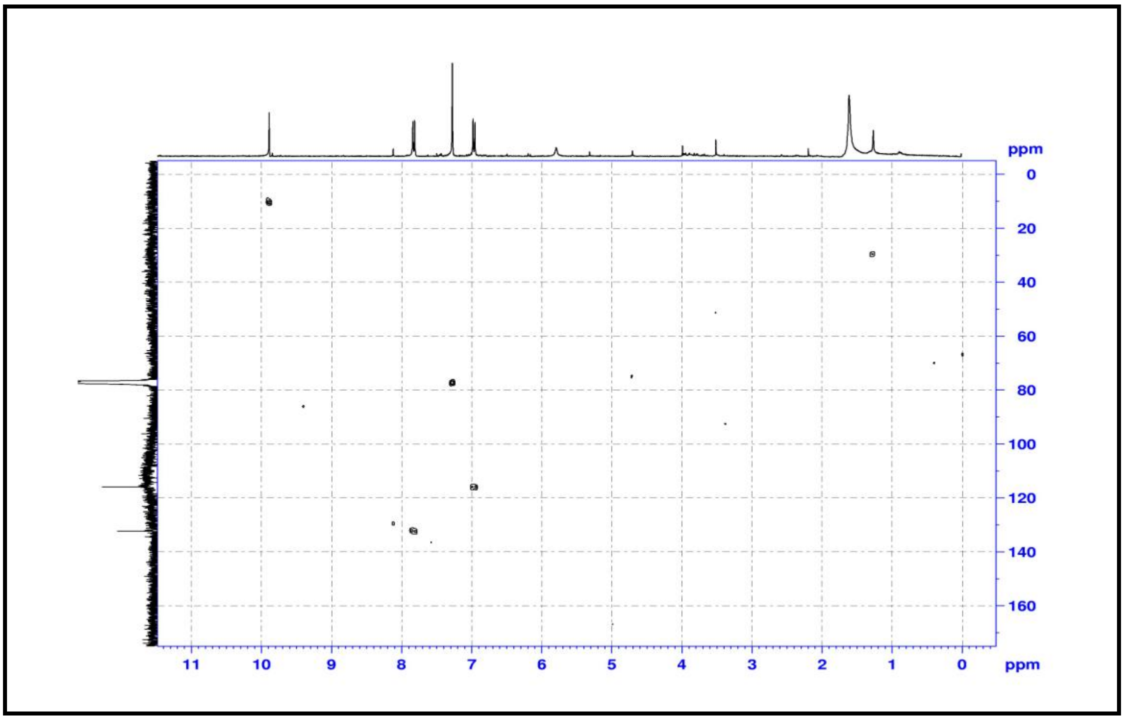


Figure A-14 HMQC spectrum (in $CDCl_3$) of **TM4** (4-Hydroxybenzaldehyde)

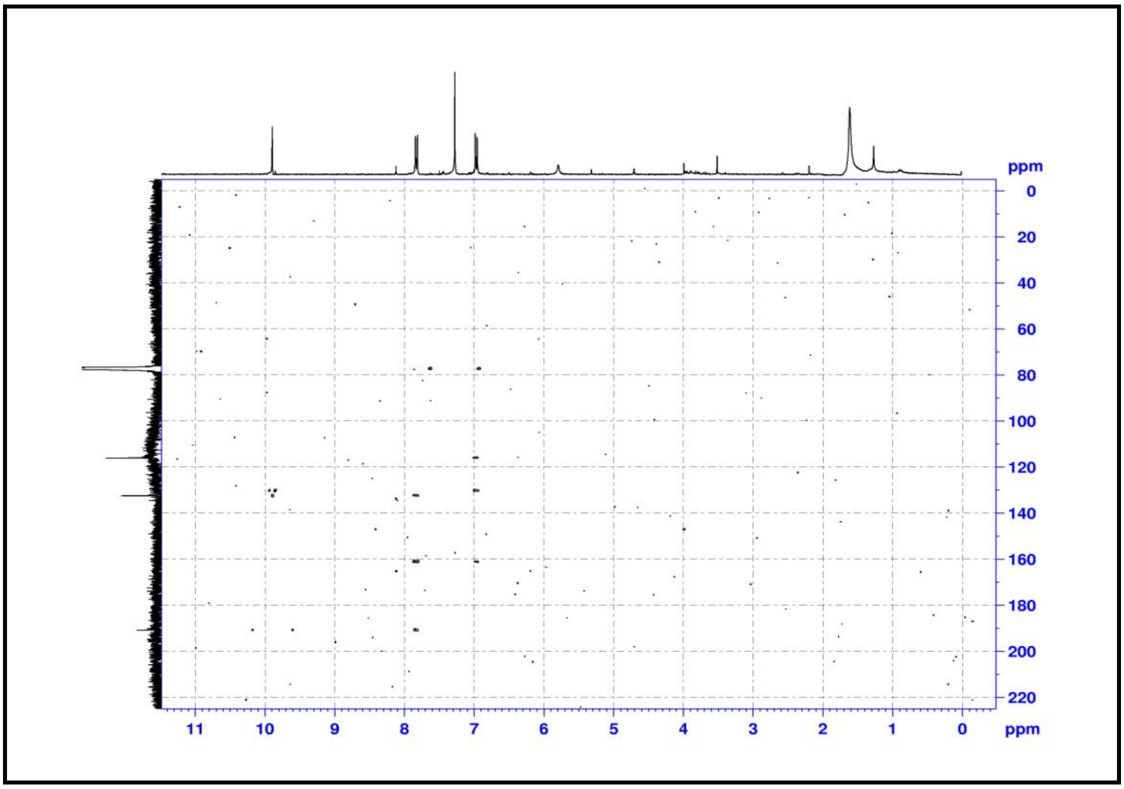


Figure A-15 HMBC spectrum (in $CDCl_3$) of **TM4** (4-Hydroxybenzaldehyde)

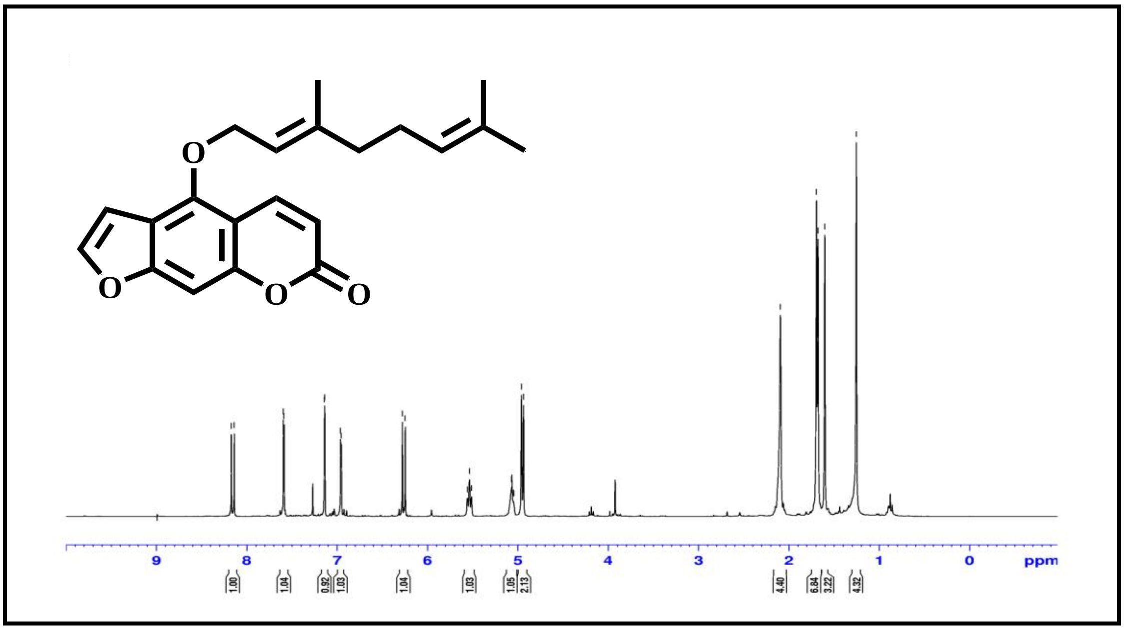


Figure A-16 ^{1}H NMR spectrum (in $CDCl_3$) of **TM5** (Bergamottin)

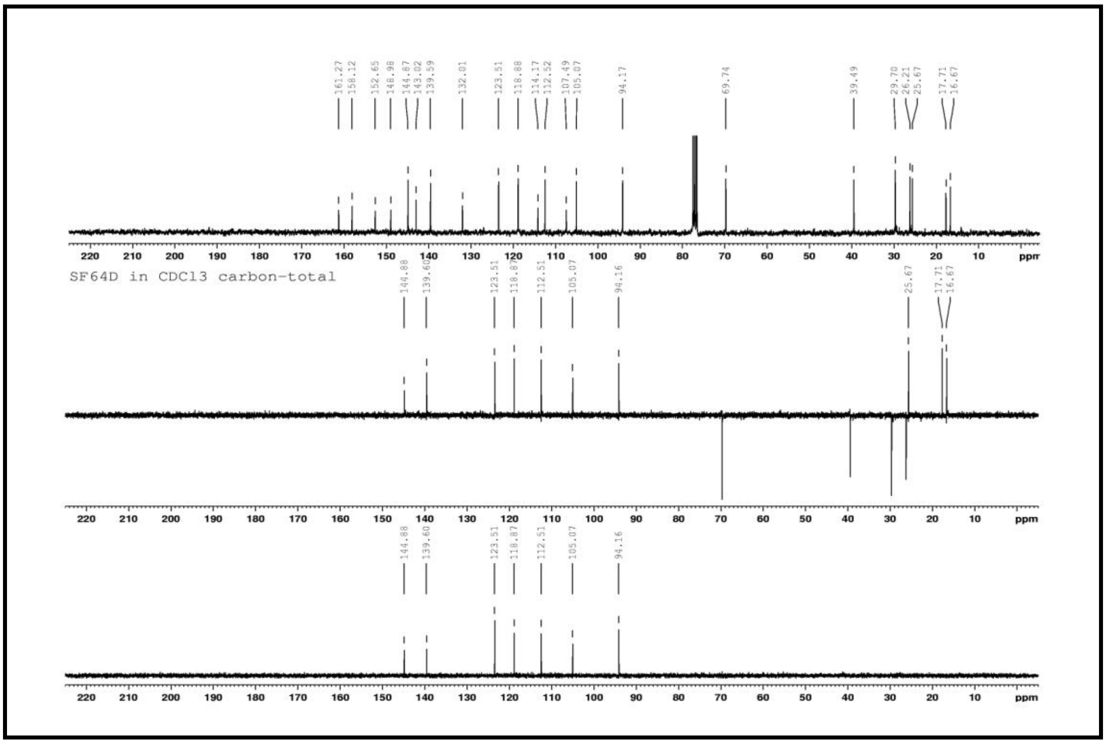


Figure A-17 ^{13}C NMR , Dept -135 and Dept-90 spectrum (in $CDCl_3$) of **TM5** (Bergamottin)

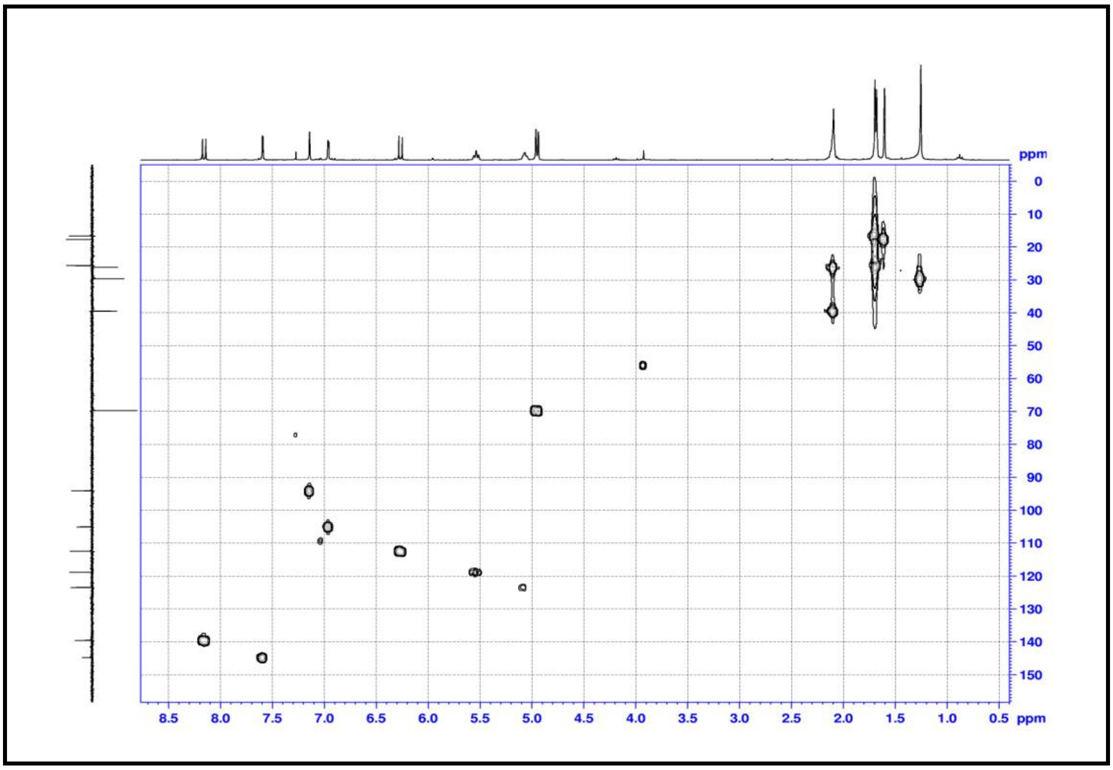


Figure A-18 HMQC spectrum (in $CDCl_3$) of **TM5** (Bergamottin)

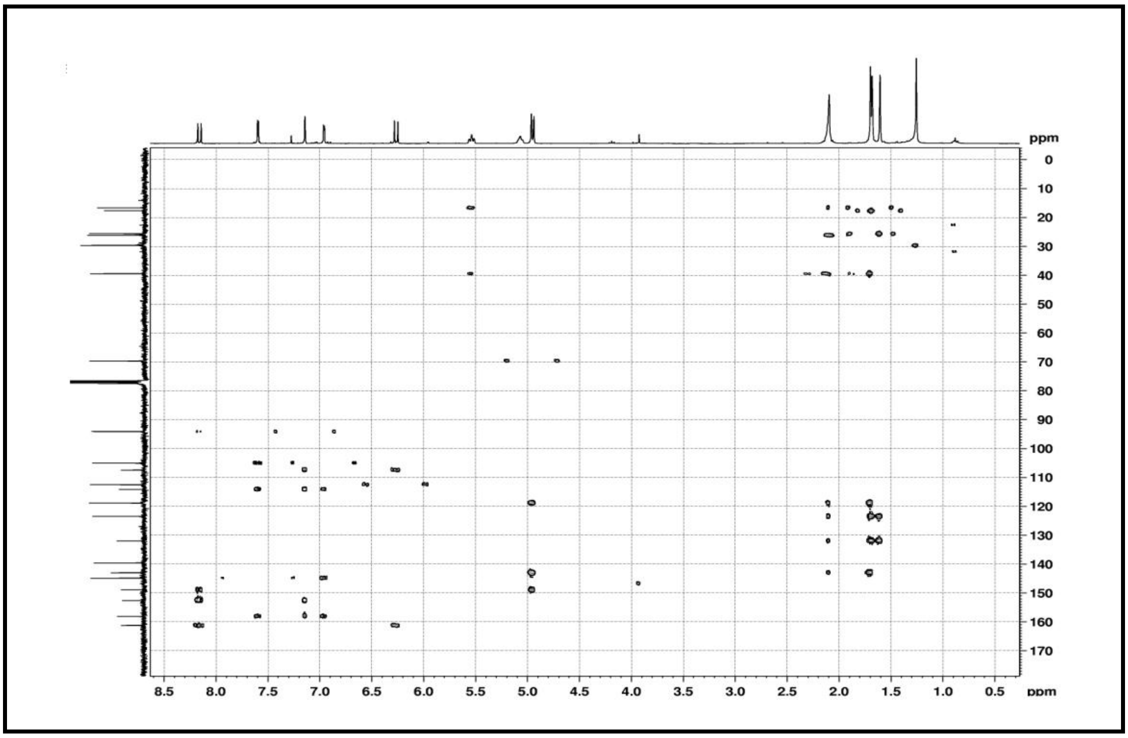


Figure A-19 HMBC spectrum (in $CDCl_3$) of **TM5** (Bergamottin)

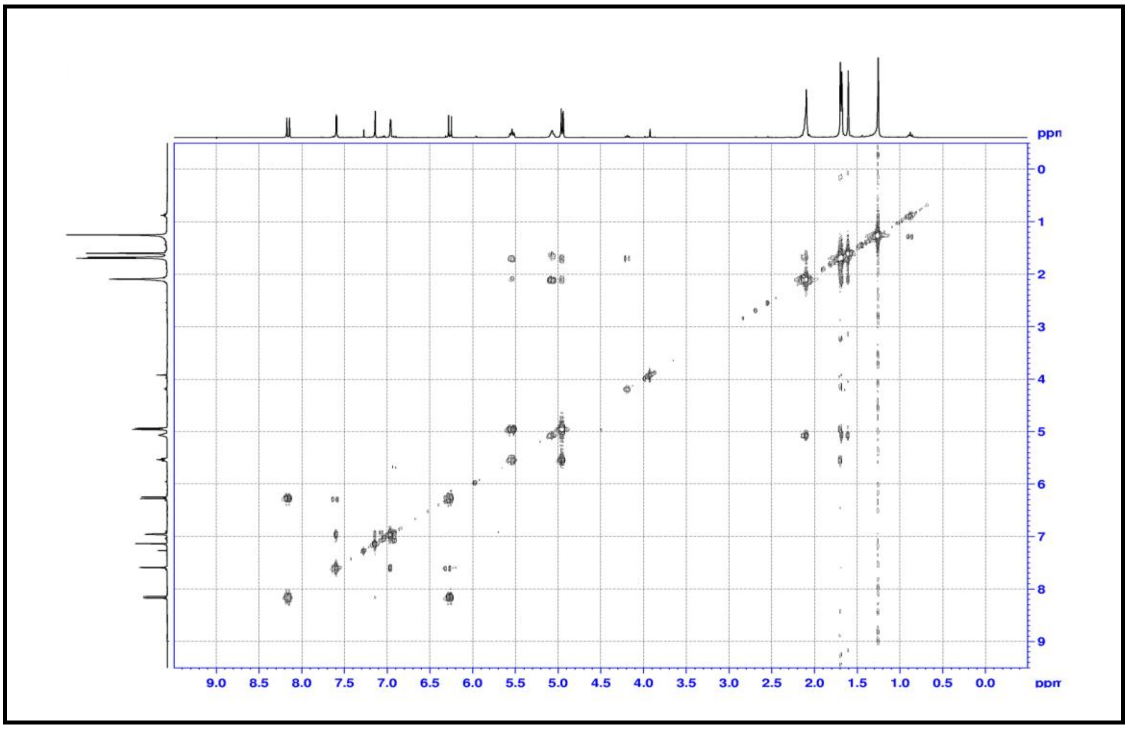


Figure A-20 COSY spectrum (in $CDCl_3$) of **TM5** (Bergamottin)

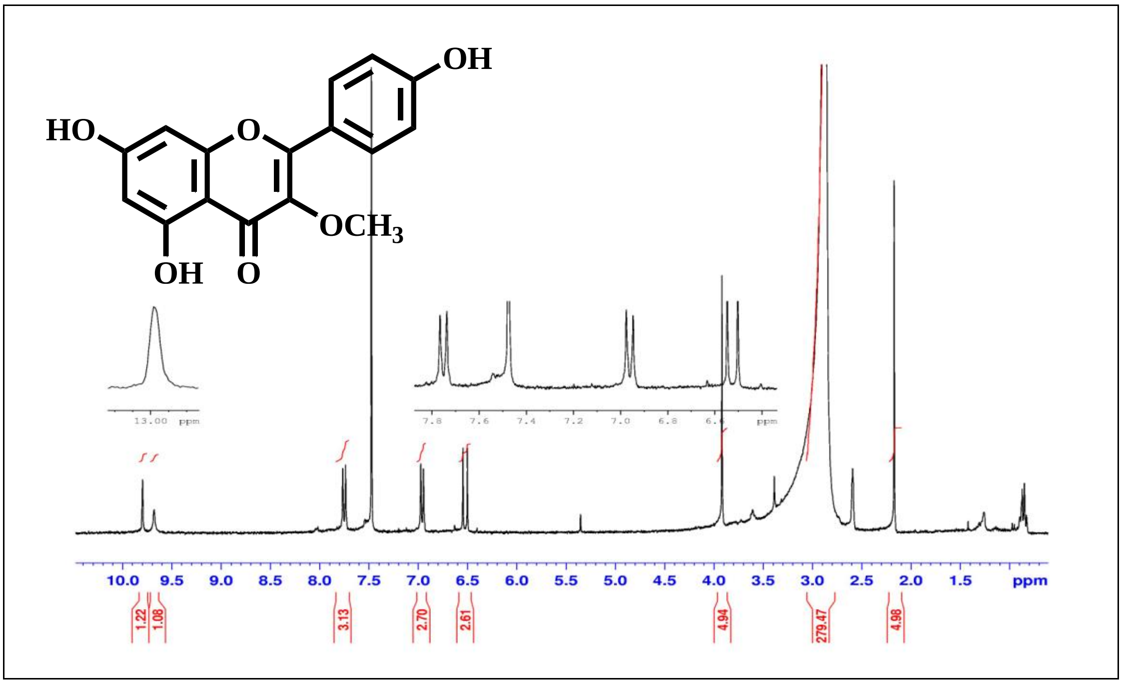


Figure A-21 ^{1}H NMR spectrum (in CDCl$_3$+ DMSO-d_6) of **TM6** (5,7,4'-Trihydroxy-3-methoxyflavone)

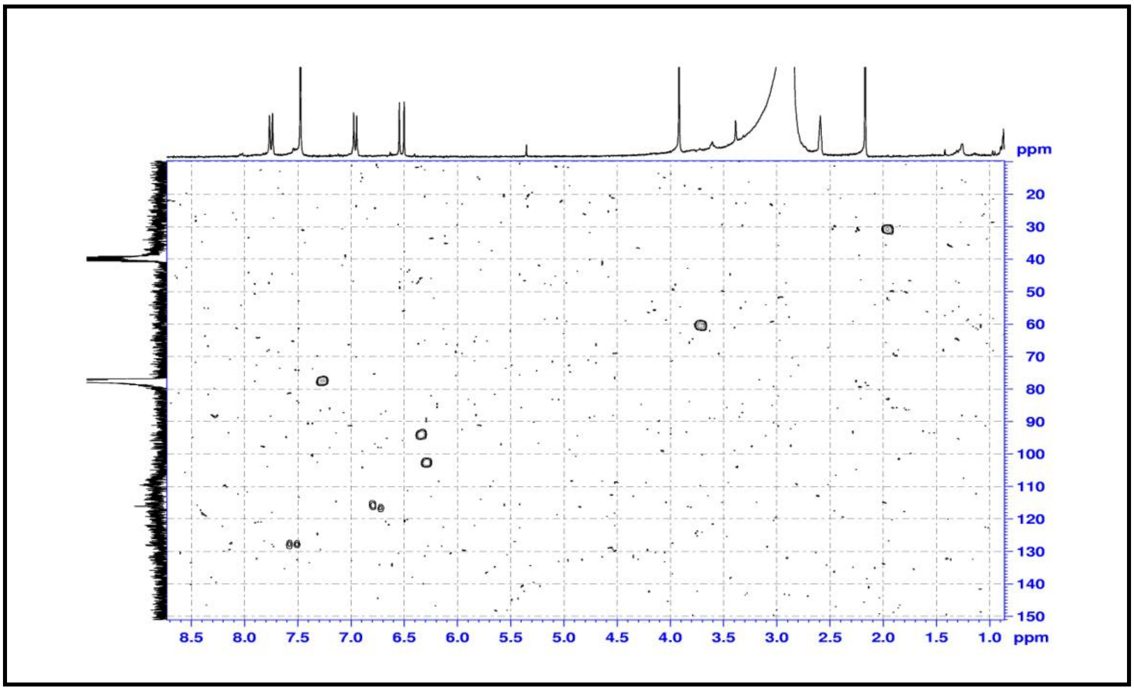


Figure A-22 HMQC spectrum (in CDCl$_3$+ DMSO-d_6) of **TM6** (5,7,4'-Trihydroxy-3-methoxyflavone)

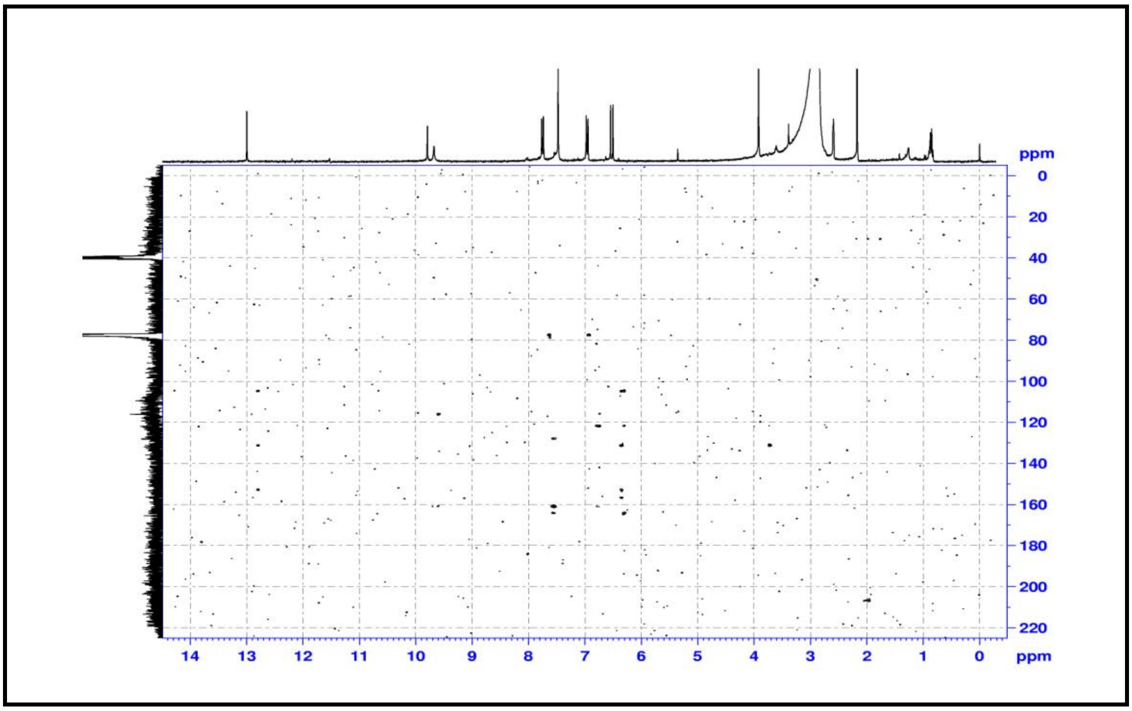


Figure A-23 HMBC spectrum (in $CDCl_3$+ DMSO-d_6) of **TM6** (5,7,4'-Trihydroxy-3-methoxyflavone)

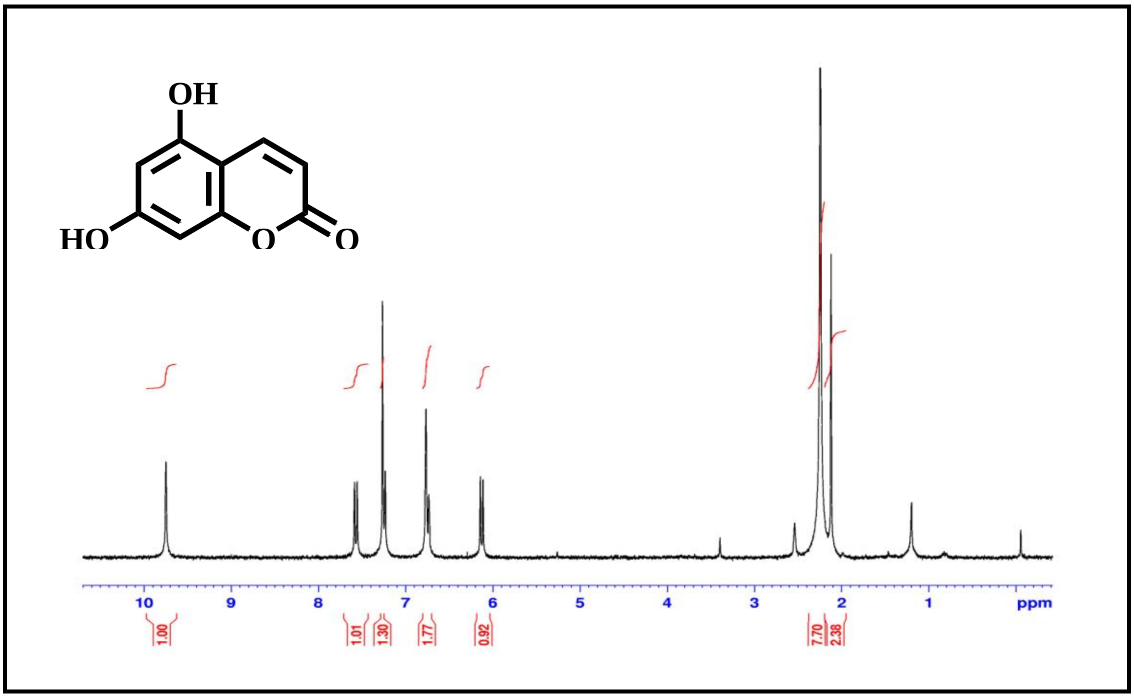


Figure A-24 ^{1}H NMR spectrum (in $CDCl_3$+ DMSO-d_6) of **TM7** (Umbelliferone)

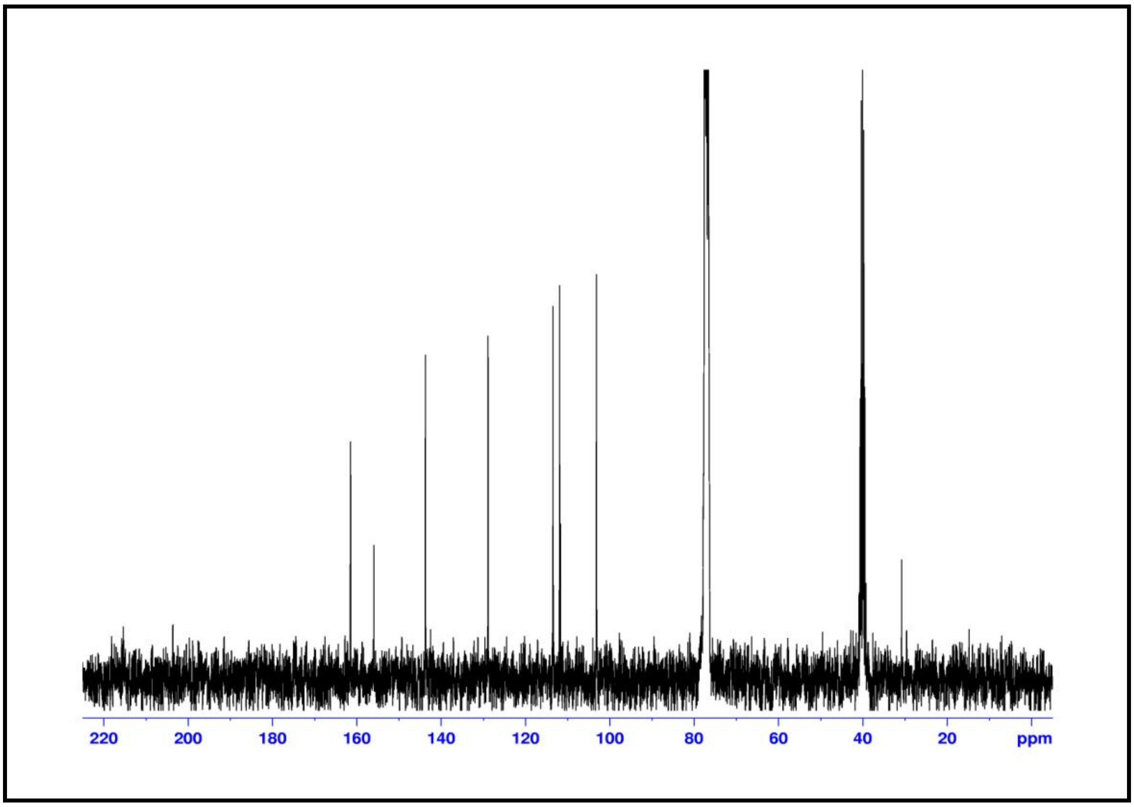


Figure A-25 ^{13}C NMR spectrum (in $CDCl_3$+ DMSO-*d*$_6$) of **TM7** (Umbelliferone)

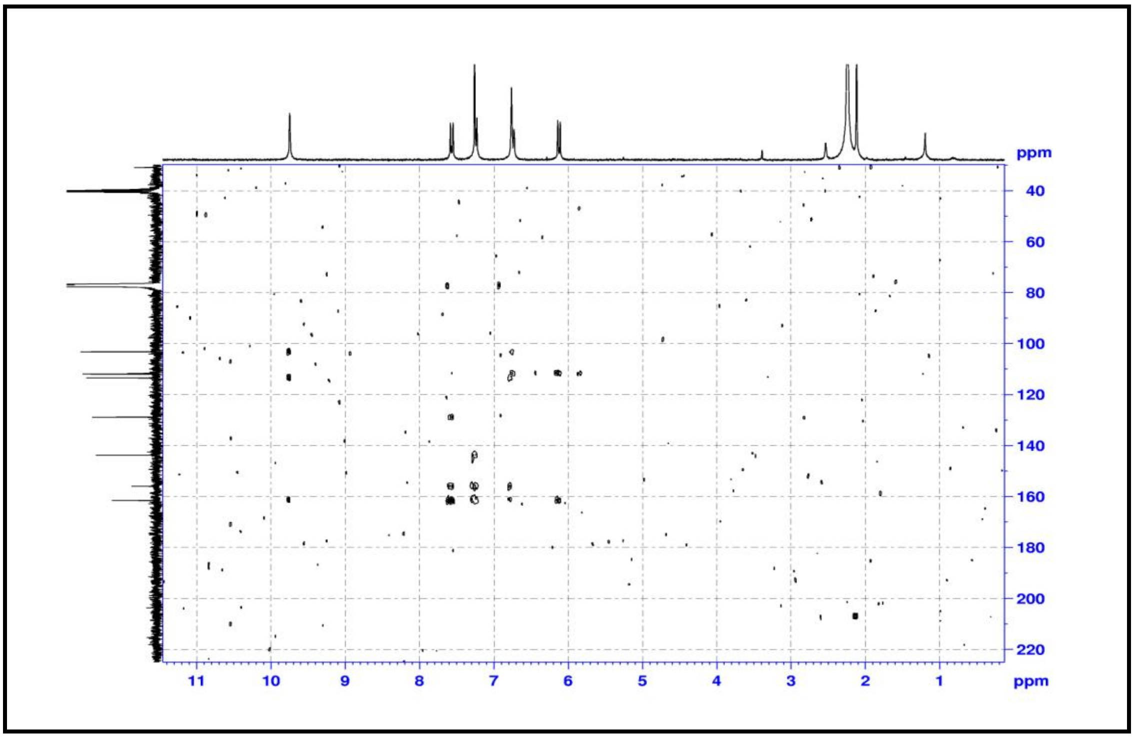


Figure A-26 HMBC spectrum (in $CDCl_3$+ DMSO-*d*$_6$) of **TM7** (Umbelliferone)

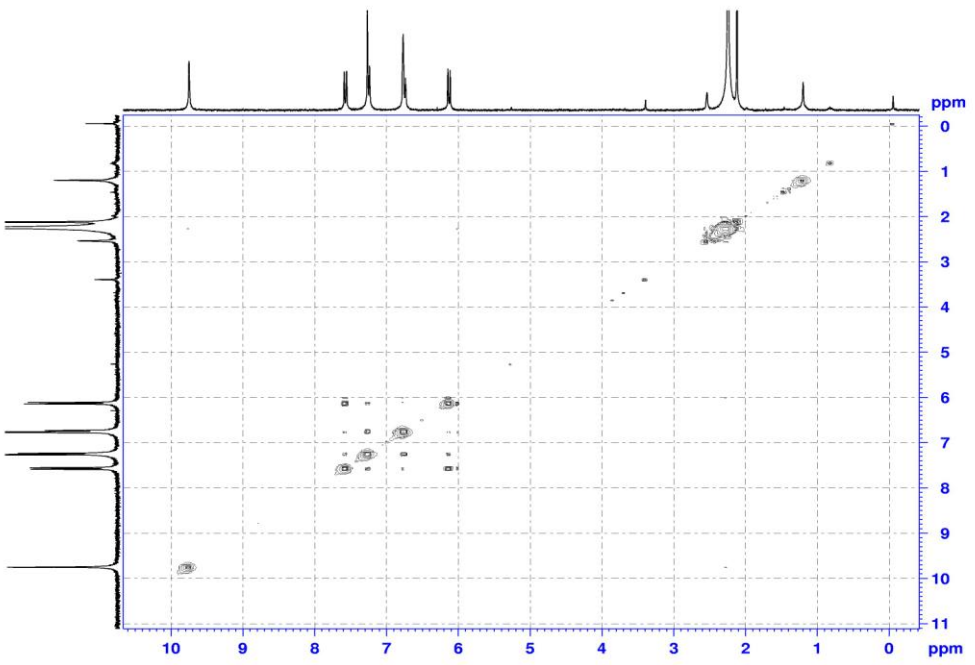


Figure A-27 COSY spectrum (in $CDCl_3$+ DMSO-d_6) of **TM7** (Umbelliferone)

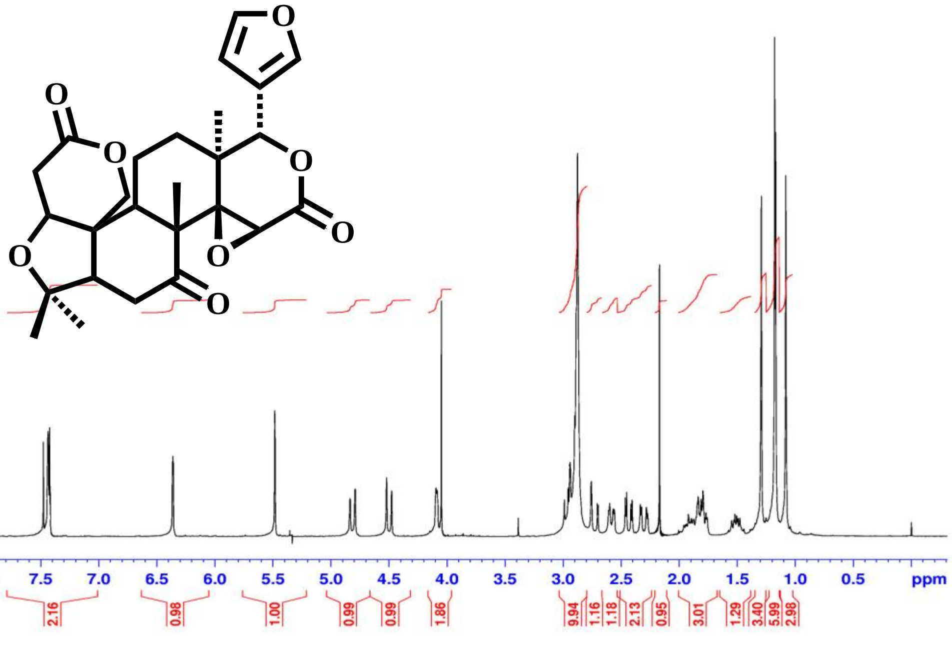


Figure A-28 ^1H NMR spectrum (in $CDCl_3$+ DMSO-d_6) of **TM8** (Limonin)

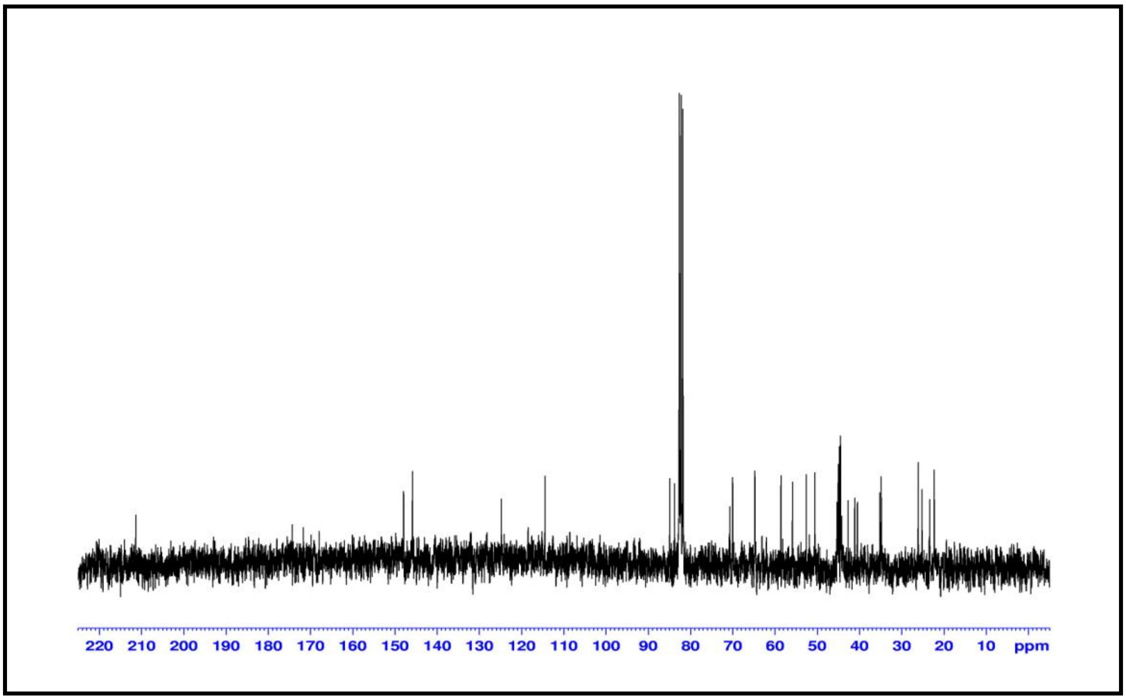


Figure A-29 ^{13}C NMR spectrum (in $CDCl_3$+ DMSO-d_6) of **TM8** (Limonin)

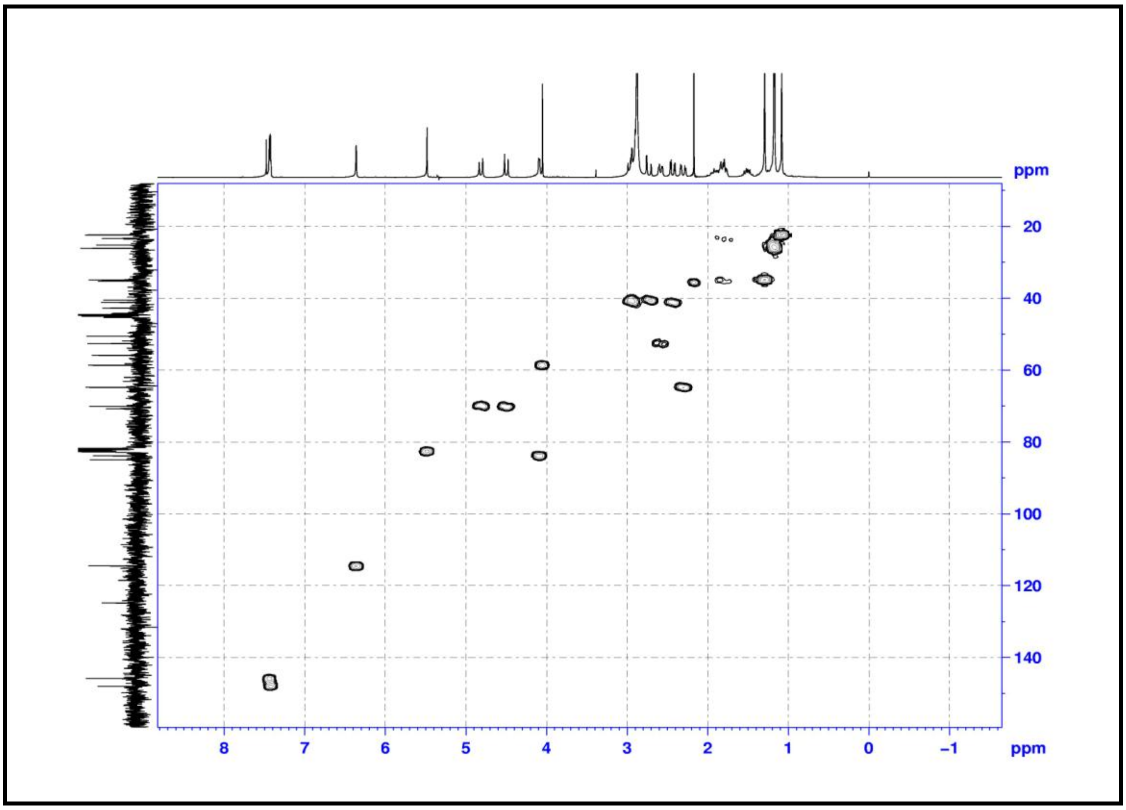


Figure A-30 HMQC spectrum (in $CDCl_3$+ DMSO-d_6) of **TM8** (Limonin)

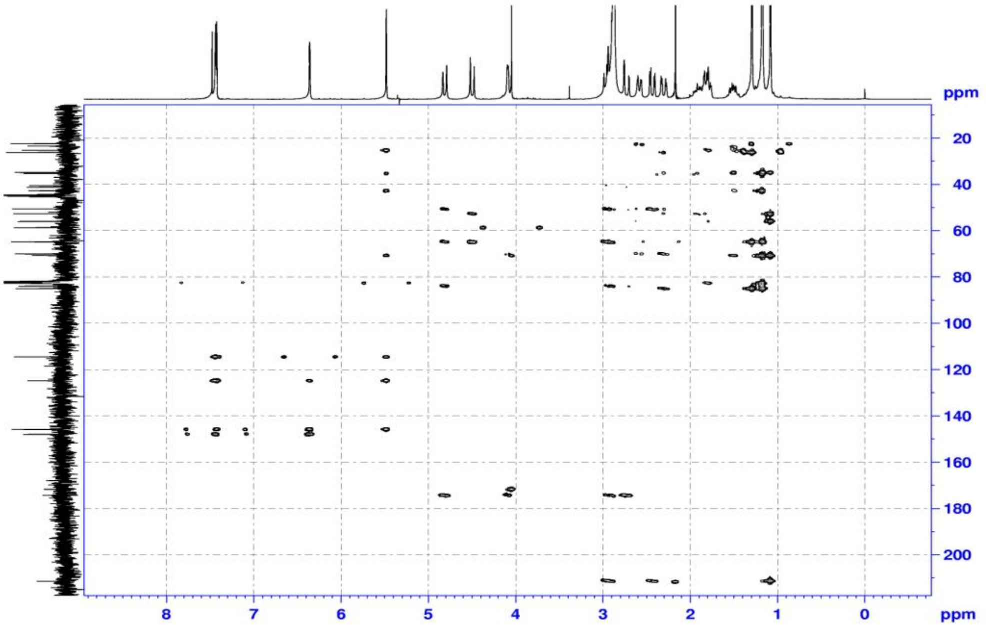


Figure A-31 HMBC spectrum (in $CDCl_3$+ DMSO-d_6) spectrum of **TM8** (Limonin)

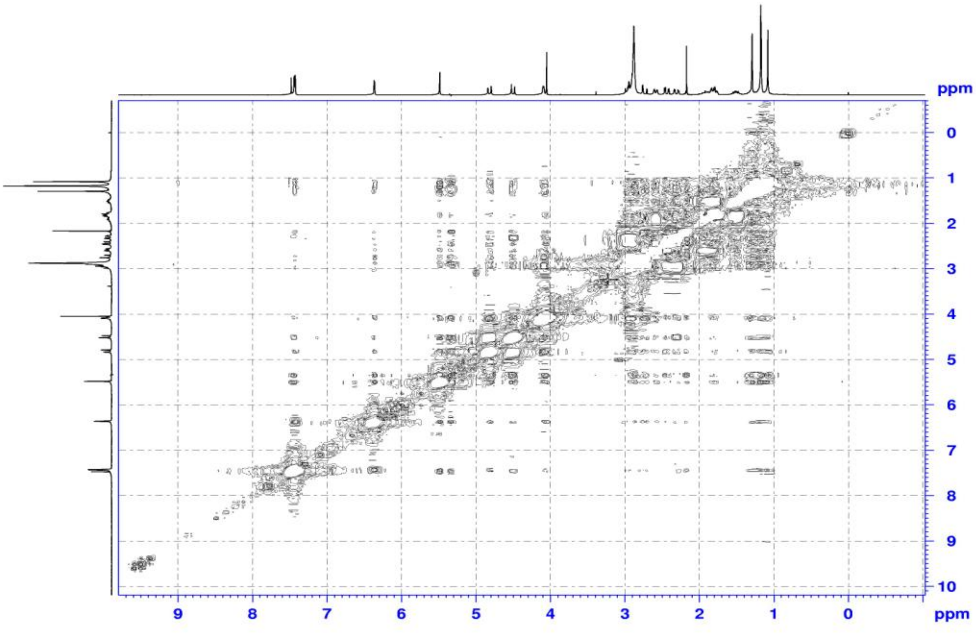


Figure A-32 COSY spectrum (in $CDCl_3$+ DMSO-d_6) of **TM8** (Limonin)

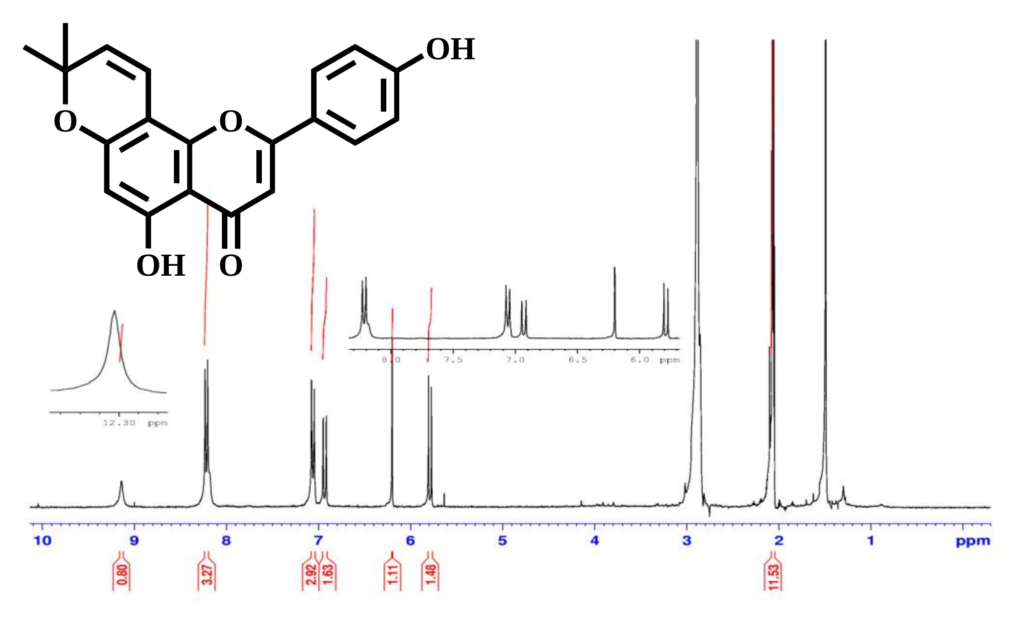


Figure A-33 ^{1}H NMR spectrum (in $CDCl_3$) of **TM9** (Atalantoflavone)

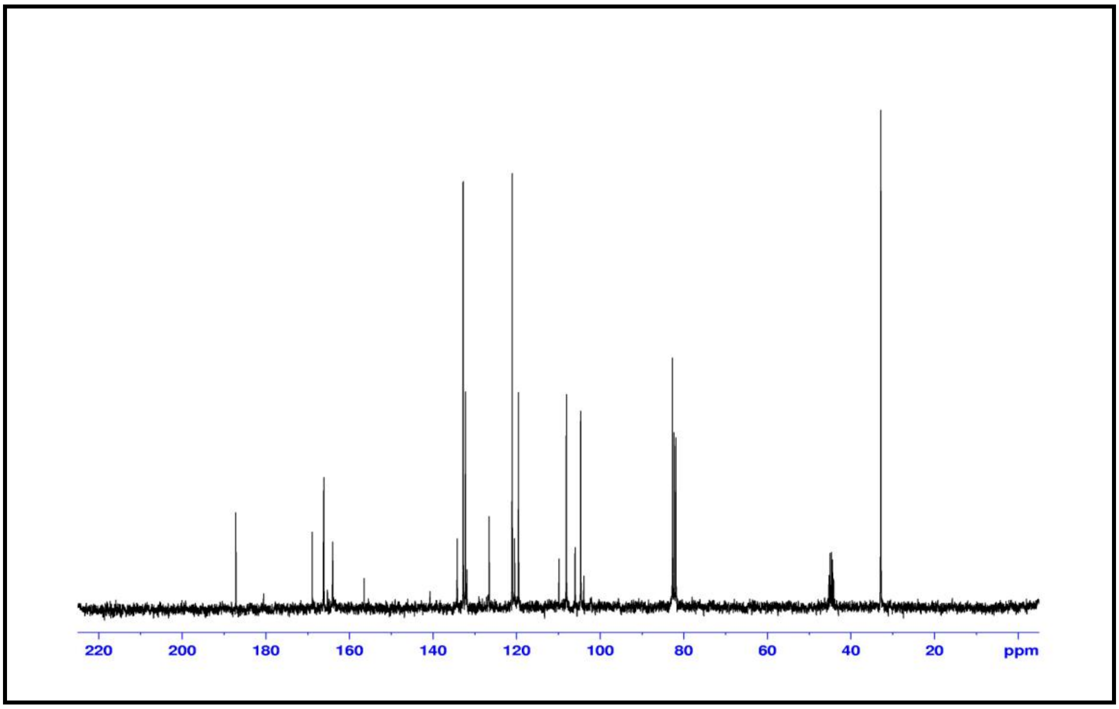


Figure A-34 ^{13}C NMR spectrum (in $CDCl_3$) of **TM9** (Atalantoflavone)

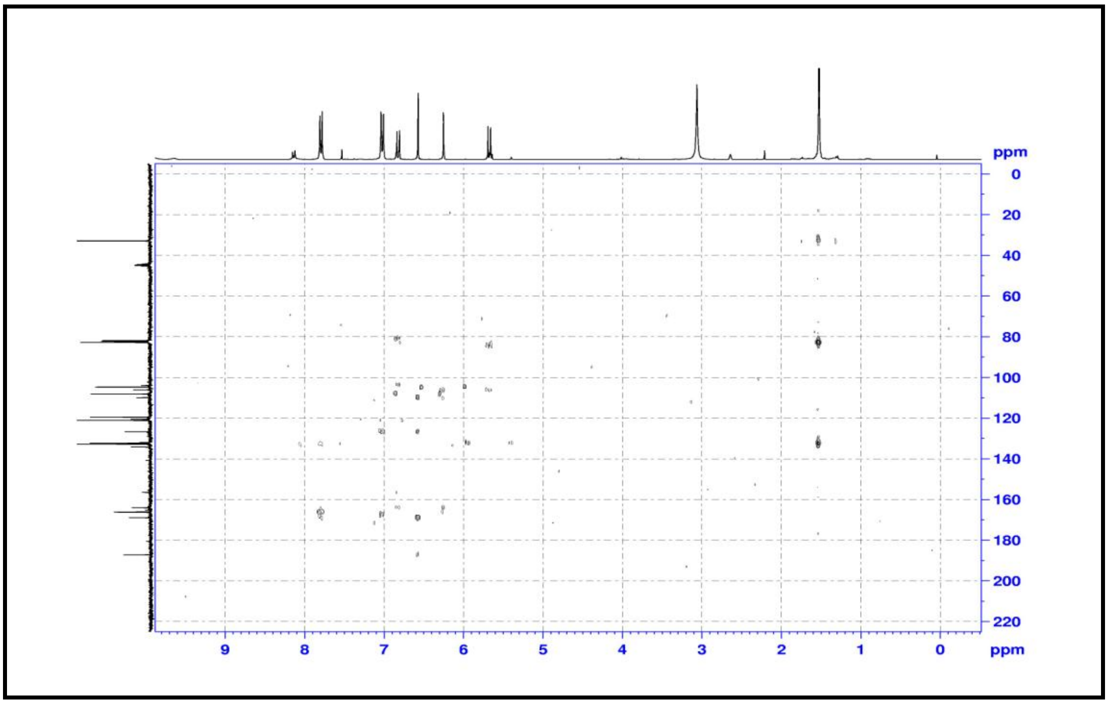


Figure A-35 HMBC spectrum (in $CDCl_3$) of **TM9** (Atalantoflavone)

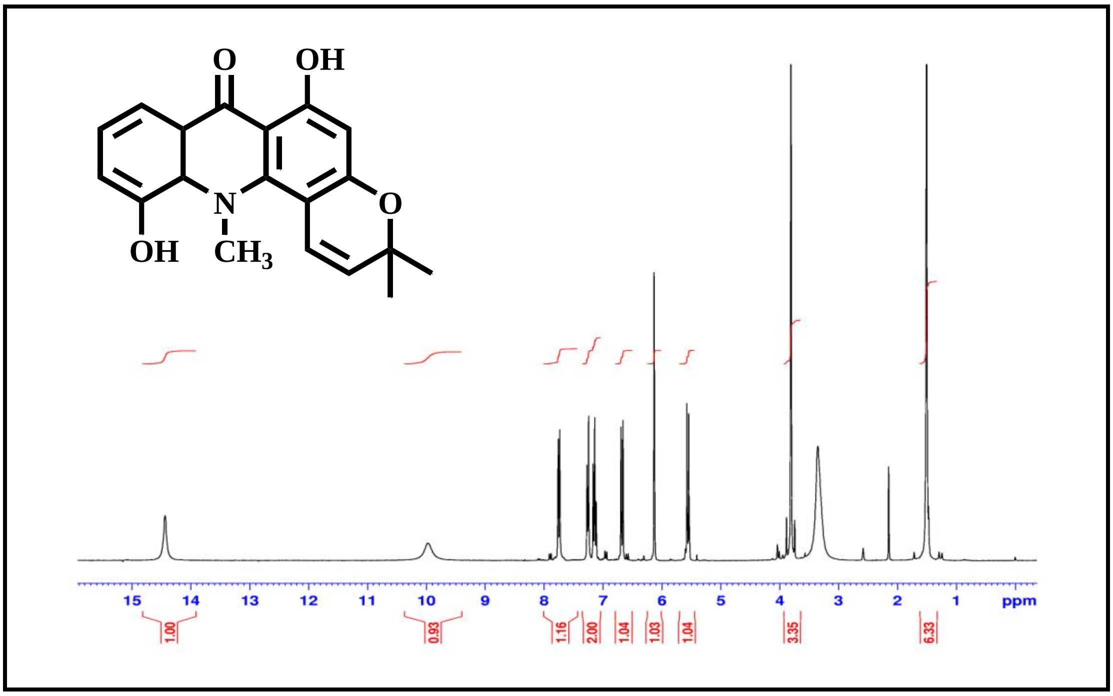


Figure A-36 ^1H NMR spectrum (in $CDCl_3$+ DMSO-d_6) of **TM10**

(5-Hydroxynoracronycine)

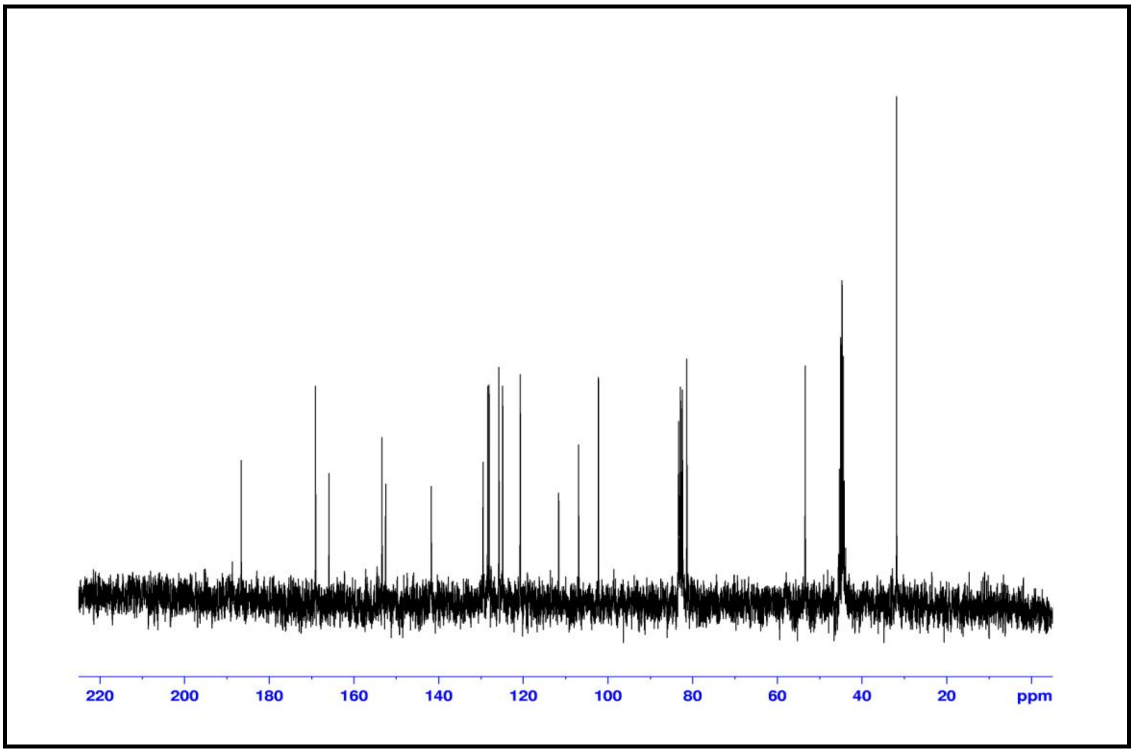


Figure A-37 ^{13}C NMR spectrum (in $CDCl_3$+ DMSO-d_6) of **TM10** (5-Hydroxynoracronycine)

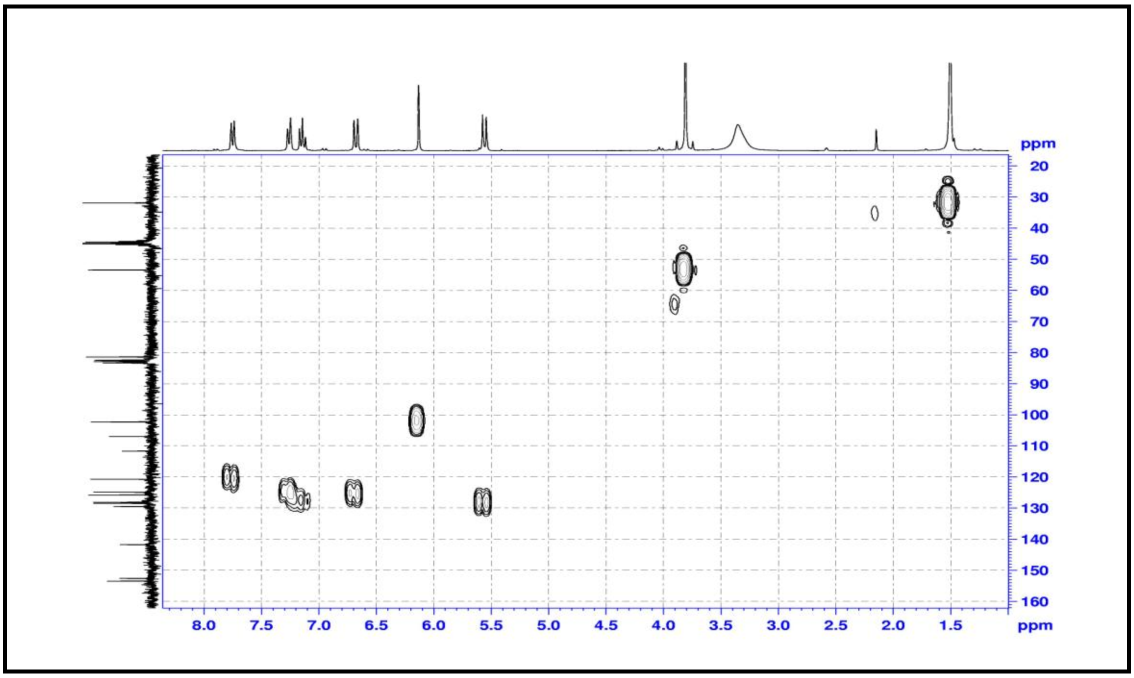


Figure A-38 HMQC spectrum (in $CDCl_3$+ DMSO-d_6) of **TM10** (5-Hydroxynoracronycine)

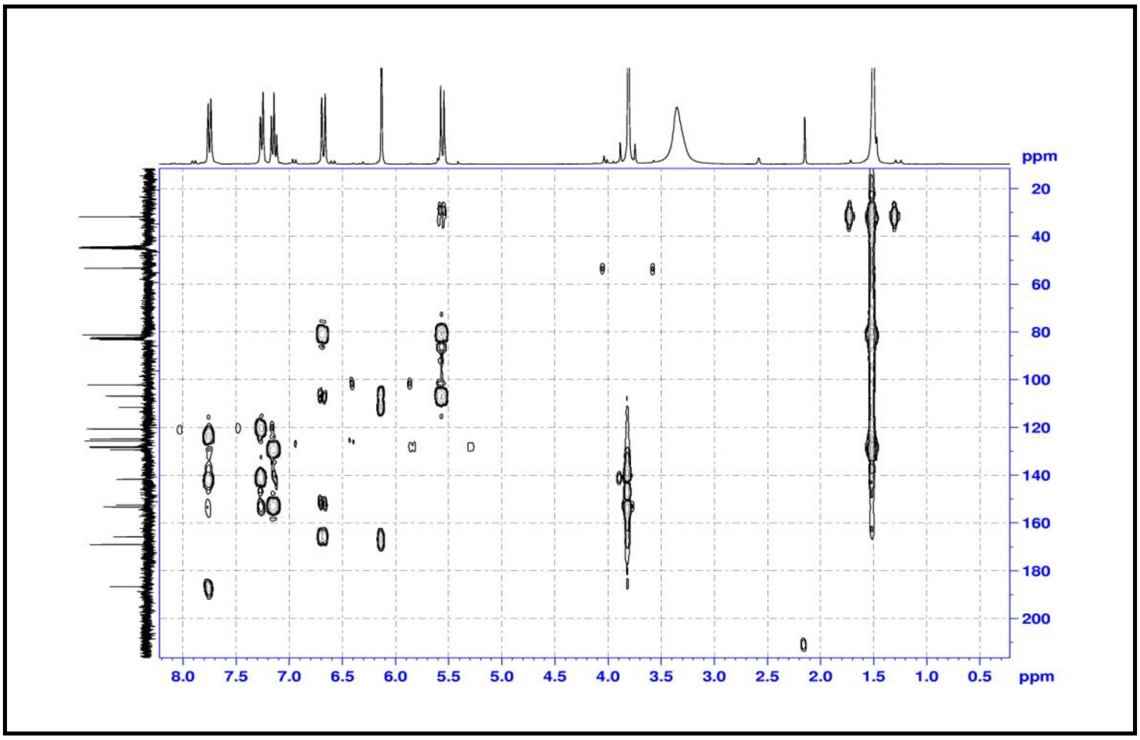


Figure A-39 HMBC spectrum (in $CDCl_3$+ DMSO-d_6) of **TM10** (5-Hydroxynoracronycine)

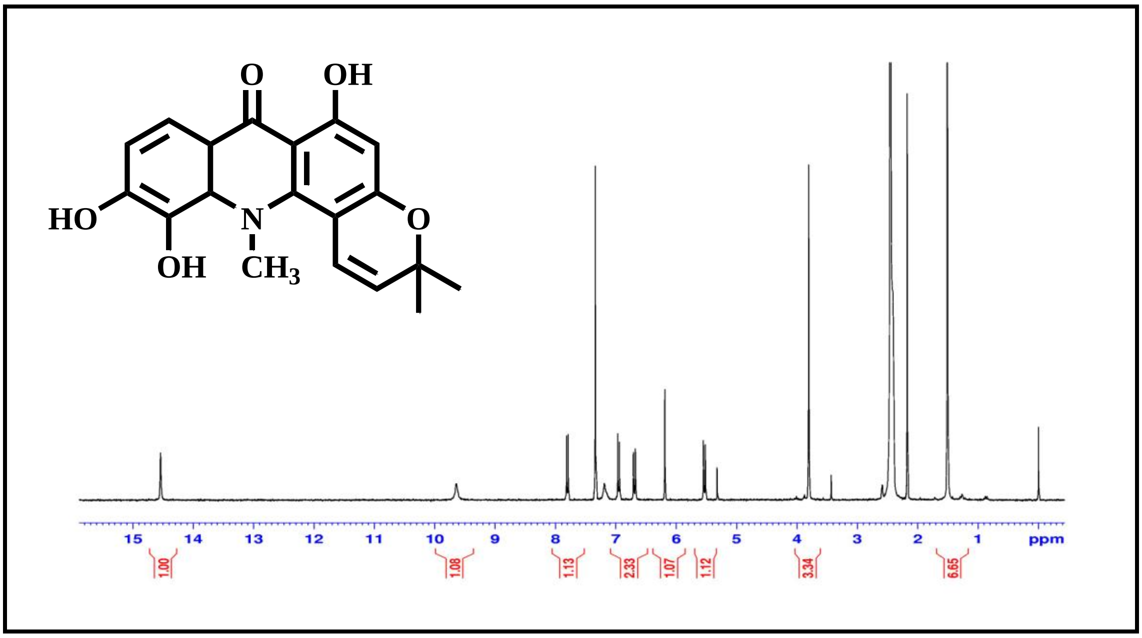


Figure A-40 ^1H NMR spectrum (in $CDCl_3$+ DMSO-d_6) of **TM11** (Citracridone-II)

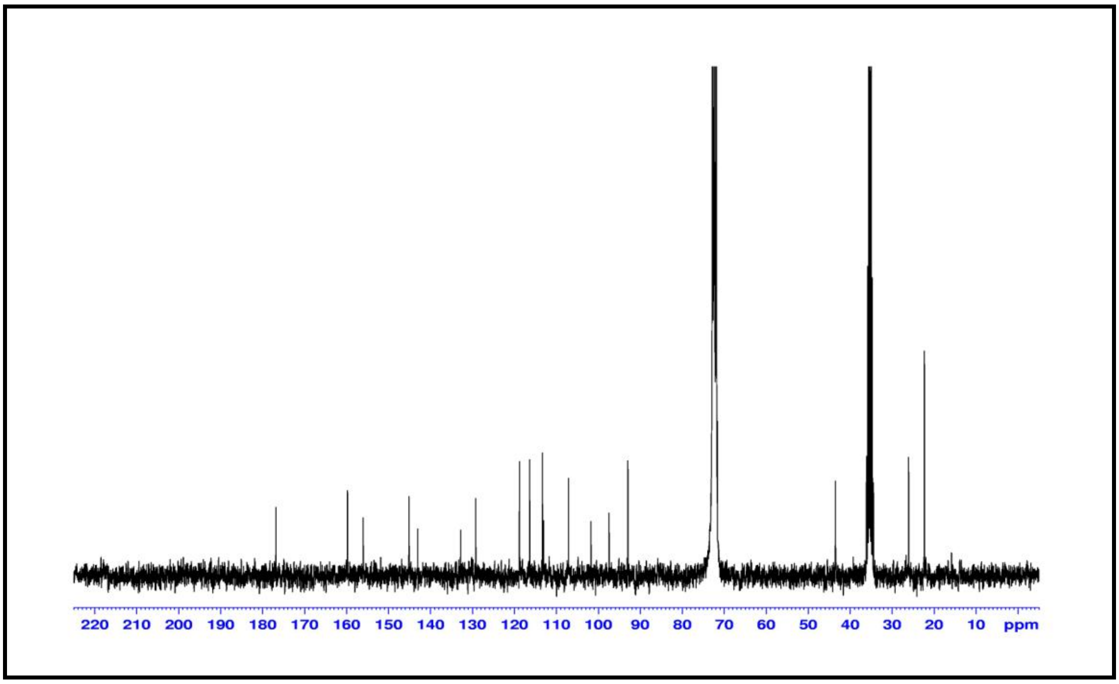


Figure A-41 ^{13}C NMR spectrum (in $CDCl_3$+ DMSO-d_6) of **TM11** (Citracridone-III)

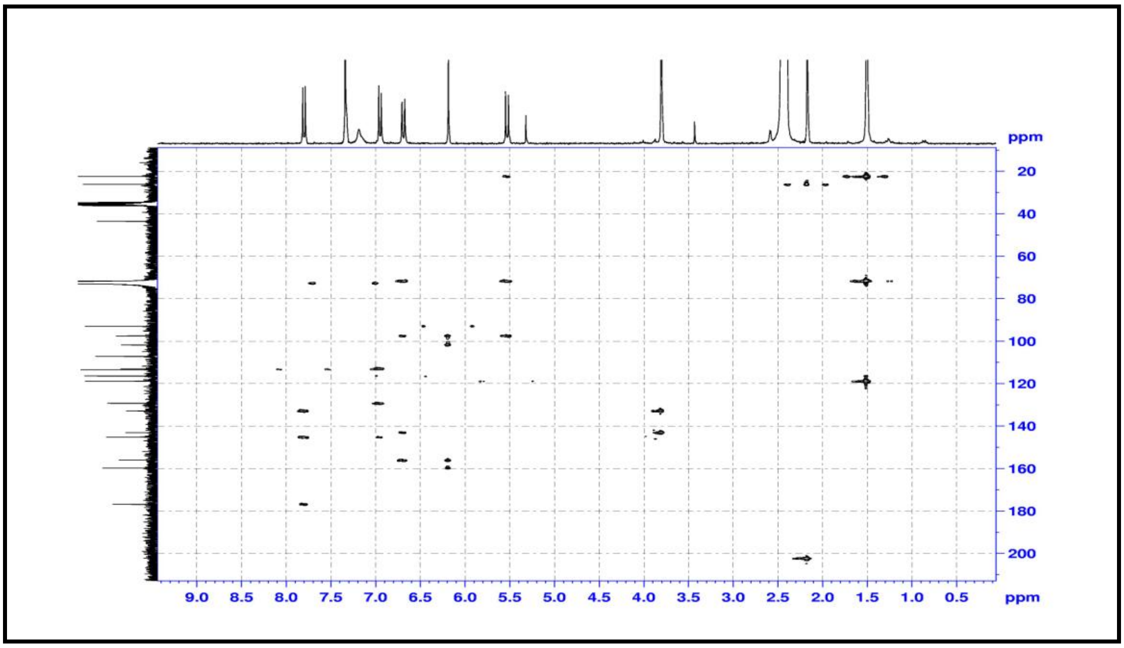


Figure A-42 HMBC spectrum (in $CDCl_3$+ DMSO-d_6) of **TM11**(Citracridone-III)

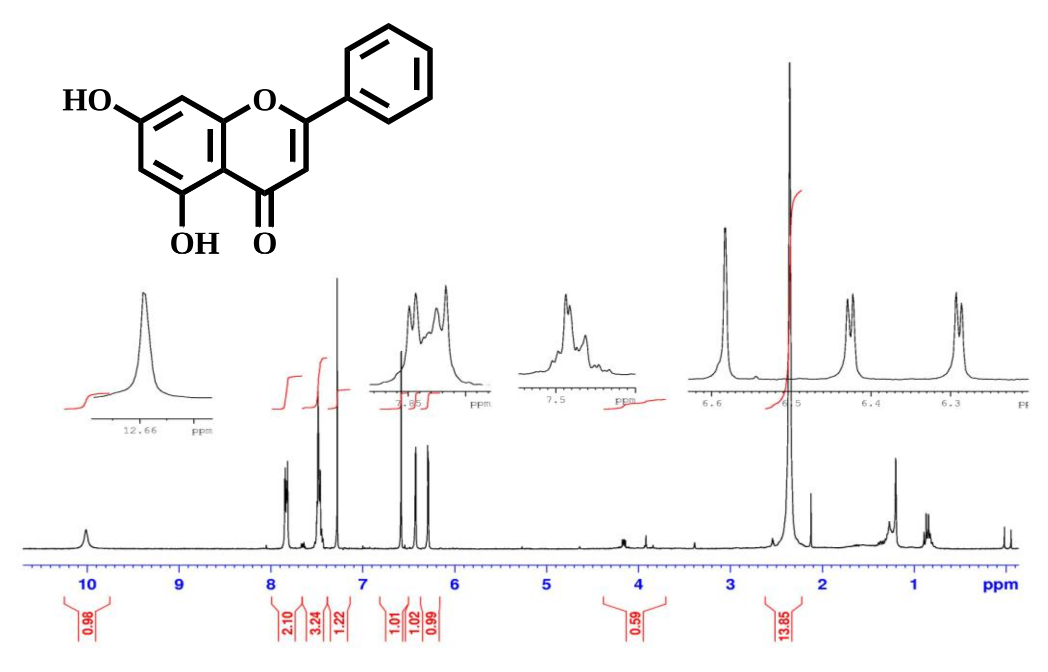


Figure A-43 ^{1}H NMR spectrum (in $CDCl_3$) of **TM12** (Chrysin)

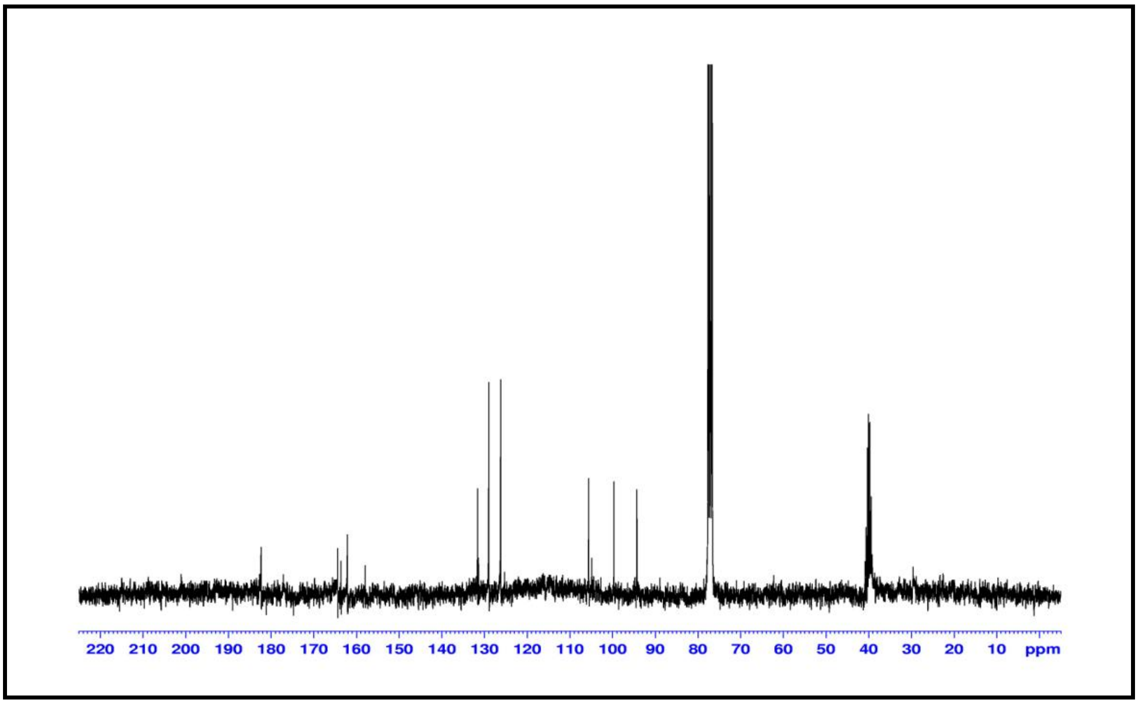


Figure A-44 ^{13}C NMR spectrum (in $CDCl_3$) of **TM12** (Chrysin)

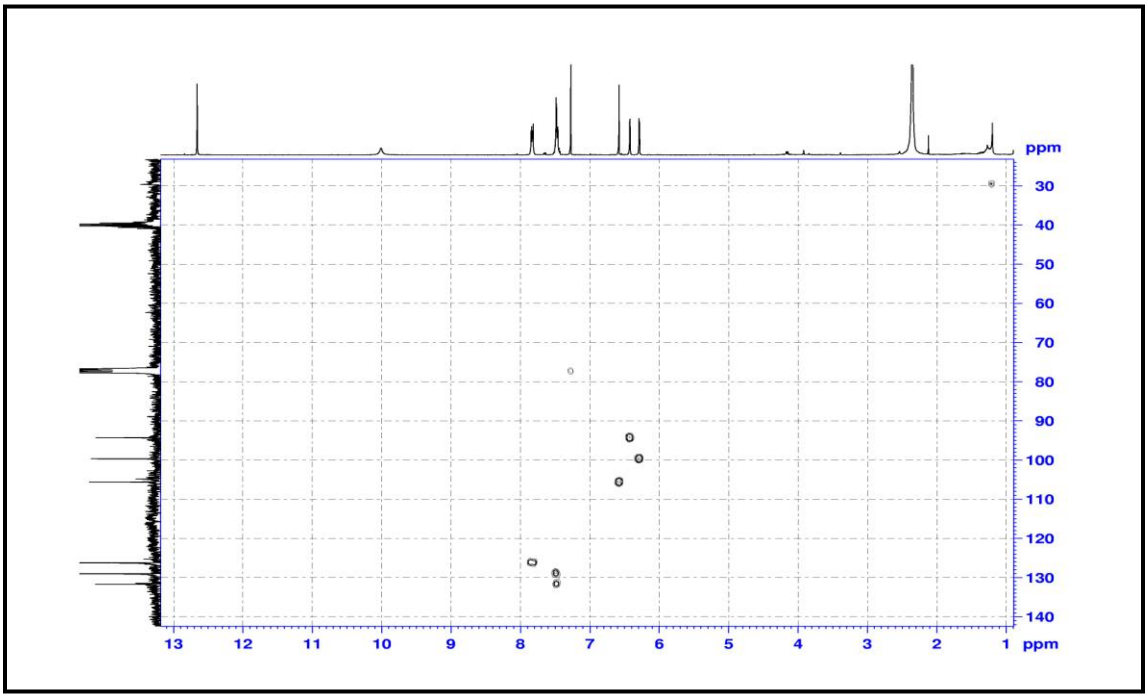


Figure A-45 HMQC spectrum (in $CDCl_3$) of **TM12** (Chrysin)

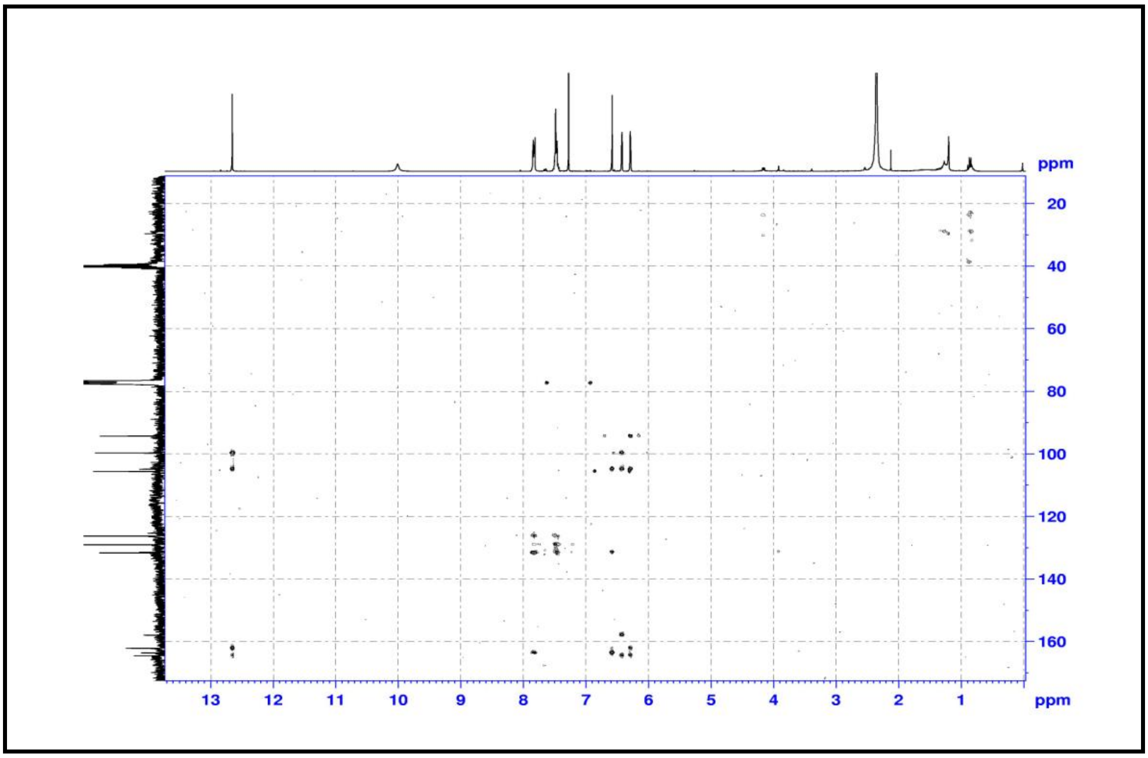


Figure A-46 HMBC spectrum (in $CDCl_3$) of **TM12** (Chrysin)

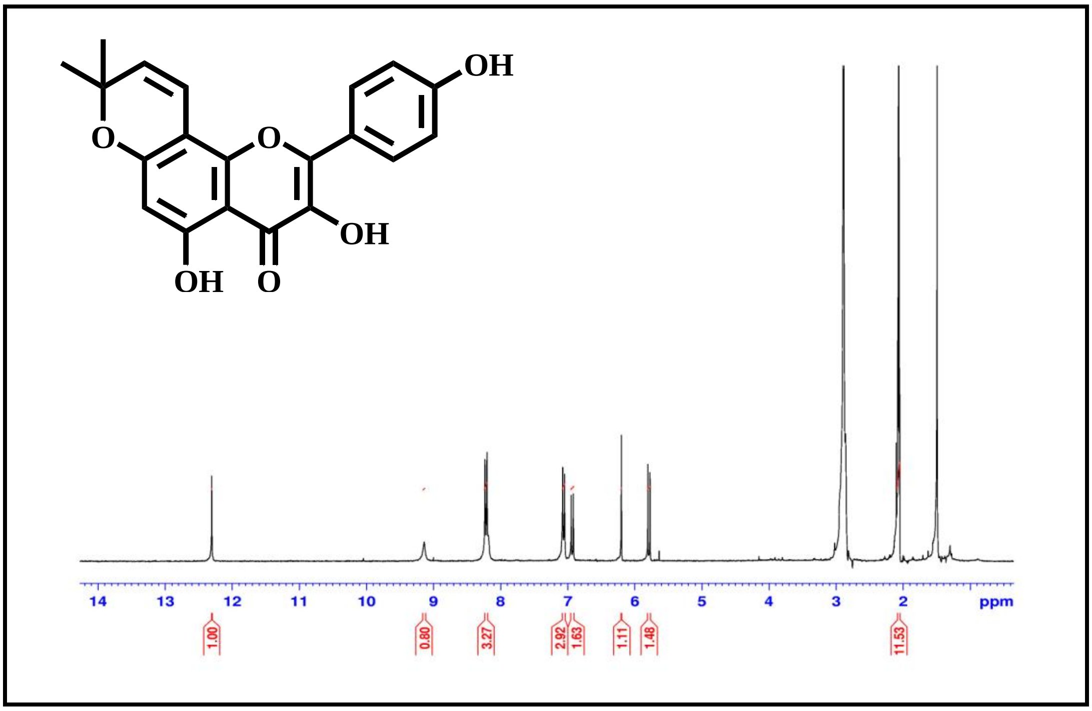


Figure A-47 ^{1}H NMR spectrum (in acetone-d_6) of **TM13** (Citrusinol)

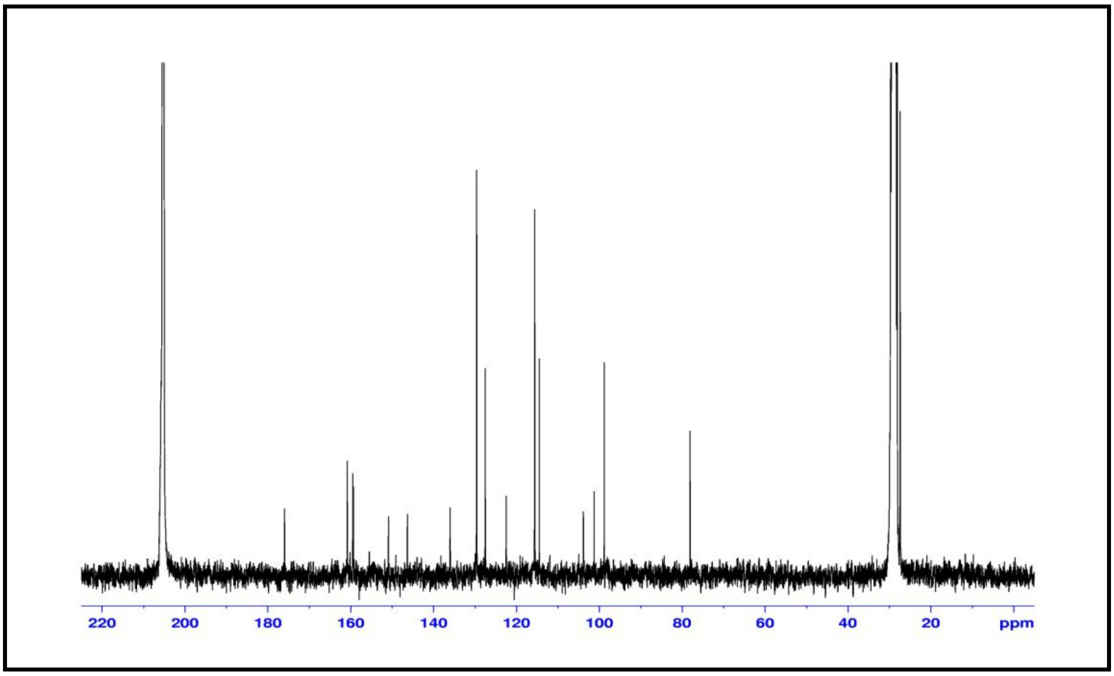


Figure A-48 ^{13}C NMR spectrum (in acetone-d_6) of **TM13** (Citrusinol)

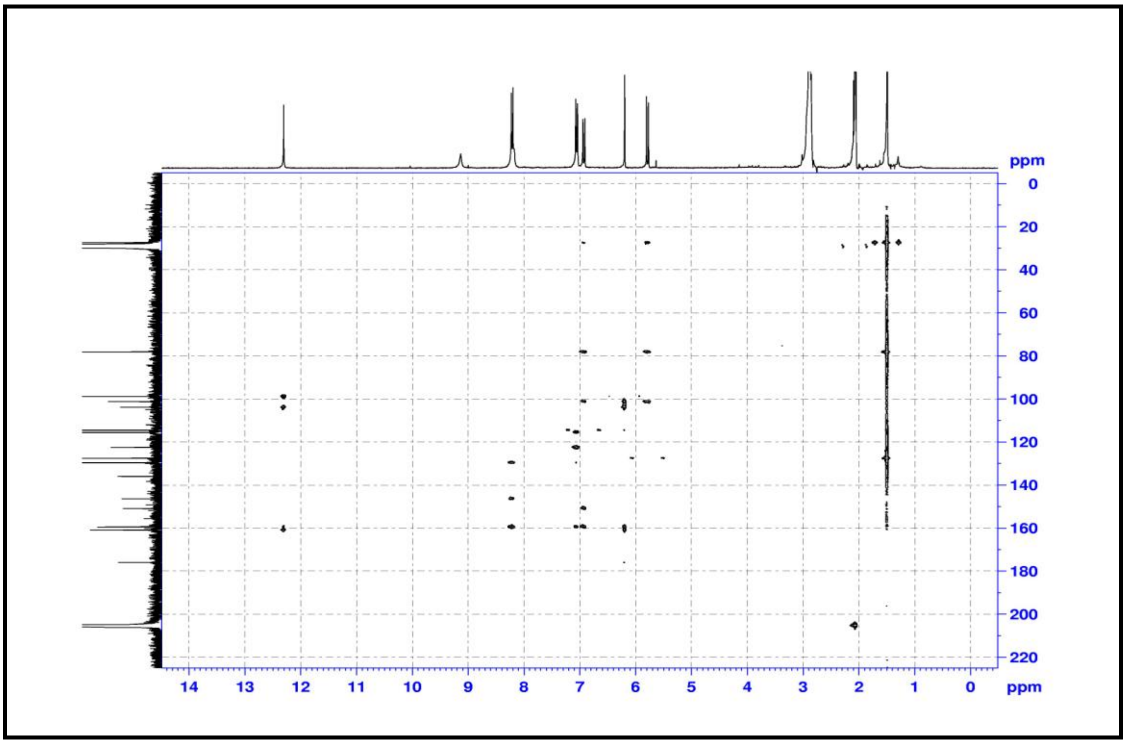


Figure A-49 HMBC spectrum (in acetone-d_6) of **TM13** (Citrusinol)

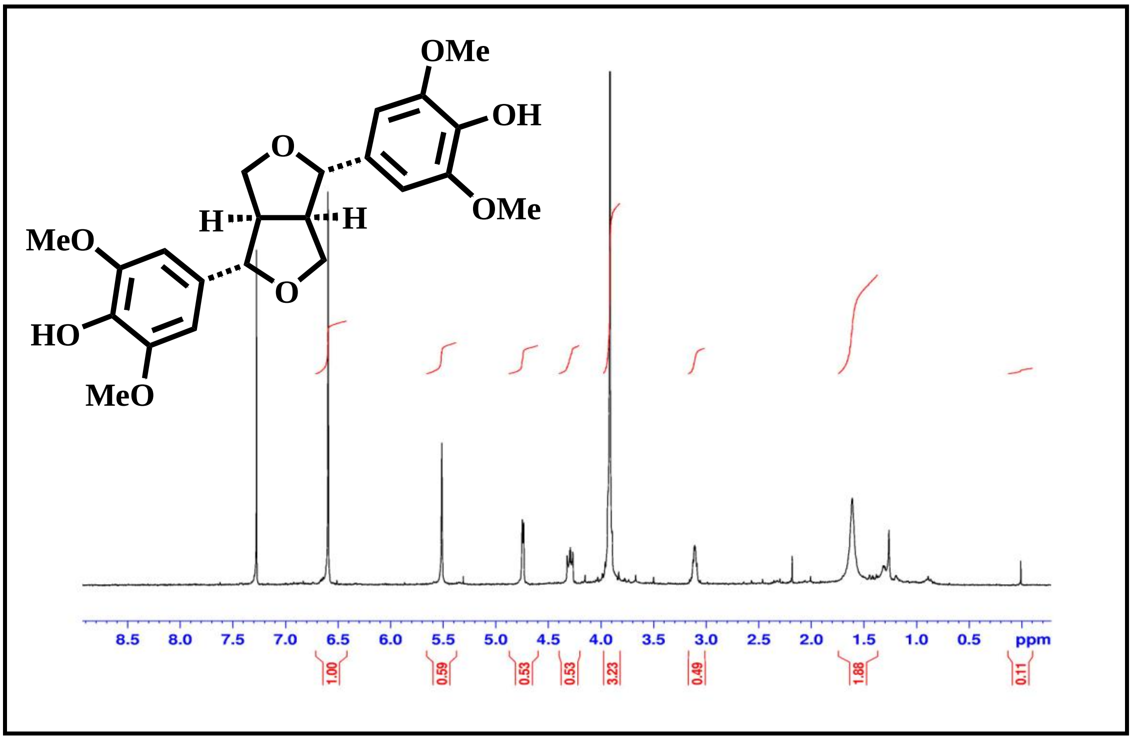


Figure A-50 ^{1}H NMR spectrum (in $CDCl_3$) of **TM14** (Syringaresinol)

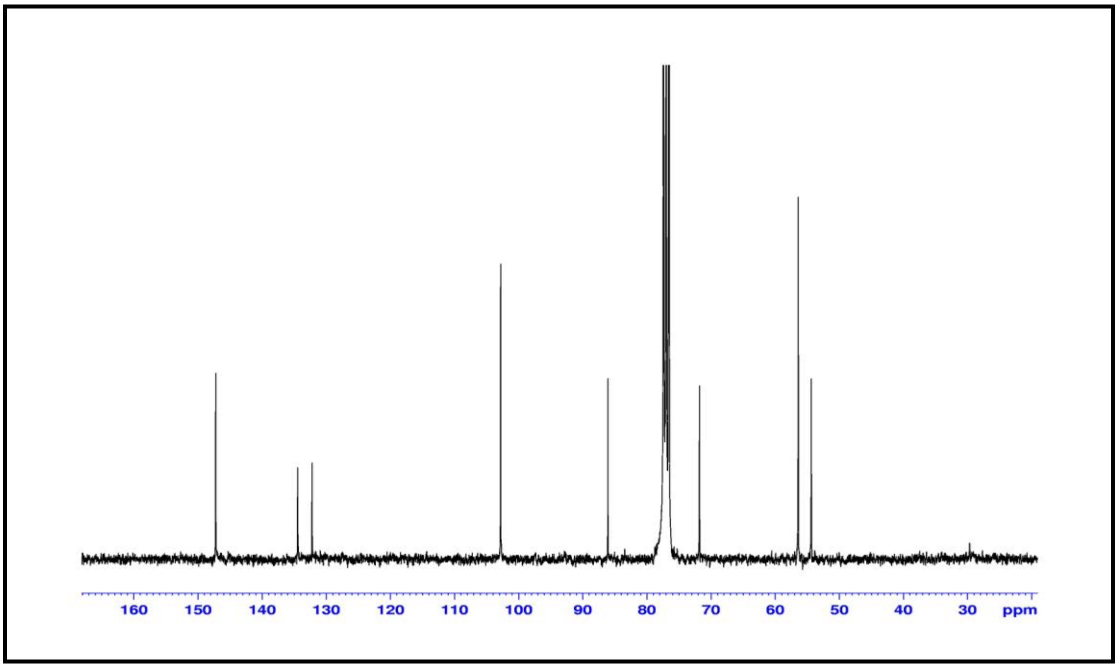


Figure A-51 ^{13}C NMR spectrum (in $CDCl_3$) of **TM14** (Syringaresinol)

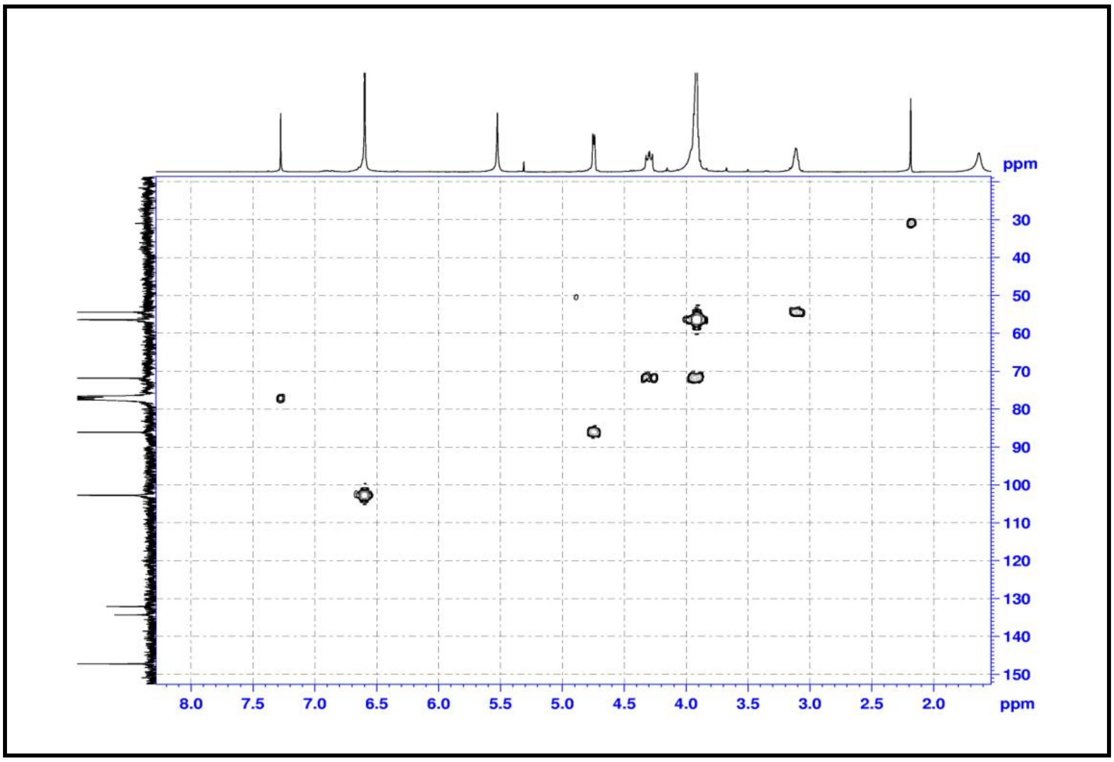


Figure A-52 HMQC spectrum (in $CDCl_3$) of **TM14** (Syringaresinol)

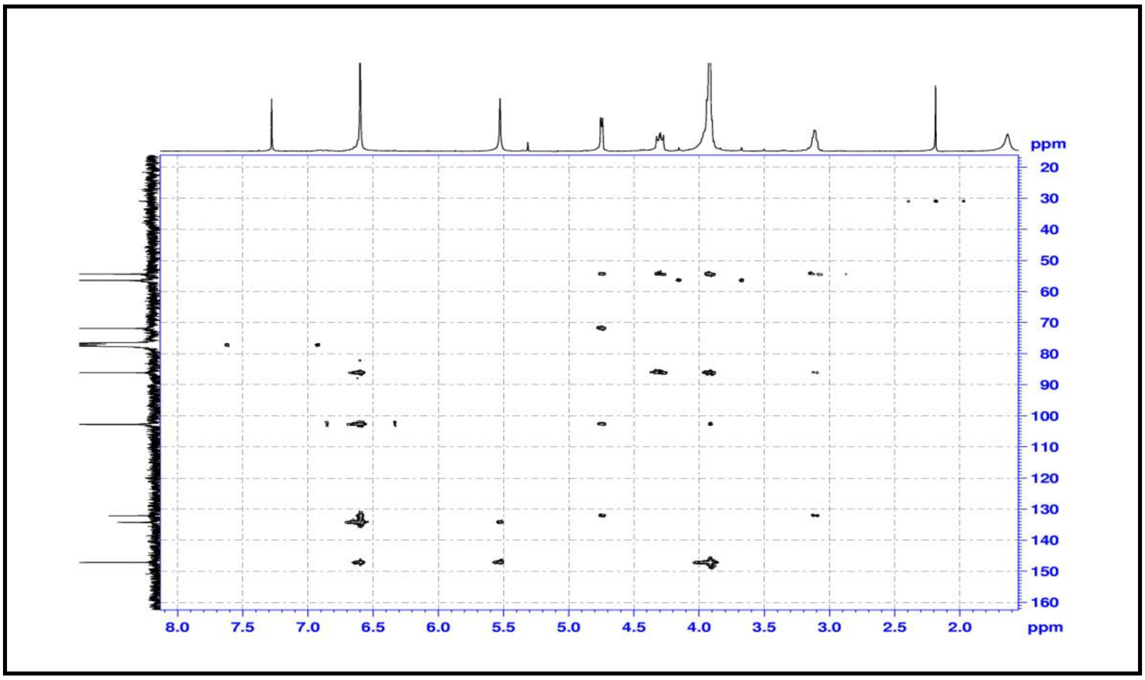


Figure A-53 HMBC spectrum (in $CDCl_3$) of **TM14** (Syringaresinol)

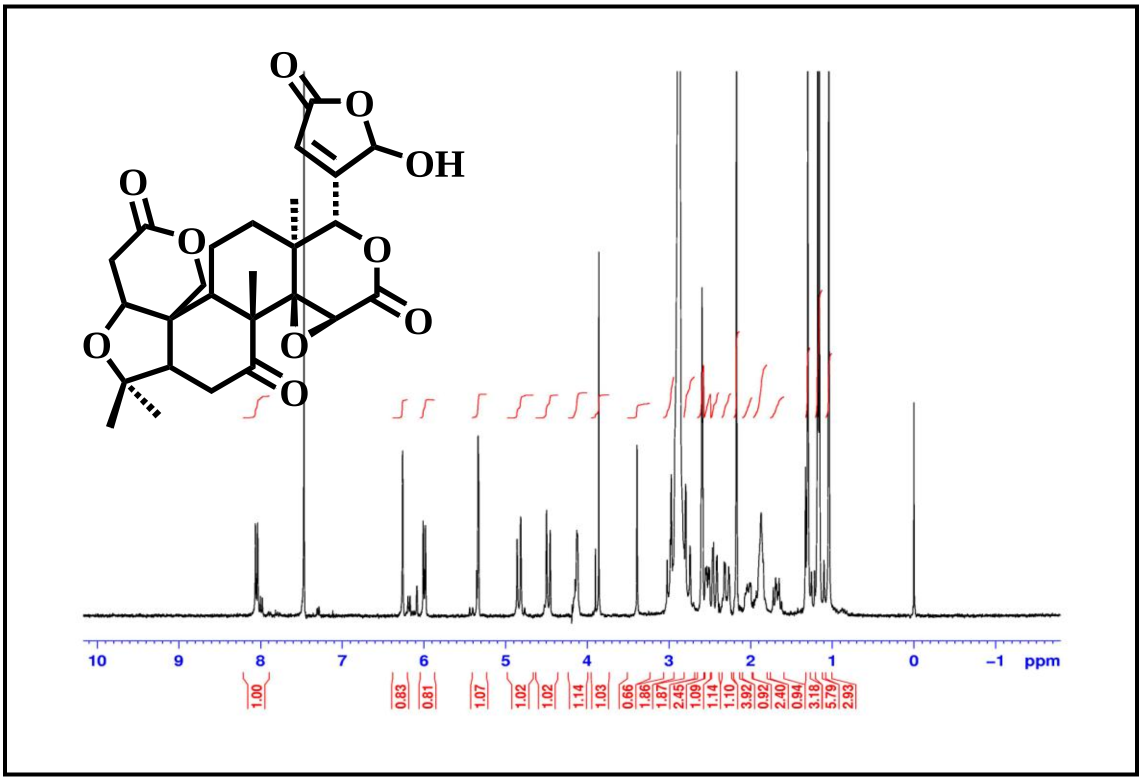


Figure A-54 ^1H NMR spectrum (in $CDCl_3$+ DMSO-d_6) of **TM15** (Limonexic acid)

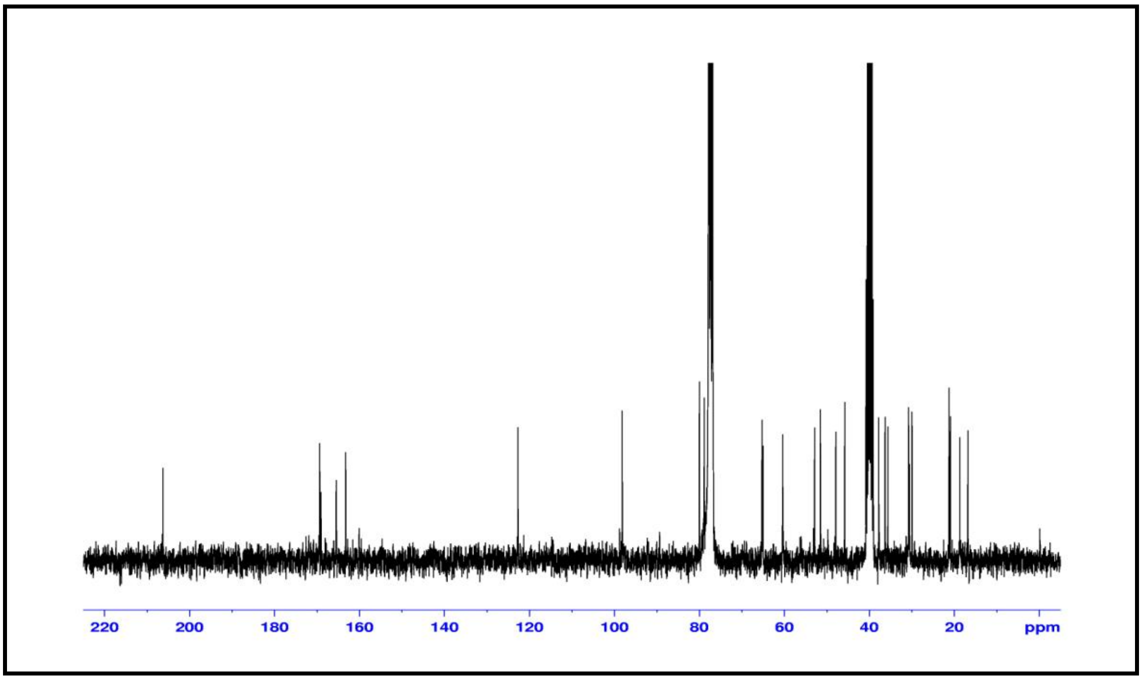


Figure A-55 ^{13}C NMR spectrum (in $CDCl_3$+ DMSO-d_6) of **TM15** (Limonexic acid)

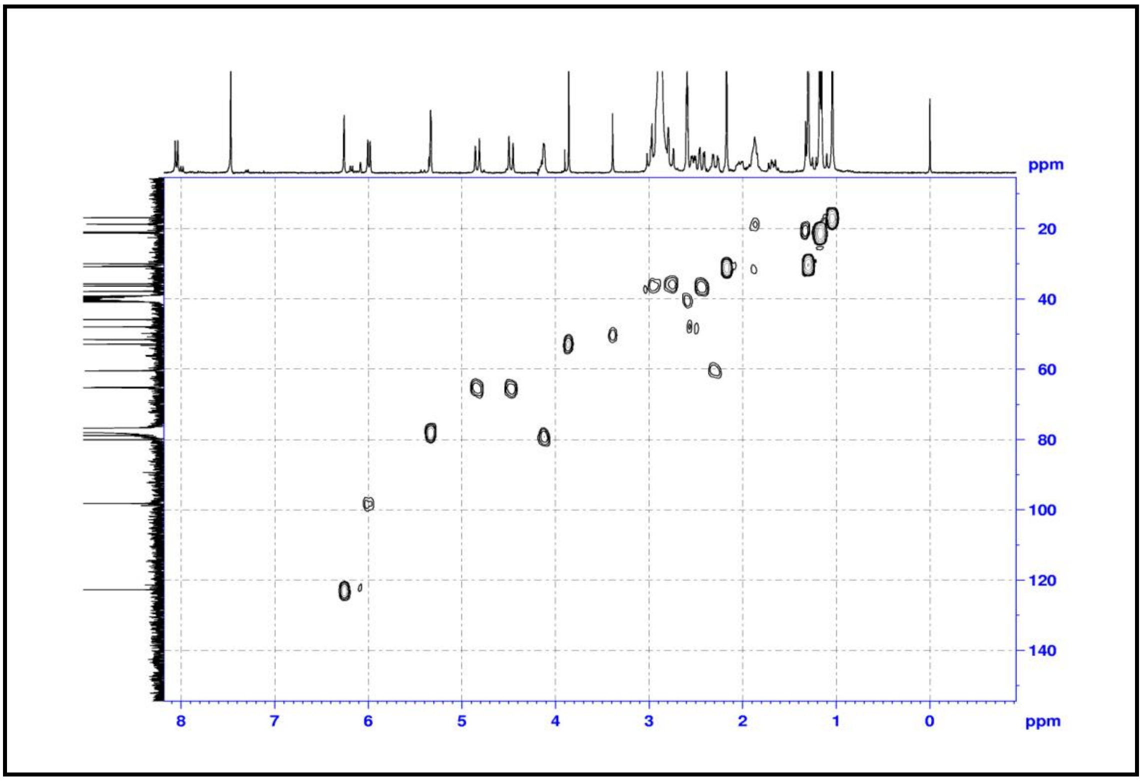


Figure A-56 HMQC spectrum (in $CDCl_3$+ DMSO-d_6) of **TM15** (Limonexic acid)

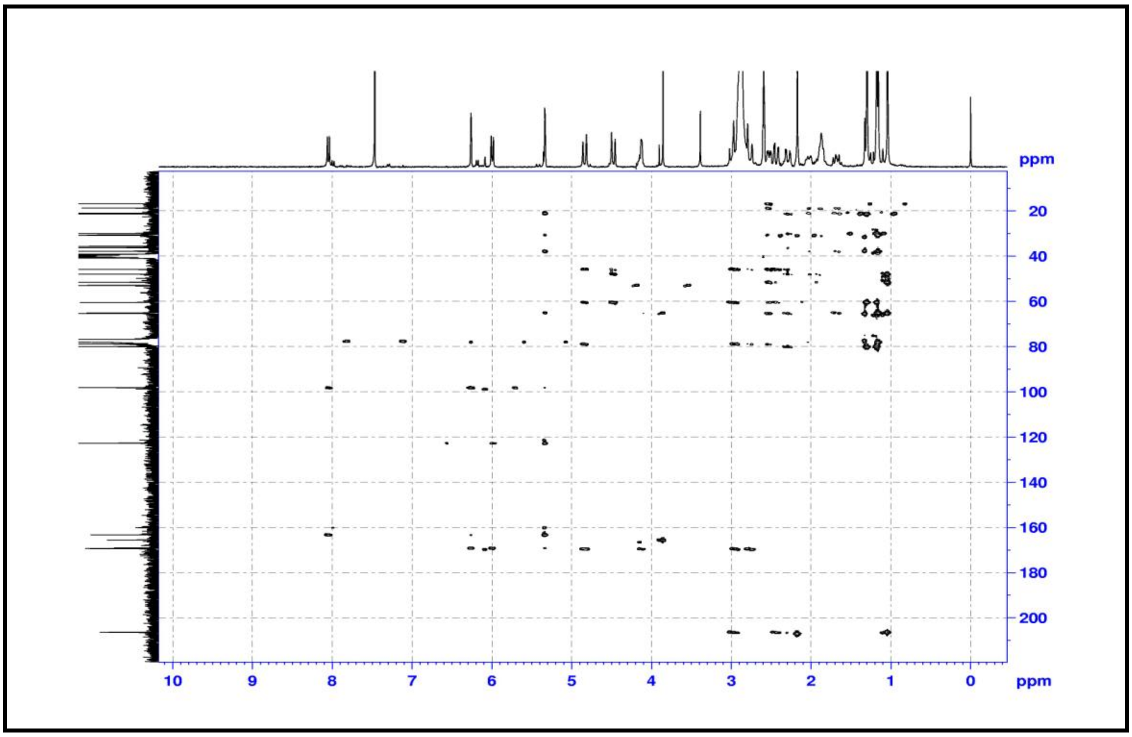


Figure A-57 HMBC spectrum (in $CDCl_3$+ DMSO-d_6) of **TM15** (Limonexic acid)

2. ^{1}H NMR and ^{13}C NMR spectral data of known compounds from literature

Table A-1 ^{1}H and ^{13}C NMR spectral data of Bergapten (Kawaii *et al*., 1999)

Position	δ_H (multiplicity)	δ_C (C-Type)
2	-	161.2 (C=O)
3	6.25 (*d*, *J* = 9.8)	112.6 (CH)
4	8.14 (*d*, *J* = 9.8)	139.2 (CH)
4a		106.4 (C)
5	-	149.6 (C)
6	-	112.7 (C)
7	-	158.4 (C)
8	7.12 (*s*)	93.9 (CH)
8a	-	156.5 (C)
2'	7.58 (*d*, *J* = 2.5)	144.8 (CH)
3'	7.00 (*d*, *J* = 2.5)	105.0 (CH)
5-OCH_3	4.25 (*s*)	60.1 (CH_3)

in $CDCl_3$

Table A-2 ^{1}H and ^{13}C NMR spectral data of Isopimpinellin (Patil *et al*., 2010)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**
2	-	162.7 (C=O)
3	6.29 (*d*, *J* = 9.8)	113.2 (CH)
4	8.24 (*d*, *J* = 9.8)	141.4 (CH)
4a		108.8 (C)
5	-	146.2 (C)
6	-	116.5 (C)
7	-	151.7 (C)
8	-	129.3 (C)
8a	-	144.9 (C)
2'	7.82 (*d*, *J* = 2.4)	147.1 (CH)
3'	7.21 (*d*, *J* = 2.4)	106.4 (CH)
5-OCH_3	4.20 (*s*)	61.5 (CH_3)
8-OCH_3	4.11 (*s*)	62.1 (CH_3)

in CD_3OD

Table A-3 ^{1}H and ^{13}C NMR spectral data of Citropten (Kawaii *et al.*, 1999)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**
2	-	161.5 (C=O)
3	6.14 (*d*, *J* = 9.7)	111.0 (CH)
4	7.95 (*d*, *J* = 9.7)	138.7 (CH)
4a	-	104.0 (C)
5	-	157.0 (C)
6	6.26 (*d*, *J* = 2.1)	94.9 (CH)
7	-	163.7 (C)
8	6.40 (*d*, *J* = 2.1)	92.8 (CH)
8a	-	156.9 (C)
5-OCH_3	3.87 (*s*)	55.9(CH_3)
7-OCH_3	3.84 (*s*)	55.8 (CH_3)

in $CDCl_3$

Table A-4 ^{1}H and ^{13}C NMR spectral data of 4-Hydroxybenzaldehyde (Jang *et al.*, 2004)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**
1	-	129.9 (C)
2/6	7.82 (*d*, *J* = 8.6)	132.5 (CH)
3/5	6.98 (*d*, *J* = 8.6)	116.0 (CH)
4	-	161.6 (C)
1'	9.86 (*s*)	191.2 (C)

in $CDCl_3$

Table A-5 ^{1}H and ^{13}C NMR spectral data of bergamottin (Kawaii *et al.*, 1999)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**
2	-	161.3 (C=O)
3	6.25 (*d*, *J* = 9.8)	112.6 (CH)
4	8.14 (*d*, *J* = 9.8)	139.6 (CH)
4a		107.6 (C)
5	-	149.0 (C)
6	-	114.3 (C)
7	-	158.2 (C)
8	7.13 (*s*)	94.3 (CH)
8a	-	152.7 (C)
2'	7.57 (*d*, *J* = 2.5)	144.9 (CH)
3'	6.94 (*d*, *J* = 2.5)	105.0 (CH)
1"	4.98 (*d*, *J* = 6.8)	69.8 (CH_2)
2"	5.51 (*m*)	118.9 (CH)
3"	-	143.0 (C)
4"	2.08 (*m*)	39.5 (CH_2)
5"	2.08 (*m*)	26.2 (CH_2)
6"	5.05 (*m*)	123.5 (CH)
7"	-	132.0 (C)
8"	1.68 (*s*)	25.7 (CH_3)
9"	1.58 (*s*)	17.7 (CH_3)
10"	1.66 (*s*)	16.7(CH_3)

in $CDCl_3$

Table A-6 ^{1}H and ^{13}C NMR spectral data of Umbelliferone
(Khalil *et al*., 2003)

Position	δ_H **(multiplicity)**	δ_C **(C-Type)**
2	-	160.6 (C=O)
3	6.19 (*d*, *J* = 9.5)	115.4 (CH)
4	7.86 (*d*, *J* = 9.5)	144.2 (CH)
4a	-	114.6 (C)
5	7.46 (*d*, *J* = 8.5)	127.8 (CH)
6	6.87 (*dd*, *J* = 8.5, 2.3)	113.5 (CH)
7	-	161.4 (C)
8	6.78 (*d*, *J* = 2.3)	105.1 (CH)
8a	-	153.9 (C)
7-OH	-	-

in CD_3OD

Table A-7 ^{1}H and ^{13}C NMR spectral data of Limonin (Khalil *et al*., 2003)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**
1	4.10 (*br s*)	78.8 (CH)
2	2.26 (*dd*, *J* = 14.8, 3.2) 2.65 (*dd*, *J* = 14.8, 3.2)	36.1 (CH_2)
3	-	170.3 (C=O)
4	-	79.5 (C)
5	2.47 (*dd*, *J* = 15.0, 2.8)	58.4 (CH)
6	2.75 (*dd*, *J* = 15.0, 2.8) 3.10 (*dd*, *J* = 15.0, 2.8)	36.6 (CH_2)
7	-	208.1 (C=O)
8	-	50.3 (C)
9	2.55 (*dd*, *J* = 10.0, 2.0)	46.9 (CH)
10	-	45.3 (C)
11	1.78 (*m*) 1.70 (*m*)	19.7 (CH_2)
12	1.26 (*m*) 1.73 (*m*)	29.3 (CH_2)
13	-	37.7 (C)
14	-	64.9 (C)
15	4.09 (*s*)	54.1 (CH)
16	-	167.4 (C)
17	5.45 (*s*)	77.9 (CH)
18	1.09 (*s*)	17.6 (CH_3)
19	4.46 (*d*, *J* = 13.0) 4.90 (*d*, *J* = 13.0)	66.7 (CH_2)

in DMSO-d_6

Table A-7 ^{1}H and ^{13}C NMR spectral data of Limonin (Khalil *et al*., 2003) (continued)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**
20	-	120.3 (C)
21	7.63 (*s*)	143.4 (CH)
22	6.48 (*d*, *J* = 3.0)	110.2 (CH)
23	7.69 (*d*, *J* = 3.0)	141.7 (CH)
24	0.98 (*s*)	17.1 (CH_3)
25	1.17 (*s*)	29.8 (CH_3)
26	1.01 (*s*)	21.4 (CH_3

in DMSO-d_6

Table A-8 ^{1}H, ^{13}C NMR and HMBC spectral data of Atalantoflavone (Bacher *et al*., 2009)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**	**HMBC**
2	-	165.19 (C=O)	-
3	6.67 (*s*)	104.14 (CH)	C-2, C-4, C-4a, C-1'
4	-	183.39 (C)	-
4a	-	105.86 (C)	-
5	-	162.77 (C)	-
6	6.16 (*s*)	100.40 (CH)	C-4a, C-5, C-7, C-8
7	-	160.28 (C)	-
8	-	102.24 (C)	-
8a	-	152.78 (C)	-
9	6.90 (*d*, J = 10.0)	115.47 (CH)	-
10	5.77 (*d*, J = 10.0)	138.72 (CH)	C-8, C-11, C-12
11	-	78.94 (C)	-
12	1.47 (*s*)	28.31 (CH_3)	C-10, C-11, C-12
1'	-	123.10 (C)	-
2',6'	7.96 (*d*, J = 8.9)	129.37 (CH)	C-2, C-2', C-4', C-6'
3',5'	7.16 (*d*, J = 8.9)	117.02 (CH)	C-1', C-3', C-4', C-5'
4'	-	162.31 (C)	-
5-OH	13.08 (*s*)	-	C-6
4'-OH	4.59 (*s*)	-	-

in Acetone-d_6

Table A-9 ^{1}H, ^{13}C NMR and HMBC spectral data of 5-Hydroxynoracronycine (Teng *et al.*, 2005)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**
1	-	164.3 (C)
2	6.10 (*s*)	98.7 (CH)
3	-	162.2 (C)
4	-	102.7 (C)
4a	-	148.8 (C)
5	-	149.4 (C)
6	7.33(*d*, *J* = 7.6)	120.9 (CH)
7	7.21 (*t*, *J* = 7.6)	124.2 (CH)
8	7.78 (*d*, *J* = 7.6)	117.1 (CH)
8a	-	124.6 (C)
9	-	182.9 (C=O)
9a	-	106.7 (C)
10a	-	138.1 (C)
1'	6.82 (*d*, *J* = 10.0)	123.0 (CH)
2'	5.66 (*d*, *J* = 10.0)	121.9 (CH)
3'	-	80.6 (C)
4'/5'	1.47 (*s*)	22.5 (CH_3)
1-OH	14.42 (*s*)	-
5-OH	9.57 (*s*)	-
N-CH_3	3.84 (*s*)	49.3 (CH_3)

in Aceton-d_6

Table A-10 ^{1}H, ^{13}C NMR and HMBC spectral data of Citracridone-III (Teng *et al.*, 2005)

Position	δ_H (multiplicity)	δ_C (C-Type)
1	-	165.5 (C)
2	6.10 (*s*)	98.2 (CH)
3	-	162.2 (C)
4	-	103.3 (C)
4a	-	148.8 (C)
5	-	132.8 (C)
6	-	149.4 (C)
7	7.21 (*d*, *J* = 7.6)	124.2 (CH)
8	7.78 (*d*, *J* = 7.6)	117.1 (CH)
8a	-	125.8 (C)
9	-	182.9 (C=O)
9a	-	107.6 (C)
10a	-	138.1 (C)
1'	6.70 (*d*, *J* = 10.0)	123.0 (CH)
2'	5.66 (*d*, *J* = 10.0)	121.9 (CH)
3'	-	80.6 (C)
4'/5'	1.47 (*s*)	22.5 (CH_3)
1-OH	14.42 (*s*)	-
5-OH	9.57 (*s*)	-
6-OH	-	-
N-CH_3	3.84 (*s*)	49.1 (CH_3)

in Aceton-d_6

Table A-11 ^{1}H and ^{13}C NMR spectral data of Chrysin (Chen *et al*., 2003)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**
2	-	1634.5 (C)
3	6.4 (*s*)	104.9 (CH)
4	-	182.7 (C=O)
4a	-	104.3 (C)
5	-	165.6 (C)
6	6.1 (*d*, *J* = 2.1)	99.3 (CH)
7	-	158.4 (C)
8	6.5 (*d*, *J* = 2.1)	95.5 (CH)
8a	-	158.4 (C)
1'	-	126.0 (C)
2',6'	7.9 (*dd*, *J* = 7.5, 1.8)	126.3 (CH)
3',5'	7.5 (*m*)	129.1 (CH)
4'	7.51-7.46 (*m*)	131.9 (CH)
5-OH	12.68 (*s*)	-
7-OH	10.09 (*s*)	-

in $CDCl_3$ + DMSO-d_6

Table A-12 ^{1}H, ^{13}C NMR and HMBC spectral data of Citrusinol (Shang *et al*., 2007).

Position	δ_H **(multiplicity)**	δ_C **(C-Type)**
2	-	145.7 (C)
3	-	136.6 (C)
4	-	176.8 (C=O)
4a	-	100.9 (C)
5	-	160.2 (C)
6	6.20 (*s*)	99.7 (CH)
7	-	159.9 (C)
8	-	104.6 (C)
8a	-	154.7 (C)
9	6.92 (*d*, *J* = 10.0)	115.4 (CH)
10	5.80 (*d*, *J* = 10.0)	128.5 (CH)
11	-	78.0 (C)
12	1.50 (*s*)	28.3 (CH_3)
1'	-	123.2 (C)
2',6'	8.23 (*d*, *J* = 8.0)	130.5 (CH)
3',5'	7.08 (*d*, *J* = 8.0)	116.4 (CH)
4'	-	159.7 (C)
3-OH	-	-
5-OH	13.08 (*s*)	-
4'-OH	4.59 (*s*)	-

in Acetone-d_6

Table A-13 ^{1}H, ^{13}C NMR and HMBC spectral data of Syringaresinol (Lin-gen *et al.*,1982)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**
1/5	3.15 (*m*)	49.5 (CH)
2/6	4.78 (*d*, *J* = 4.0)	84.1 (CH)
$4_a/8_a$	4.31 (*m*)	68.7 (CH_2)
$4_e/8_e$	3.95 (*m*)	68.7 (CH_2)
1'/1"	-	130.0 (C)
2'/2", 6'/6"	6.62 (*s*)	103.0 (CH)
3'/3", 5'/5"	-	147.0 (C)
4'/4"	-	133.8 (C)
3'/3", 5'/5"-OCH_3	3.91 (*s*)	56.4 (4×OCH_3)
4'/4"-OH	5.53 (*br s*)	-

in $CDCl_3$

Table A-14 ^{1}H and ^{13}C NMR spectral data of Limonexic acid (Khalil *et al.*, 2003)

Position	**δ_H (multiplicity)**	**δ_C (C-Type)**
1	4.11 (*br s*)	78.3 (CH)
2	2.26 (*dd*, *J* = 14.8, 3.2)	35.6 (CH_2)
	2.65 (*dd*, *J* = 14.8, 3.2)	
3	-	170.1 (C=O)
4	-	83.8 (C)
5	2.49 (*m*)	45.8 (CH)
6	2.99 (*m*)	36.1 (CH_2)
	3.12 (*t*, *J* = 15.0)	
7	-	207.8 (C=O)
8	-	50.2 (C)
9	2.50 (*dd*, *J* = 10.0, 2.0)	46.3 (CH)
10	-	45.2 (C)
11	1.78 (*m*)	17.7 (CH_2)
	1.70 (*m*)	
12	1.26 (*m*)	28.7 (CH_2)
	1.73 (*m*)	
13	-	37.8 (C)
14	-	66.1 (C)
15	3.81 (*s*)	52.6 (CH)
16	-	167.2 (C)
17	5.10 (*s*)	77.6 (CH)
18	1.09 (*s*)	19.7 (CH_3)
19	4.42 (*d*, *J* = 13.0)	64.8 (CH_2)
	4.92 (*d*, *J* = 13.0)	
20	-	161.5 (C)

in DMSO-d_6

Table A-14 ^{1}H and ^{13}C NMR spectral data Of Limonexic acid (Khalil *et al*., 2003) (continued)

Position	δ_H **(multiplicity)**	δ_C **(C-Type)**
21	5.96 (*br s*)	103.1 (CH)
22	6.36 (*br s*)	123.1 (CH)
23	-	168.7 (C=O)
24	1.12 (*s*)	16.4 (CH_3)
25	1.44 (*s*)	29.6 (CH_3)
26	0.98 (*s*)	21.4 (CH_3)
21-OH	8.10 (*br s*)	-

in DMSO-d_6

VITAE

Name	Miss Tareeyah Malee
Student ID	5210220131

Educational Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Science (General Science)	Prince of Songkla University	2008

Scholarship Awards during Enrolment

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

List of Publication and Proceedings

Tareeyah Malee and Wilawan Mahabusarakam. "Chemical constituent from the leaves of *Citrus aurantifolia* Swingle". Proceeding of 2011 International Conference on Alternative Energy in Developing Countries and Energing Economic, JB Hotel, Hadyai, Songkla, Thailand, 25-28 May 2011.(Poster presentation).

Tareeyah Malee and Wilawan Mahabusarakam. "Chemical constituent from the leaves and stems of *Citrus aurantifolia* Swingle". Proceeding of 1st International Congress on Natural Products, Phang Nga, Thailand, 17-18 October 2011.(Poster presentation).