สารจับอนุมูลอิสระจากใบปาหนันขี้แมว

นางสาว ฉวีวรรณ คล่องศิริเวช

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FREE RADICAL SCAVENGERS FROM *GONIOTHALAMUS TENUIFOLIUS* LEAVES

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การศึกษาทางพฤกษเคมีของใบปาหนันขี้แมว สามารถแยกได้สารบริสุทธิ์ 9 ชนิด ประกอบด้วยสารกลุ่ม 3-methoxyflavone 8 ชนิด และสารบริสุทธิ์ *trans*-cinnamic acid อีก 1 ชนิด โดยสารในกลุ่ม3-methoxyflavone มีสาร 1 ชนิด เป็นสารที่พบครั้งแรกในธรรมชาติคือ 3'-hydroxy-3,5,7,4'-tetramethoxyflavone และอีก 7 ชนิดเป็นสารที่เคยมีรายงานมาก่อนได้แก่ retusine, pachypodol, kumatakenin, 5,7,3',4'-tetrahydroxy-3-methoxyflavone, 3',4'-dihydroxy-3,5,7-trimethoxyflavone, 3,5,7,3',4'-pentamethoxyflavone, 4'-hydroxy-3,5,7,3'-tetramethoxyflavone การพิสูจน์โครงสร้างทางเคมีของสารที่แยกได้นี้ อาศัยการวิเคราะห์สเปคตรัมของ UV, IR, MS และ NMR ร่วมกับการเปรียบเทียบข้อมูลของสารที่ทราบโครงสร้างแล้ว และได้ทำการทดสอบฤทธิ์จับอนุมูลอิสระของสารบริสุทธิ์แต่ละชนิดที่แยกได้ โดยวิธี DPPH radical scavenging assay พบว่า มีสาร 3 ชนิดที่แสดงฤทธิ์จับอนุมูลอิสระ ได้แก่ สาร kumatakenin, 5,7,3',4'-tetrahydroxy-3-methoxyflavone และ 3',4'-dihydroxy-3,5,7-trimethoxyflavone โดยมีค่า IC$_{50}$ เท่ากับ 5.8, 6.4 และ 6.7 μM ตามลำดับ เมื่อเปรียบเทียบกับ quercetin ซึ่งมีค่า IC$_{50}$ เท่ากับ 6.6 μM

ภาควิชาเภสัชเวท ลายมือชื่อนิสิต... ....
สาขาวิชาเภสัชเวท ลายมือชื่ออาจารย์ที่ปรึกษา..
ปีการศึกษา 2546 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....................................
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KEY WORD: *GONIOTHALAMUS TENUIFOLIUS* / FREE REDICAL SCAVENGING ACTIVITY/ FLAVONOIDS

CHAWEEWAN KLONGSIRIWET: FREE RADICAL SCAVENGERS FROM *GONIOTHALAMUS TENUIFOLIUS* LEAVES. THESIS ADVISOR: ASSOC. PROF. KITTISAK LIKHITWITAYAWUID, Ph. D., THESIS CO-ADVISORS: ASSOC. PROF. SUMPHAN WONGSERIPIPATANA, Ph.D. AND ASST. PROF. VICHIEN JONGBUNPRASERT, M. Sc. in Pharm. 136 PP. ISBN 974-17-3502-2

Phytochemical study of the leaves of *Goniothalamus tenuifolius* led to the isolation of nine pure compounds, which included a new natural product namely 3'-hydroxy-3,5,7,4'-tetramethoxyflavone and eight known compounds including *trans*-cinnamic acid, retusine, pachypodol, kumatakenin, 5,7,3',4'-tetrahydroxy-3-methoxyflavone, 3',4'-dihydroxy-3,5,7-trimethoxyflavone, 3,5,7,3',4'-pentamethoxyflavone, 4'-hydroxy-3,5,7,3'-tetramethoxyflavone. The structures of all of these isolates were determined by interpretation of their spectroscopic data (UV, IR, MS and NMR) and comparison of the spectral properties with previously reported values. Each of these compounds was evaluated for free radical scavenging activity on DPPH decoloration test. The active compounds were kumatakenin, 5,7,3',4'-tetrahydroxy-3-methoxyflavone and 3',4'-dihydroxy-3,5,7-trimethoxyflavone with IC_{50} values of 5.8, 6.4 and 6.7 μM, respectively, whereas quercetin (positive control) showed an IC_{50} value of 6.6 μM.

Department Pharmacognosy	Student's signature..
Field of study Pharmacognosy	Advisor's signature..
Academic year 2003	Co-advisor's signature..
	Co-advisor's signature..

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CONTENTS

CONTENTS (continued)

CONTENTS (continued)

LIST OF TABLES

Table **Page**

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF SCHEMES

LIST OF ABBREVIATIONS AND SYMBOLS

α = alpha

$ABTS^{\cdot+}$ = 2,2'- azinobis (3-ethylbenzothiazoline-6-sulfonic acid)

Acetone-d_6 = deuterated acetone

β = beta

br = broad (for NMR spectra)

BHT = butylated hydroxytoluene

calcd = calculated

cm = centimeter

C = concentration

°C = degree celsius

CCl_4 = carbontetrachloride

$CDCl_3$ = deuterated chloroform

$CHCl_3$ = chloroform

^{13}C-NMR = carbon-13 nuclear magnetic resonance

1-D = one-dimension

2-D = two-dimension

d = doublet (for NMR spectra)

dd = doublet of doublets (for NMR spectra)

DEPT = distortionless enhancement by polarization transfer

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

DMSO-d_6 = deuterated dimethylsulfoxide

DPPH = 1,1-diphenyl-2-picrylhydrazyl

ESIMS = electrospray ionization mass spectrometry

EtOAc = ethyl acetate

g = gram

hr = hour

^{1}H-NMR = proton nuclear magnetic resonance

HMBC = ^{1}H-detected heteronuclear multiple bond coherence

HMQC = ^{1}H-detected heteronuclear multiple quantum coherence

HRESIMS = High resolution electrospray ionization mass spectrometry

Hz = hertz

IC$_{50}$ = median inhibitory concentration

IR = infrared spectrum

J = coupling constant

kg = kilogram

L = liter

δ = chemical shift

λ_{max} = wavelength at maximal absorption

ε = molar absorptivity

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

ν_{max} = wave number at maximal absorption

m = meta

m = meter

m = multiplet (for NMR spectra)

mg = milligram

μg = microgram

ml = milliliter

μl = microliter

μM = micromolar

min = minute

mult = multiplicity

m/z = mass to charge ratio

M^+ = molecular ion

MAD = malonaldehyde

MeOH = methanol

MHz = megahertz

MS = mass spectrometry

MW = molecular weight

nm = nanometer

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

NMR = nuclear magnetic resonance

NOESY = nuclear overhauser effect spectroscopy

o = ortho

p = para

ppm = part per million

spp. = species

s = singlet (for NMR spectra)

TEAC = trolox equivalent antioxidant capacity

TLC = thin layer chromatography

*t*BH = *tert*-butyl hydroperoxide

UV = ultraviolet

UV-VIS = ultraviolet and visible spectrophotometry

VLC = vacuum liquid column chromatography

CHAPTER I

INTRODUCTION

Oxidation is the transfer of electrons from one atom to another and represents an essential part of aerobic life and our metabolism, since oxygen is the ultimate electron acceptor in the electron flow system that produces energy in the form of ATP. However, problems may arise when the electron flow becomes uncoupled (transfer of unpaired single electron), generating free radicals (Pietta, 2000). Unpaired electrons usually seek other electrons to become paired. Thus, free radicals are in general reactive and attack other molecules, although some radicals are not reactive but stable enough to have long life (Papas, 1999).

Active oxygen species (also known as reactive oxygen species) and related species play an important physiological role and at the same time, they may exert toxic effects as well. The active oxygen species are essential for production of energy, synthesis of biologically essential compounds and phagocytosis, a criticle process of our immune system (Papas, 1999).

Humans have evolved with antioxidant systems to protect against free radicals. These systems include some antioxidants produced in the body (endogenous) and others obtained from the diet (exogenous) (Pietta, 2000). Such exogenous antioxidants are commonly obtained from food and include vitamins C and E, β-carotene and a variety of phenolic compounds, including flavonoids (Campos *et al.*, 2003).

Plants of the genus *Goniothalamus* are reported to contain compounds with a wide range of biological activity such as cytotoxic, antitumor, pesticidal, abortifacient, teratogenic and embryototic effects (Hasan *et al.,* 1994). Ethnobotanical uses of several species of the genus *Goniothalamus* are well known in Malaysia; many of these plants have provided bioactive acetogenins, alkaloids, stryl-lactones and flavonoids (Cao *et al.*, 1998).

Goniothalamus tenuifolius King (Annonaceae), locally known as “Panan Kee Meaw,” is a shrub or small tree growing in several parts of Thailand (ปิยะ เฉลิมกลิ่น, 2544). It belongs to the tribe Mitrephoreae. Sinclair (1955) described the characteristics of this plant as follows.

Shrub or small tree 2-7 m high. *Young twigs* slender, pubescent, later glabrous and striate. *Leaves* membranous, varying considerably in shape and size, lanceolate or oblong lanceolate, acuminate, base acute, rarely rounded, the margins sometimes slightly undulate, glabrous or pubescenct on the midrib and veins beneath; main nerves 8-11 pairs, fine, curving and interarching 5 mm from margin; reticulations faint and lax; length 8-18.5 cm; breadth 2-6 cm; petiole 5-8 mm long, glabrous or pubescent. *Flowers* solitary, axillary, pendulous. *Pedicels* 5 mm- 2mm long, glabrous or pubescent with 2-3 minute bracts at base. *Sepals* ovate, acute or acuminate, membranous, several-nerved and reticulate, persistent, varying much in size, 7 mm - 2.7 cm long and 6 mm- 2.2 cm broad. *Petals* yellowish to pinkish, thinly coriaceous, pubescent, outer broadly lanceolate, acuminate, much contracted at the base, varying much in length with age, 2-3 cm long, inner ovate, acuminate, 1 cm long or less. *Stamens* 2 mm long, numerous with flat-topped or convex connectives. *Ovaries* about 3 mm long, narrow; style filiform, stigma funnel-shaped, split down the inner side. *Ripe carpels* ovoid, slightly apiculate, pubescent or glabrescent, 1-1.2 cm long; stalks 4-5 mm long. *Seeds* 1 rarely 2.

A previous phytochemical study of this plant has shown the presence of antimalarial aristolactam alkaloids in the stembark (Likhitwitayawuid *et al.*, 1997). Our preliminary screening of the leaves of this plant showed that the ethyl acetate and methanol extracts had free radical scavenging activity with DPPH in a TLC autobiographic assay. Up to the present time, there has been no study on the chemical constituents of the leaves of this plant.

The main objectives in this investigation are as follows.

1. to isolate and purify compounds possessing free radical scavenging activity from the leaves of *G. tenuifolius*.

2. to determine the chemical structure of each isolated compound.

3. to evaluate the free radical scavenging activity of each isolated compound.

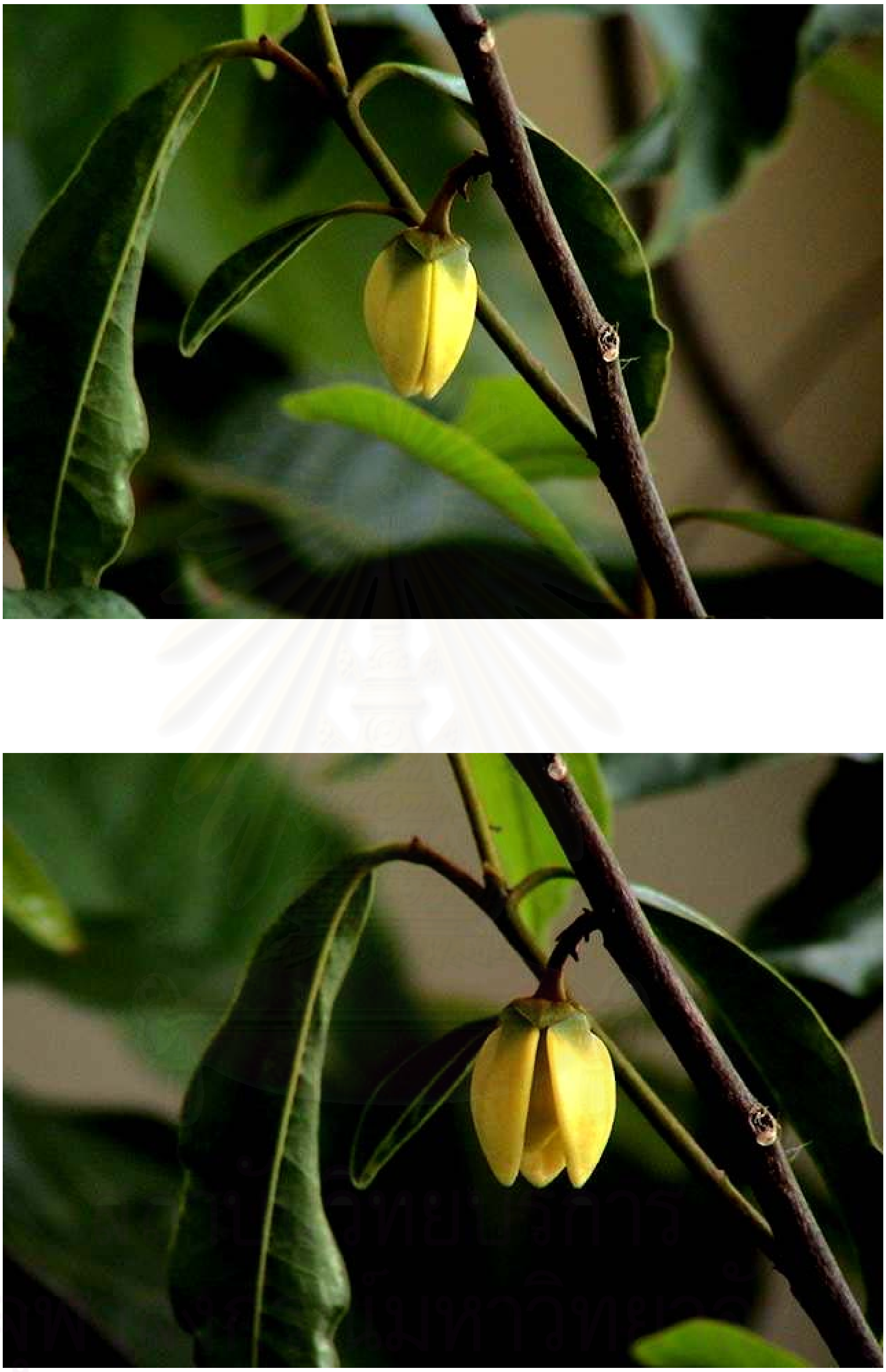


Figure 1 *Goniothalamus tenuifolius* King

(Photographed by Mr.Tanawat Chaowasku)

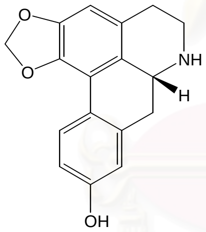
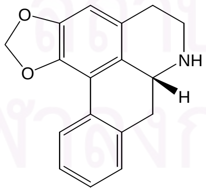
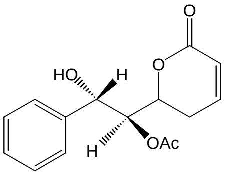
CHAPTER II

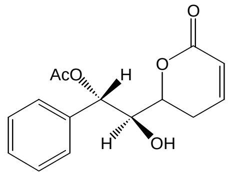
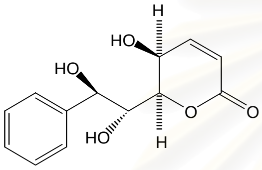
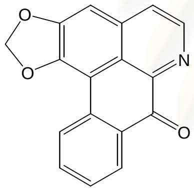
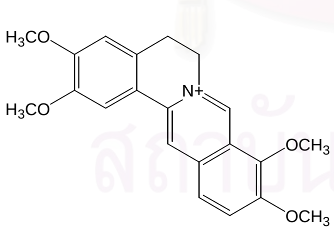
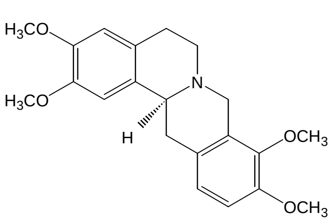
HISTORICAL

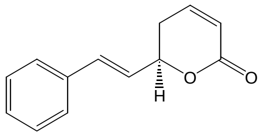
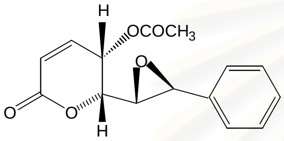
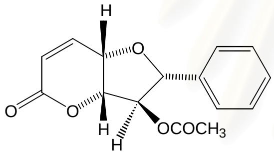
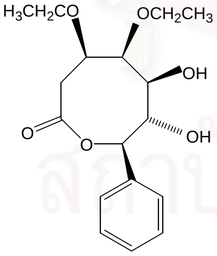
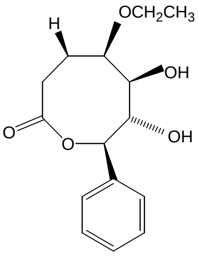
1.Chemical constituents of *Goniothalamus* spp.

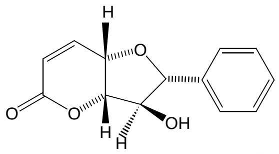
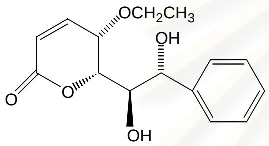
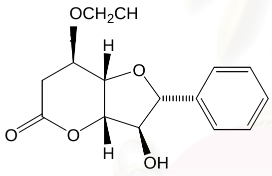
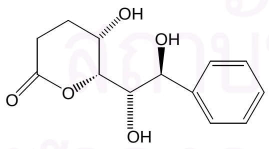
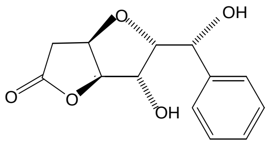
The chemical constituents of plants in the genus *Goniothalamus* can be classified into eleven groups namely acetogenins, alkaloids, aza-anthraquinones, benzenoids, flavonoids, naphthoquinones, styrylpyrones, sterols, styrene derivatives, terpenoids and miscellaneous compounds as shown in Table 1.

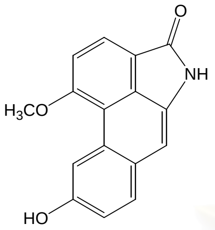
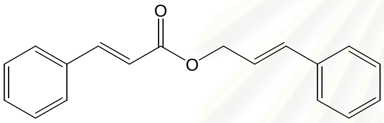
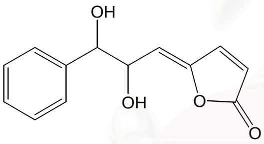
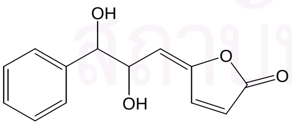
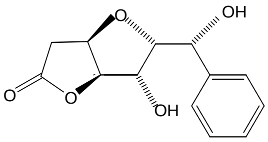
Table 1 Distribution of chemical constituents in the genus *Goniothalamus*.

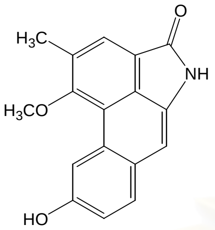
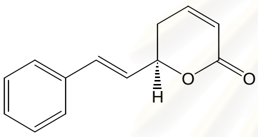
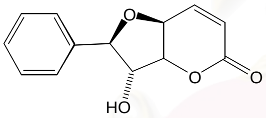
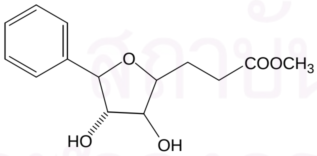
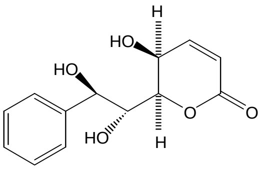
Plant & chemical compounds	Category	Plant part	References
Goniothalamus amuyon			
(-)-Anolobine **[1]**  	Aporphine alkaloid	Wood	Lu, Wu and Leou, 1985
(-)-Anonaine **[2]**  	Aporphine alkaloid	Wood	Lu, Wu and Leou, 1985
Goniodiol-7-monoacetate **[3]**  	Styrylpyrone	Leave	Wu, Duh, Chang and Chang, 1991

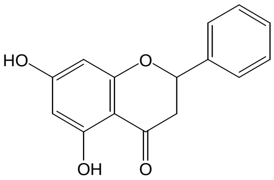
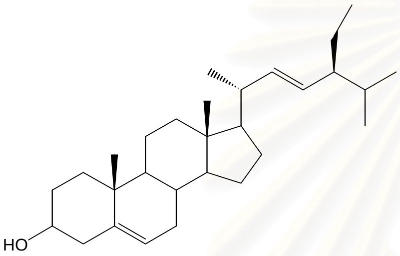
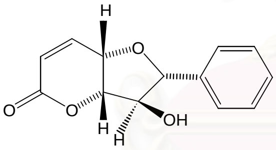
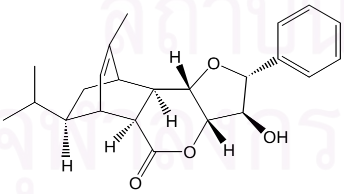
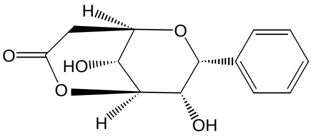
Plant & chemical compounds	Category	Plant part	References
G. amuyon			
Goniodiol-8-monoacetate **[4]** 	Styrylpyrone	Leave	Wu *et al.*, 1992
Goniotriol **[5]** 	Styrylpyrone	Leave	Wu *et al.*, 1992
Liriodenine **[6]** 	Oxoaporphine alkaloid	Stem bark	Lu, Wu and Leou, 1985
Palmatine **[7]** 	Protoberberine alkaloid	Stem bark	Lu, Wu and Leou, 1985
(-)-Tetrahydropalmatine **[8]** 	Tetrahydro protoberberine alkaloid	Stem bark	Lu, Wu and Leou, 1985

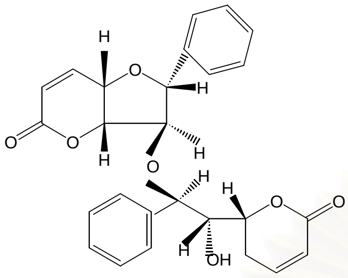
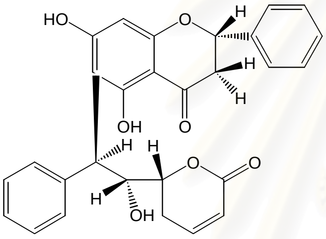
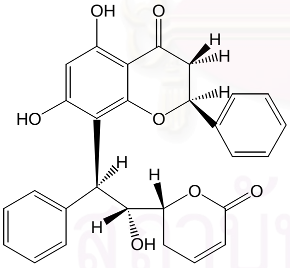
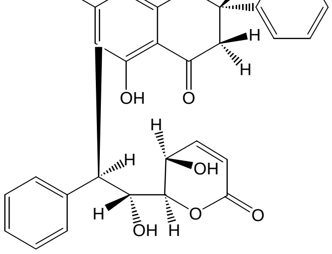
Plant & chemical compounds	Category	Plant part	References
G. andersonii (+)-Goniothalamin **[9]** 	Styrylpyrone	Leave, Fruit, Root, Stem	Jewers , Davis, Dougan and Manchanda, 1972
G. arvensis 5-Acetoxyisogoniothalamin oxide **[10]** 	Styrylpyrone	Stem bark	Peris *et al.*, 2000
3-Acetylaltholactone **[11]** 	Styrylpyrone	Stem bark	Peris *et al.*, 2000
Almuheptolide-A **[12]** 	Benzenoid	Stem bark	Bermojo *et al.*, 1998
Almuheptolide-B **[13]** 	Benzenoid	Stem bark	Bermojo *et al.*, 1998

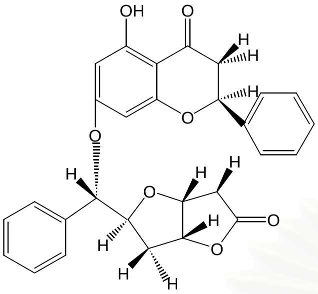
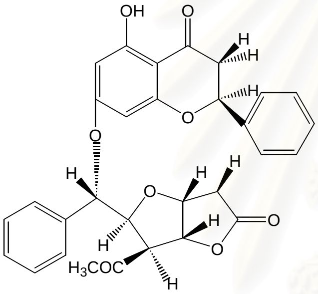
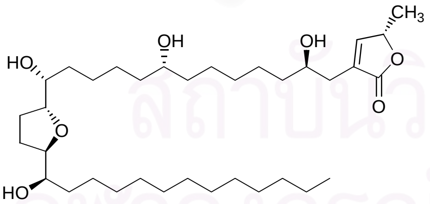
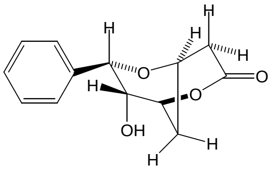
Plant & chemical compounds	Category	Plant part	References
G. arvensis Altholactone **[14]** (Synoname : (+)-Goniothalenol) 	Styrylpyrone	Stem bark	Peris *et al.*, 2000
(+)-Etharvendiol **[15]** 	Styrylpyrone	Stem bark	Bermejo, Blazquez, Rao and Cortest, 1998
(-)-Etharvensis **[16]** 	Styrylpyrone	Stem bark	Bermejo *et al.,* 1997
(+)-Garvensintriol **[17]** 	Styrylpyrone	Stem bark	Bermejo, Blazquez, Rao and Cortest, 1998
(+)-Goniofufurone **[18]** 	Styrene derivative	Stem bark	Bermejo, Blazquez, Rao and Cortest, 1998

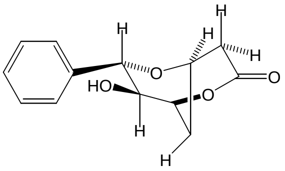
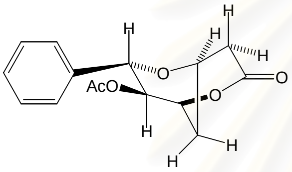
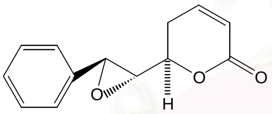
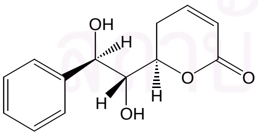
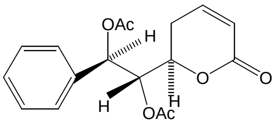
Plant & chemical compounds	Category	Plant part	References
G. borneensis			
Aristolactam- AIII **[19]** 	Aristolactam alkaloid	Bark	Cao *et al.*, 1998
Cinnamyl cinnamate **[20]** 	Miscellaneous	Bark	Cao *et al.*, 1998
Goniobutenolide A **[21]** 	Styrene derivative	Bark	Cao *et al.*, 1998
Goniobutenolide B **[22]** 	Styrene derivative	Bark	Cao *et al.*, 1998
Goniofufurone **[18]** 	Styrene derivative	Bark	Cao *et al.*, 1998

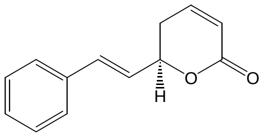
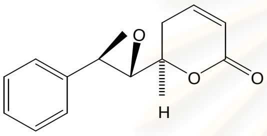
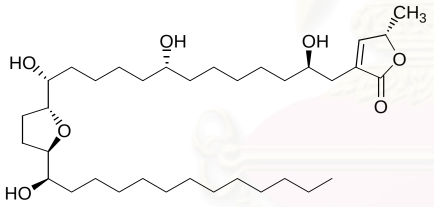
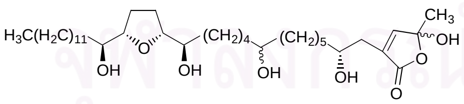
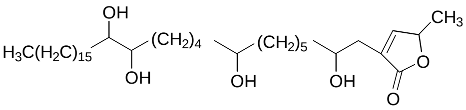
Plant & chemical compounds	Category	Plant part	References
G. borneensis Goniothalactam **[23]** 	Aristolactam alkaloid	Bark	Cao *et al.*, 1998
Goniothalamin **[9]** 	Styrylpyrone	Bark	Cao *et al.*, 1998
(+)-Goniothalenol **[14]** 	Styrylpyrone	Bark	Cao *et al.*, 1998
Goniothalesdiol **[24]** 	Styrene derivative	Bark	Cao *et al.*, 1998
Goniotriol **[5]** 	Styrylpyrone	Bark	Cao *et al.*, 1998

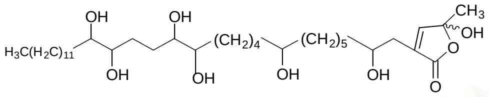
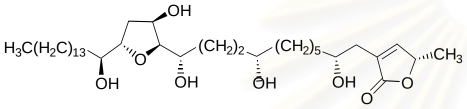
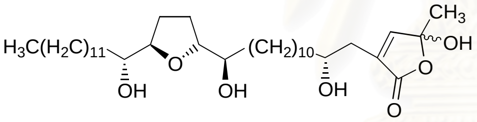
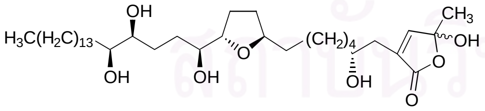
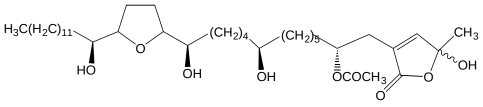
Plant & chemical compounds	Category	Plant part	References
G. borneensis Pinocembrin **[25]** 	Flavonoid	Bark	Cao *et al.*, 1998
Stigmasterol **[26]** 	Sterol	Bark	Cao *et al.*, 1998
G. cardiopetalus Altholactone **[14]** 	Styrylpyrone	Stem bark	Hisham *et al.*, 2000
Cardiopetalolactone **[27]** 	Styrylpyrone	Stem bark	Hisham *et al.*, 2000
Goniopyrone **[28]** 	Styrylpyrone	Stem bark	Hisham *et al.*, 2000

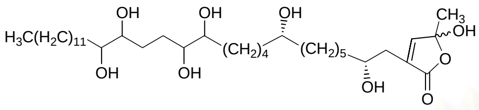
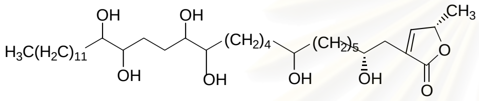
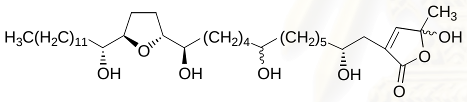
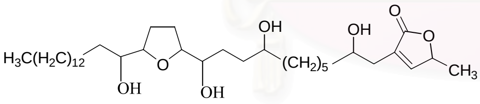
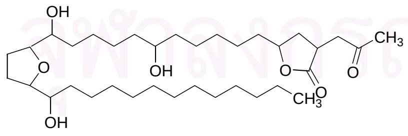
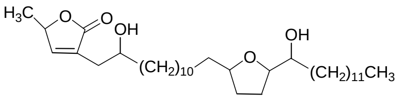
Plant & chemical compounds	Category	Plant part	References
G. cheliensis Goniolactone A **[29]** 	Bi-styrylpyrone	Root	Wang, Zhang, Chen and Yu, 2002
Goniolactone B **[30]** 	Flavanone-styrylpyrone	Root	Wang, Zhang, Chen and Yu, 2002
Goniolactone C **[31]** 	Flavanone-styrylpyrone	Root	Wang, Zhang, Chen and Yu, 2002
Goniolactone D **[33]** 	Flavanone-styrylpyrone	Root	Wang, Zhang, Chen and Yu, 2002

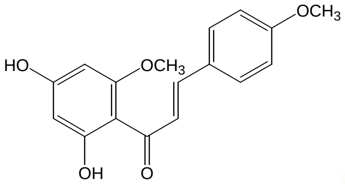
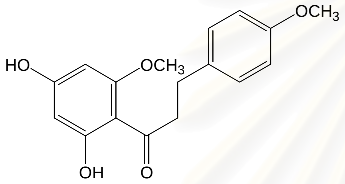
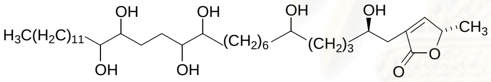
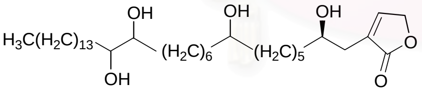
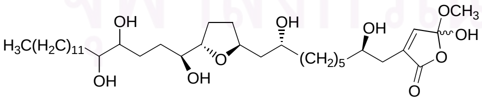
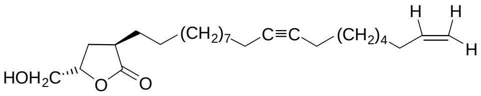
Plant & chemical compounds	Category	Plant part	References
G. cheliensis Goniolactone E **[33]** 	Flavanone-styrylpyrone	Root	Wang, Zhang, Chen and Yu, 2002
Goniolactone F **[34]** 	Flavanone-styrylpyrone	Root	Wang, Zhang, Chen and Yu, 2002
G. dolichocarpus (+)-Annonacin **[35]** 	Acetogenin	Stem bark	Goh, Ee, Chuah and Wei, 1995
(+)-5-Deoxygoniopypyrone **[36]** 	Styrylpyrone	Stem bark	Goh, Ee, Chuah and Wei, 1995

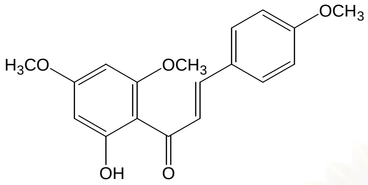
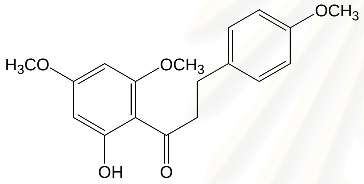
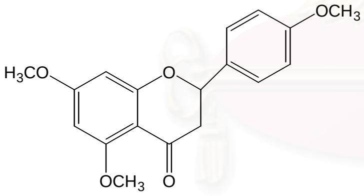
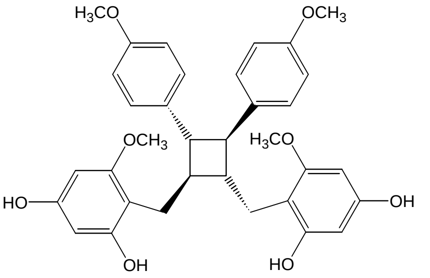
Plant & chemical compounds	Category	Plant part	References
G. dolichocarpus (-)-Iso-5-Deoxygoniopypyrone **[37]** 	Styrylpyrone	Stem bark	Goh, Ee, Chuah and Wei, 1995
(-)-Iso-5-deoxygoniopypyrone acetate **[38]** 	Styrylpyrone	Stem bark	Goh, Ee, Chuah and Wei, 1995
Isogoniothalamin epoxide **[39]** 	Styrylpyrone	Stem bark	Goh, Ee, Chuah and Wei, 1995
(+)-Goniodiol **[40]** 	Styrylpyrone	Stem bark	Goh, Ee, Chuah and Wei, 1995
(+)-Goniodiol diacetate **[41]** 	Styrylpyrone	Stem bark	Goh, Ee, Chuah and Wei, 1995

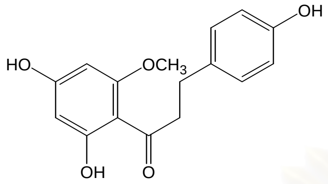
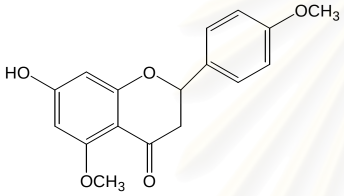
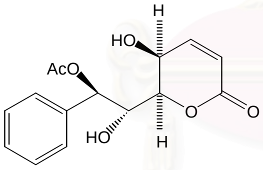
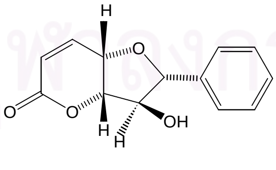
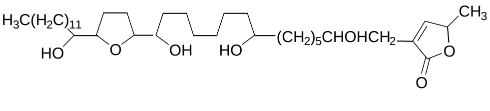
Plant & chemical compounds	Category	Plant part	References
G. dolichocarpus (+)-Goniothalamine **[9]** 	Styrylpyrone	Stem bark	Goh, Ee, Chuah and Wei, 1995
(+)-Goniothalamine epoxide **[42]** 	Styrylpyrone	Stem bark	Goh, Ee, Chuah and Wei, 1995
G. donnaiensis (+)-Annonacin **[36]** 	Acetogenin	Root	Jiang and Yu, 1997
cis-Goniodonin **[43]** and 34-*epi*-*cis*-goniodonin **[44]** 	Acetogenin	Root	Jiang, Chen, Chen and Yu, 1997
Donbutocin **[45]** 	Acetogenin	Root	Jiang, Chen, Chen and Yu, 1998a

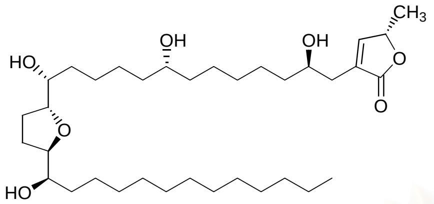
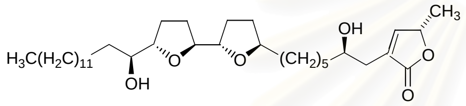
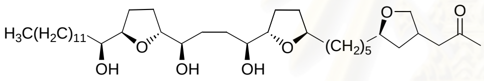
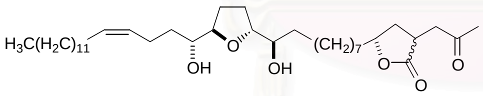
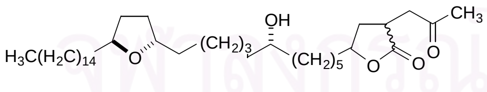
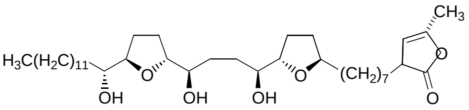
Plant & chemical compounds	Category	Plant part	References
G. donnaiensis Donhepocin **[46]** and 34-*epi*-donhepocin **[47]** 	Acetogenin	Root	Jiang, Chen, Chen and Yu, 1998a
Donaienin **[48]** 	Acetogenin	Root	Jiang, Chen, Chen and Yu, 1998b
Donnaienin A **[49]** and 34-*epi*-donnaienin A **[50]** 	Acetogenin	Root	Jiang and Yu, 1997
Donnaienin B **[51]** and 34-*epi*-donnaienin B **[52]** 	Acetogenin	Root	Jiang and Yu, 1997
Donaienin C **[53]** and 34-*epi*-donaienin C **[54]** 	Acetogenin	Root	Jiang, Chen, Chen and Yu, 1998c

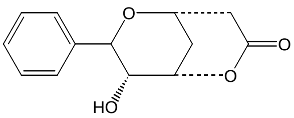
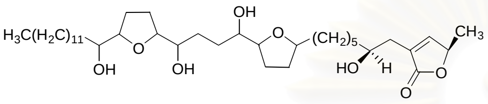
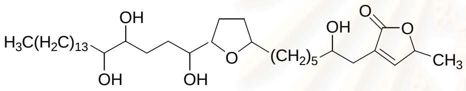
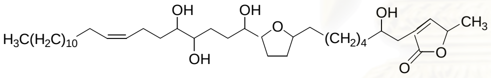
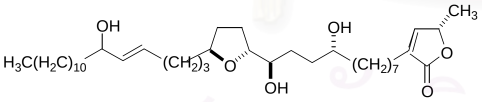
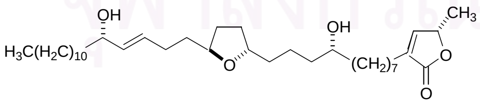
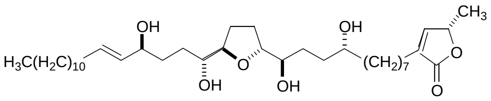
Plant & chemical compounds	Category	Plant part	References
G. donnaiensis Donaienin D **[55]** and 34-*epi*-donaienin D **[56]** 	Acetogenin	Root	Jiang, Chen, Chen and Yu, 1998c
Donhexocin **[57]** 	Acetogenin	Root	Jiang, Chen, Chen and Yu, 1998a
Goniodonin **[58]** and 34-*epi*-goniodonin **[59]** 	Acetogenin	Root	Jiang, Chen, Chen and Yu, 1997
Goniothalamicin **[60]** 	Acetogenin	Root	Jiang and Yu, 1997
Isoannonacin **[61]** 	Acetogenin	Root	Jiang and Yu, 1997
Murisolin **[62]** 	Acetogenin	Root	Jiang and Yu, 1997

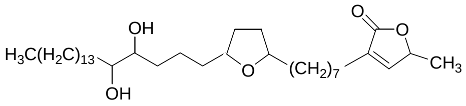
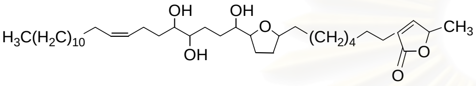
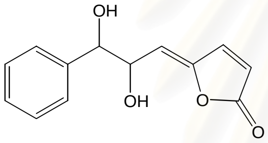
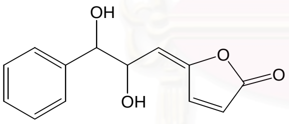
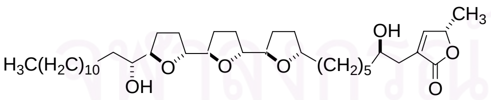
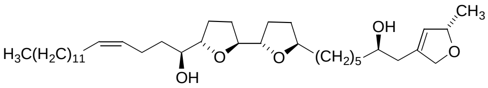
Plant & chemical compounds	Category	Plant part	References
G. gardneri 2', 4'- Dihydroxy-4 ,6'-dimethoxychalcone **[63]** 	Flavonoid	Aerial part	Seidal, Bailleul and Waterman, 2000
2', 4'- Dihydroxy-4,6'-dimethoxydihydrochalcone **[64]** 	Flavonoid	Aerial part	Seidal, Bailleul and Waterman, 2000
Gardnerilins A **[65]** 	Acetogenin	Root	Chen, Jiang, Chen and Yu, 1998a
Gardnerilins B **[66]** 	Acetogenin	Root	Chen, Jiang, Chen and Yu, 1998a
Gardnerinin **[67]** and 34-*epi*-gardnerinin **[68]** 	Acetogenin	Root	Chen, Chen, Jiang and Yu, 1998b
Goniothalamusin **[69]** 	Acetogenin	Aerial part	Seidal, Bailleul and Waterman, 1999

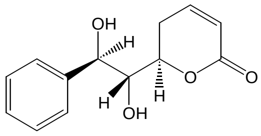
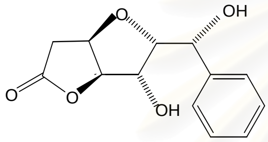
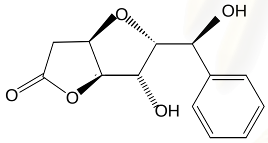
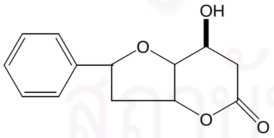
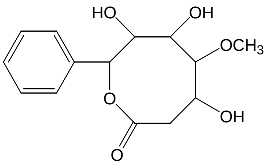
Plant & chemical compounds	Category	Plant part	References
G. gardneri 2'- Hydroxy-4,4',6'-trimethoxychalcone **[70]** 	Flavonoid	Aerial part	Seidal, Bailleul and Waterman, 2000
2'- Hydroxy-4,4',6'-trimethoxydihydrochalcone **[71]** 	Flavonoid	Aerial part	Seidal, Bailleul and Waterman, 2000
Narigenin trimethyl ether **[72]** 	Flavonoid	Aerial part	Seidal, Bailleul and Waterman, 2000
Rel-(1β, 2α) di-(2,4- dihydroxy-6-methoxybenzoyl)-(3β-4α)-di-(4-methoxyphenyl)-cyclobutane **[73]** 	Flavonoid	Aerial part	Seidal, Bailleul and Waterman, 2000

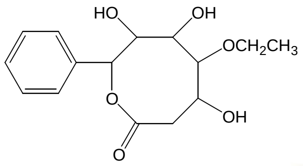
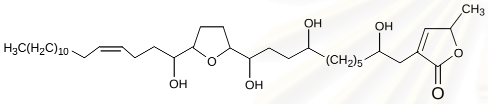
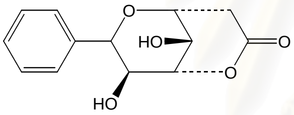
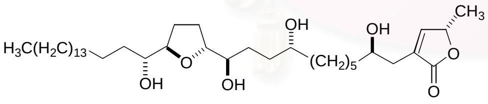
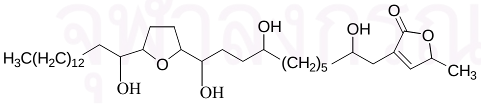
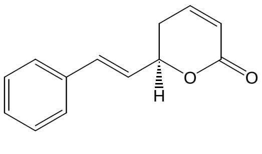
Plant & chemical compounds	Category	Plant part	References
G. gardneri 2',4,4',- Trihydroxy-6'-methoxydihydrochalcone **[74]** 	Flavonoid	Aerial part	Seidal, Bailleul and Waterman, 2000
Tsugafolin **[75]** 	Flavonoid	Aerial part	Seidal, Bailleul and Waterman, 2000
G. giganteus 8-Acetylgoniotriol **[76]** 	Styrylpyrone	Stem bark	Fang *et al.,* 1990
Altholactone **[14]** 	Styrylpyrone	Stem bark	El-Zayat *et al.*, 1985
Annomontacin **[77]** 	Acetogenin	Stem bark	Fang *et al.*, 1992a

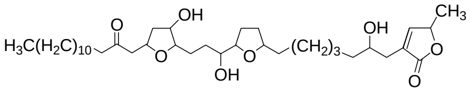
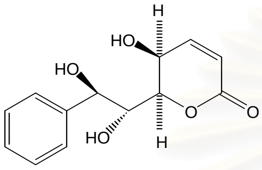
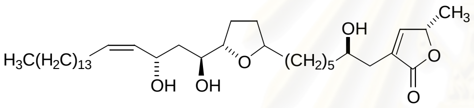
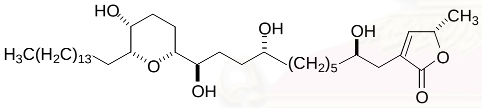
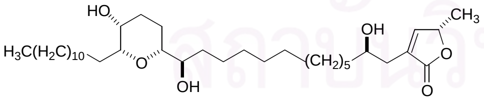
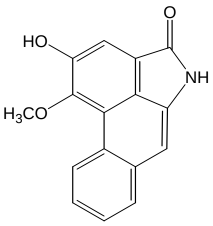
Plant & chemical compounds	Category	Plant part	References
G. giganteus Annonacin **[35]** 	Acetogenin	Stem bark	Alkofahi *et al.,* 1988
Asimilobin **[78]** 	Acetogenin	Stem bark	Zhang *et al.*, 1995
2,4-*cis* and *trans*-Gigantecin **[79]** 	Acetogenin	Stem bark	Alali, Zhang, Rogers and McLaughlin, 1997
2,4-*cis* and *trans*-Gonioneninone **[80]** 	Acetogenin	Stem bark	Alali, Zhang, Rogers and McLaughlin 1998
2,4-*cis* and *trans*-Xylomaticinone **[81]** 	Acetogenin	Stem bark	Alali, Rogers, Zhang and McLaughlin 1999
4-Deoxygigantecin **[82]** 	Acetogenin	Stem bark	Alali, Zhang, Rogers and McLaughlin, 1997

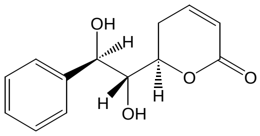
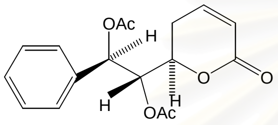
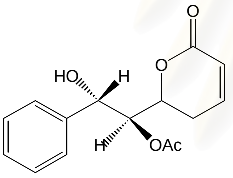
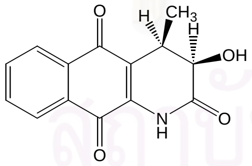
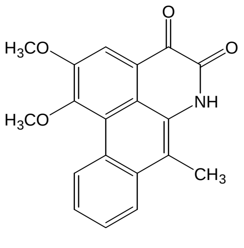
Plant & chemical compounds	Category	Plant part	References
G. giganteus 9-Deoxy-goniopypyrone **[83]** 	Styrylpyrone	Stem bark	Fang *et al.*, 1991a
Gigantecin **[84]** 	Acetogenin	Stem bark	Alkofahi *et al.,* 1990
Gigantetrocin **[85]** 	Acetogenin	Stem bark	Fang *et al.*, 1991
Gigantetronenin **[86]** 	Acetogenin	Stem bark	Fang *et al.*, 1992a
Gigantransenin A **[87]** 	Acetogenin	Stem bark	Zeng, Zhang and McLaughlin 1996
Gigantransenin B **[88]** 	Acetogenin	Stem bark	Zeng, Zhang and McLaughlin 1996
Gigantransenin C **[89]** 	Acetogenin	Stem bark	Zeng, Zhang and McLaughlin 1996

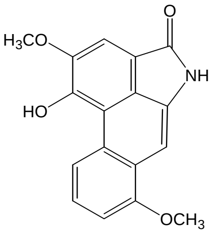
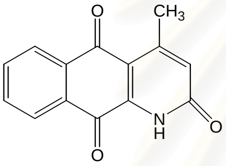
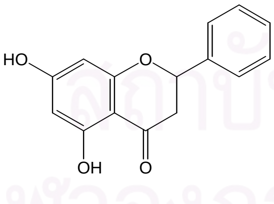
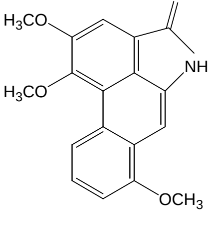
Plant & chemical compounds	Category	Plant part	References
G. giganteus Gigantriocin **[90]** 	Acetogenin	Stem bark	Fang *et al.*, 1991
Gigantrionenin **[91]** 	Acetogenin	Stem bark	Fang *et al.*, 1992a
Goniobutenolide A **[21]** 	Styrene derivative	Stem bark	Fang *et al.*, 1991b
Goniobutenolide B **[22]** 	Styrene derivative	Stem bark	Fang *et al.*, 1991b
Goniocin **[92]** 	Acetogenin	Stem bark	Gu, Fang, Zeng and McLaughlin, 1994
Goniodenin **[93]** 	Acetogenin	Stem bark	Zhang *et al.*, 1995

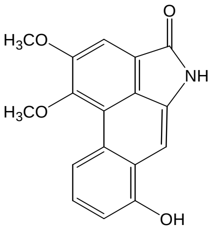
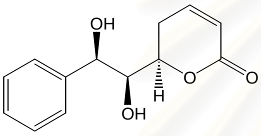
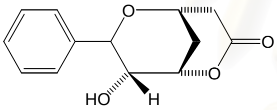
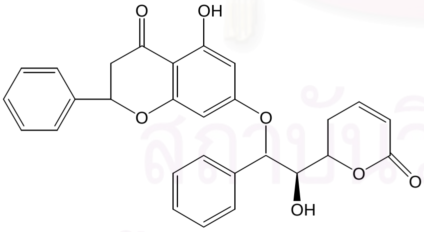
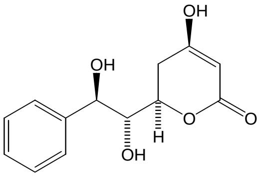
Plant & chemical compounds	Category	Plant part	References
G. giganteus			
Goniodiol **[40]** 	Styrylpyrone	Stem bark	Fang *et al.*, 1991a
Goniofufurone **[18]** 	Styrene derivatives	Stem bark	Fang *et al.*, 1990
7-*epi*-Goniofufurone **[94]** 	Styrene derivative	Stem bark	Fang *et al.*, 1991b
Goniofupyrone **[95]** 	Styrylpyrone	Stem bark	Fang *et al.*, 1991b
Gonioheptolide A **[96]** 	Benzenoid	Stem bark	Fang *et al.*, 1993

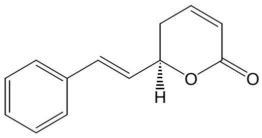
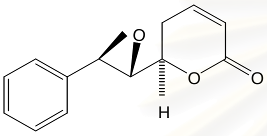
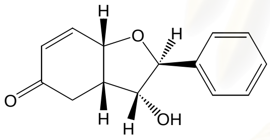
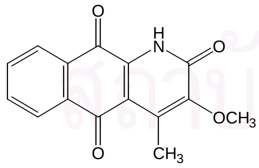
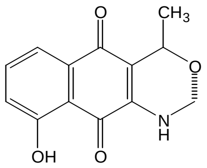
Plant & chemical compounds	Category	Plant part	References
G. giganteus Goniheptolide B **[97]** 	 Benzenoid	 Stem bark	 Fang *et al.*, 1993
Gonionenin **[98]** 	Acetogenin	Stem bark	Gu, Fang, Zeng and McLaughlin, 1994
Goniopypyrone **[99]** 	Styrylpyrone	Stem bark	Fang *et al.,* 1990
Goniotetracin **[100]** 	Acetogenin	Stem bark	Alali, Zhang, Rogers and McLaughlin, 1998
Goniothalamicin **[60]** 	Acetogenin	Stem bark	Alkofahi *et al.*, 1988 and Fang *et al.*, 1992b
Goniothalamin **[9]** 	Styrylpyrone	Stem bark	El-Zayat *et al.*, 1985

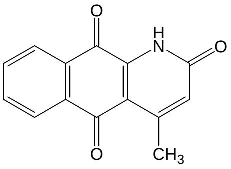
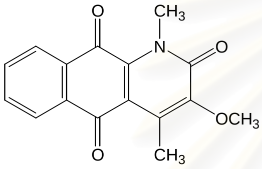
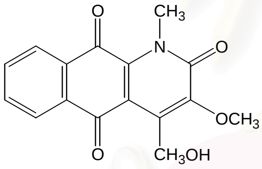
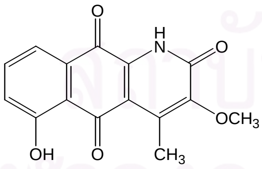
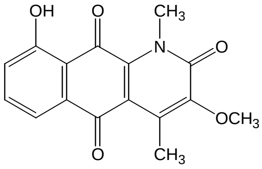
Plant & chemical compounds	Category	Plant part	References
G. giganteus Goniotriocin **[101]** 	Acetogenin	Stem bark	Alali, Rogers, Zhang and McLaughlin, 1999
Goniotriol **[5]** 	Styrylpyrone	Stem bark	Alkofahi *et al.,* 1989
Goniotrionin **[102]** 	Acetogenin	Stem bark	Alali, Zhang, Rogers and McLaughlin, 1998
Pyragonicin **[103]** 	Acetogenin	Stem bark	Alali, Zhang, Rogers and McLaughlin, 1998
Pyranicin **[104]** 	Acetogenin	Stem bark	Alali, Zhang, Rogers and McLaughlin, 1998
G. griffithii Aristolactam A-II **[105]** 	Aristolactam alkaloid	Root	Zhang, Kong, Chen and Yu, 1999

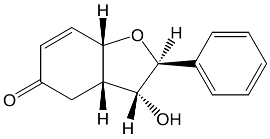
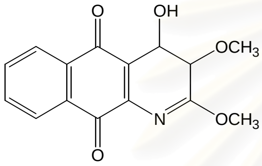
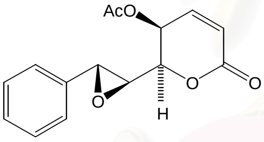
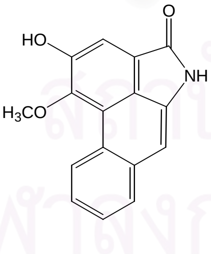
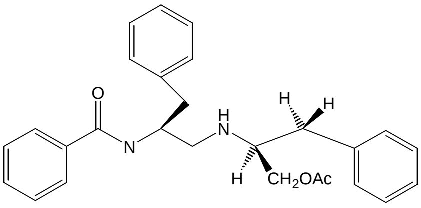
Plant & chemical compounds	Category	Plant part	References
G. griffithii Goniodiol **[40]** 	Styrylpyrone	Stem bark	Talapatra *et al.*, 1985
Goniodiol diacetate **[41]** 	Styrylpyrone	Stem bark	Talapatra *et al.*, 1985
Goniodiol-7-monoacetate **[3]** 	Styrylpyrone	Stem bark	Talapatra *et al.*, 1985
Griffithazanone A **[106]** 	Aza-anthraquinone	Root	Zhang, Kong, Chen and Yu, 1999
Griffithdione **[107]** 	4,5-Dioxo-aporphine alkaloid	Root	Zhang, Kong, Chen and Yu, 1999

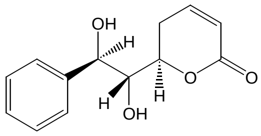
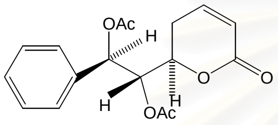
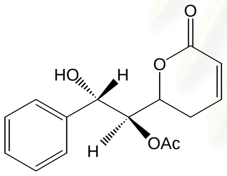
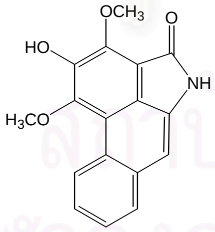
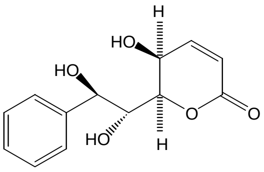
Plant & chemical compounds	Category	Plant part	References
G. griffithii Griffithinam **[108]** 	Aristolactam alkaloid	Root	Zhang, Kong, Chen and Yu, 1999
4-Methyl-2,9,10-(2H)-1-azaanthracenetrione **[109]** 	Aza-anthraquinone	Root	Zhang, Kong, Chen and Yu, 1999
Nor-cepharanone B **[110]**	Alkaloid	Root	Zhang, Kong, Chen and Yu, 1999
Pinocembrin **[25]** 	Flavonoid	Stem bark	Talapatra, Deb and Tarapatra, 1985
Taliscanine **[111]** 	Aristolactam alkaloid	Root	Zhang, Kong, Chen and Yu, 1999

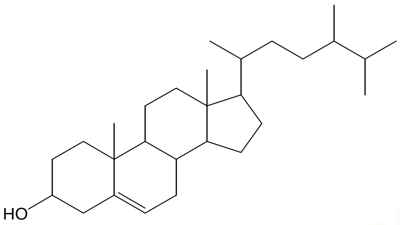
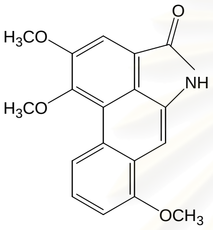
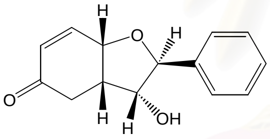
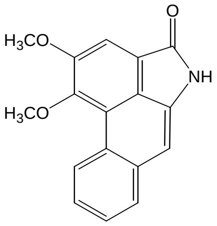
Plant & chemical compounds	Category	Plant part	References
G. griffithii Velutinam **[112]** 	Aristolactam alkaloid	Root	Zhang, Kong, Chen and Yu, 1999
G. leiocarpus 7-*epi*-Goniodiol **[113]** 	Styrylpyrone	Stem bark	Mu *et al.*, 1999
Leiocarpin A **[114]** 	Styrene derivative	Stem bark	Mu *et al.*, 1999
Leiocarpin B **[115]** 	Flavanone-styrylpyrone	Stem bark	Mu *et al.*, 1999
Leiocarpin C **[116]** 	Styrylpyrone	Stem bark	Mu *et al.*, 1999

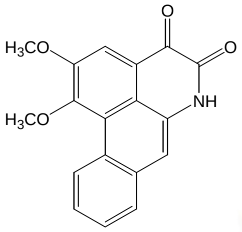
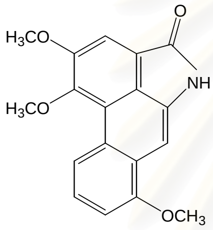
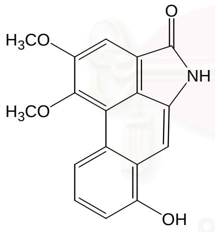
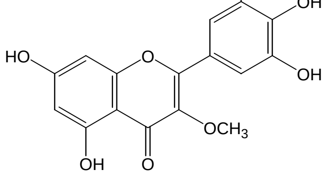
Plant & chemical compounds	Category	Plant part	References
G. macrophyllus (+)-Goniothalamin **[9]** 	Styrylpyrone	Stem bark, Root	Sam *et al.*, 1987
Goniothalamin oxide **[42]** 	Styrylpyrone	Stem bark, Root	Sam *et al.*, 1987
G. malayanus (+)-Isoaltholactone **[117]** 	Styrene derivative	Stem bark	Colegate *et al.*, 1990
G. marcanii Dielsiquinone **[118]** 	Aza-anthraquinone	Stem bark	Soonthorn-chareonnon *et al.*, 1999
5-Hydroxy-3-amino-2-aceto-1,4-napthoquinone **[119]** 	Naphtho-quinone	Stem bark	Soonthorn-chareonnon *et al.*, 1999 ref 22

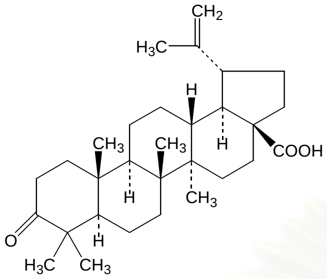
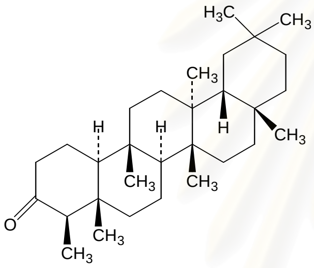
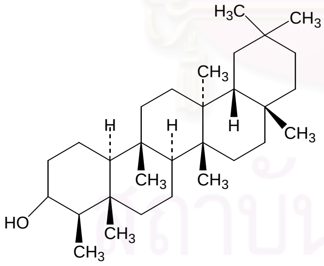
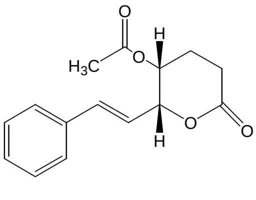
Plant & chemical compounds	Category	Plant part	References
G. marcanii Marcanine A **[120]** 	Aza-anthraquinone	Stem bark	Soonthorn-chareonnon *et al.*, 1999
Marcanine B **[121]** 	Aza-anthraquinone	Stem bark	Soonthorn-chareonnon *et al.*, 1999
Marcanine C **[122]** 	Aza-anthraquinone	Stem bark	Soonthorn-chareonnon *et al.*, 1999
Marcanine D **[123]** 	Aza-anthraquinone	Stem bark	Soonthorn-chareonnon *et al.*, 1999
Marcanine E **[124]** 	Aza-anthraquinone	Stem bark	Soonthorn-chareonnon *et al.*, 1999

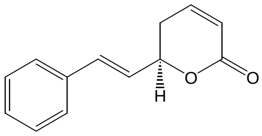
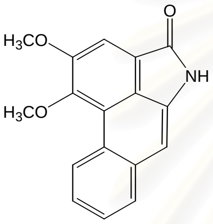
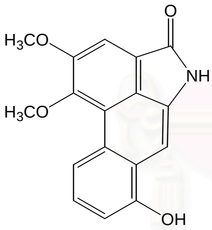
Plant & chemical compounds	Category	Plant part	References
G. montanus (+)-Isoaltholactone **[117]** 	Styrene derivative	Stem bark	Colegate *et al.*, 1990
G. scortechinii Scornazanone **[125]** 	Aza-anthraquinone	Root	Din, Colegate and Razak, 1990
G. sesquipedalis 5-Acetoxyisogoniothalamin oxide **[126]** 	Styrylpyrone	Stem bark	Hasan, Mia, Rashid and Connolly, 1994
Aristolactam A-II **[105]** 	Aristolactam alkaloid	Leave, Twig	Talapatra, Basu, Chattopadhyay and Tarapatra, 1988
Aurantiamid acetate **[127]** 	Miscellaneous	Leave, Twig	Talapatra, Basu, Chattopadhyay and Tarapatra, 1988

Plant & chemical compounds	Category	Plant part	References
G. sesquipedalis (+)-Goniodiol **[40]** 	Styrylpyrone	Leave, Twig	Talapatra *et al.*, 1985
(+)-Goniodiol diacetate **[41]** 	Styrylpyrone	Leave, Twig	Talapatra *et al.*, 1985
Goniodiol-7-monoacetate **[3]** 	Styrylpyrone	Leave, Twig	Talapatra *et al.*, 1985
Goniopedaline **[128]** 	Aristolactam alkaloid	Leave, Twig	Talapatra, Basu, Chattopadhyay and Tarapatra, 1988
Goniotriol **[5]** 	Styrylpyrone	Leave, Twig	Talapatra *et al.*, 1985

Plant & chemical compounds	Category	Plant part	References
G. sesquipedalis *β*-Sitosterol **[129]** 	Steroid	Leave, Twig	Talapatra, Basu, Chattopadhyay and Tarapatra, 1988
Taliscanine **[111]** 	Aristolactam alkaloid	Leave, Twig	Talapatra, Basu, Chattopadhyay and Tarapatra, 1988
G. tapis Isoaltholactone [118] 	Styrene derivative	Stembark	Colegate *et al.*, 1990
G. tenuifolius Aristolactam A-II **[105]** 	Aristolactam alkaloid	Stem bark	Likhitwitayawuid *et al.*, 1997
Cepharanone B **[130]** 	Aristolactam alkaloid	Stem bark	Likhitwitayawuid *et al.*, 1997

Plant & chemical compounds	Category	Plant part	References
G. tenuifolius Norcepharadione B **[131]** 	4,5-Dioxo-aporphine alkaloid	Stem bark	Likhitwitayawuid *et al.*, 1997
Taliscanine **[111]** 	Aristolactam alkaloid	Stem bark	Likhitwitayawuid *et al.*, 1997
Velutinam **[112]** 	Aristolactam alkaloid	Stem bark	Likhitwitayawuid *et al.*, 1997
G. thwaitesii Annulatin **[132]** 	Flavonoid	Aerial part	Seidel, Bailleul and Waterman, 2000

Plant & chemical compounds	Category	Plant part	References
G. thwaitesii Betulinic acid **[133]** 	Triterpene	Aerial part	Seidel, Bailleul and Waterman, 2000
Friedelin **[134]** 	Triterpene	Aerial part	Seidel, Bailleul and Waterman, 2000
Friedelinol **[135]** 	Triterpene	Aerial part	Seidel, Bailleul and Waterman, 2000
G. uvarioides 5-Acetylgoniothlamin **[136]** 	Styrylpyrone	Root	Ahmad, Tukol, Omar and Sharif 1991

Plant & chemical compounds	Category	Plant part	References
G. uvarioides Goniothlamin **[9]**  	Styrylpyrone	Root	Ahmad, Tukol, Omar and Sharif 1991
G. velutinus Cepharanone B **[131]**  	Aristolactam alkaloid	Stem bark	Omar *et al.*, 1992
Velutinam **[113]**  	Aristolactam alkaloid	Stem bark	Omar *et al.*, 1992

2. Free radical scavenging activity from natural sources.

Examples of oxygen centered free radicals, known as reactive oxygen species (ROS) include superoxide ($O_2^{\cdot -}$), peroxyl ($ROO^{\cdot}$), alkoxyl ($RO^{\cdot}$), hydroxyl ($OH^{\cdot}$) and nitric oxide ($NO^{\cdot}$) (Pietta, 2000). Five groups of natural products have been shown to possess free radical scavenging activity. They can be classified as:

2.1 Flavonoids

Flavonoids are naturally occurring phenolics which are widely distributed in a variety of plants at high levels and are commonly ingested from vegetables, fruits and beverages (tea and wine) (Haraguchi, 2001). Flavonoids have been shown to elicit antitumoral, antiplatelet, antiischemic, antiallergic and antiinflammatory activities. Along with these activities, flavonoids have also been shown to inhibit the activity of several enzymes, including lipoxygenase and cycloxygenase, monoxygenase, xanthine oxidase, mitocondrial succinoxidase and NADH-oxidase, phospholipase A_2 and protein kinase. The biological activities of the flavonoids are thought to be the result of their antioxidant properties, where the inhibition of the enzyme by flavonoids could be attributed to their ability to react with reactive oxygen species (ROS) formed at or near the reaction center (Dugas *et al.*, 2000). Flavonoids as free radical scavengers have been grouped, as follows:

2.1.1 Flavone derivatives

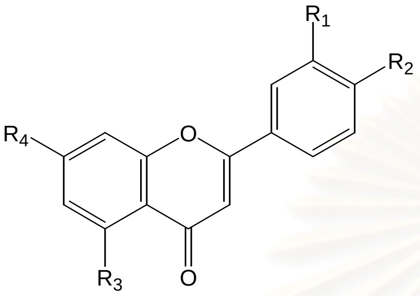
Flavones from leaves of *Ginkgo biloba* have been evaluated for their antilipid peroxidation which was induced by *tert* - butyl hydroperoxide (*t* BH). Lipid peroxidation was monitored by the production of malonaldehyde (MAD).

Table 2 Antilipidperoxidation of flavones from *Ginkgo biloba* leaves

Compounds	**MAD** (nmoles /10^6 cells)	**DPPH** (% decolouration at 10^{-4}M)
Positive control	0.35 ± 0.07	0
t BH 1.5 mM	10.42 ± 0.33	0
Luteolin **(137)**	0.70 ± 0.18***	59
Apigenin **(138)**	7.13 ± 0.48*	0
Flavone **(139)**	11.11 ± 0.32	0
Acacetin **(140)**	6.97 ± 0.51*	0
Chrysin **(141)**	6.93 ± 1.79***	0

* $p < 0.05$, ** $p < 0.01$, *** $p< 0.001$

Table 2 shows the results obtained on MAD production and DPPH (1,1-diphenyl-2-picrylhydrazyl) decolouration test. At 100 µM, only luteolin **(137)** produced a decolouration of DPPH radical and significantly reduced MAD production. The other flavones are not active with the DPPH radical and are only weak antilipidperoxidants (Joyeux *et al.,* 1995).



	R_1	R_2	R_3	R_4
Luteolin **(137)**	OH	OH	OH	OH
Apigenin **(138)**	H	OH	OH	OH
Flavone **(139)**	H	H	H	H
Acacetin **(140)**	H	OCH_3	OH	OH
Chrysin **(141)**	H	H	OH	OH

Figure 2 Structures of flavones from of *Ginkgo biloba*

Flavones have been studied for the relationships between their structures and radical scavenging activity. The Trolox equivalent antioxidant capacity (TEAC) is defined as the concentration of Trolox with the same antioxidant capacity as 1 mM concentration of the antioxidant under investigation. This assay is based on the ability of an antioxidant to scavenge (at pH 7.4) a preformed radical cation chromophore of 2,2'- azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) in relation to that of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), an aqueous soluble vitamin E analogue (Pietta, 2000), as shown in Table 3.

Table 3 Flavones as $ABTS^{\cdot+}$ cation scavengers

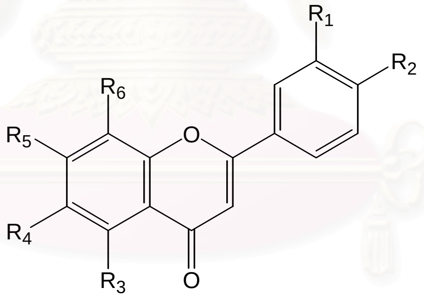
Compounds	**TEAC** (mM)
Luteolin **(137)**	2.09
Luteolin 4'-glucoside **(142)**	1.74
Apigenin **(138)**	1.45
Chrysin **(141)**	1.43

Four flavone compounds were isolated from the dried leaves of *Polygonum hydropiper* (Laksa leaves), and evalutated for antioxidant activity by using TEAC (Peng *et al.*, 2003), as shown in Table 4.

Table 4 TEAC values of the flavones isolated from Laksa leaves

Compounds	**TEAC** (mM)
6-Hydroxyluteolin 7-O'-D-glucopyranoside **(143)**	2.87 ± 0.04
6-Hydroxyluteolin; 3',4',5,6,7-pentahydroxyflavone **(144)**	2.33 ± 0.04
Scutillarein **(145)**	2.16 ± 0.05
Scutillarein 7-O-β-D-glucopyranoside **(146)**	1.98 ± 0.08

The antilipid peroxidative effect of some flavones were investigated using CCl_4-induced lipid peroxidation in rat microsomes. The active compounds were luteolin **(137)**, apigenin **(138)** and gardenin D **(147)** with IC_{50} values of 70.4 ± 1.7, 79.1 ± 0.8 and 84.6 ± 1.7 µM, respectively (Choli, Paya and Alcaraz, 1991).



	R_1	R_2	R_3	R_4	R_5	R_6
Compound **(143)**	OH	OH	OH	OH	GlcO	H
Compound **(144)**	OH	OH	OH	OH	OH	H
Compound **(145)**	H	OH	OH	OH	OH	H
Compound **(146)**	H	OH	OH	OH	GlcO	H
Compound **(147)**	OH	OCH_3	OH	OCH_3	OCH_3	OCH_3

Figure 3 Structures of flavones **143** to **147**

2.1.2 Flavonol derivatives

Flavonols which inhibited CCl_4-induced lipid peroxidation were dasticetin **(148)**, morin **(149)**, galangin **(150)** with IC_{50} values of 39.5 ± 0.8, 48.5 ± 0.9, 68.9 ± 1.3 μM, respectively (Cholbi, Paya and Alcaraz, 1991). Flavonols from leaves of *Ginkgo biloba* have been evaluated for their antilipidperoxidation which was induced by *tert*-butyl hydroperoxide (*t*BH) and for their DPPH radical scavenging activity as shown in Table 5 (Joyeux *et al.*, 1995).

Table 5 Antilipidperoxidation of flavonols from *Ginkgo biloba* leaves

Compounds	**MAD** (nmoles /10^6 cells)	**DPPH** (% decolouration at 10^{-4}M)
Positive control	1.42 ± 0.31	0
*t*BH 1.5 mM	8.74 ± 0.86	0
Myricetin **(151)** 50μM	0.48 ± 0.06***	63
Quercetin **(152)** 50μM	1.33 ± 0.07***	58
Fisetin **(153)** 50μM	1.95 ± 0.10***	44
Kaempferol **(154)** 50μM	1.12 ± 0.19***	37
Rutin **(155)** 100μM	7.33 ± 1.91	36

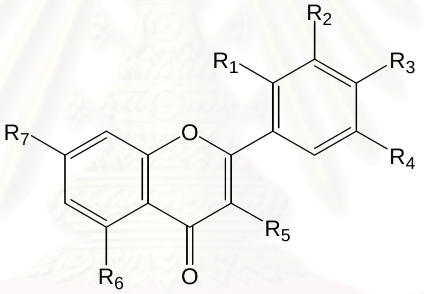
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Five flavonols from *Polygonum hydropiper* (Laksa leaves) showed $ABTS^{\cdot+}$ cation scavenging activity, which was expressed as the Trolox equivalent antioxidant capacity value (TEAC) (Peng *et al.*, 2003), as shown in Table 6.

Table 6 TEAC values of the flavonols isolated from Laksa leaves

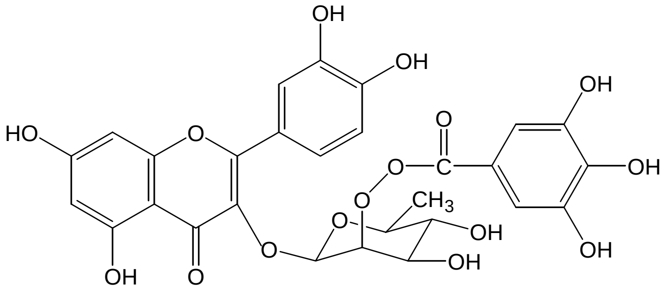
Compounds	**TEAC** (mM)
2"-*O*-(3,4,5-Trihydroxybenzoyl) quercitrin; galloyl quercitrin **(156)**	6.14 ± 0.06
Quercetin-3-*O*-β-D-glucuronide **(157)**	5.08 ± 0.09
Quercetin **(152)**	4.65 ± 0.07
3-*O*-α-L-Rhamnopyranosyloxy-3', 4', 5, 7-tetrahydroxyflavone (quercitin) **(158)**	3.46 ± 0.11
3-*O*-β-D-Glucopyranosyloxy-4', 5, 7-trihydroxyflavone (kaempferol 3-glucoside) **(159)**	1.39 ± 0.07

From Table 6, the order of effectiveness in scavenging the $ABTS^{\cdot+}$ radicals is as follows: Compound 156 > 157 > 152 > 158 >159

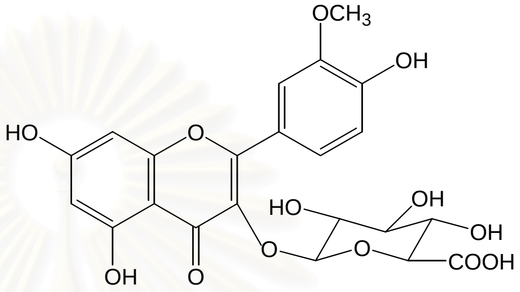


	R$_1$	**R$_2$**	**R$_3$**	**R$_4$**	**R$_5$**	**R$_6$**	**R$_7$**
Compound **(148)**	OH	H	H	H	OH	OH	OH
Compound **(149)**	H	H	H	H	OH	OH	OH
Compound **(150)**	OH	H	OH	H	OH	OH	OH
Compound **(151)**	H	OH	OH	OH	OH	OH	OH
Compound **(152)**	H	OH	OH	H	OH	OH	OH
Compound **(153)**	H	OH	OH	H	OH	H	OH
Compound **(154)**	H	H	OH	H	OH	OH	OH
Compound **(155)**	H	OH	OH	H	O-Rha-Glu	OH	OH

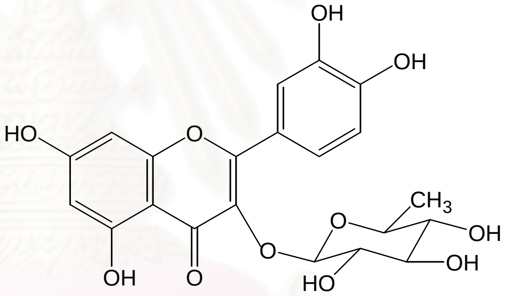
Compound **(156)**



Compound **(157)**



Compound **(158)**



Compound **(159)**

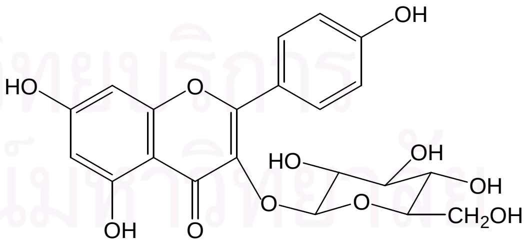


Figure 4 Structures of flavonols with free radical scavenging activity (compounds **148 -159**)

2.1.3 Flavanone derivatives

Eriodictyol **(160)** showed antilipid peroxidative effect in rat liver microsome induced by CCl_4 with IC_{50} of 78.9 ± 1.3 μM (Choli, Paya and Alcaraz, 1991). This compound **(160)** and several flavanone derivatives, including hesperetin **(161)**, naringenin **(162)** and naringenin 7-rutinoside **(163)** exhibited ABTS$^{\cdot+}$ cation scavenging activity with TEAC values at 1.8, 1.4, 1.5 and 0.8 mM, respectively (Pietta, 2000). Four flavanones have been compared in a dose-response manner with vitamin C, vitamin E and β-carotene by using an *in vitro* lipoprotein oxidation model. The IC_{50} data are shown in Table 7 (Vinson *et al.*, 1995). Hesperetin **(161)** was the only compound which possessed significant antioxidant activity.

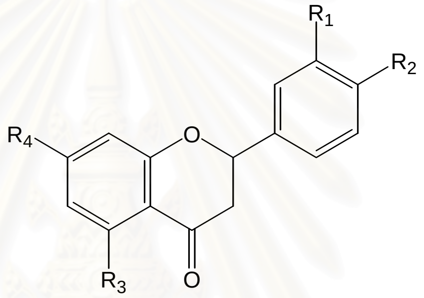
Table 7 Comparison of antioxidant effectiveness of vitamins and flavanones

Compounds	**IC_{50}** (μM)
Vitamin C	1.45
Vitamin E	2.40
β-carotene (provitamin A)	4.30
Hesperetin **(161)**	3.66
Hesperidin (hesperetin rutinoside) **(164)**	> 16
Neohesperidin **(165)**	> 16
Naringenin **(162)**	> 16

Burda and Oleszek (2001) studied the relationships between the structures of flavanones and their antiradical activity by the DPPH decoloration test, as shown in Table 8

Table 8 Antiradical activities of flavanones (3.3 x 10^{-5}μM) in methanol solution of DPPH

Compounds	**Antiradical activity (%)**
Hesperetin **(161)**	30.0
Naringenin **(162)**	6.3
Naringin **(166)**	4.7
Flavanone **(167)**	2.6



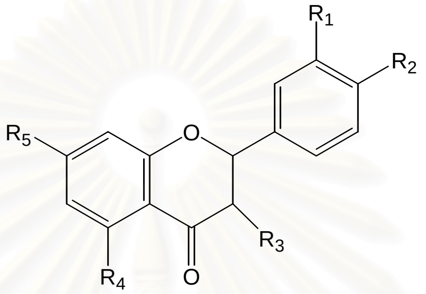
	R_1	R_2	R_3	R_4
Compound **(160)**	OH	OH	OH	OH
Compound **(161)**	OH	OCH_3	OH	OH
Compound **(162)**	H	OH	OH	OH
Compound **(163)**	H	OH	OH	O-Glu-Rha
Compound **(164)**	OH	OCH_3	OH	O-Glu-Rha
Compound **(165)**	OH	OCH_3	OH	O-Glu-Rha
Compound **(166)**	H	OH	OH	O-neohesp
Compound **(167)**	H	H	H	H

Figure 5 Structures of flavanones with free radical scavenging activity (compounds **160 -167**)

2.1.4 Flavanonol derivatives

Taxifolin **(168)** inhibited CCl_4- induced rat liver microsome lipid peroxidation at IC_{50} 100 µM (Choli, Paya, Alxaraz, 1991). TEAC values of taxifolin **(168)** and dihydrokaempferol **(169)** have been reported at IC_{50} 1.9 and 1.3 mM, respectively (Pietta, 2000).

Taxifolin **(168)** and fustin (dihydroquercetin) **(170)** on DPPH decoloration test, showed 94.8 and 91.9 % activity at the concentration of 3.3 x 10^{-5} µM (Burda and Oleszek, 2001).



	R_1	R_2	R_3	R_4	R_5
Compound (**168**)	OH	OH	OH	OH	OH
Compound **(169)**	OH	H	OH	OH	OH
Compound **(170)**	OH	OH	OH	H	OH

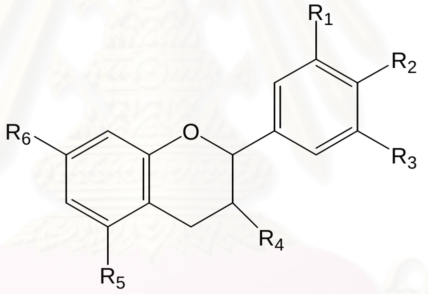
Figure 6 Structures of flavanonols with free radical scavenging activity (compounds **168-170**)

2.1.5 Flavan derivatives

Catechin **(171)** exhibited antilipid peroxidative effect in rat liver microsome, induced by CCl_4 at IC_{50} 87.1 ± 1.7 µM (Choli, Paya, Alcaraz, 1991). The $ABTS^{\cdot+}$ cation scavenging activities of (+)-catechin **(171)**, (-)-epicatechin **(172)**, (-)-epigallocatechin **(173)**, (-)-epicatechin-3-gallate **(174)** and (-)-epigallocatechin-3-gallate **(175)** have been shown as TEAC values at 2.4, 2.5, 3.8, 4.93 and 4.75 mM, respectively (Pietta, 2000). These compounds **(171 -175)** also inhibited lipid peroxidation by using an *in vitro* lipoprotein oxidation model. This model simulates the oxidation of low-density lipoproteins, which results in atherosclerosis. The IC_{50} data are shown in Table 9 (Vinson *et al.*, 1995). Among the compounds tested, epigallocatechin-3-gallate **(175)** was the most potent, being 20 times more potent than the best vitamin, ascorbic acid.

Table 9 Comparison of antioxidant effectiveness of vitamins and flavanols

Compounds	**IC_{50}** (μM)
Vitamin C	1.45
Vitamin E	2.40
β-carotene (provitamin A)	4.30
Catechin **(171)**	0.187
Epigallocatechin **(173)**	0.097
Epicatechin-3-gallate **(174)**	0.142
Epigallocatechin-3-gallate **(175)**	0.075

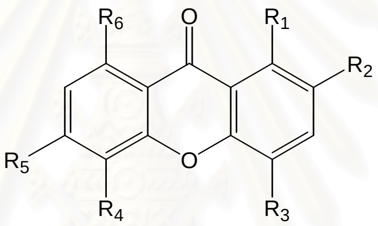


	R_1	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**
Compound **(171)**	OH	OH	H	(*β*)OH	OH	OH
Compound **(172)**	OH	OH	H	(α)OH	OH	OH
Compound **(173)**	OH	OH	OH	(α)OH	OH	OH
Compound **(174)**	OH	OH	H	O-Gall	OH	OH
Compound **(175)**	OH	OH	OH	O-Gall	OH	OH

Figure 7 Structures of flavans with free radical scavenging activity (compounds **171 -175**)

2.2 Xanthones

Since most xanthones have phenolic functional groups on a linear tricyclic ring, they often exhibit a wide range of biological and pharmacological activities (Minami *et al.*, 1994). Four xanthones have been isolated from the wood of *Garcinia subelliptica*. Antioxidative properties of all compounds have been evalulated *in vitro* using three assay systems to measure antilipidperoxidation and free radical and superoxide anion scavenging activity. Among them, 1,2-dihydroxy-5,6-dimethoxyxanthone **(176)** and 1,8-dihydroxy-6-methoxyxanthone **(177)** were effective in preventing lipid peroxidation in rat brain homogenates. 1,2,5-Trihydroxyxanthone **(178)** was a potent scavenger against DPPH radical and O_2^- derived from xanthine-xanthine oxidase system. Globuxanthone **(179)** was also effective in scavenging O_2^- and preventing lipid peroxidation.



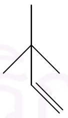
	R_1	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**
Compound **(176)**	OH	OH	H	OCH_3	OCH_3	H
Compound (**177**)	OH	H	H	H	OCH_3	OH
Compound **(178**)	OH	OH	H	OH	H	H
Compound **(179)**	OH	OH		OH	H	H

Figure 8 Structures of xanthones with free radical scavenging activity (compounds **176 -179**)

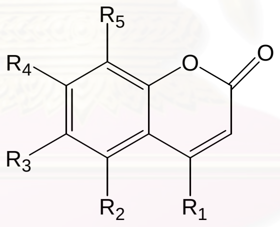
2.3 Courmarins

The coumarins (also known as benzopyrones) consist of fused benzene and α-pyrone rings, and form a large class of phenolic compounds occurring in plants. Six coumarins (compounds **180**, **181**, **182**, **183**, **184** and **185**), all of which have two hydroxyl groups, were effective inhibitors of Fe^{3+}-ascorbate induced microsomal lipid peroxidation, as shown in Table 10.

Table 10 Inhibition of microsomal lipid peroxidation by coumarins

Compounds	% Inhibition at 100 μM	IC_{50}(μM)
7,8-Dihydroxy-6-methoxycoumarin (fraxetin) **(180)**	100	3.3
6,7-Dihydroxycoumarin (esculetin) **(181)**	100	13.0
6,7-Dihydroxy-4-methylcoumarin (4-methylesculetin) **(182)**	100	8.0
5,7-Dihydroxy-4-methylcoumarin **(183)**	100	12.0
7,8-Dihydroxycoumarin (daphnetin) **(184)**	100	18.0
7,8-Dihydroxy-4-methylcoumarin (4-methyldaphnetin) **(185)**	100	2.8

The dihydroxylated coumarins were all active as inhibitors of lipid peroxidation, with *ortho*-dihydroxy being more favorable than *meta*-dihydroxy (compounds **182** and **185** *vs* **183**). Addition of a further 4-methyl substituent increases potency (**182** *vs* **181** and **185** *vs* **184**), presumably by enhancing lipid solubility (Paya, Halliwell and Hoult, 1992).



	R_1	**R_2**	**R_3**	**R_4**	**R_5**
Compound **(180)**	H	H	CH_3	OH	OH
Compound **(181)**	H	H	H	OH	OH
Compound **(182)**	CH_3	H	H	OH	OH
Compound **(183)**	CH_3	OH	H	OH	H
Compound **(184)**	H	H	H	OH	OH
Compound **(185)**	CH_3	H	H	OH	OH

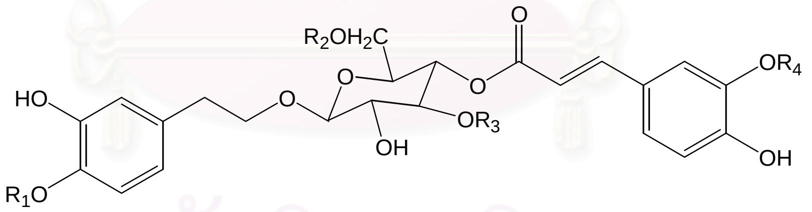
Figure 9 Structures of coumarins with free radical scavenging activity (compounds **180 -185**)

2.4 Phenylpropanoids

Phenylpropanoids are also widely distributed in edible plants and foodstuff derived from plants (Haraguchi, 2001). Five phenylpropanoid glycosides, verbascoside **(186)**, pedicularioside A **(187)**, pedicularioside M **(188)**, pedicularioside N **(189)**, leucosceptoside A **(190)** and martynoside **(191)**, isolated from *Pedicularis* plants, have been studied for the scavenging activity on superoxide anion and hydroxy radicals, as shown in Table 11. The results demonstrated that their radical scavenging activities are related to the number of phenolic hydroxy groups in the structures (Wang *et al.*, 1996).

Table 11 Percentage of scavenging activity of phenylpropanoid glycosides on hydroxyl radical

Compounds	**No. of Ph-OH**	**Concentration (mM)**	**% Radical scavenging**
Verbascoside **(186)**	4	1.53	79.4 ± 2.1
Pedicularioside A **(187)**	4	1.50	77.8 ± 2.8
Pedicularioside M **(188)**	3	1.73	55.1 ± 1.7
Pedicularioside N **(189)**	2	1.56	33.0 ± 1.2
Leucosceptoside A **(190)**	3	1.57	17.5 ± 4.1
Martynoside **(191)**	2	1.50	28.3 ± 1.3



	R_1	**R_2**	**R_3**	**R_4**
Compound **(186)**	H	H	H	H
Compound **(187)**	H	Rha	Api	H
Compound **(188)**	H	Rha	Api	CH_3
Compound **(189)**	CH_3	Rha	Api	CH_3
Compound **(190)**	H	H	Rha	CH_3
Compound **(191)**	CH_3	H	Rha	CH_3

Figure 10 Structures of phenylpropanoid glycosides with free radical scavenging activity (compounds **186 - 191**)

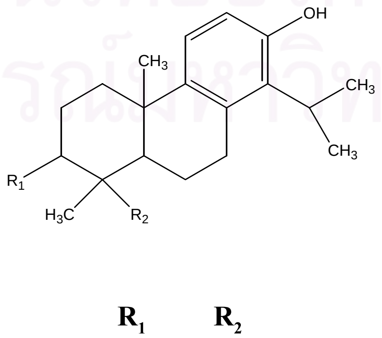
2.5 Terpenoids

Terpenoids are also widely distributed in a variety of plants. In comparison to hydrophillic flavonoids, lipophilic terpenoids have been revealed to possess potent antioxidative activities and protective effects against oxidative stresses in mitochondria (Haraguchi, 2001).

Diterpenes from *Podocarpus nagi* showed potent inhibition of rat liver microsomal lipid peroxidation. Totarol **(192)**, totaradiol **(193)** and 19-hydroxytotarol **(194)** exhibited almost complete inhibition at 19 μM. Totaral **(195)**, 4*β*-carboxy-19-nortotarol **(196)** and sugiol **(197)** also inhibited microsomal lipid peroxidation over 50% at 35 μM. Their IC_{50} values are shown in Table 12 with that of BHT, a common synthetic antioxidant (positive control) (Haraguchi, Ishikawa and Kubo, 1997).

Table 12 Antioxidative activities of diterpenoids from *Podocarpus nagi*.

Compounds	**IC_{50} (μM)**
Totarol **(192)**	4.79 ± 0.16
Totaradiol **(193)**	6.69 ± 0.22
19-Hydroxytotarol **(194)**	6.55 ±0.18
Totaral **(195)**	12.25 ±0.63
4*β*-carboxy-19-nortotarol **(196)**	18.96 ± 0.55
Sugiol **(197)**	31.24 ± 1.36
BHT	1.92 ± 0.07
Myricetin	41.70 ± 2.61



	R_1	**R_2**
Compound **(192)**	H	CH_3
Compound **(193)**	OH	CH_3

Compound **(194)**	H	CH_2OH
Compound **(195**)	H	CHO
Compound **(196)**	H	COOH

Compound **(197)**

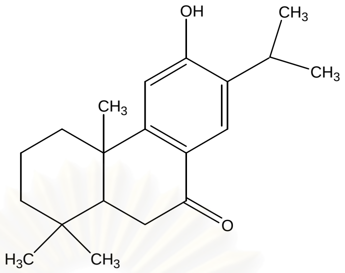


Figure 11 Structures of diterpenoids with free radical scavenging activity (compounds **192** - **197**)

CHAPTER III

EXPERIMENTAL

1. Source of plant material

The leaves of *Goniothalamus tenuifolius* were collected from Kaengkrachan, Phetchaburi province, Thailand. The plant was identified by comparison with herbarium specimens in the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Co-operatives, Bangkok. A voucher specimen (VJ 04-2538) is retained at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

2. General techniques

2.1 Analytical thin-layer chromatography

Technique : One dimention, ascending

Adsorbent : Silica gel 60G F254 (E.Merck) precoated plate

Layer thickness : 0.2 mm

Distance : 6 cm

Temperature : Laboratory temperature (30-35 °C)

Detection : 1.Ultraviolet light (254 and 365 nm)
2.Anisaldehyde and heating at 105°C for 10 min

2.2 Column chromatography

2.2.1 Vacuum liquid column chromatography

Adsorbent : Silica gel 60 (No. 7734) particle size 0.063-0.200 mm (70-230 mesh ASTM) (E. Merck)

Packing method : Dry packing

Sample loading : The sample was dissolved in an organic solvent, mixed with the adsorbent, triturated, dried and then placed gently on top of the column.

Detection : Fractions were examined by TLC observing under UV light (254 and 365 nm)

2.2.2 Flash column chromatography

Adsorbent : Silica gel 60 (No. 9385) particle size 0.040-0.063 mm (230-400 mesh ASTM) (E. Merck)

Packing method : Wet packing

Sample loading : The sample was dissolved in a small amount of eluent and then applied gently on top of the column.

Detection : Fractions were examined in the same manner as described in section 2.2.1

2.2.3 Gel Filtration chromatography

Gel filter : Sephadex LH 20 (Pharmacia)

Packing method : Gel filter was suspended in the eluent and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tightly.

Sample loading : The sample was dissolved in a small amount of eluent and applied on top of the column.

2.3 Spectroscopy

2.3.1 Ultraviolet (UV) absorption spectra

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

2.3.2 Infrared (IR) absorption spectra

IR spectra (film) were recored on a JASCO FT/IR-300E spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.3.3 Mass spectra

Electrospray ionization mass spectra (ESIMS) were measured on a mass spectrometer LCT (LCMS) Micromass (National Center for Genetic Engineering and Biotechnology, BIOTEC (NSTDA, Science Park, Pathumthani, Thailand).

2.3.4 Proton and Carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra

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

Solvents for NMR were deuterated acetone (acetone-d_6), deuterated chloroform ($CDCl_3$) and deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts were reported in ppm scale using the chemical shifts of the solvent as the reference signal.

2.4 Solvents

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

2.5 Microtiter plate reading

Microtiter plate reading was performed on an Anthos HTL instrument (Department of Biochemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

3. Extraction and isolation

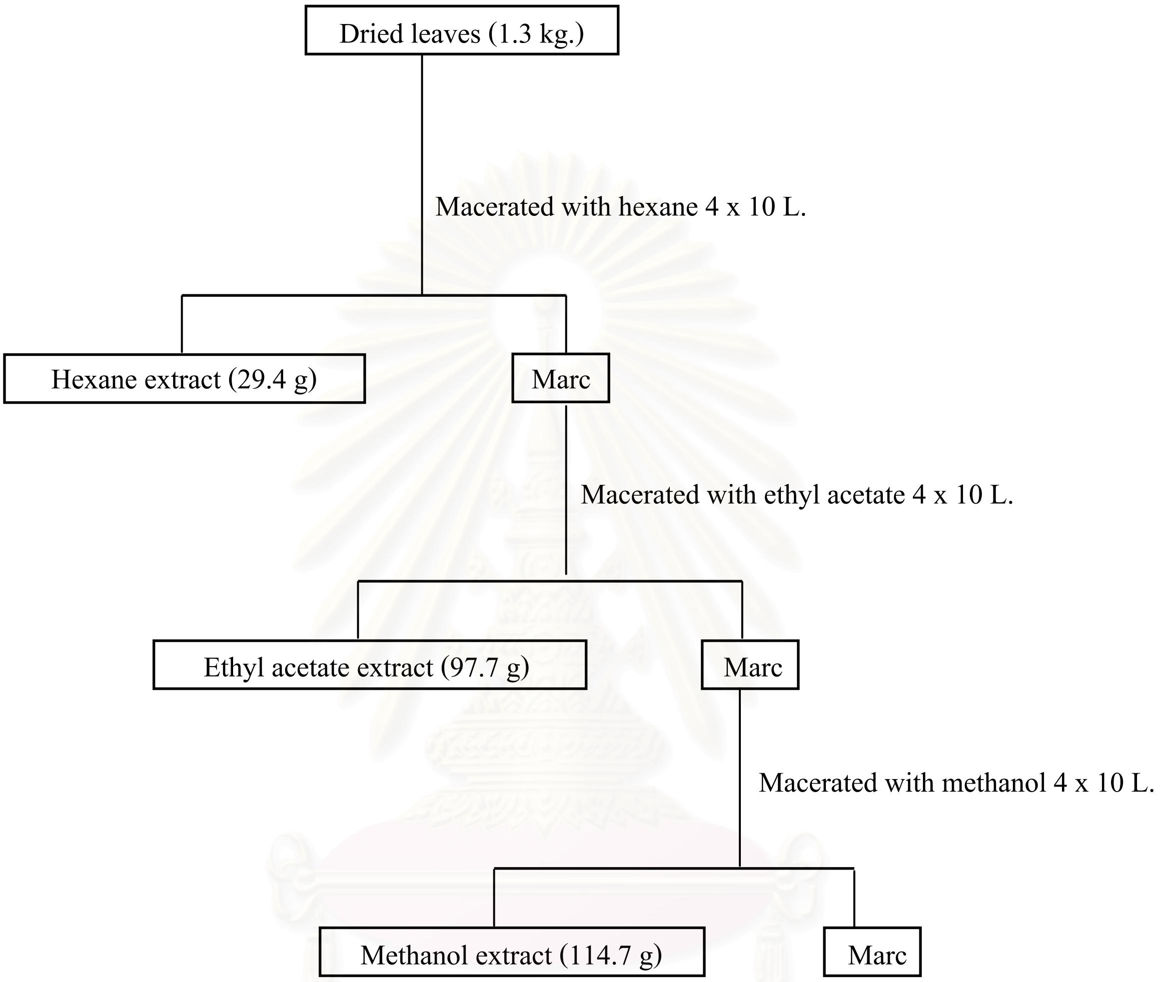
3.1 Extraction and isolation of compounds from *Goniothalamus tenuifolius*

3.1.1 Extraction

The dried leaves of *Goniothalamus tenuifolius* (1.3 kg) were chopped, ground and then extracted four times with hexane (10 L, 3 days each) and then filtered. The filtrate was pooled and evaporated under reduced pressure to yield a hexane extract (syrupy mass 29.4 g, 2.26 % based on dried weight of leaves).

The marc was repeatedly extracted four times with ethyl acetate (10 L, 3 days each) and then filtered. The filtrate was pooled and evaporated under reduced pressure to yield an ethyl acetate extract (syrupy mass 97.7 g, 7.51 % based on dried weight of leaves).

Finally, the marc was extracted four times with methanol (10 L, 3 days each) and then filtered. The filtrate was pooled and evaporated under reduced pressure to yield a methanol extract (syrupy mass 114.7 g, 8.82 % based on dried weight of leaves). Each extract was subjected to free radical scavenging activity evaluation as described in Section 5.



Scheme 1 Extraction of *Goniothalamus tenuifolius* King

3.1.2 Isolation

The ethyl acetate extract, which was found be active against DPPH (see Section 5), was selected for further separation by vacuum liquid column chromatography using a sintered glass filter column of silica gel (No. 7734). The ethyl acetate extract (45 g) was dissoved in a small amount of ethyl acetate, triturated with silica gel (No.7734, 45 g) and dried under vacuum. The mixture was then applied on the top of the column. Elution was performed in a polarity gradient manner with pet-ether, ethyl acetate and methanol as the solvent. The eluate was collected 500 ml per fraction and examined by TLC (silica gel, pet ether: ethyl acetate 1:4). Fractions with similar chromatographic patterns were combined to yield 10 fractions: fraction A (2.0 g), fraction B (460 mg), fraction C (1.2 g), fraction D (5.1 g), fraction E (1.6 g), fraction F (4.7 g), fraction G (2.4 g), fraction H (1.5 g), fraction I (6.3 g), fraction J (8.0 g).

3.1.2.1 Isolation of compound GT-1 [198]

Fraction B (460 mg) was divided into two portions. Each portion was separated by gel filtration chromatography using a column of Sephadex LH 20 with a mixture of methanol and chloroform (1.5:1) as the eluent. The eluates were collected and combined according to their TLC chromatographic patterns (silica gel, methanol: chloroform 1:19) to give 5 fractions (1-B1 to 1-B5). Fraction 1-B4 (106 mg) was further separated in a similar manner to give 5 fractions (2-B1 to 2-B5). Fraction 2-B4 (40 mg) was re-separated on Sephadex LH 20 to give compound GT-1 (5 mg, R_f value = 0.57, silica gel, ethyl acetate: chloroform 2:3) as pale yellow needles. This compound was later identified as retusine (5-hydroxy-3,7,3',4'-tetramethoxyflavone) **[198]**.

3.1.2.2 Isolation of compound GT-2 [199]

Fraction 1-B5 (83 mg) was separated on Sephadex LH 20 (methanol and chloroform 1.5: 1). Fourteen fractions (20 ml, each) were collected and combined based on their chromatographic patterns (silica gel, methanol: chloroform 1:19) to yield 4 fractions (4-B1 to 4-B4). Fraction 4-B4 (61 mg) was re-separated in a similar manner to give 4 fractions (5-B1 to 5-B4). Fraction 5-B3 (43 mg) was further separated on Sephadex LH 20 (methanol and ethyl acetate 1:1). Eluates with similar TLC behavior (silica gel, ethyl acetate: chloroform 2:3) were pooled to give 4 fractions (6-B1 to 6-B4). Fraction 6-B3 gave compound GT-2 (2 mg, R_f value = 0.29, silica gel, ethyl acetate: chloroform 2:3) as pale yellow needles. Compound GT-2 (10 mg) was also obtained from fraction 6-B2 (34 mg) by re-separation on Sephadex LH 20 (methanol and chloroform 1.5:1). This compound was identified as *trans*-cinnamic acid **[199].**

3.1.2.3 Isolation of compound GT-3 [200]

Compound GT-3 was obtained as yellow needles from fraction C through recrytallization from a mixture of chloroform and methanol (10 mg, R_f value = 0.49, silica gel, ethyl acetate: chloroform 2:3). It was later identified as pachypodol (5,4'-dihydroxy-3,7,3'-trimethoxyflavone) **[200]**.

3.1.2.4 Isolation of compound GT-4 [201] and compound GT-5 [202]

Fraction D (5.1g) was recrystallized from a mixture of chloroform and acetone to yield Re-Fr.D (128 mg) which was then separated by gel filtration chromatography using a column of Sephadex LH 20 with methanol. The eluates were collected and combined according to their TLC chromatographic patterns (silica gel, acetone: chloroform 3:7) to give 6 fractions (1-D1 to 1-D6). Fraction 1-D3 gave compound GT-4 (86 mg, R_f value = 0.19, silica gel, ethyl acetate: chloroform 2:3) as yellow needles. This compound was later identified as kumatakenin (5,3',4'-trihydroxy-3,7-dimethoxyflavone) **[201]**.

Fraction 1-D5, after removal of the solvent, gave compound GT-5 (8 mg, R_f value = 0.06, silica gel, ethyl acetate: chloroform 2:3) as yellow needles. It was subsequently identified as 5,7,3',4'-tetrahydroxy-3-methoxyflavone **[202]**.

3.1.2.5 Isolation of compound GT-6 [203] and compound GT-7 [204]

Fraction G (2.4 g) was fractionated by vacuum liquid chromatograpy (silica gel No.7734). Elution was performed in a polarity gradient manner with mixtures of pet-ether: ethyl acetate (100:0 to 0:100) and ethyl acetate: acetone (100:0 to 0:100). Twenty fractions (200 ml, each) were collected and combined based on their TLC behavior (silica gel, acetone: chloroform 1:4) leading to 7 fractions: fractions 1-G1 to 1-G7.

Fraction 1-G3 (1.4 g) was recrystallized from chloroform and methanol to yield Re-Fr.1-G3 (20 mg) and then re-purified on Sephadex LH 20 with mixtures of methanol and ethyl acetate (1:1). Eluates with similar TLC behavior (silica gel, ethyl acetate: chloroform 1:1) were pooled to give 5 fractions (2-G1 to 2-G5). Fraction 2-G4 gave compound GT-6 (6 mg, R_f value = 0.05, silica gel, ethyl acetate: chloroform 2:3) which was identified as a 3',4'-dihydroxy-3,5,7-trimethoxyflavone **[203]**.

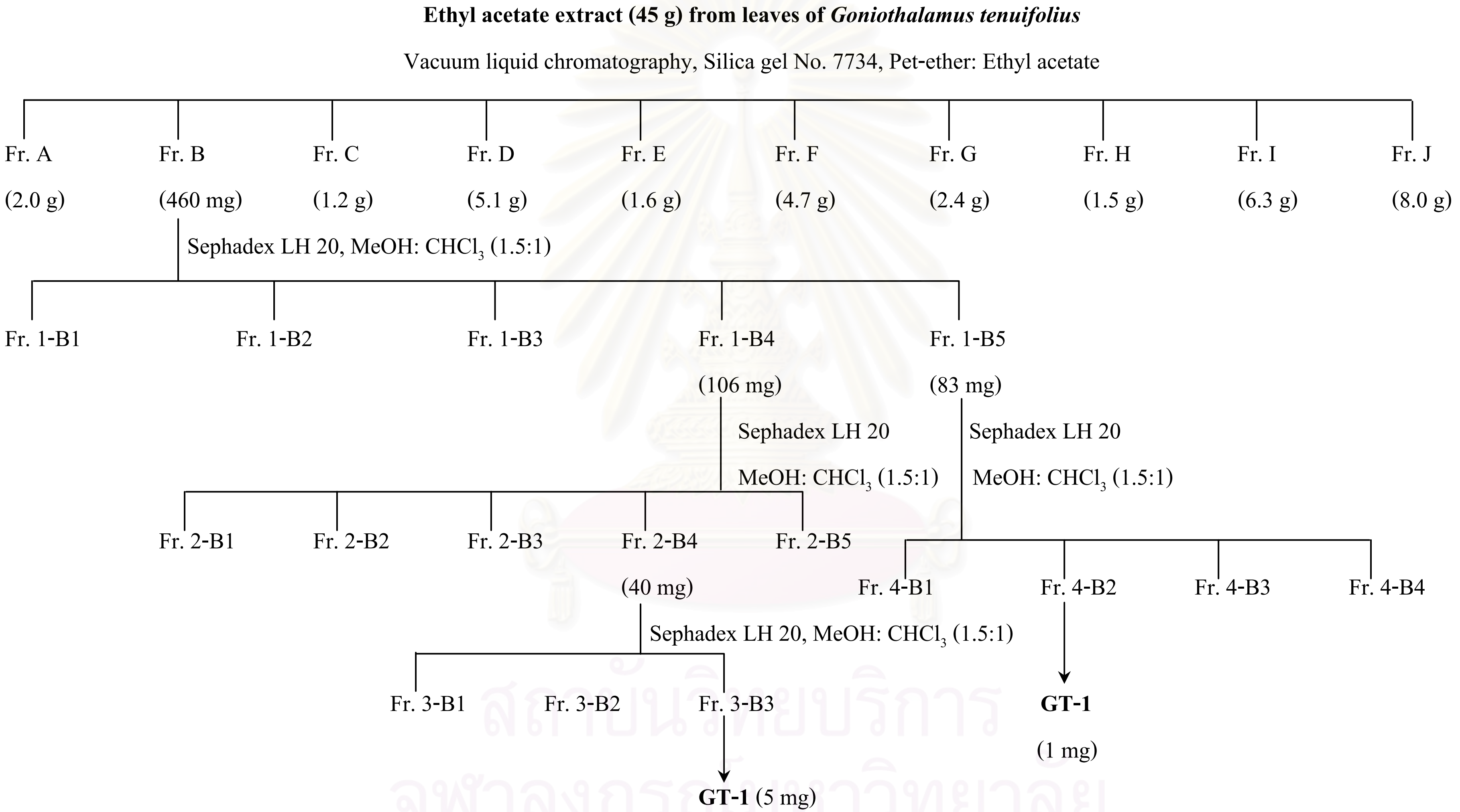
The mother liquor was dried and fractionated on a column using silica gel 60 (No. 7734) as the adsorbent. Isocratic elution with a mixture of acetone and chloroform (1:9) was performed. Fractions with similar chromatographic patterns (silica gel, acetone: chloroform 1:9) were combined to yield 6 major fractions: fractions 3-G1 to 3-G6.

Fraction 3-G2 was recrystallized from a mixture of chloroform and methanol to give compound GT-7 (220 mg, R_f value = 0.25, silica gel, ethyl acetate: chloroform 2:3) as pale yellow needles. This compound was identified as 3,5,7,3',4'-pentamethoxyflavone **[204]**.

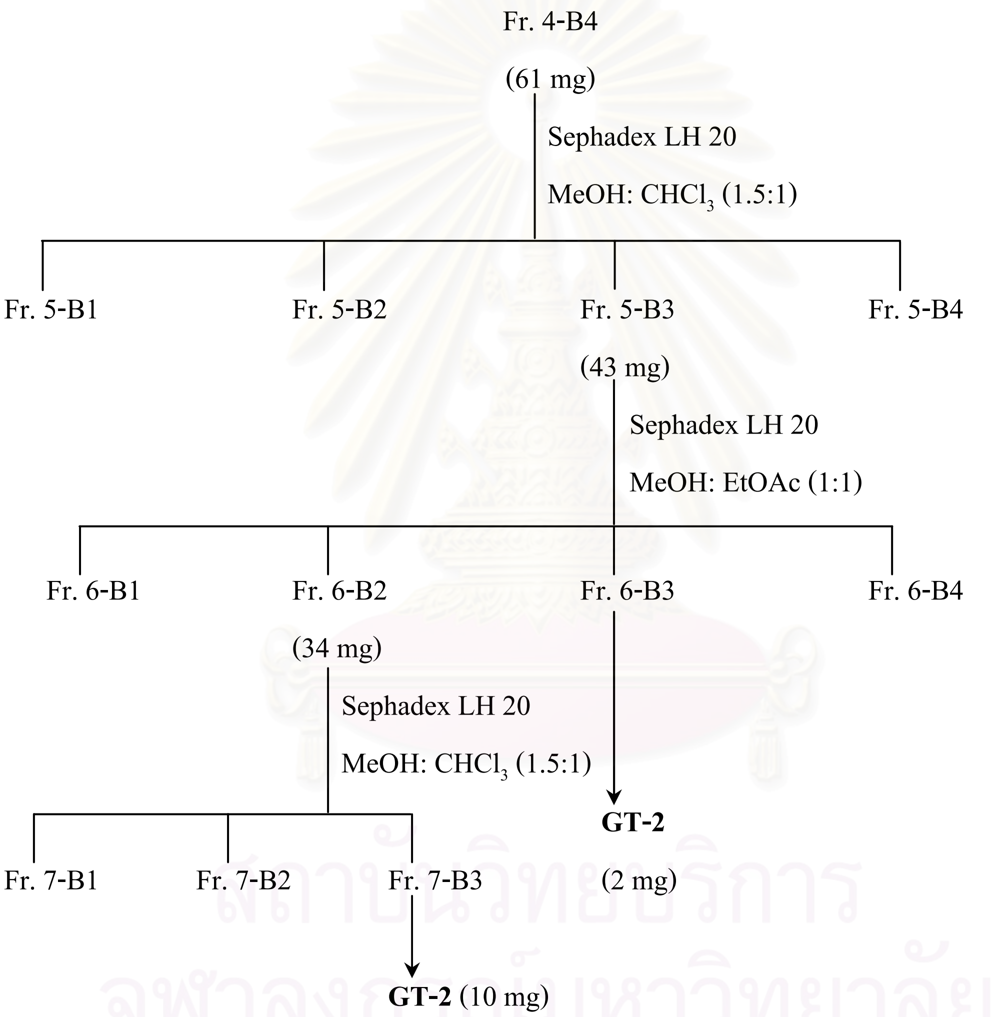
3.1.2.6 Isolation of compound GT-8 [205] and compound GT-9 [206]

Fraction 3-G3 (44 mg) was dissolved in chloroform. The solution, after removal of insoluble substances (Re-Fr. 3-G3), was dried and separated by gel filtration chromatography using a column of Sephadex LH 20 with acetone as the eluent. Twenty-four fractions (20 ml, each) were collected and combined according to their TLC behavior (silica gel, acetone: chloroform 1:4) to yield 4 fractions (4-G1 to 4-G4). Fraction 4-G3 (16 mg) was subjected to separation in a similar manner to give 2 fractions (5-G1 and 5-G2). Fraction 5-G1 (13 mg) was further separated on Sephadex LH 20 with ethyl acetate: chloroform (1:1). The eluates were collected and combined based on their TLC chromatographic patterns (silica gel, acetone: chloroform 3:7) to give 2 fractions: (6-G1 and 6-G2). Fraction 6-G1, after removal of the solvent, gave compound GT-8 (5 mg, R_f value = 0.17, silica gel, ethyl acetate: chloroform 2:3) as pale yellow needles. Fraction 6-G2 (7 mg) was combined with Re-Fr. 3-G3 (5 mg) and then fractionated on Sephadex LH 20 with ethyl acetate: chloroform (1:1) to give GT-8 (2 mg, R_f value =0.17, silica gel, ethyl acetate: chloroform 2:3) and GT-9 (2 mg, R_f value = 0.16, silica gel, ethyl acetate: chloroform 2:3).

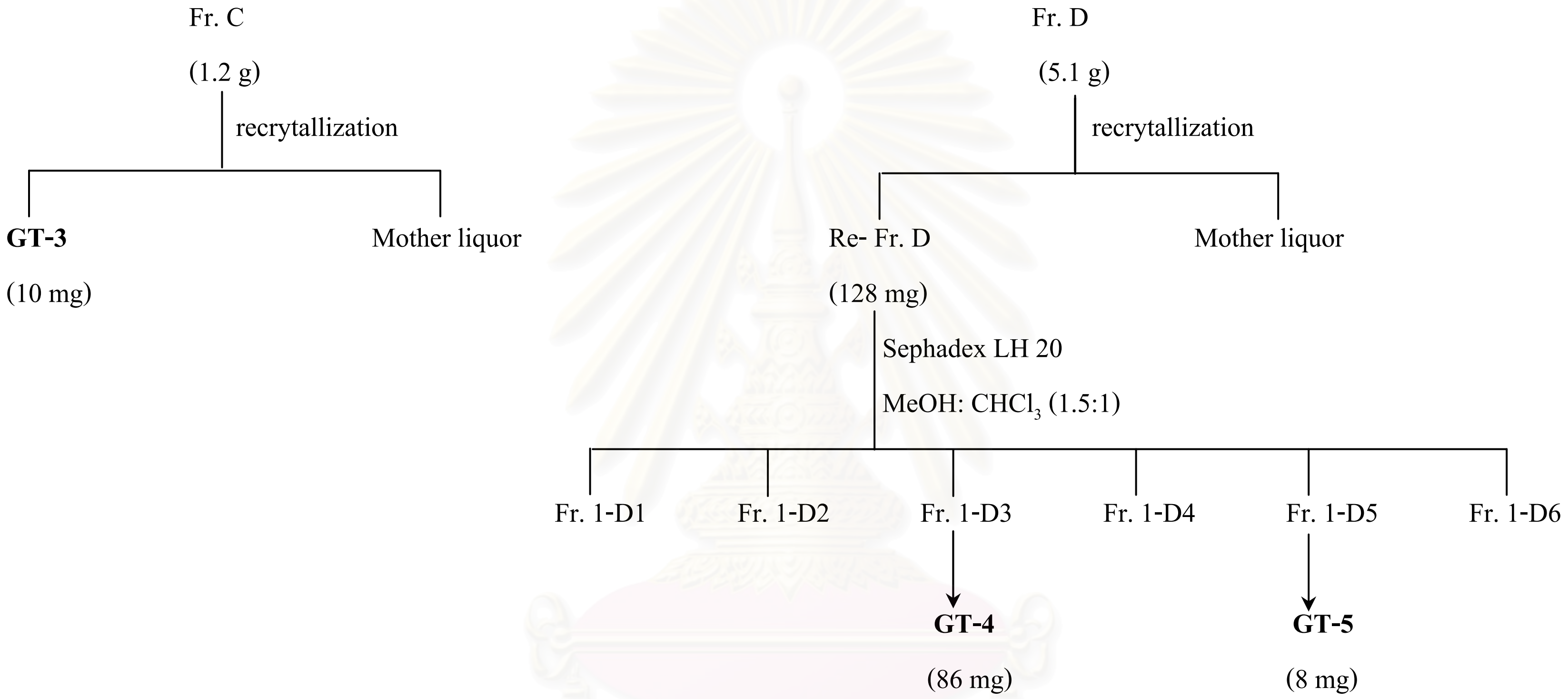
Compound GT-9 was also obtained from fraction 3-G4 (16 mg) through recrystallization from a mixture of ethyl acetate and chloroform (5 mg, R_f value = 0.16, silica gel, ethyl acetate: chloroform 2:3). GT-8 was identified as a 4'-hydroxy-3,5,7,3'-tetramethoxyflavone **[205]** whereas GT-9 was identified as a 3'-hydroxy-3,5,7,4'-tetramethoxyflavone **[206]**



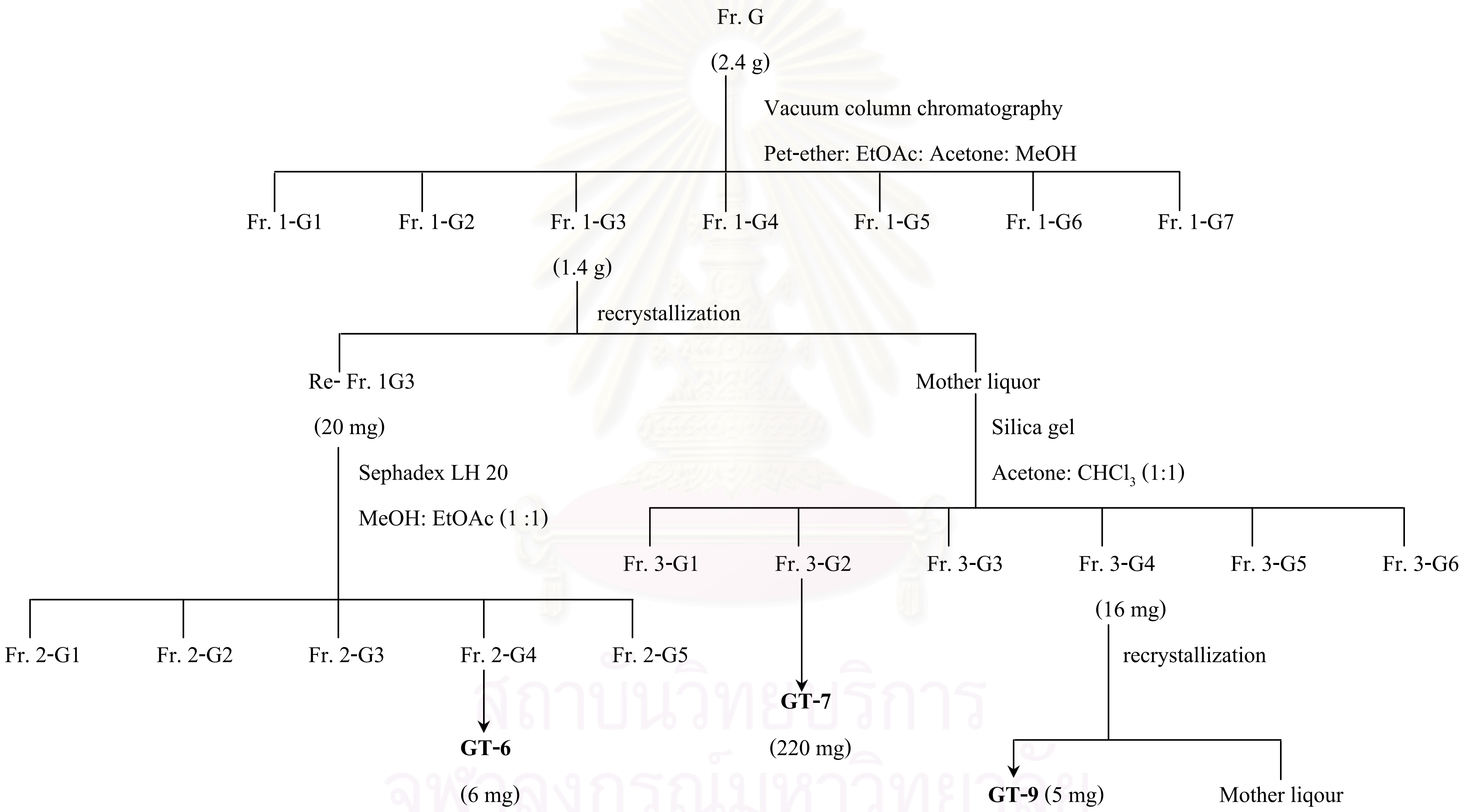
Scheme 2 Separation of the ethyl acetate extract of the leaves of *Goniothalamus tenuifolius*



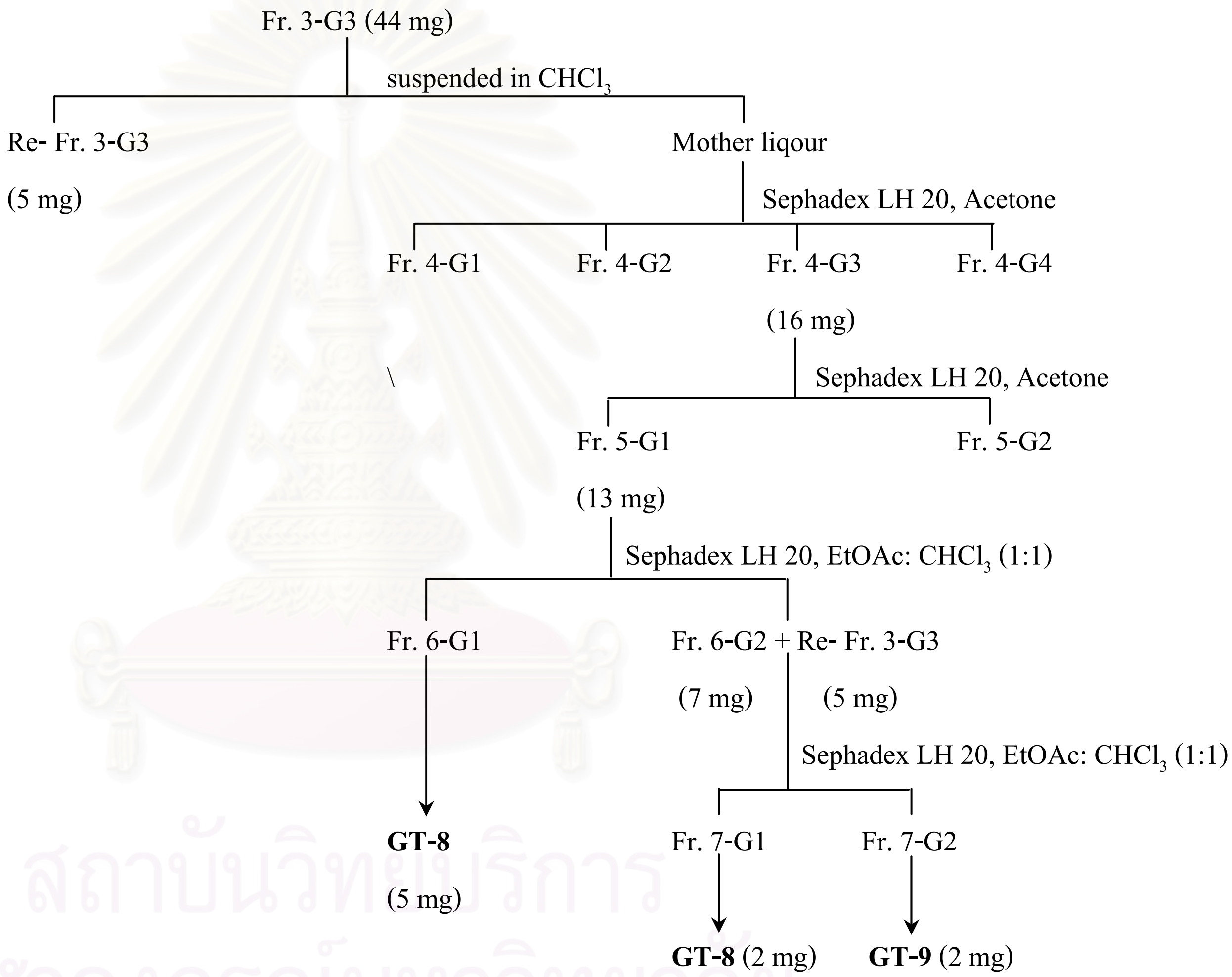
Scheme 3 Separation of fraction 4-B4 of the ethyl acetate extract



Scheme 4 Separation of fractions C and D of the ethyl acetate extract



Scheme 5 Separation of fraction G of the ethyl acetate extract



Scheme 6 Separation of fraction 3-G3 of the ethyl acetate extract

4. Physical and spectral data of isolated compounds

4.1 Compound GT-1 [198]

Compound GT-1 was obtained as pale yellow needles, soluble in chloroform (6 mg, 5.38×10^{-4} % based on dried weight of leaves).

ESIMS : $[M+H]^+$ *m/z* 359 (positive ion mode); Figure 16

UV : λ_{max} nm (log ε), in methanol; Figure 14

252 (4.30), 351 (4.28)

IR : ν_{max} cm^{-1}, film; Figure 15

3448, 2920, 1648, 1589, 1495, 1233, 1149, 812, 796

^{1}H-NMR : δ ppm, 300 MHz, in $CDCl_3$; Figure 17, Table 14

^{13}C-NMR : δ ppm, 75 MHz, in $CDCl_3$; Figure 18, Table 14

4.2 Compound GT-2 [199]

Compound GT-2 was obtained as pale yellow needles, soluble in chloroform (12 mg, 9.23×10^{-4} % based on dried weight of leaves).

ESIMS : $[M-H]^-$ *m/z* 147 (negative ion mode); Figure 21

UV : λ_{max} nm (log ε), in methanol; Figure 19

214 (4.23), 270 (4.25)

IR : ν_{max} cm^{-1}, film; Figure 20

3432, 1681, 1629, 1448, 1286, 933

^{1}H-NMR : δ ppm, 300 MHz, in $CDCl_3$; Figure 22, Table 15

^{13}C-NMR : δ ppm, 75 MHz, in $CDCl_3$; Figure 23, Table 15

4.3 Compound GT-3 [200]

Compound GT-3 **[200]** was obtained as yellow needles, soluble in chloroform (10 mg, 7.69×10^{-4} % based on dried weight of leaves).

ESIMS : $[M+H]^+$ *m/z* 345.12 (positive ion mode); Figure 26

UV : λ_{max} nm (log ε), in methanol; Figure 24

253 (4.40), 356 (4.39)

IR : ν_{max} cm^{-1}, film; Figure 25

3432, 2927, 1656, 1593, 1496, 1212, 811

^{1}H-NMR : δ ppm, 300 MHz, in $CDCl_3$; Figure 27, Table 16

^{13}C-NMR : δ ppm, 75 MHz, in $CDCl_3$; Figure 28, Table 16

4.4 Compound GT-4 [201]

Compound GT-4 **[201]** was obtained as yellow needles, soluble in acetone and methanol (86 mg, 6.61×10^{-3} % based on dried weight of leaves).

ESIMS : $[M+H]^+$ *m/z* 331.11 (positive ion mode); Figure 34

UV : λ_{max} nm (log ε), in methanol; Figure 32

256 (4.51), 356 (4.48)

IR : ν_{max} cm^{-1}, film; Figure 33

3431, 3181, 1662, 1590, 1497, 1309, 823

^{1}H-NMR : δ ppm, 300 MHz, in acetone-d_6 ; Figure 35, Table 17

: δ ppm, 300 MHz, in DMSO-d_6 ; Figure 36, Table 17

^{13}C-NMR : δ ppm, 75 MHz, in acetone-d_6 ; Figure 37, Table 17

: δ ppm, 75 MHz, in DMSO-d_6 ; Figure 38,Table 17

4.5 Compound GT- 5 [202]

Compound GT-5 was obtained as yellow needles, soluble in acetone and methanol (8 mg, 6.15 x 10^{-4} % based on dried weight of leaves).

ESIMS : $[M+H]^+$ *m/z* 317.10 (positive ion mode); Figure 44

UV : λ_{max} nm (log ε), in methanol; Figure 42

256 (4.25), 358 (4.22)

IR : ν_{max} cm^{-1}, film; Figure 43

3177, 1653, 1605, 1504, 1167, 799

^{1}H-NMR : δ ppm, 300 MHz, in DMSO-d_6 ; Figure 45, Table 18

^{13}C-NMR : : δ ppm, 75 MHz, in DMSO-d_6 ; Figure 46, Table 18

4.6 Compound GT-6 [203]

Compound GT-6 was obtained as pale yellow needles, soluble in acetone and methanol (6 mg, 4.61 x 10^{-4} % based on dried weight of leaves).

ESIMS : $[M+H]^+$ *m/z* 345.14 (positive ion mode); Figure 52

UV : λ_{max} nm (log ε), in methanol; Figure 51

251 (4.22), 345 (4.20)

IR : ν_{max} cm^{-1}, film; Figure 50

3438, 2915, 1603, 1457, 1217, 823

^{1}H-NMR : δ ppm, 300 MHz, in acetone-d_6 ; Figure 53, Table 19

4.7 Compound GT-7 [204]

Compound GT-7 was obtained as pale yellow needles, soluble in chloroform (220 mg, 1.69 x 10^{-2} % based on dried weight of leaves).

ESIMS : $[M+H]^+$ *m/z* 373.12 (positive ion mode); Figure 56

UV : λ_{max} nm (log ε), in methanol; Figure 54

247 (4.60), 339 (4.58)

IR : ν_{max} cm^{-1}, film; Figure 55

2936, 1627, 1604, 1515, 1216, 822, 750

^{1}H-NMR : δ ppm, 300 MHz, in $CDCl_3$; Figure 57, Table 20

^{13}C-NMR : δ ppm, 75 MHz, in $CDCl_3$; Figure 58, Table 20

4.8 Compound GT-8 [205]

Compound was obtained as pale yellow needles, soluble in chloroform (7 mg, 5.38 x 10^{-4} % based on dried weight of leaves).

ESIMS : $[M+H]^+$ *m/z* 359.10 (positive ion mode); Figure 64

UV : λ_{max} nm (log ε), in methanol; Figure 62

248 (4.181), 342 (4.286)

IR : ν_{max} cm^{-1}, film; Figure 63

3439, 2926, 1623, 1604, 1458, 1216, 823, 754

^{1}H-NMR : δ ppm, 300 MHz, in $CDCl_3$; Figure 65, Table 21

^{13}C-NMR : δ ppm, 75 MHz, in $CDCl_3$; Figure 66, Table 21

4.9 Compound GT-9 [206]

Compound GT-9 was obtained as pale yellow needles, soluble in chloroform (7 mg, 5.38×10^{-4} % based on dried weight of leaves).

HRESIMS : $[M+H]^+$ at *m/z* 359.1129 (positive ion mode)

calcd for $C_{19}H_{18}O_7$ 359.1131

ESIMS : $[M+H]^+$ *m/z* 359.03 (positive ion mode); Figure 71

UV : λ_{max} nm (log ε), in methanol; Figure 69

250 (4.27), 343 (4.24)

IR : ν_{max} cm^{-1}, film; Figure 70

3421, 2919, 1631, 1603, 1437, 1023, 802

^{1}H-NMR : δ ppm, 300 MHz, in $CDCl_3$; Figure 72, Table 22

^{13}C-NMR : δ ppm, 75 MHz, in $CDCl_3$; Figure 73, Table 22

5. Determination of free radical scavenging activity

5.1 TLC screening assay (Calis *et al*., 1999)

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

5.2 Free radical scavenging activity assay (Sritularak, 2002)

5.2.1 Preparation of the test sample

Pure compounds from *G. tenuifolius* were first tested at 40 µg/ml. Compounds exhibiting more than 50% inhibition were further analyzed for their IC_{50} values. Each test sample was prepared as an ethanolic solution with initial concentration of 400 µg/ml. For IC_{50} analysis, serial dilution was performed to give seven concentrations (200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml). Assays were carried out in duplicate. The test sample (20µl) was added to the reaction mixture (180 µl) to furnish the total volume of 200 µl. The final concentration (Table 13) was calculated by the formula below.

$$N_1V_1 = N_2V_2$$

N_1 = Beginning concentration (µM)

V_1 = Beginning volume (µl)

N_2 = Final concentration (µM)

V_2 = Final volume (µl)

For example, 20 µl of test sample (400 µg/ml) was added to the reaction mixture to furnish the total volume of 200 µl.

Thus, final concentration of test sample = 400 µg/ml x 20 µl / 200 µl

= 40 µg/ml

Table 13 The initial and final concentrations (µg/ml) of test sample

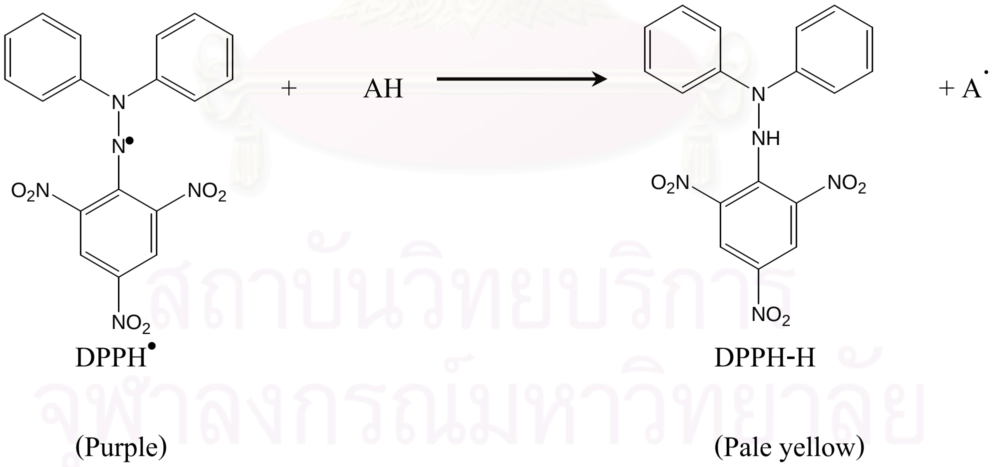
Initial concentration (µg/ml)	400	200	100	50	25	12.5	6.25	3.125
Final concentration (µg/ml)	40	20	10	5	2.5	1.25	0.625	0.312

5.2.2 Preparation of the DPPH solution (50 µM)

Two mg of DPPH (MW 394.32) was dissolved in 100 ml of ethanol and the solution was subsequently stirred for 30 min.

5.2.3 Measurement of activity

The test sample (20 µl) was added to 180 µl of DPPH solution (50 µM) in a 96-well microtiter plate. The reaction mixture was incubated at 37° C for 30 min, and then the absorbance of each well was measured at 510 nm. The DPPH solution was used as negative control. Quercetin was used as reference compound.



DPPH = 1,1-diphenyl-2-picrylhydrazyl

AH = antioxidant

Scheme 7 Structure of DPPH and reaction with antioxidant

5.2.4 Calculation of percentage of free radical scavenging activity

The percentage of scavenging activity was calculated as follows.

$$\% \text{ DPPH reduction} = \left[\frac{A - B}{A} \right] \times 100$$

A : The absorbance of DPPH solution after incubation at 510 nm.

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

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

CHAPTER IV

RESULTS AND DISCUSSION

The dried leaves of *Goniothalamus tenuifolius* King (1.3 kg) were extracted with pet ether, ethyl acetate and methanol to give a pet ether extract (29.4 g), ethyl acetate extract (97.7 g) and methanol extract (114.7 g). Each extract was subjected to free radical scavenging activity evaluation. The ethyl acetate and methanol extracts were equally active with the DPPH assay. The ethyl acetate extract was further separated using several chromatographic techniques to yield nine compounds (compounds GT-1 to GT-9). The structures of all of the isolates were determined by interpretation of their UV, IR, NMR and MS data and comparison with previously reported values.

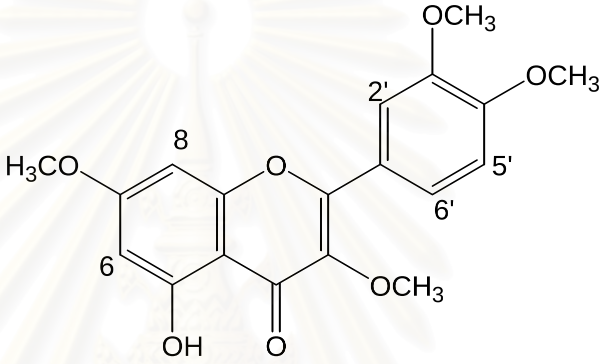
1. Structure determination of isolated compounds

1.1 Structure determination of compound GT-1

Compound GT-1 was obtained as pale yellow needles. The positive ESIMS (Figure 16) exhibited its $[M+H]^+$ ion at *m/z* 359, suggesting the molecular formula $C_{19}H_{18}O_7$. The IR sprectrum (Figure 15) showed absorption bands at 3448 (O-H stretching, H-bonded), 2920 (C-H stretching), 1648 (C=O stretching), 1589 and 1495 (C=C aromatic ring), 1149 (C-O stretching), 812 and 796 (=C-H bending) cm^{-1}. The UV absorptions at 351, 252 nm (Figure 14) and the presence of a phenolic proton signal in the ^1H NMR spectrum at δ 12.61 ppm indicated a 5-hydroxyflavone compound (Vidari, Finzi and Bernardi, 1971).

The ^1H-NMR spectrum (Figure 17) revealed the presence of four methoxyl groups at δ 3.84, 3.86, 3.94 and 3.95 (3H each, s). The substitution pattern of the A ring showed an AB system of two aromatic protons (H-6 and H-8) with typical *meta*-coupling constant at δ 6.34 (d, J = 2.1 Hz, H-6) and δ 6.43 (d, J = 2.1 Hz, H-8). The remaining three aromatic protons formed the characteristic pattern for a 3', 4'-disubstituted B ring (Harborne and Mabry, 1982). The signals of the H-2' at δ 7.67 (d, J = 1.8 Hz) and H-6' at δ 7.72 (dd, J = 8.5, 1.8 Hz) were overlapping. The doublet signal at δ 6.97 could be assigned to H-5'.

Compound GT-1 was identified as retusine (5-hydroxy-3,7,3', 4'-tetramethoxyflavone) **[198]**, based on the above spectral data. The ^{1}H-NMR and ^{13}C-NMR spectra were in close agreement with previously published values (Ohashi *et al.*, 1999 and Dong *et al.*, 1999) as shown in Table 14.



Retusine **[198]**

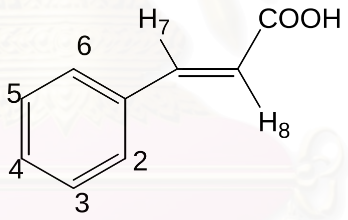
Table 14 NMR Spectral data of compound GT-1 and retusine

Position	Compound GT-1 ($CDCl_3$)		Retusine ($CDCl_3$)	
	^1H (mult, *J* in Hz)	^{13}C	^1H (mult, *J* in Hz)	^{13}C
2	-	155.8	-	155.6
3	-	139.0	-	138.8
4	-	178.7	-	178.6
5	-	156.7	-	156.6
6	6.34 (d, 2.1)	97.8	6.36 (d, 2.2)	97.7
7	-	165.4	-	165.3
8	6.43 (d, 2.1)	92.2	6.45 (d, 2.2)	92.1
9	-	162.0	-	161.9
10	-	106.0	-	105.9
1'	-	122.9	-	122.8
2'	7.67 (d, 1.8)	110.8	7.69 (d, 2.2)	110.7
3'	-	148.8	-	148.6
4'	-	151.4	-	151.3
5'	6.97 (d, 8.5)	111.3	7.00 (d, 8.4)	110.4
6'	7.72 (dd, 8.5, 1.8)	122.1	7.74 (dd, 8.4, 2.2)	122.1
OCH_3, OCH_3, OCH_3, OCH_3	3.84 (s), 3.86 (s) 3.94 (s), 3.95 (s)	60.1, 55.8 55.9, 56.0	3.87, 3.89 3.97, 3.98	60.9, 55.7, 55.8, 55.9
OH	12.64 (br s)	-	12.65 (br s)	-

1.2 Structure determination of compound GT-2

Compound GT-2 was obtained as pale yellow needles. The negative ESIMS (Figture 21) showed its $[M-H]^-$ ion at *m/z* 147 indicated the molecular formula $C_9H_8O_2$. The UV spectrum (Figure 19) displayed absorptions at 214 and 270 nm. The IR spectrum (Figure 20) exhibited absorption bands at 3022 (O-H stretching), 1681 (C=O stretching), 1629 and1448 (C=C aromatic ring), 1286 (C-O stretching) and 933 (*trans* -CH=CH-) cm^{-1}.

In the ^1H-NMR spectrum (Figure 22), *trans*-olefinic protons were observed at δ 6.44 (H-8) and δ 7.78 (H-7) (each d, J = 15.9 Hz). In the aromatic region, a set of three aromatic protons including H-3, H-4 and H-5 showed as multiplet signals at δ 7.52-7.54 ppm. The complex signal at δ 7.38-7.40 ppm belonged to H-2 and H-6. These data indicated that this compound was *trans*-cinnamic acid **[199]**. Comparison of the ^1H-NMR and ^{13}C-NMR spectra of this compound with those of *trans*-cinnamic acid (Hanai *et al.*, 2001) comfirmed the structure (Table 15).



trans-Cinnamic acid **[199]**

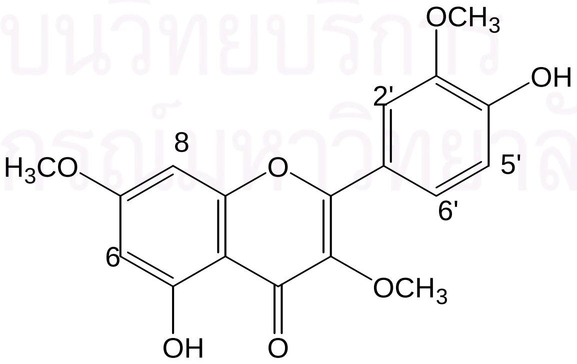
Table 15 NMR Spectral data of compound GT-2 and *trans*-cinnamic acid

Position	Compound GT-2 ($CDCl_3$)		*trans*-Cinnamic acid ($CDCl_3$)	
	^{1}H (mult, *J* in Hz)	^{13}C	^{1}H (mult, *J* in Hz)	^{13}C
1	-	133.9	-	134.0
2	7.52 -7.55 (m)	128.2	7.54-7.57 (m)	128.3
3	7.38-7.40 (m)	128.8	7.40-7.41 (m)	128.9
4	7.38-7.40 (m)	130.5	7.40-7.41 (m)	130.7
5	7.38-7.40 (m)	128.8	7.40-7.41 (m)	128.9
6	7.52 -7.55 (m)	128.2	7.54-7.57 (m)	128.3
7	7.78 (d, 15.9)	146.8	7.80 (d, 16.1)	147.1
8	6.44 (d, 15.9)	117.2	6.46 (d, 16.1)	117.3
9	-	171.9	-	172.6

1.3 Structure determination of compound GT-3

Compound GT-3 was obtained as yellow needles. The positive ESIMS (Figure 26) exhibited its $[M+H]^+$ ion at *m/z* 345, suggesting the molecular formula $C_{18}H_{16}O_7$. The UV spectrum displayed absorptions at 253 and 356 nm (Figure 24). The IR sprectrum (Figure 25) showed absorption bands at 3432 (O-H stretching, H-bonded), 2927 (C-H stretching), 1656 (C=O stretching), 1593 and 1496 (C=C aromatic ring), 1212 (C-O stretching), 811 and 783 (=C-H bending) cm^{-1}. The presence of IR absorption band at 3432 cm^{-1} and the ^1H-NMR signal at δ 12.61 ppm suggested a 5- hydroxyflavone structure.

The ^1H-NMR spectrum (Figure 27) showed the presence of three methoxyl groups at δ 3.83, 3.85 and 3.96 ppm (3H each, s). They were located at C-3, C-7 and C-3' respectively based on their correlations in a NOESY experiment. The A ring displayed signals for protons with *meta*-coupling at δ 6.33 (d, J = 1.8 Hz, H-6,) and δ 6.42 (d, J = 1.8 Hz, H-8). The B ring protons showed an ABX system at δ 7.02 (d, J = 8.4 Hz, H-5'), with overlaping signals for H-6' and H-2' at δ 7.63 and 7.67 ppm, respectively. The siglet signal at δ 6.01 could be assigned to the OH group at C-4' position. The ^1H- and ^{13}C-NMR assignments of this compound were thoroughly studied by ^{13}C-NMR, DEPT 90, DEPT 135 (Figure 28), HMQC (Figure 30), HMBC (Figure 31) and NOESY (Figure 29) experiments. Based on the above spectral data, compound GT-3 was identified as 5,4'-dihydroxy-3,7,3'-trimethoxyflavone (pachypodol) **[200]**, which was previously reported by Itokawa *et al.* (Itokawa, Suto and Takeya, 1981). The earlier assignments of three methoxyl groups were revised in this study, based on the 2D-NMR data (Table 16).



Pachypodol **[200]**

Table 16 NMR Spectral data of compound GT-3 and pachypodol

Position	Compound GT-3 ($CDCl_3$)		Pachypodol (DMSO-d_6)	
	^1H (mult, J in Hz)	^{13}C	^1H (mult, J in Hz)	^{13}C
2	-	155.8	-	155.8
3	-	138.7	-	137.9
4	-	178.6	-	178.0
5	-	161.9	-	160.9
6	6.33 (d, 1.8)	97.7	6.34 (d, 2.0)	97.8
7	-	165.3	-	165.1
8	6.42 (d, 1.8)	92.1	6.74 (d, 2.0)	92.4
9	-	156.6	-	156.3
10	-	105.9	-	105.2
1'	-	122.3	-	120.7
2'	7.67	110.8	7.66	112.1
3'	-	146.3	-	147.5
4'	-	148.3	-	150.0
5'	7.02 (d, 8.4)	114.5	6.95 (d, 8.0)	115.7
6'	7.63 (dd, 8.4, 1.8)	122.6	7.61 (dd, 9.0, 2.0)	122.3
3-OCH_3	3.83*	60.1	3.89	59.7
7-OCH_3	3.84*	55.7	3.87	56.2
3'-OCH_3	3.95*	56.0	3.84	55.9

* Revised assignments

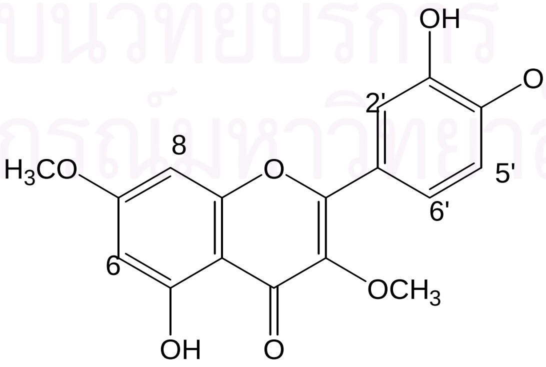
1.4 Structure determination of compound GT-4

Compound GT-4 was obtained as yellow needles. The positive ESIMS (Figure 34) showed its $[M+H]^+$ ion at *m/z* 331 suggested the molecular formula $C_{17}H_{14}O_7$. The UV spectrum (Figure 32) exhibited absorptions at 256 and 356 nm. The IR sprectrum (Figure 33) displayed absorption bands at 3431 (O-H stretching, H-bonded), 3181 (C-H stretching), 1662 (C=O stretching), 1590 and 1497 (C=C aromatic ring), 1309 (C-O stretching), 823 (=C-H bending) cm^{-1}. The ^{1}H-NMR spectrum (Figures 35 and 36) presented a phenolic proton at δ 12.70 ppm indicating a 5- hydroxyflavone structure, similar to compounds GT-1 and GT-3.

The ^{1}H-NMR (DMSO-d_6) spectrum (Figure 35) also exhibited signals for protons of ring A at δ 6.30 (br s, H-6), 6.63 (br s, H-8). The B ring showed a splitting pattern for ABX system at δ 6.89 (d, J = 8.1 Hz, H-5'), 7.45 (d, J = 8.1 Hz, H-6') and 7.58 (br s, H-2').

The first methoxyl at δ 3.78 ppm could be placed at C-3 according to its NOESY correlation peak with H-2' and H-6' and its HMBC correlation with C-3. The second methoxyl at δ 3.82 ppm was located at C-7, as shown by its NOESY interaction with H-6 and H-8 and its HMBC correlation with C-7. The NOESY and HMBC data suggested a revision for the earlier assignment of the methoxyl groups (Urbatsch *et al.*, 1976).

From the above ^{1}H-NMR data, together with the information from ^{13}C-NMR (Figures 37 and 38), HMQC (Figure 40), HMBC (Figure 41) and NOESY (Figure 39) experiments, compound GT-4 was identified as 5-3',4'-trihydroxy-3,7-dimethoxyflavone (kumatakenin) **[201]**. This compound has been isolated from *Ericameria diffusa* (Compositae) (Urbatsch *et al.*, 1976). The ^{1}H- and ^{13}C-NMR data are demonstrated in Table 17.



Kumatakenin **[201]**

Table 17 NMR Spectral data of compound GT-4 and kumatakenin

Position	Compound GT-4				Kumatakenin	
	^{1}H (mult, J in Hz)		^{13}C		^{1}H (mult, J in Hz)	^{13}C
	acetone-d_6	DMSO-d_6	acetone-d_6	DMSO-d_6	DMSO-d_6	DMSO-d_6
2	-	-	157.6	156.0	-	156.1
3	-	-	140.0	137.6	-	137.8
4	-	-	179.5	177.7	-	177.9
5	-	-	162.7	160.7	-	160.8
6	6.29 (d, 1.8)	6.30 (br s)	98.4	97.5	6.17 (d, 2.5)	97.6
7	-	-	166.5	164.8	-	165.0
8	6.62 (d, 1.8)	6.63 (br s)	92.7	92.0	6.45 (d, 2.5)	92.1
9	-	-	156.9	155.7	-	155.8
10	-	-	106.5	105.0	-	105.0
1'	-	-	122.8	120.6	-	120.6
2'	7.70 (d, 1.8)	7.58 (br s)	116.3	115.4	7.66 (d, 2.0)	115.4
3'	-	-	145.9	145.0	-	145.1
4'	-	-	149.2	148.6	-	148.7
5'	6.97 (d, 8.4)	6.89 (d, 8.1)	116.3	115.6	6.80 (d, 8.5)	115.6
6'	7.57 (dd, 8.4, 1.8)	7.45 (d, 8.1)	122.1	120.5	7.43 (dd, 8.5, 2.0)	120.5
3-OCH$_3$	3.82 (s)*	3.78 (s)*	60.1	59.6	3.84	59.5
7-OCH$_3$	3.95 (s) *	3.82 (s)*	56.3	56.0	3.78	55.9
OH	12.64 (br s)	12.66 (br s)	-	-	-	-

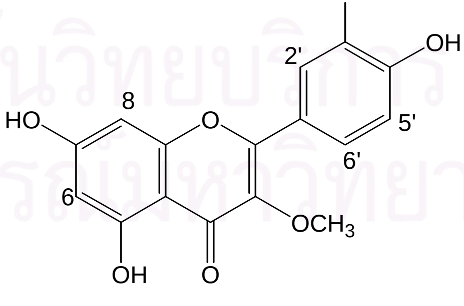
* Revised assignments

1.5 Structure determination of compound GT-5

Compound GT-5 was obtain as yellow needles. The UV spectrum (Figure 42) displayed absorptions at 256 and 358 nm. The IR sprectrum (Figure 43) exhibited absorption bands at 3177 (O-H stretching, H-bonded), 1653 (C=O stretching), 1605 and 1504 (C=C aromatic ring), 1167 (C-O stretching) and 799 (=C-H bending) cm^{-1}. The positive ESIMS (Figure 44) showed its $[M+H]^+$ ion at *m/z* 317, suggesting the molecular formula $C_{16}H_{12}O_7$.

The ^{1}H-NMR spectrum (Figure 45) showed a H-bonded phenolic proton at δ 12.69 ppm, indicating a 5- hydroxyflavone structure. It also exhibited two doublet signals at δ 6.17 and 6.39 (each d, *J* = 1.5 Hz), assignable to H-6 and H-8 of ring A. The assignments of H-6 and H-8 were based on its HMQC correlation to C-6 (δ 98.6) and C-8 (δ 93.6) respectively. The ^{1}H-NMR spectral data, furthermore, revealed the presence of a methoxyl group at δ 3.76 (3H, s) which was placed at C-3 according to its NOESY correlation with H-2' and its HMBC correlation with C-3. For ring B, the presence of an ABX spin system at δ 6.89 (d, *J* = 8.4 Hz, H-5'), δ 7.43 (dd, *J* = 8.4, 1.8 Hz, H-6') and δ 7.53 (d, *J* = 1.8 Hz, H-2') in the ^{1}H-NMR spectrum, placed the hydroxy groups at C-3' and C-4' positions.

Based on the above spectral evidence, and comparison of the ^{1}H-and ^{13}C-NMR spectral of compound GT-5 with those of the previously reported structure (Urbatsch, Bacon and Mabry, 1975 and Wang, Hamburger, Gueho and Hostettmann, 1989), the compound was identified as 5,7,3',4'-tetrahydroxy-3-methoxyflavone **[202]**, as shown in Table 18.



5,7,3',4'-Tetrahydroxy-3-methoxyflavone **[202]**

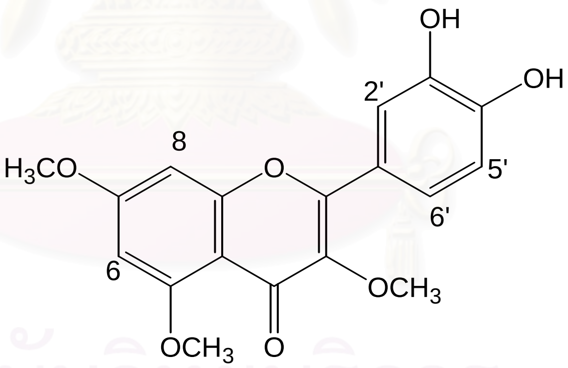
Table 18 NMR Spectral data of compound GT-5 and 5,7,3',4'-tetrahydroxy-3-methoxyflavone

Position	Compound GT-5		5,7,3',4'-Tetrahydroxy-3-methoxyflavone	
	^{1}H (mult, *J* in Hz)	^{13}C	^{1}H (mult, *J* in Hz)	^{13}C
	DMSO-d_6	DMSO-d_6	CCl_4 (OCH_3 in C_6D_6)	DMSO-d_6
2	-	155.6	-	156.2
3	-	137.7	-	137.6
4	-	177.9	-	177.8
5	-	161.3	-	161.2
6	6.17 (d, 1.5)	98.6	6.16 (d, 2.5)	98.4
7	-	164.2	-	164.0
8	6.39 (d, 1.5)	93.6	6.47 (d, 2.5)	93.5
9	-	156.4	-	155.5
10	-	104.2	-	104.1
1'	-	120.8	-	120.7
2'	7.53 (d, 1.8)	115.4	7.77 (d. 2.0)	115.3
3'	-	145.3	-	145.1
4'	-	148.7	-	148.6
5'	6.89 (d, 8.4)	115.8	6.38 (d, 9.0)	115.6
6'	7.43 (dd, 8.4, 1.8)	120.6	7.58 (dd, 9.0, 2.0)	120.5
3-OCH_3	3.76 (s)	59.7	3.87	59.5
OH	12.69 (br s)	-	-	-

1.6 Structure determination of compound GT-6

Compound GT-6, pale yellow needles, showed its $[M+H]^+$ ion at *m/z* 345 in th ESIMS, corresponding to the molecular formula $C_{18}H_{16}O_7$. The IR bands at 3438 (O-H stretching), 2915 (C-H stretching), 1603 (C=O stretching), 1457 (conjugated C=C), 1217 (C-O stretching) and 823 (=C-H bending) cm^{-1} (Figure 51) and the UV absorptions at 251 and 345 nm (Figure 50) were indicative of a flavone skeleton (Markham, 1982).

The ^{1}H-NMR spectrum (Figure 53) exhibited the signals similar to those of compound GT-4. The A ring protons appeared at δ 6.43 (d, *J* = 2.2 Hz, H-6), 6.69 (d, *J* = 2.2 Hz, H-8). Ring B showed signals for three protons at δ 6.94 (d, *J* = 8.5 Hz, H-5'), δ 7.55 (dd, *J* = 8.5, 2.0 Hz, H-6') and δ 7.68 (d, *J* = 2.0 Hz, H-2'). However, GT-6 had no H-bonded phenolic group, but possessed a methoxyl group at C-5. This was supported by the resonances of three methoxyls at δ 3.77, 3.88 and 3.93 ppm. Compound GT-6 was identified as 3',4'-dihydroxy-3,5,7-trimethoxyflavone **[203]**. It has been reported as a diacetate derivative (Ohashi *et al.*, 1999). This work is the first report of the ^{1}H-NMR spectral data (Table 19).



3',4'-dihydroxy-3,5,7-trimethoxyflavone **[203]**

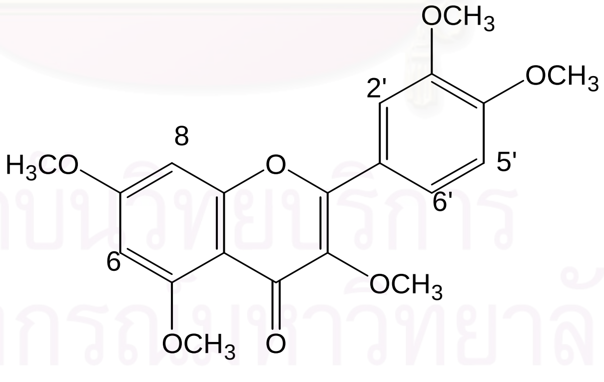
Table 19 NMR Spectral data of compound GT-6 (acetone-d_6)

Position	Compound GT-6
	^{1}H (mult, J in Hz)
2	-
3	-
4	-
5	-
6	6.45 (d, 2.2)
7	-
8	6.72 (d, 2.2)
9	-
10	-
1'	-
2'	7.69 (d, 2.0)
3'	-
4'	-
5'	6.97 (d, 8.5)
6'	7.55 (dd, 8.5, 2.0)
OCH_3	3.93 (s)
OCH_3	3.88 (s)
OCH_3	3.79 (s)

1.7 Structure determination of compound GT-7

Compound GT-7 appeared as pale yellow needles. The positive ESI mass spectrum (Figure 56) displayed its $[M+H]^+$ ion at *m/z* 373, suggesting the molecular formula $C_{20}H_{20}O_7$. The UV spectrum (Figure 54) showed λ_{max} at 247 and 339 nm. The IR sprectrum (Figure 55) exhibited absorption bands at 2936 (C-H stretching), 1627 (C=O stretching), 1604 and 1515 (C=C aromatic ring), 1216 (C-O stretching) and 822, 750 (=C-H bending) cm^{-1}, suggestive of a flavone skeleton.

The ^{1}H-NMR spectrum (Figure 57) revealed the presence of five methoxyl groups at δ 3.86, 3.88, 3.93, 3.93, 3.93 (3H each, s). The A ring showed an AB coupling system of the two aromatic protons (H-6 and H-8) at δ 6.33 (d, J = 1.8 Hz) and 6.48 (d, J = 1.8 Hz), respectively. Ring B exhibited signals for an ABX system at δ 6.95 (d, J = 8.4 Hz, H-5'), 7.66 (br s, H-6') and 7.69 (br s, H-2'). Compound GT-7 was determined as 3,5,7,3',4'-pentamethoxyflavone **[204]**, a flavone previously isolated from *Amomum koenigii* (Dong *et al.*, 1999). The ^{1}H-and ^{13}C-NMR data (Figures 57 and 58) obtained in this study suggested that the assignments of H-2', H-6' and the methoxyl groups at C-3, C-7 and C-3' should be revised. The successful revision was accomphished by application of 2D-NMR, including NOESY (Figure 59), HMQC (Figure 60) and HMBC (Figure 61) experiments.



3,5,7,3',4'-Pentamethoxyflavone **[204]**

Table 20 NMR Spectral data of compound GT-7 ($CDCl_3$) and 3,5,7,3',4'-pentamethoxyflavone ($CDCl_3$)

Position	^{1}H (mult, *J* in Hz)		Position	^{13}C	
	Compound GT-7	3,5,7,3',4'-Pentamethoxyflavone		Compound GT-7	3,5,7,3',4'-Pentamethoxyflavone
2	-	-	2	152.5	152.6
3	-	-	3	141.1	141.2
4	-	-	4	174.0	174.0
5	-	-	5	**161.0**	**158.8**
6	6.33 (d, 1.8)	6.32	6	95.7	95.8
7	-	-	7	163.8	163.9
8	6.48 (d, 1.8)	6.48	8	92.8	92.5
9	-	-	9	**158.7**	**161.1**
10	-	-	10	109.5	109.5
1'	-	-	1'	123.4	123.4
2'	**7.69** (br s)	**7.67**	2'	111.3	111.3
3'	-	-	3'	148.7	148.7
4'	-	-	4'	150.8	150.9
5'	6.95 (d, 8.4)	6.93	5'	110.8	110.8
6'	**7.66 (br s)**	**7.68**	6'	121.6	121.6
3-OCH_3	**3.93 (s)**	**3.88**	OCH_3	59.9	59.9
5-OCH_3	**3.93 (s)**	**3.91**	OCH_3	55.7	55.8
7-OCH_3	**3.88 (s)**	**3.97**	OCH_3	55.9	55.9
3'-OCH_3	**3.86 (s)**	**3.97**	OCH_3	56.0	56.1
4'-OCH_3	**3.93 (s)**	**3.97**	OCH_3	56.3	56.4

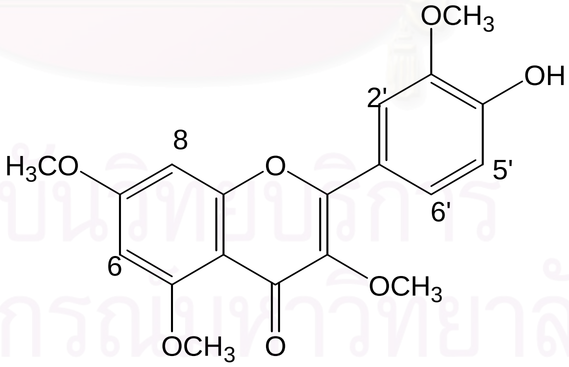
- : The bold values are revised assignments.

1.8 Structure determination of compound GT-8

Compound GT-8 was obtained as yellow needles. The UV spectrum (Figure 62) exhibited absorptions at 248 and 342 nm. The positive ESI mass spectrum (Figure 64) showed its $[M+H]^+$ ion at *m/z* 359 corresponding to the molecular formula $C_{19}H_{18}O_7$. The IR sprectrum (Figure 63) displayed absorption bands at 3439 (O-H stretching), 2926 (C-H stretching), 1623 (C=O stretching), 1604 and 1458 (C=C aromatic ring), 1216 (C-O stretching), 823 and 754 (=C-H bending) cm^{-1}, characteristic of a flavone skeleton (Markham, 1982).

The ^1H-NMR sprectrum (Figure 65) displayed a broad OH proton signal at δ 5.97 ppm. This spectrum also exhibited the presence of four methoxy groups at δ 3.84, 3.88, 3.94 and 3.94 (each 3H, s). In the aromatic region, signals for the A ring protons appeared at δ 6.32 (d, *J* = 2.1 Hz, H-6) and 6.48 (d, *J* = 2.1 Hz, H-8). An ABX system at δ 7.00 (d, *J* = 8.4 Hz, H-5'), 7.61(dd, *J* = 8.4, 1.8 Hz, H-6') and 7.72 (d, *J* = 1.8 Hz, H-2') were observed for ring B.

The results from NOESY and HMQC experiments indicated that four methoxyl groups were on C-3, C-5, C-7 and C-3' position. Consequently, this compound was determined to be 4'-hydroxy-3,5,7,3'-tetramethoxyflavone **[205]**. It has been previously isolated from *Melicope triphylla* (Higa, Miyagi, Yogi and Hokama, 1987). The present work is the first report of the ^{13}C-NMR spectral data of this compound (Table 21).



4'-Hydroxy-3,5,7,3'-tetramethoxyflavone **[205]**

Table 21 NMR Spectral data of compound GT-8 ($CDCl_3$) and 4'-hydroxy-3,5,7,3'-tetramethoxyflavone ($CDCl_3$)

Position	Compound GT-8		Position	4'-Hydroxy-3,5,7,3'-tetramethoxyflavone
	^1H (mult, J in Hz)	^{13}C		^1H (mult, J in Hz)
2	-	152.4	2	-
3	-	140.8	3	-
4	-	173.7	4	-
5	-	160.8	5	-
6	6.32 (d, 2.1)	95.7	6	6.32 (d, 2.5)
7	-	163.6	7	-
8	6.48 (d, 2.1)	92.3	8	6.48 (d, 2.5)
9	-	158.8	9	-
10	-	109.4	10	-
1'	-	122.7	1'	-
2'	7.72 (d, 1.8)	110.8	2'	7.67 (d, 2.0)
3'	-	146.1	3'	-
4'	-	147.5	4'	-
5'	7.00 (d, 8.4)	114.3	5'	7.02 (d, 9.0)
6'	7.61 (dd, 8.4, 1.8)	121.9	6'	7.59 (dd, 9.0, 2.0)
3-OCH_3	3.84 (s)	59.9	OCH_3	3.84
5-OCH_3	3.94 (s)	56.1	OCH_3	3.94
7-OCH_3	3.88 (s)	55.7	OCH_3	3.84
3'-OCH_3	3.94 (s)	56.6	OCH_3	3.94
4'-OH	5.97 (br s)	-	OH	-

1.9 Structure determination of compound GT-9

Compound GT-9 was obtained as pale yellow needles. The IR sprectrum (Figure 70) showed absorption bands at 3421 (O-H stretching), 2919 (C-H stretching), 1631 (C=O stretching), 1603 and 1437 (C=C aromatic ring), 1023 (C-O stretching), 802 (=C-H bending) cm^{-1}. The positive ESIMS (Figure 71) displayed its $[M+H]^+$ ion at *m/z* 359, suggesting the molecular formula $C_{19}H_{18}O_7$. The UV spectrum (Figure 69) exhibited absorption bands at 250, 343 nm, indicative of flavone skeleton (Markham, 1982).

The ^1H-NMR spectrum (Figure 72) showed signals for a set of protons with *meta* - coupling at δ 6.31 (d, J = 2.1 Hz, H-6) and 6.48 (d, J = 2.1 Hz, H-8). On ring B, an ABX splitting pattern consisting of overlapping signals appeared at δ 7.65 (d, J = 2.1 Hz, H-2') and 7.69 (dd, J = 8.4, 2.1 Hz, H-6') and a broad doublet at 6.93 (d, J = 8.4 Hz, H-5'). The sharp proton singlet at δ 5.67 could be assigned to the hydroxyl group at C-3'. The ^{13}C-NMR (Figure 73) showed 19 signals, corresponding to four methoxyls, five methines and ten quaternary carbons.

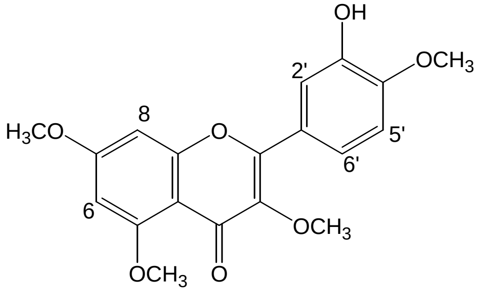
Further analysis of the ^1H-and ^{13}C-NMR spectra (Figures 72 and 73, respectively) revealed the presence of four methoxyl groups at δ_H 3.85, 3.93, 3.87, 3.95 (3H each, s) and δ_C 59.8 (3-OCH_3), 56.3 (5-OCH_3), 55.7 (7-OCH_3) and 56.0 (4'-OCH_3), respectively. The assignments of these methoxyl groups were based on the NOESY (Figure 74), HMQC (Figure 75) and HMBC (Figure 76) experiments.

The first methoxyl group at δ 3.85 ppm could be placed at C-3 according to its NOESY correlation peak with H-6' and H-2' and HMBC correlation of the methoxyl group with C-3. The second methoxyl group at δ 3.87 ppm was located at C-7, as shown by its NOESY interaction with H-8 and H-6 and the HMBC correlation of the methoxyl group with C-7. The third methoxyl group at δ 3.93 should be assigned at C-5 based on the NOESY correlation peak with H-6 and the HMBC correlation of the methoxyl group with C-5. The last methoxyl group at δ 3.95 was placed at C-4', as exhibited by its NOESY interaction with H-5' and the HMBC correlation of the methoxyl group with C-4'.

Thus, compound GT-9 was identified as 3'-hydroxy-3,5,7,4'-tetramethoxyflavone **[206]**. Although this compound has been earlier synthesized (Bouktaib, Lebrun, Atmani and Rolando, 2002, Beutler *et al.*, 1998, Parmar *et al.*, 1996, Parmar *et al.*, 1994, Wang, Hamburger, Gueho and Hostettmann, 1989), this is the first time it has been found as a naturally occuring compound. Prior to this study, the ^{13}C data of this compound have not been reported.

Table 22 NMR Spectral data of compound GT-9 ($CDCl_3$) and 3'-hydroxy-3,5,7,4'-tetramethoxyflavone ($CDCl_3$)

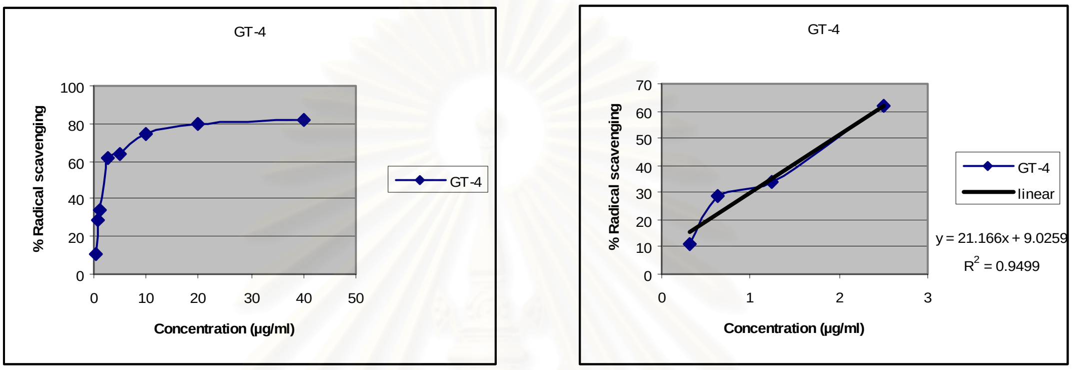
Position	Compound GT-9		Position	3'-Hydroxy-3,5,7,4'-tetramethoxyflavone
	^{1}H (mult, *J* in Hz)	^{13}C		^{1}H (mult, *J* in Hz)
2	-	152.3	2	-
3	-	141.3	3	-
4	-	174.0	4	-
5	-	161.0	5	-
6	6.31 (d, 2.1)	95.7	6	6.35 (s)
7	-	163.8	7	-
8	6.48 (d, 2.1)	92.4	8	6.55 (s)
9	-	158.8	9	-
10	-	109.5	10	-
1'	-	124.1	1'	-
2'	7.65 (d, 2.1)	114.1	2'	7.50-7.65 (m)
3'	-	145.4	3'	-
4'	-	148.2	4'	-
5'	6.93 (d, 8.4)	110.3	5'	6.95 (d, 9.0)
6'	7.69 (dd, 8.4, 2.1)	121.2	6'	7.50-7.65 (m)
3-OCH_3	3.85 (s)	59.8	OCH_3	3.77
5-OCH_3	3.93 (s)	56.3	OCH_3	3.88
7-OCH_3	3.87 (s)	55.7	OCH_3	3.90
4'-OCH_3	3.95 (s)	56.0	OCH_3	3.93
3'-OH	5.67 (s)	-	OH	-



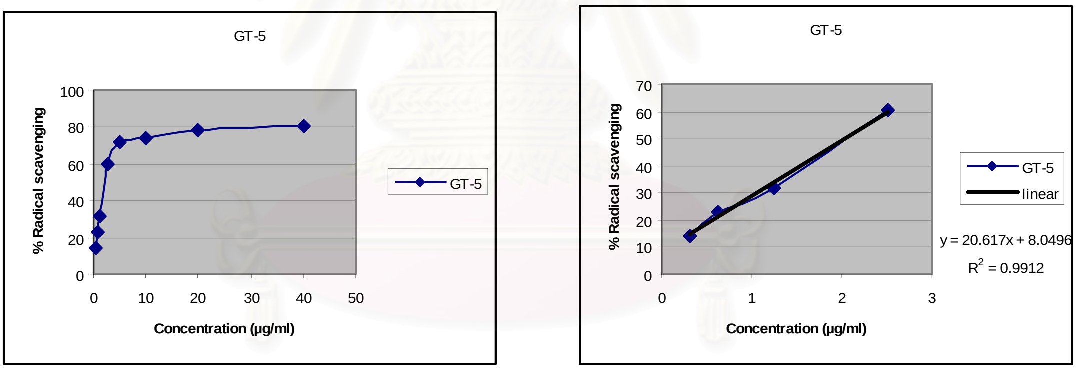
3'-Hydroxy-3,5,7,4'-tetramethoxyflavone **[206]**

2. Free radical scavenging activity

By TLC screening assay, the ethyl acetate and methanol extracts from the leaves of *Goniothalamus tenuifolius* showed free radical scavenging activity. Pure compounds from *G. tenuifolius* were first tested at 40 µg/ml. Compounds exhibiting more than 50% inhibition were further analyzed for their IC$_{50}$ values (Figure 12). Quercetin was used as positive control. The results are summarized in Table 23



A.



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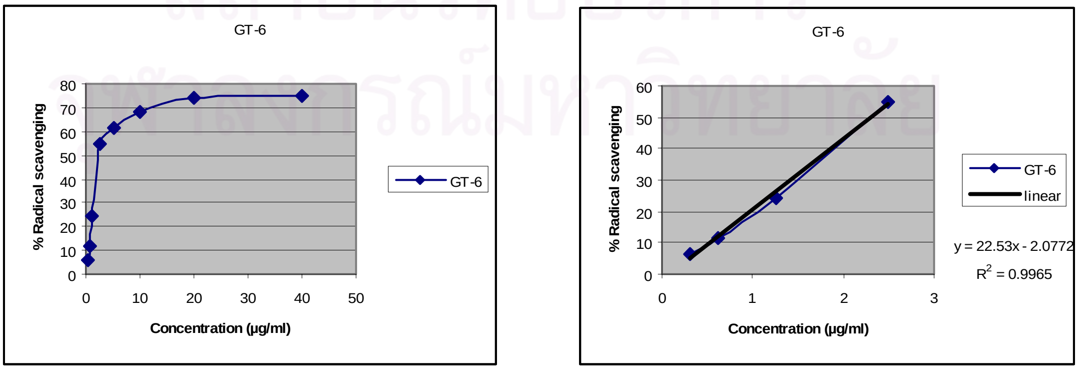
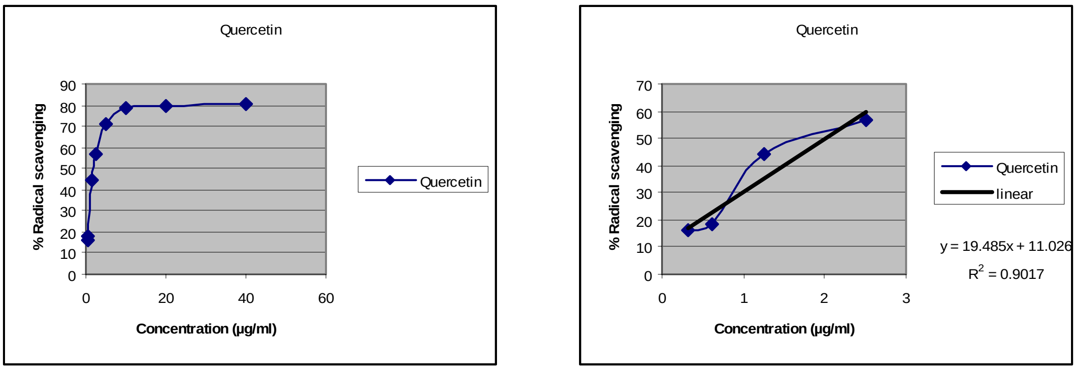


C.



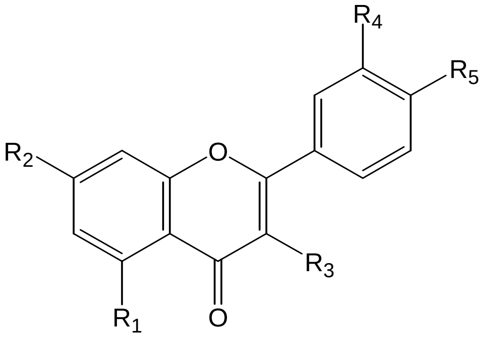
D.

Figure 12 Free radical scavenging activity of compounds GT-4, GT-5 and GT-6 on DPPH radical. Measurement at 510 nm, determination after 30 min. and linear equation for analysis IC_{50} values (µg/ml). A: GT-4, B: GT-5, C: GT-6, D: Quercetin

Table 23 Percentage of free radical scavenging activity by pure compounds isolated from *G. tenuifolius*

Compounds	% Scavenging activity (40 µg/ml)	IC_{50}	
		(µg/ml)	(µM)
GT-1 **[198]**	4.27	-	-
GT-2 **[199]**	6.20	-	-
GT-3 **[200]**	13.69	-	-
GT-4 **[201]**	82.15	1.93	5.8
GT-5 **[202]**	80.31	2.03	6.4
GT-6 **[203]**	75.06	2.31	6.7
GT-7 **[204]**	0.61	-	-
GT-8 **[205]**	19.07	-	-
GT-9 **[206]**	11.49	-	-
Quercetin	80.19	2.00	6.6

From Table 23, only three compounds (GT-4, GT-5, GT-6) showed free radical scavenging activity. The structures of these compounds were composed of free hydroxyl groups at C-3' and C-4'. This 3',4'-diphenolic partial structure should therefore be important for the activity.



	R_1	**R_2**	**R_3**	**R_4**	**R_5**
Compound [198]	OH	OCH_3	OCH_3	OCH_3	OCH_3
Compound [200]	OH	OCH_3	OCH_3	OCH_3	OH
Compound [201]	OH	OCH_3	OCH_3	OH	OH
Compound [202]	OH	OH	OCH_3	OH	OH
Compound [203]	OCH_3	OCH_3	OCH_3	OH	OH
Compound [204]	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3
Compound [205]	OCH_3	OCH_3	OCH_3	OCH_3	OH
Compound [206]	OCH_3	OCH_3	OCH_3	OH	OCH_3

Compound [199]

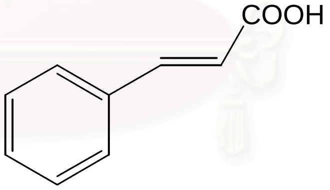


Figure 13 Structures of compounds isolated from the leaves of *G. tenuifolius* with free radical scavenging activity (compounds **198**-**206**)

CHAPTER V

CONCLUSION

From the leaves of *Goniothalamus tenuifolius* King (Annonaceae), eight 3-methoxyflavones have been isolated, together with *trans*-cinnamic acid. They were identified as 5-hydroxy-3,7,3',4'-tetramethoxyflavone**[198]**, *trans*-cinnamic acid **[199]**, pachypodol **[200]**, kumatakenin **[201]**, 5,7,3',4'-tetrahydroxy-3-methoxyflavone **[202]**, 3',4'-dihydroxy-3,5,7-trimethoxyflavone **[203]**, 3,5,7,3',4'-pentamethoxyflavone **[204]**, 4'-hydroxy-3,5,7,3'-tetramethoxyflavone **[205]** and 3'-hydroxy-3,5,7,4'-tetramethoxyflavone **[206]**, respectively. Compound **[206]** is a new natural product. The unambiguous ^{1}H-NMR assignments of 3',4'-dihydroxy-3,5,7-trimethoxyflavone **[203]** and the ^{13}C-NMR data of 4'-hydroxy-3,5,7,3'-tetramethoxyflavone **[205]** and 3'-hydroxy-3,5,7,4'-tetramethoxyflavone **[206]** were also obtained for the first time in this study. All of the isolates have been tested for free radical scavenging activity, using the DPPH assay. The active compounds were kumatakenin **[201]**, 5,7,3',4'-tetrahydroxy-3-methoxyflavone **[202]** and 3',4'-dihydroxy-3,5,7-trimethoxyflavone **[203]** with IC$_{50}$ values of 5.8, 6.4 and 6.7 µM, respectively, whereas quercetin (positive control) showed an IC$_{50}$ value of 6.6 µM.

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APPENDIX

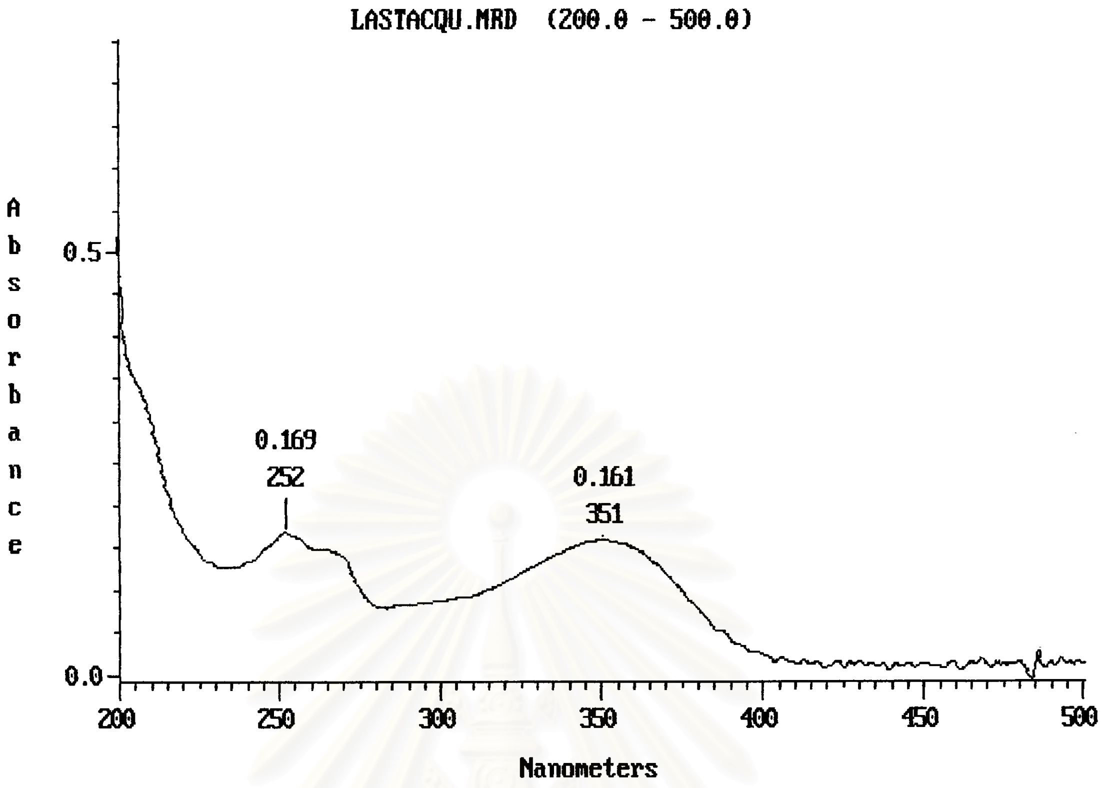

Figure 14 UV Spectrum of compound GT-1 (methanol)

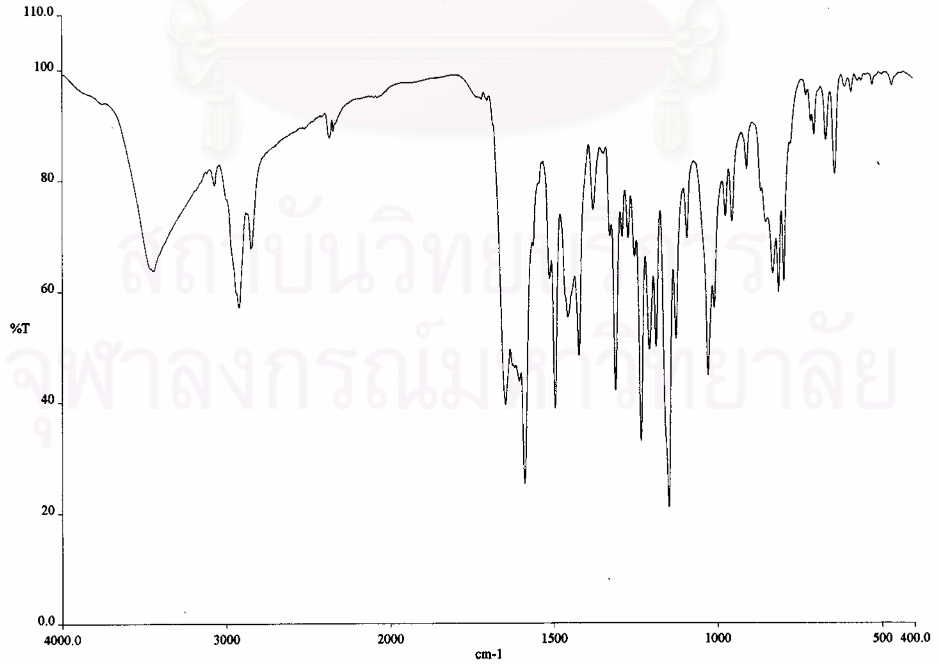

Figure 15 IR Spectrum of compound GT-1 (film)

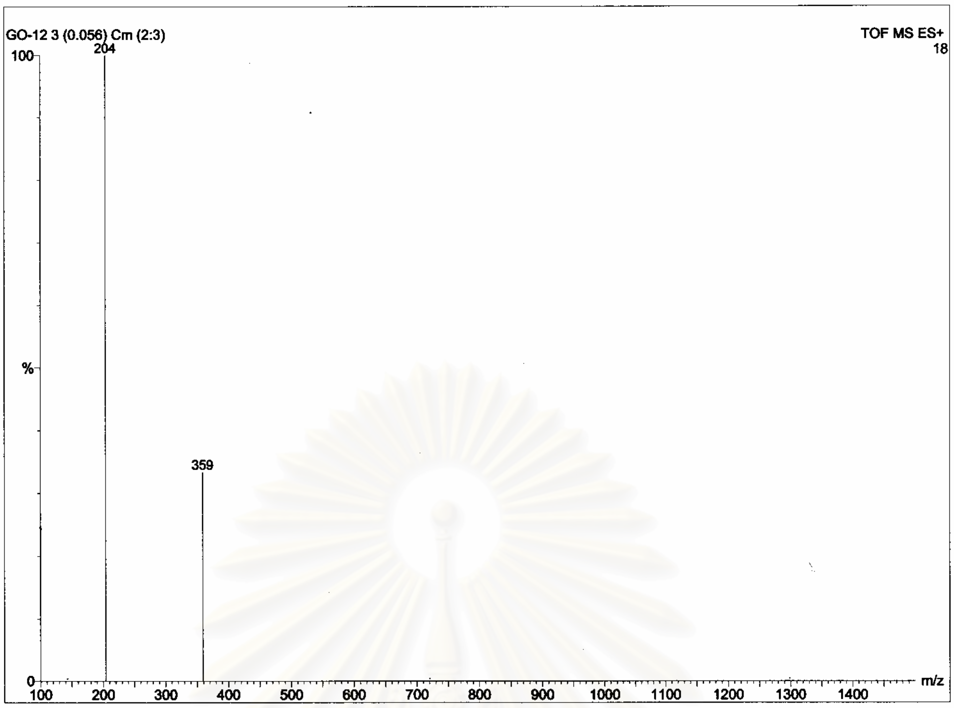

Figure 16 Mass spectrum of compound GT-1

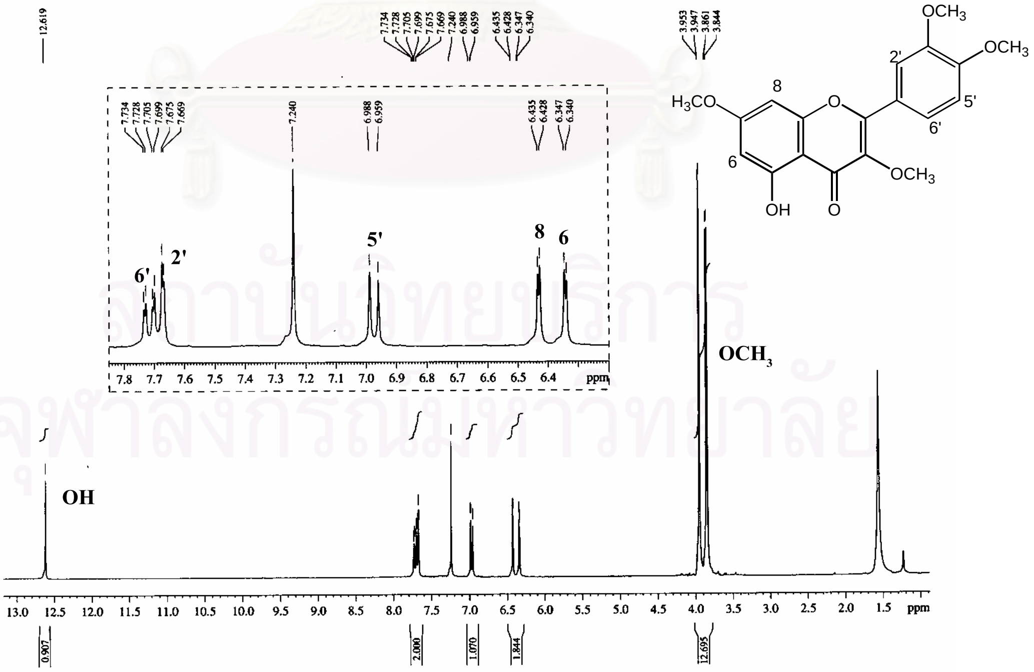

Figure 17 ^{1}H-NMR (300 MHz) Spectrum of compound GT-1 ($CDCl_3$)

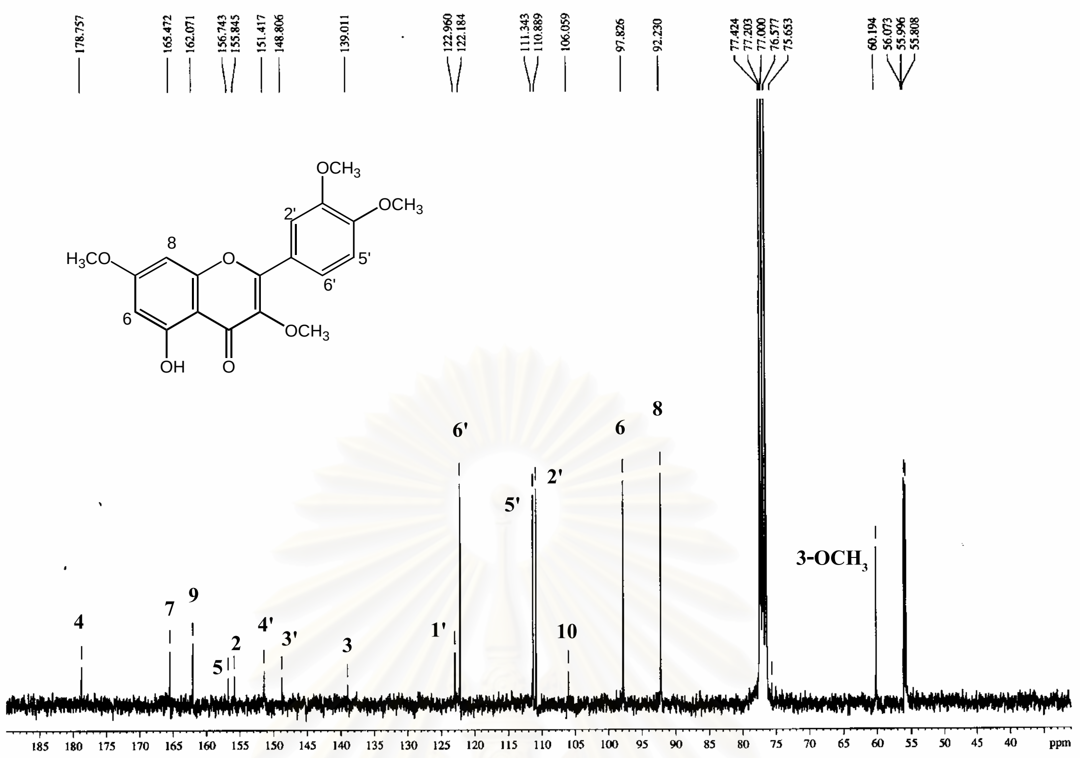

Figure 18 ^{13}C-NMR (75 MHz) Spectrum of compound GT-1 (CDCl$_3$)

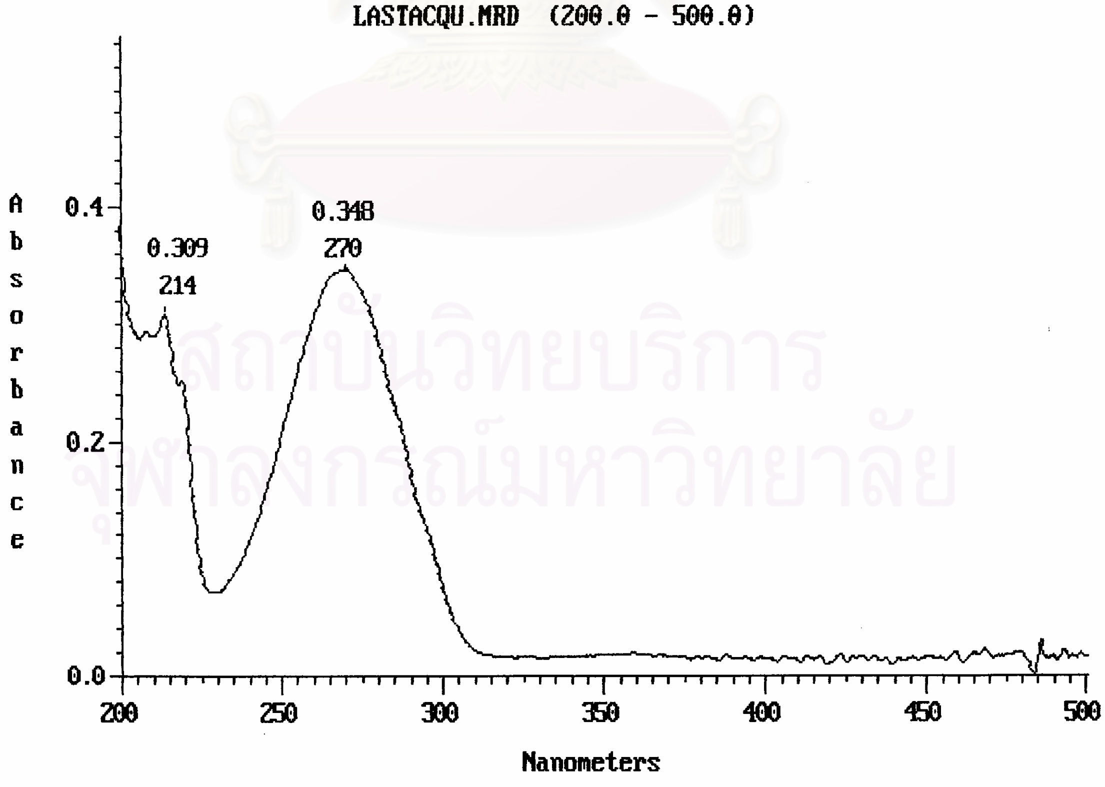

Figure 19 UV Spectrum of compound GT-2 (methanol)

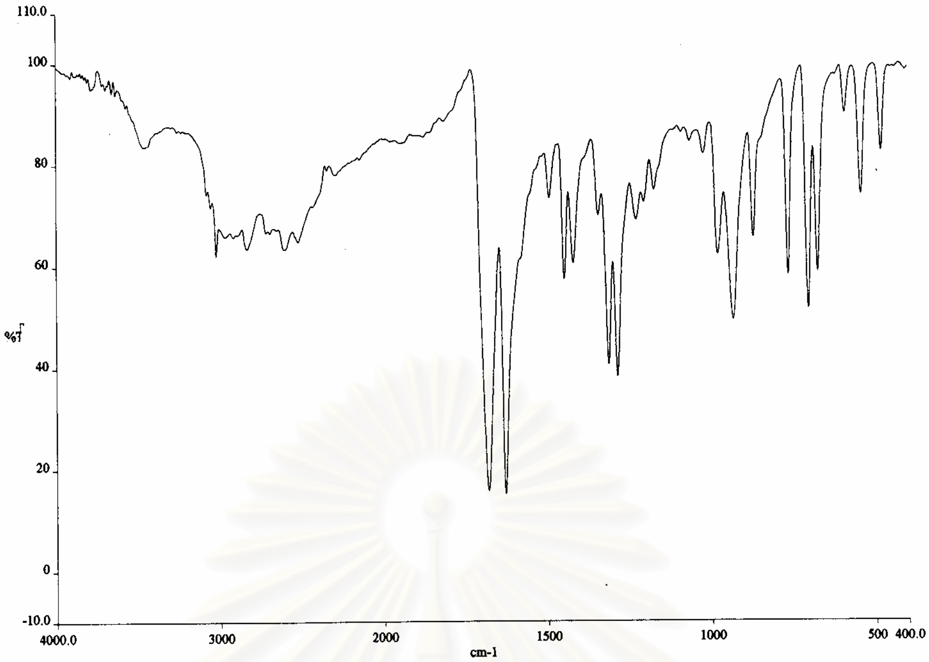

Figure 20 IR Spectrum of compound GT-2 (film)

Figure 21 Mass spectrum of compound GT-2

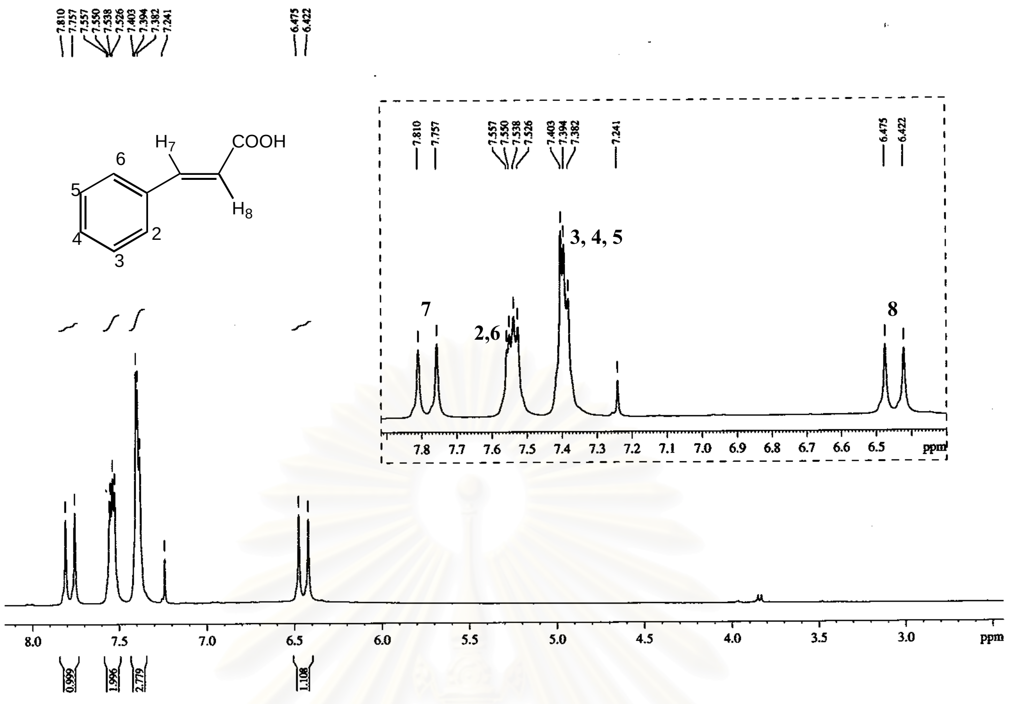

Figure 22 ^{1}H-NMR (300 MHz) Spectrum of compound GT-2 ($CDCl_3$)

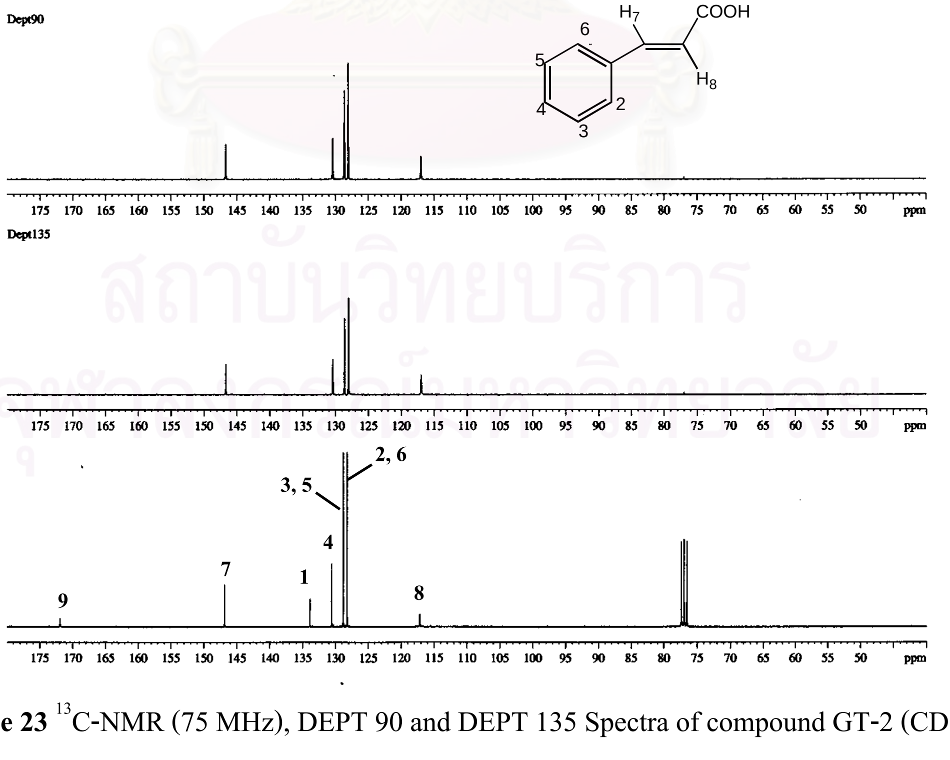

Figure 23 ^{13}C-NMR (75 MHz), DEPT 90 and DEPT 135 Spectra of compound GT-2 ($CDCl_3$)

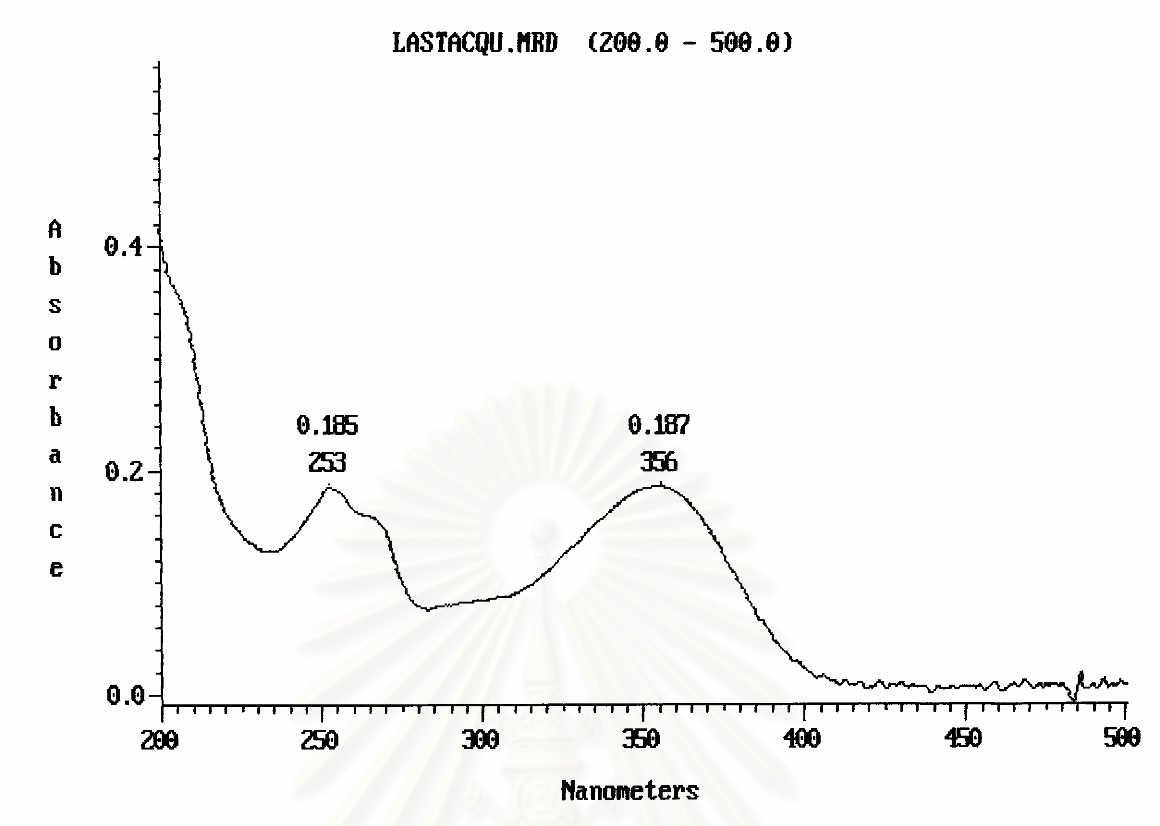

Figure 24 UV Spectrum of compound GT-3 (methanol)

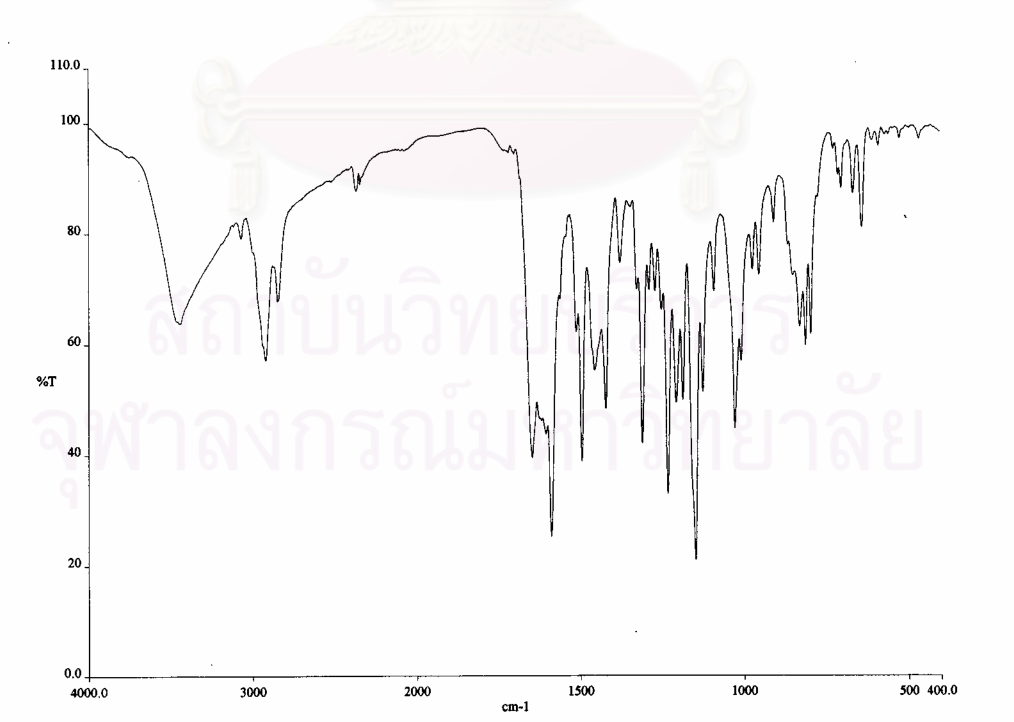

Figure 25 IR Spectrum of compound GT-3 (film)

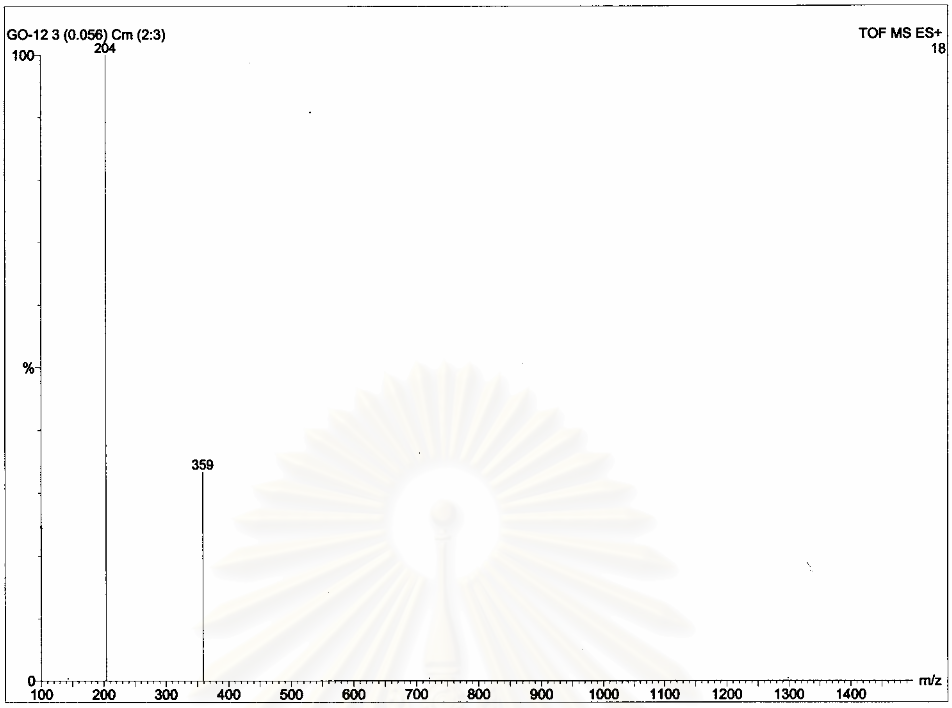

Figure 26 Mass spectrum of compound GT-3

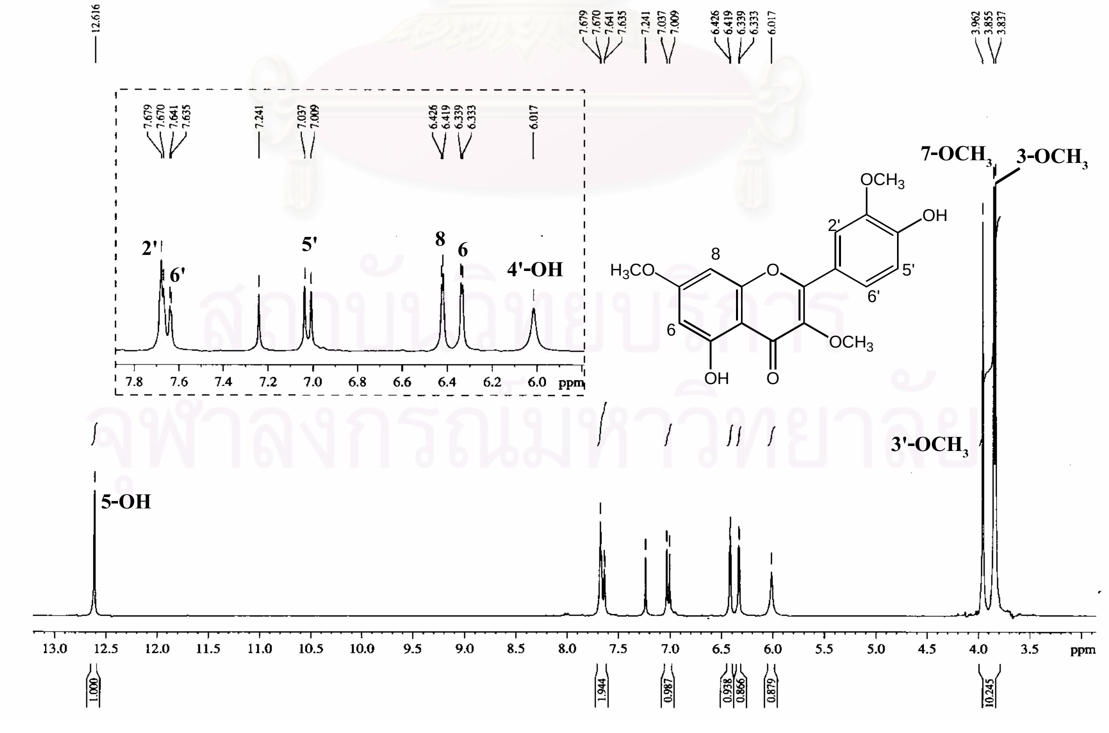

Figure 27 ^{1}H-NMR (300 MHz) Spectrum of compound GT-3 ($CDCl_3$)

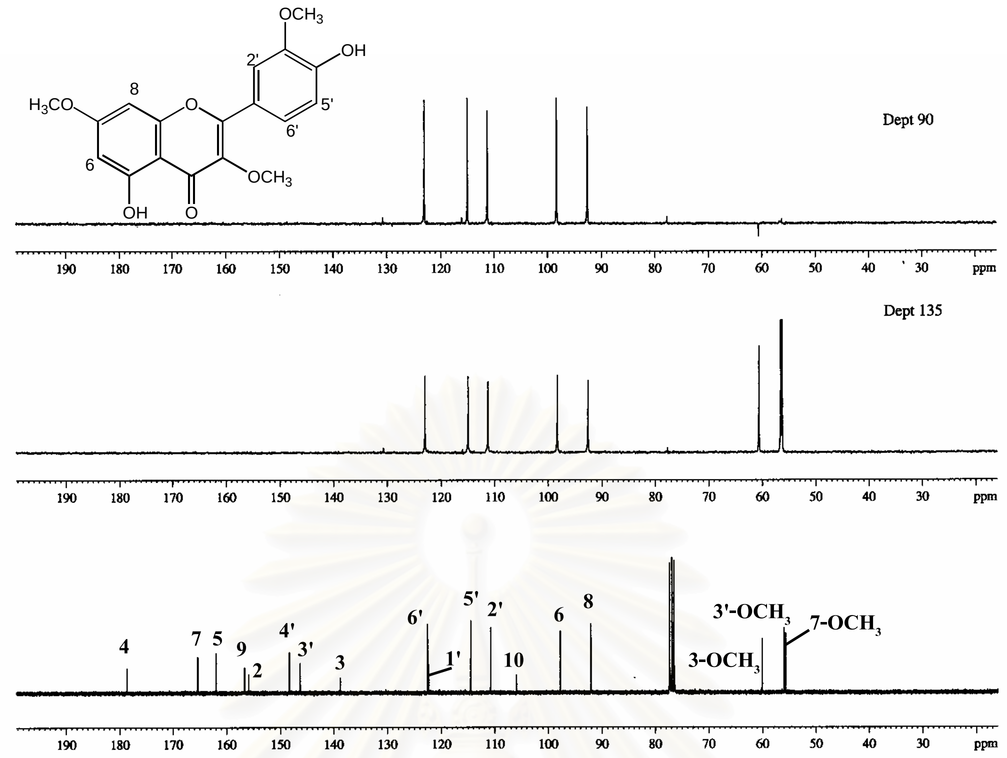

Figure 28 ^{13}C-NMR (75 MHz), DEPT 90 and DEPT 135 Spectra of compound GT-3 (CDCl$_3$)

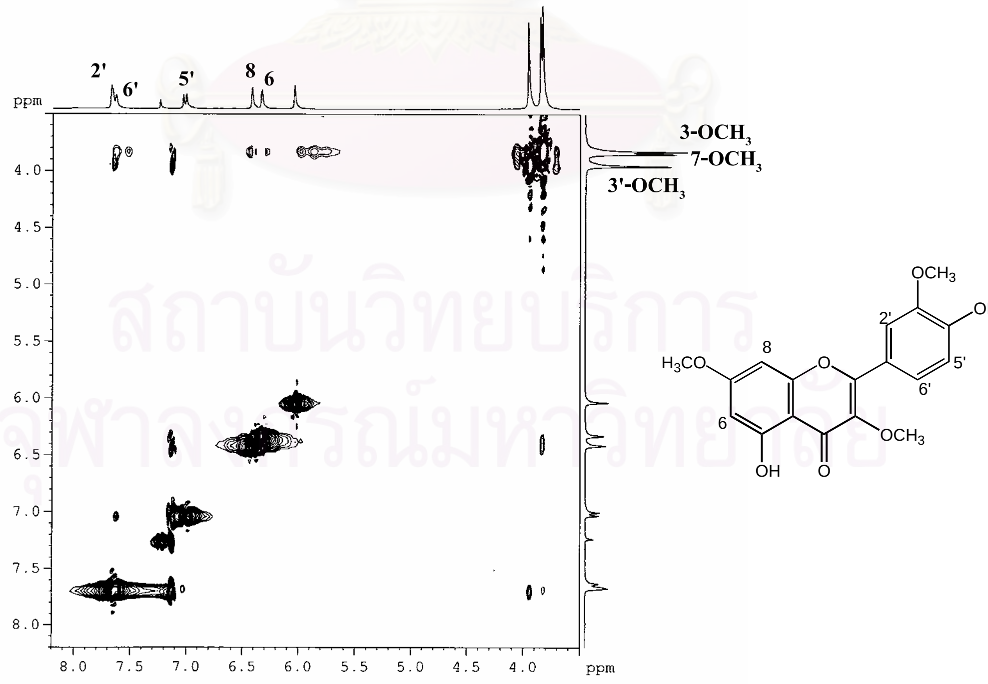

Figure 29 NOESY Spectrum of compound GT-3 (CDCl$_3$)

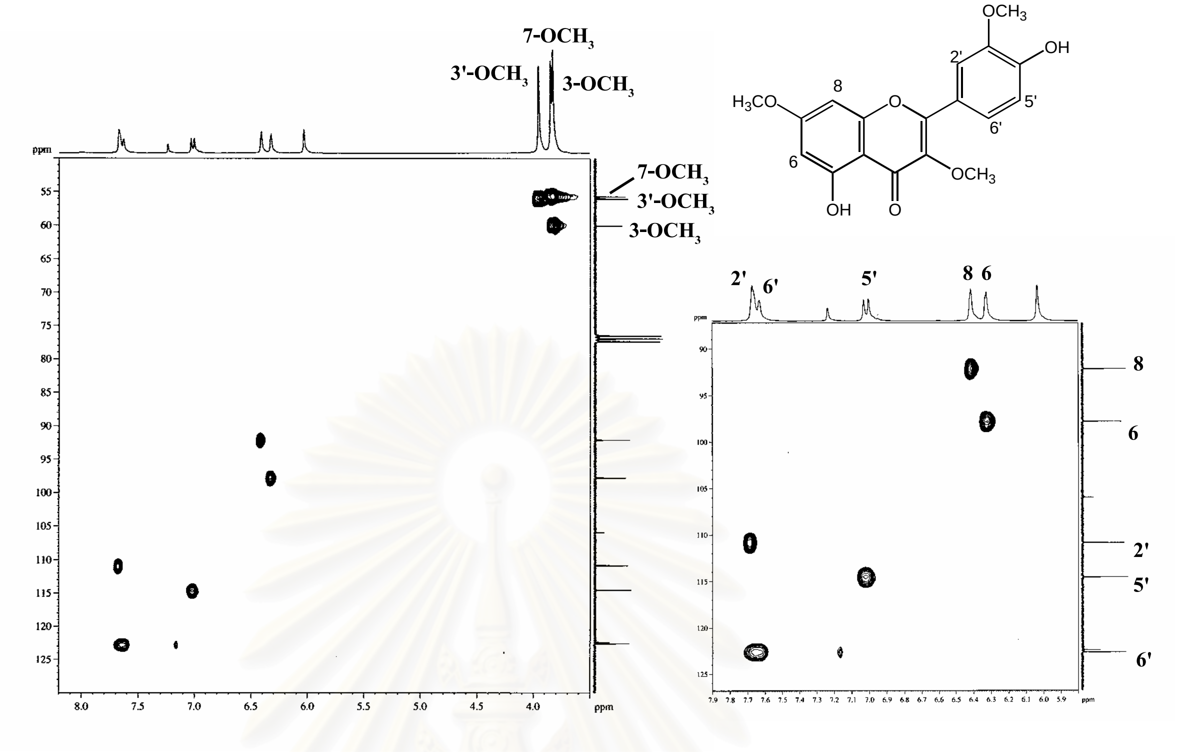

Figure 30 HMQC Spectrum of compound GT-3 (CDCl$_3$)

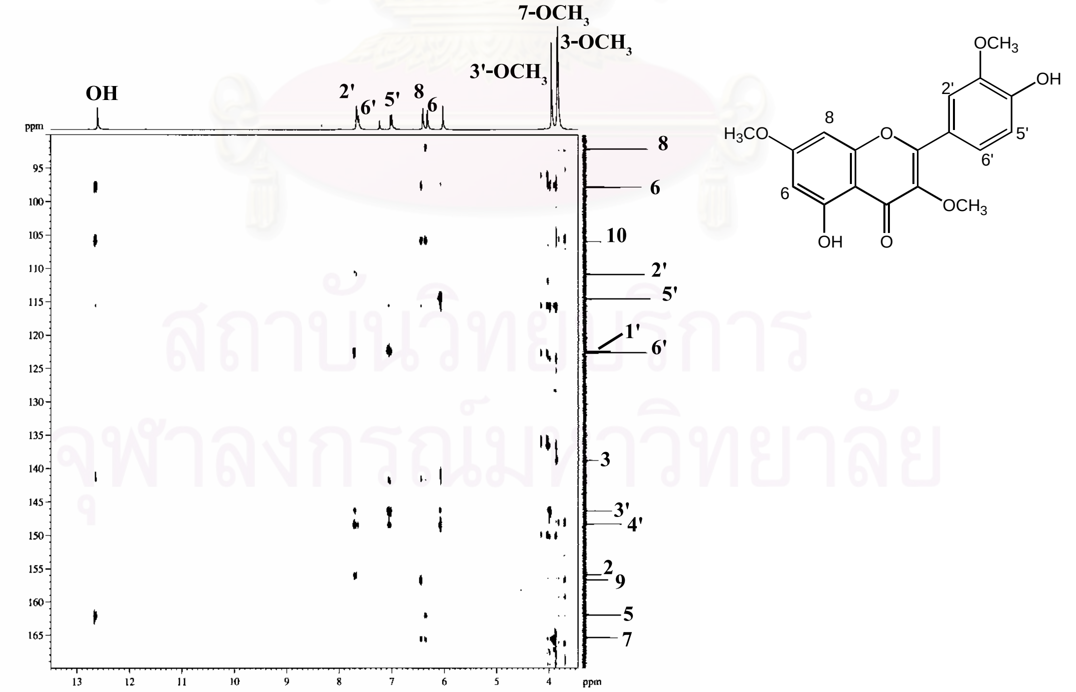

Figure 31 HMBC Spectrum of compound GT-3 (CDCl$_3$)

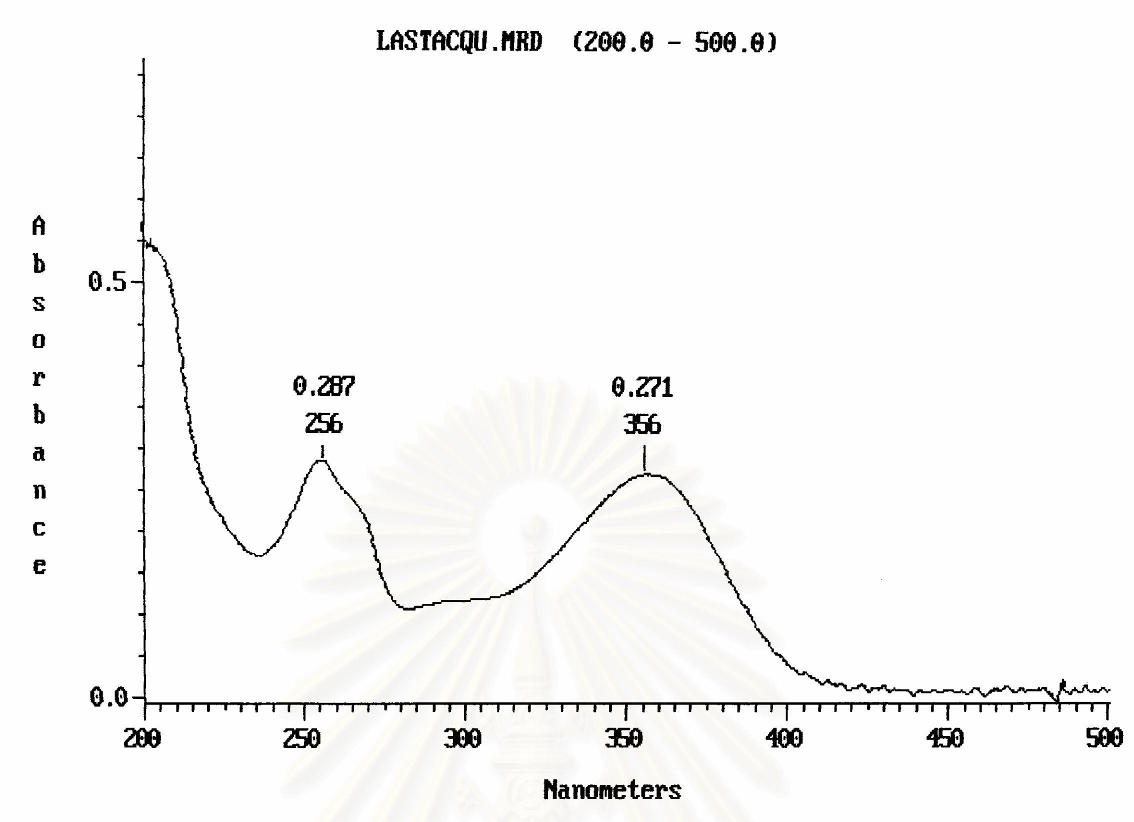

Figure 32 UV Spectrum of compound GT-4 (methanol)

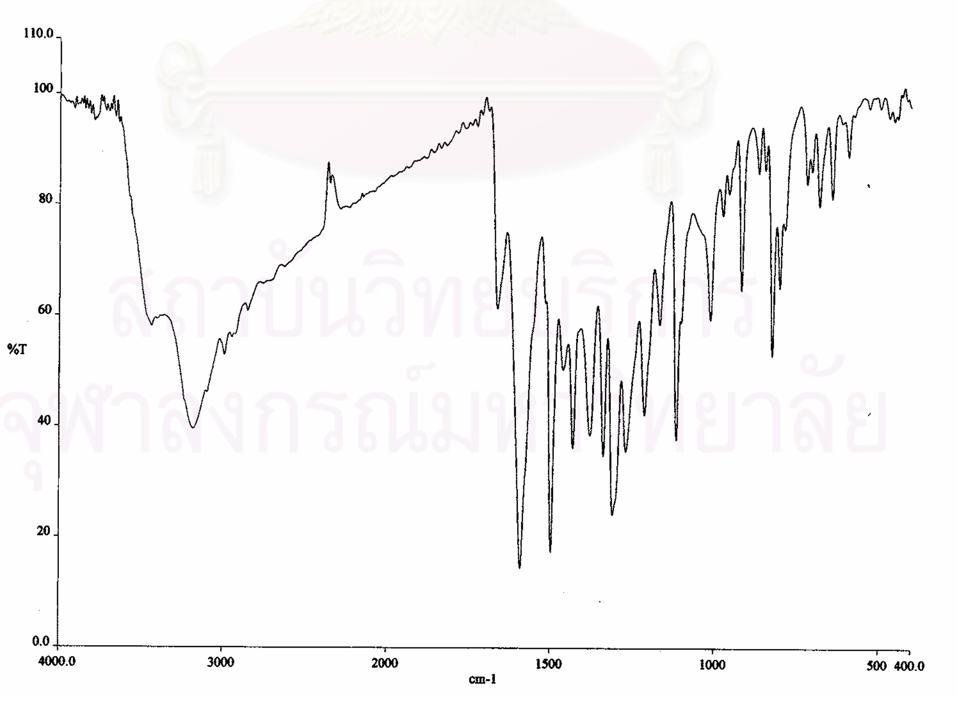

Figure 33 IR Spectrum of compound GT-4 (film)

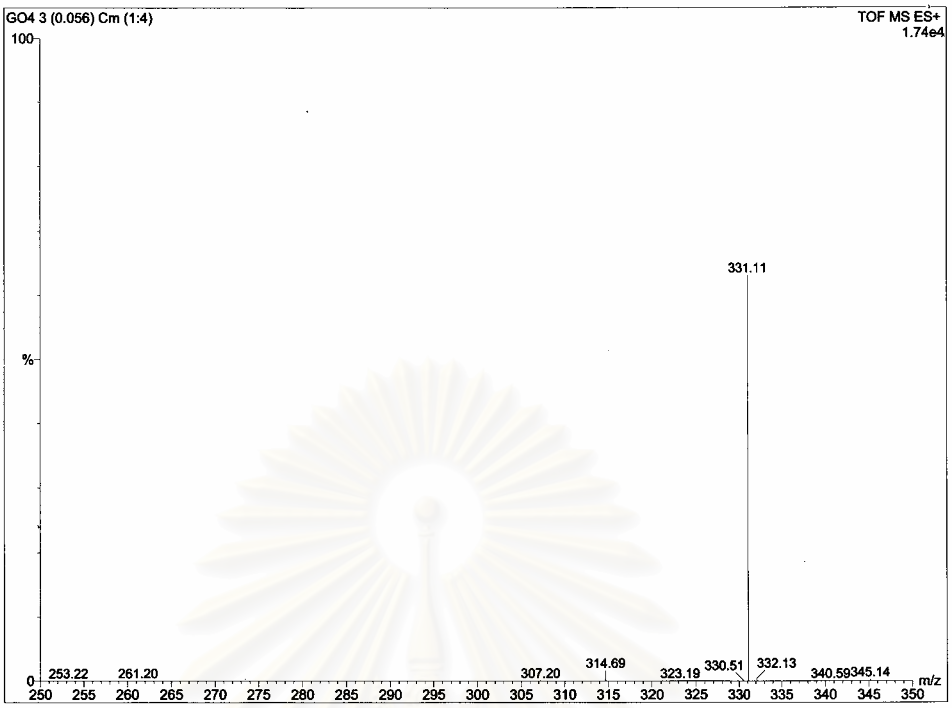

Figure 34 Mass spectrum of compound GT-4

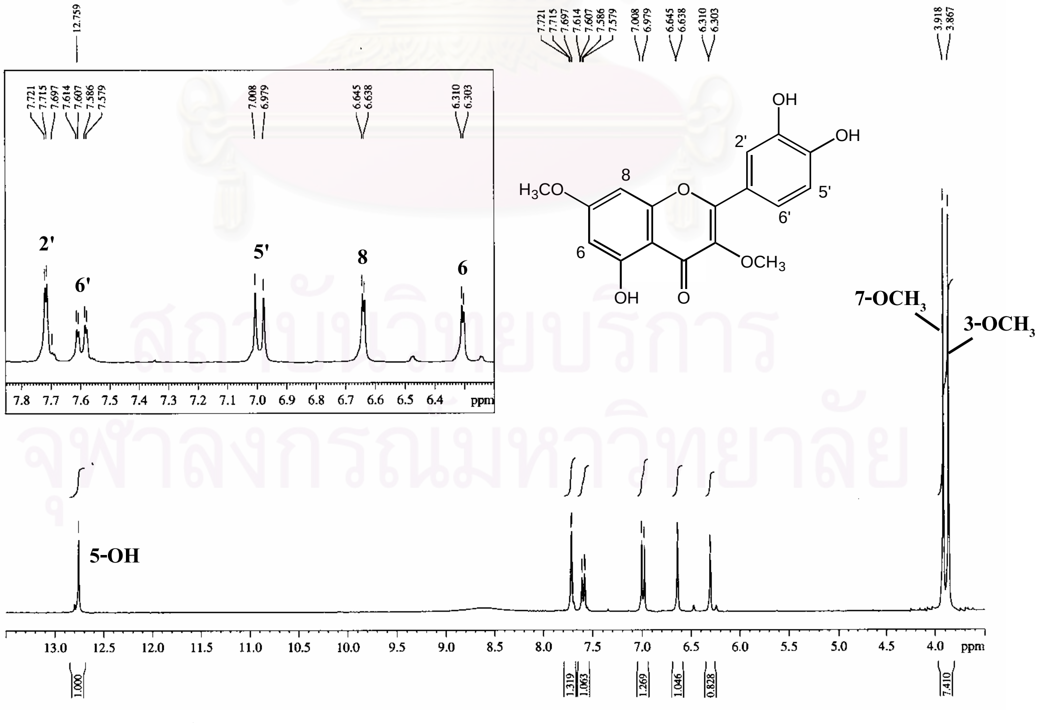

Figure 35 ^{1}H-NMR (300 MHz) Spectrum of compound GT-4 (acetone- d_6)

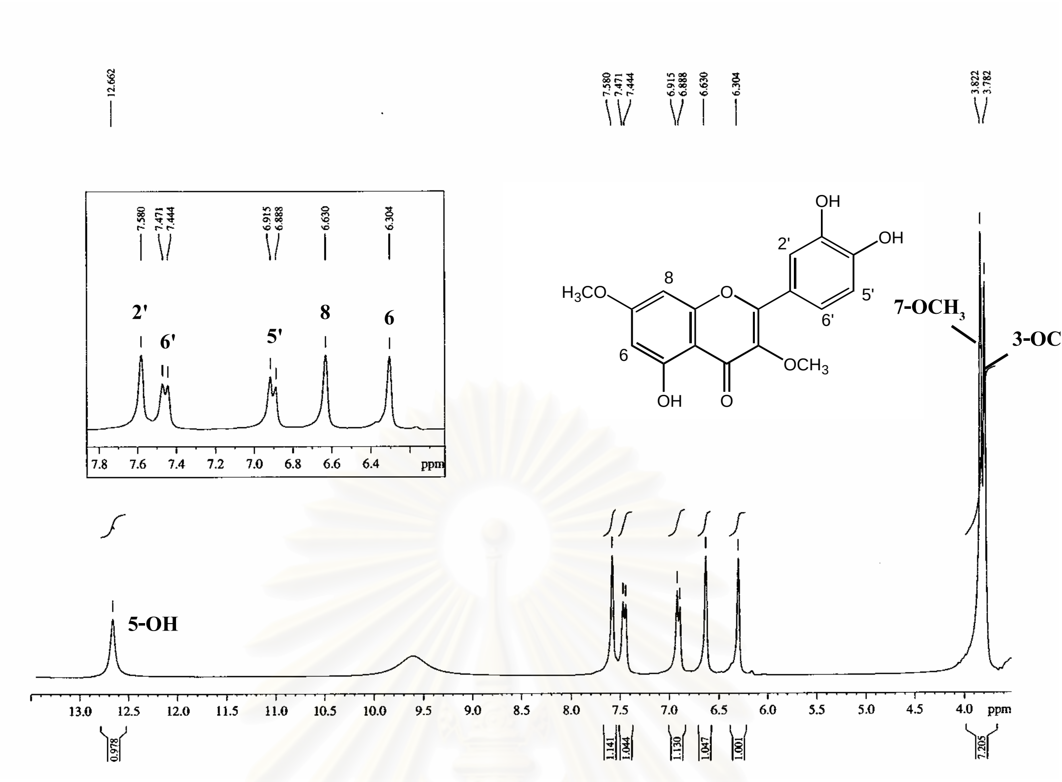

Figure 36 ^{1}H-NMR (300 MHz) Spectrum of compound GT-4 (DMSO-d_6)

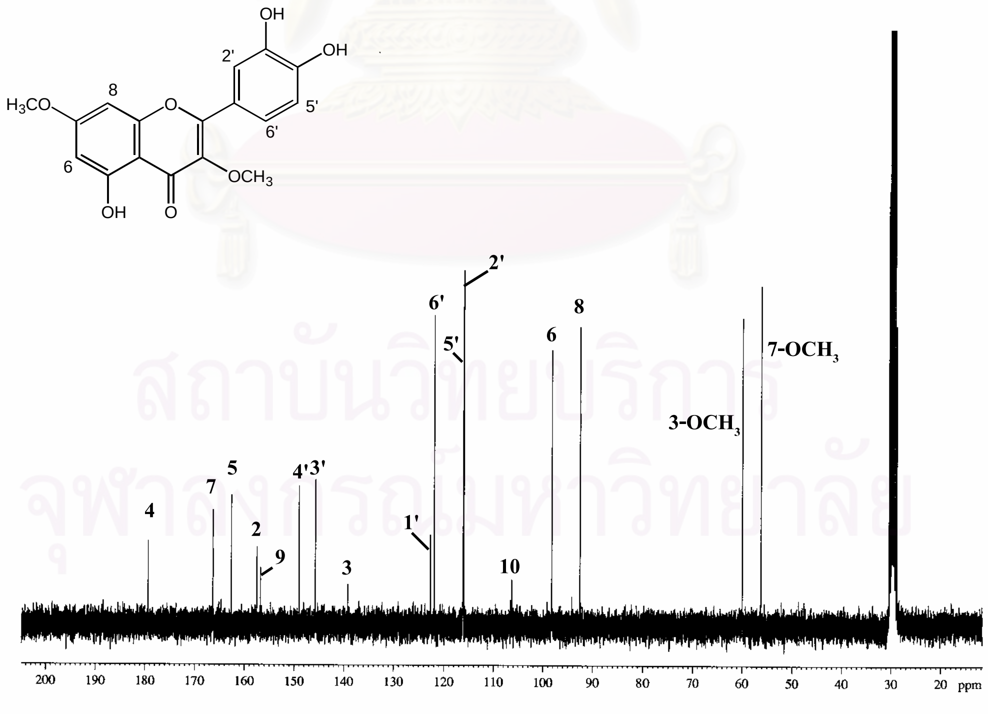

Figure 37 ^{13}C-NMR (75 MHz) Spectrum of compound GT-4 (acetone-d_6)

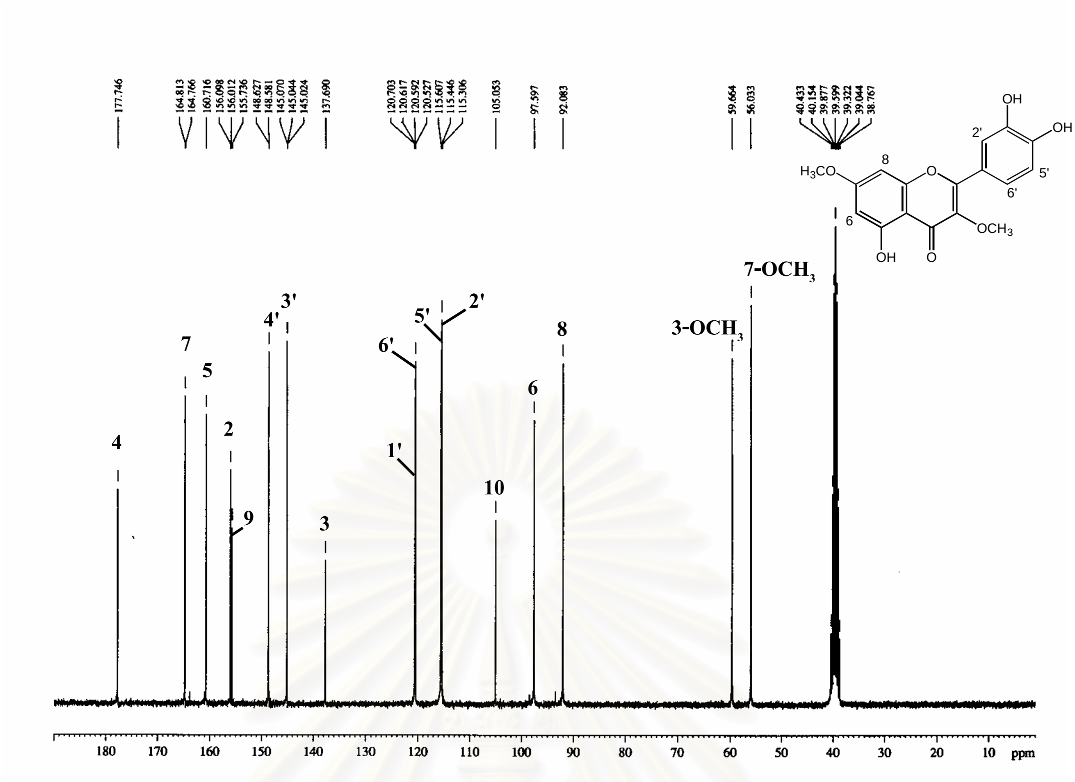

Figure 38 ^{13}C-NMR (75 MHz) Spectrum of compound GT-4 (DMSO-d_6)

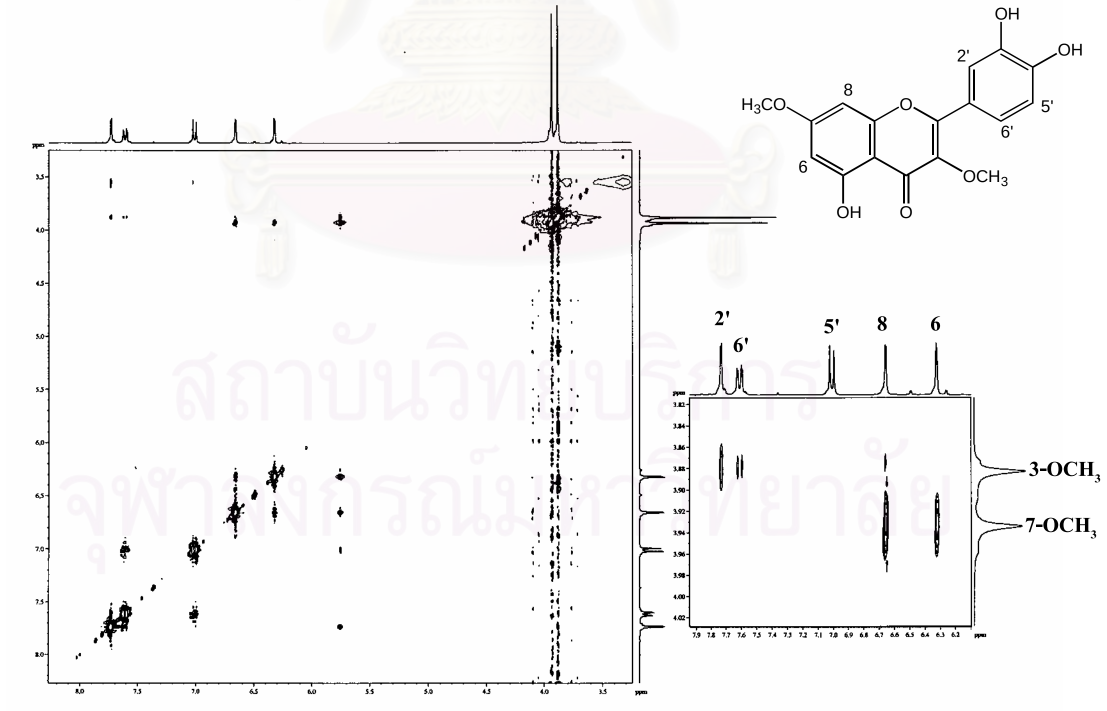

Figure 39 NOESY Spectrum of compound GT-4 (acetone- d_6)

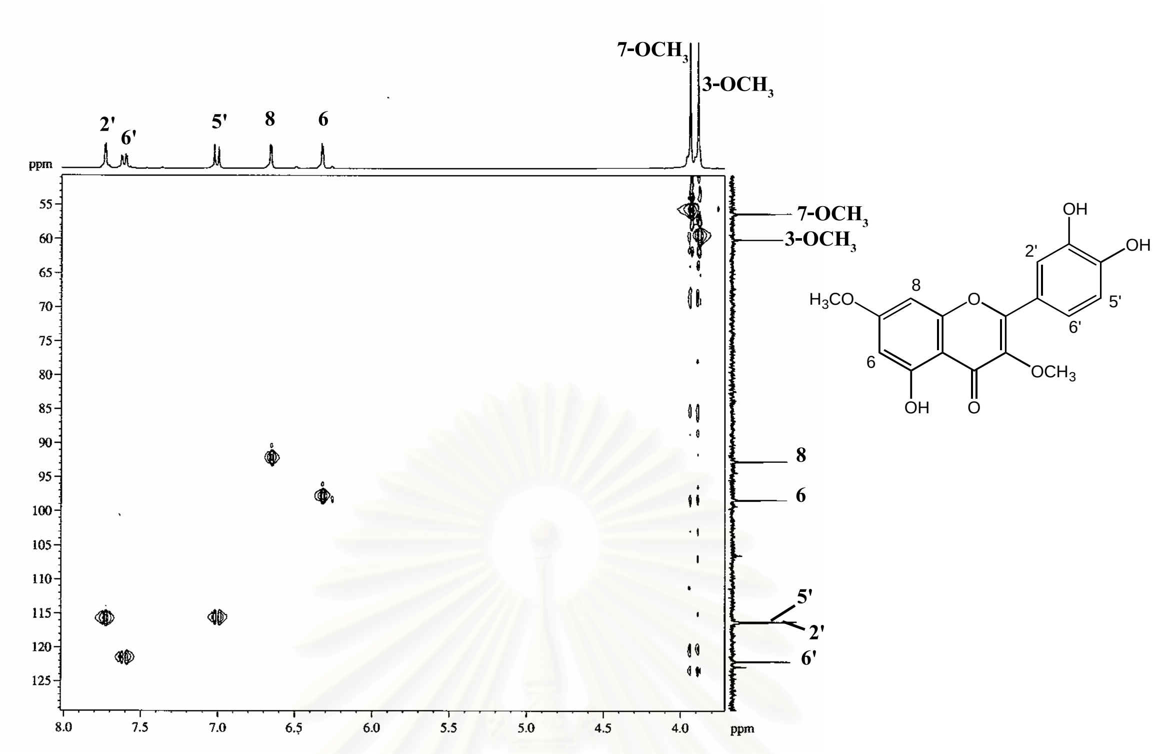

Figure 40 HMQC Spectrum of compound GT-4 (acetone- d_6)

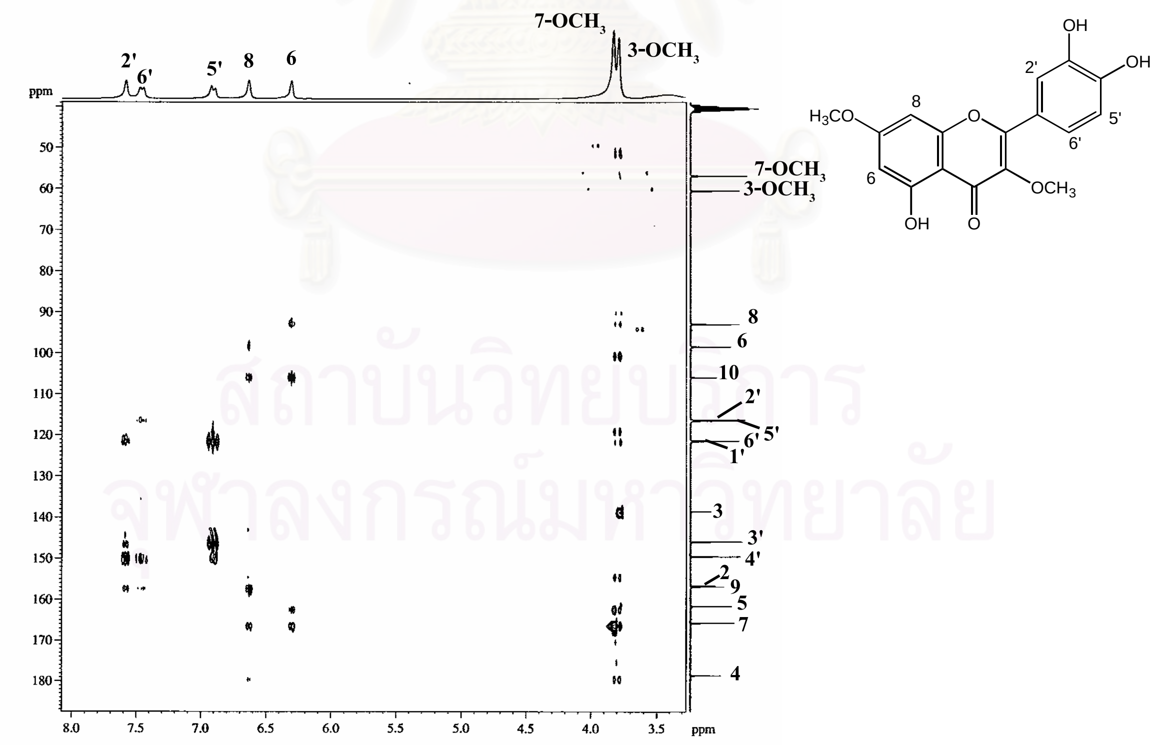

Figure 41 HMBC Spectrum of compound GT-4 (DMSO-d_6)

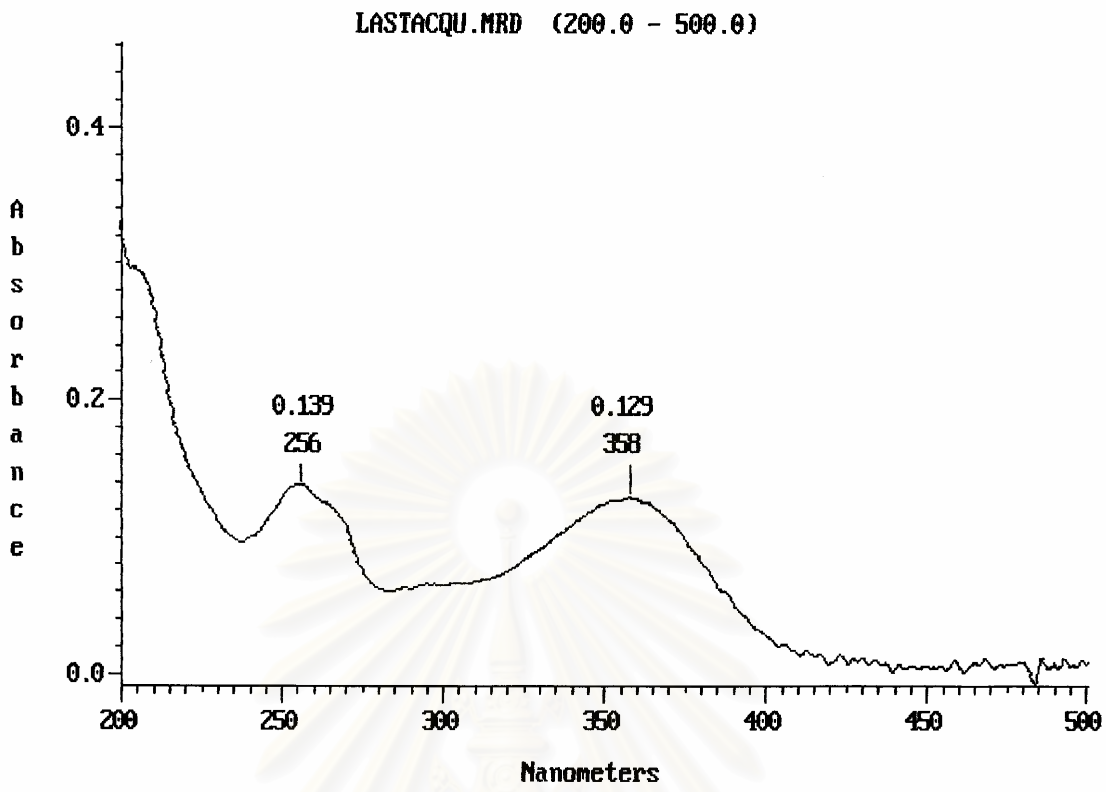

Figure 42 UV Spectrum of compound GT-5 (methanol)

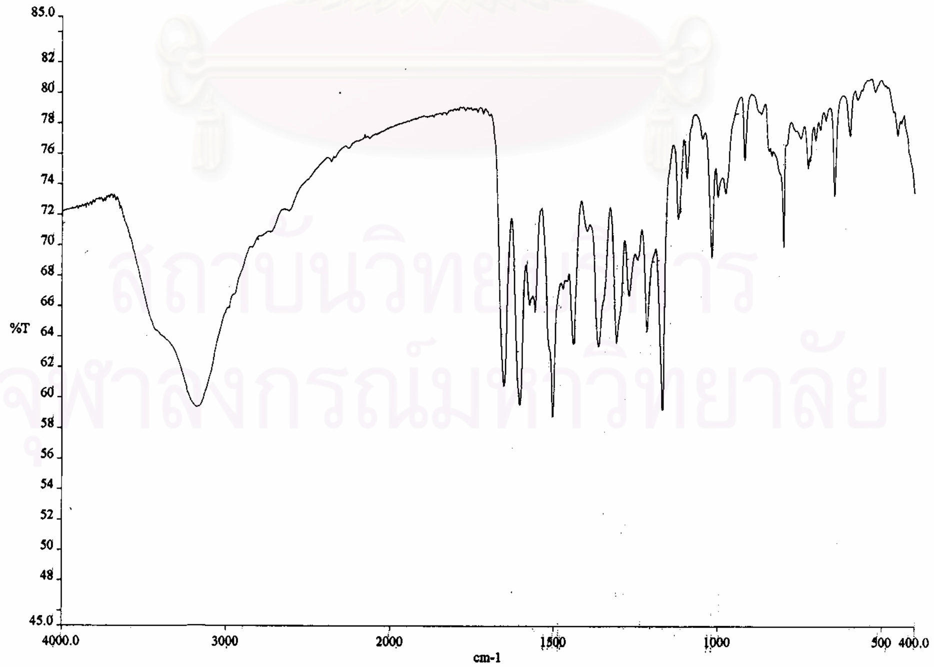

Figure 43 IR Spectrum of compound GT-5 (film)

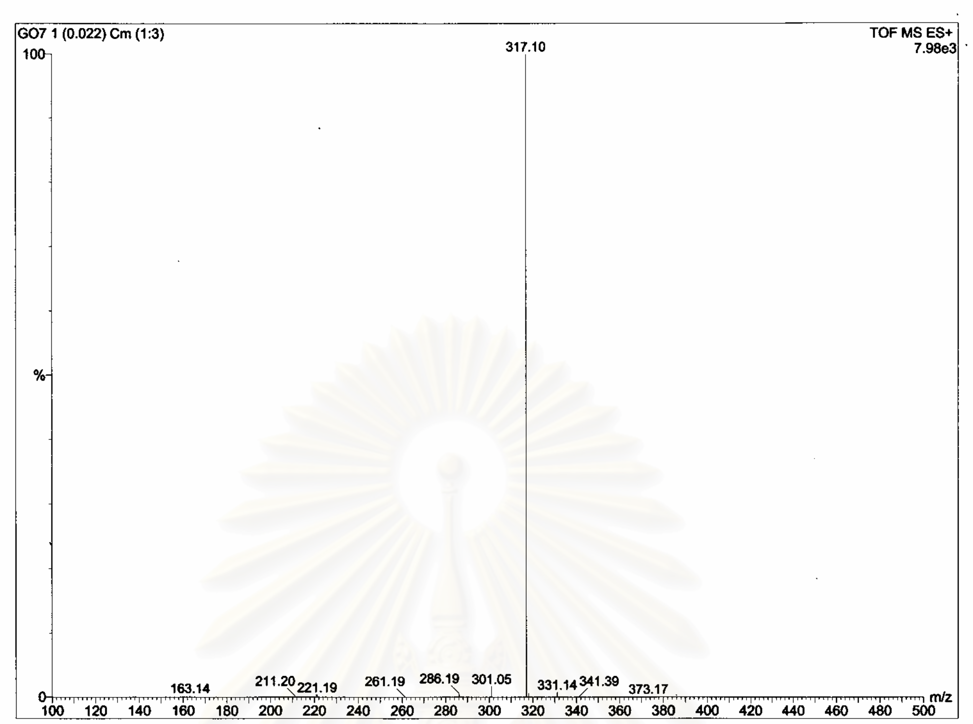

Figure 44 Mass spectrum of compound GT-5

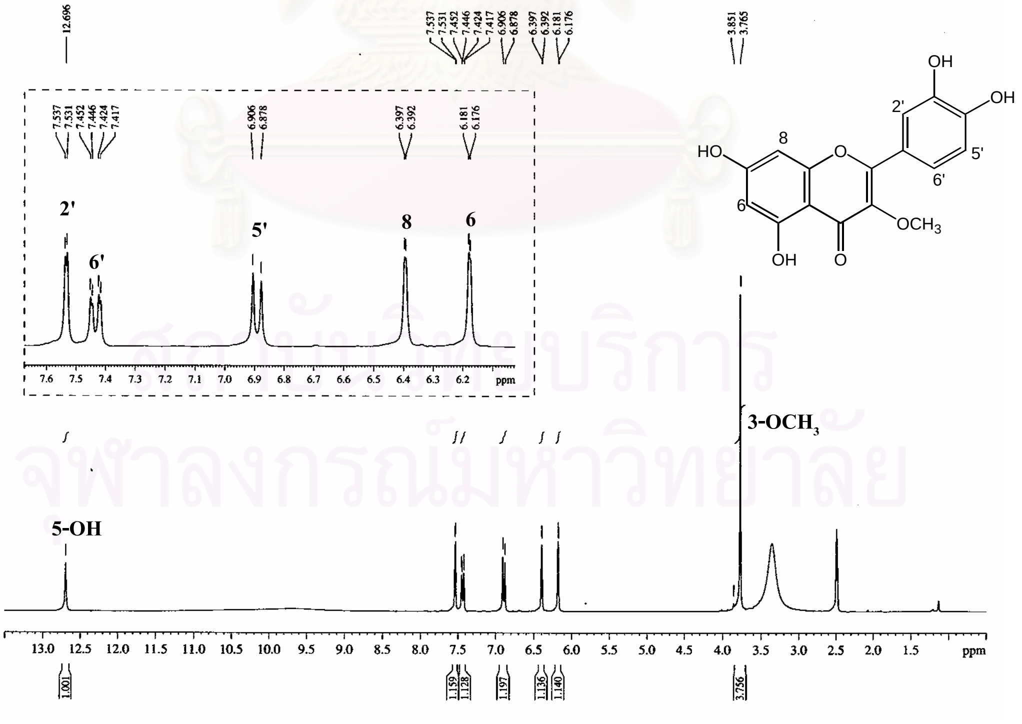

Figure 45 ^{1}H-NMR (300 MHz) Spectrum of compound GT-5 (DMSO-d_6)

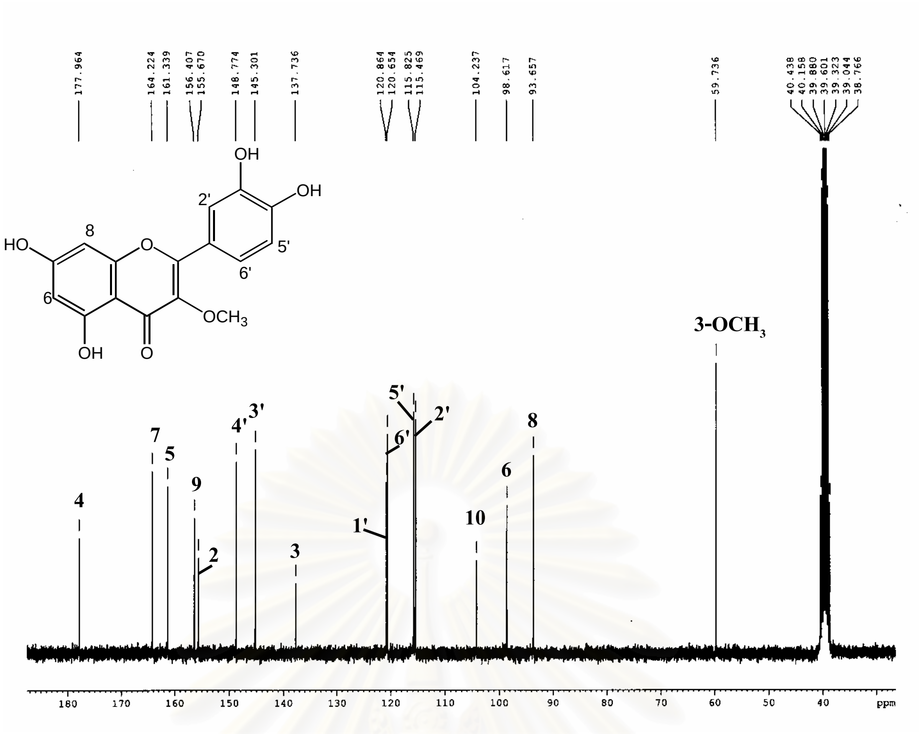

Figure 46 ^{13}C-NMR (75 MHz) Spectrum of compound GT-5 (DMSO-d_6)

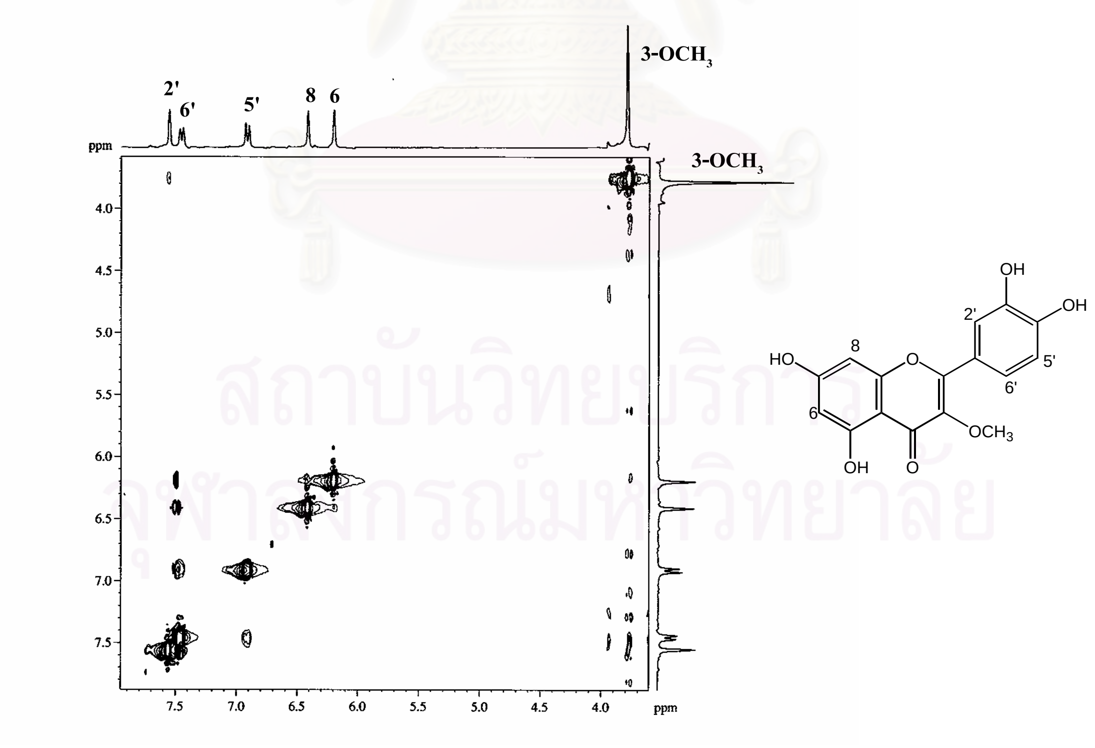

Figure 47 NOESY Spectrum of compound GT-5 (DMSO-d_6)

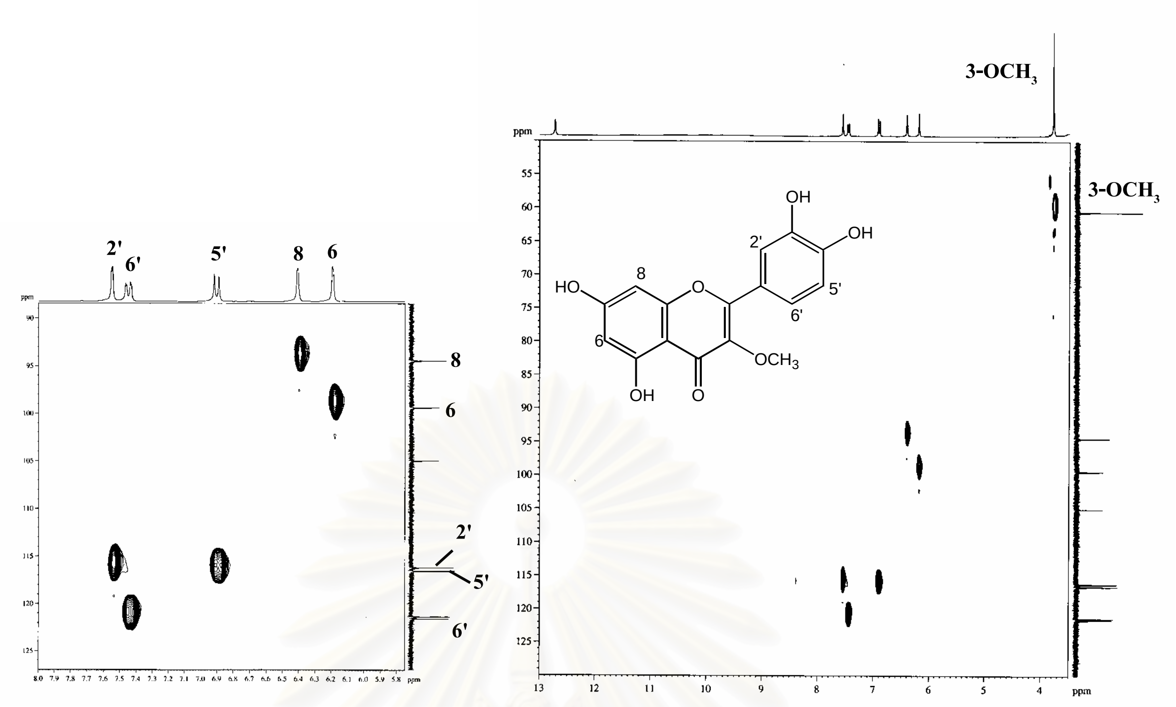

Figure 48 HMQC Spectrum of compound GT-5 (DMSO-d_6)

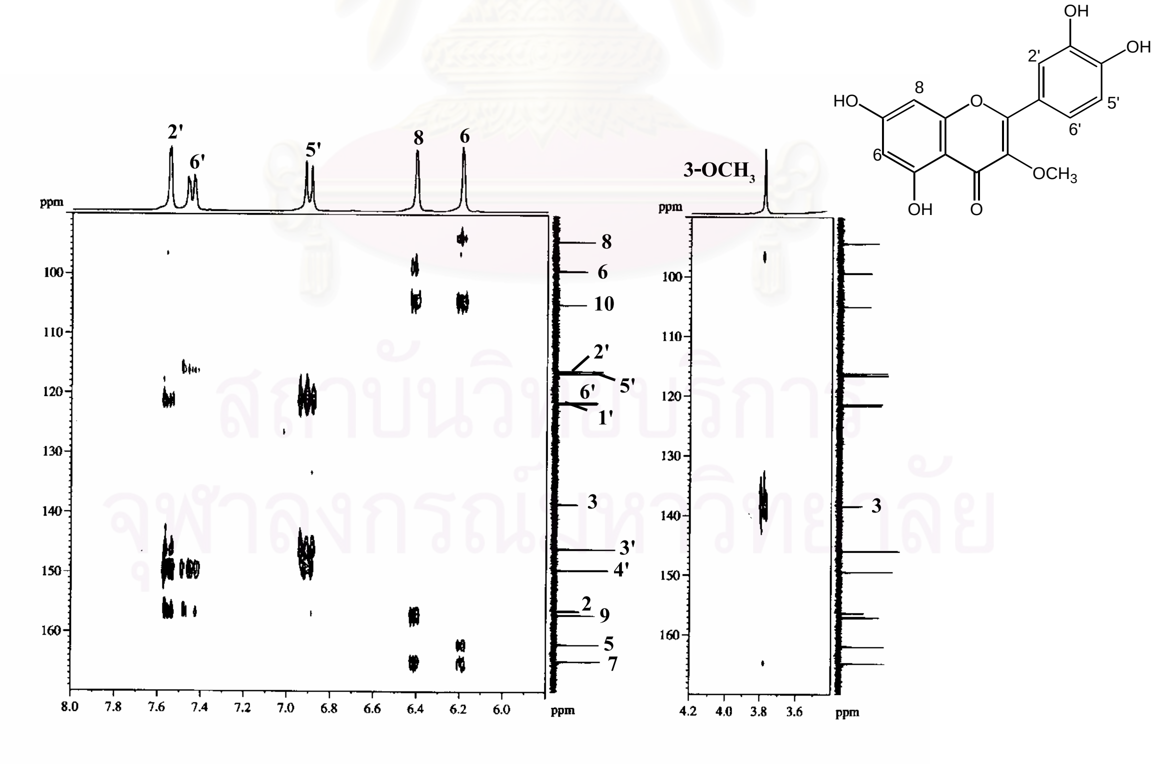

Figure 49 HMBC Spectrum of compound GT-5 (DMSO-d_6)

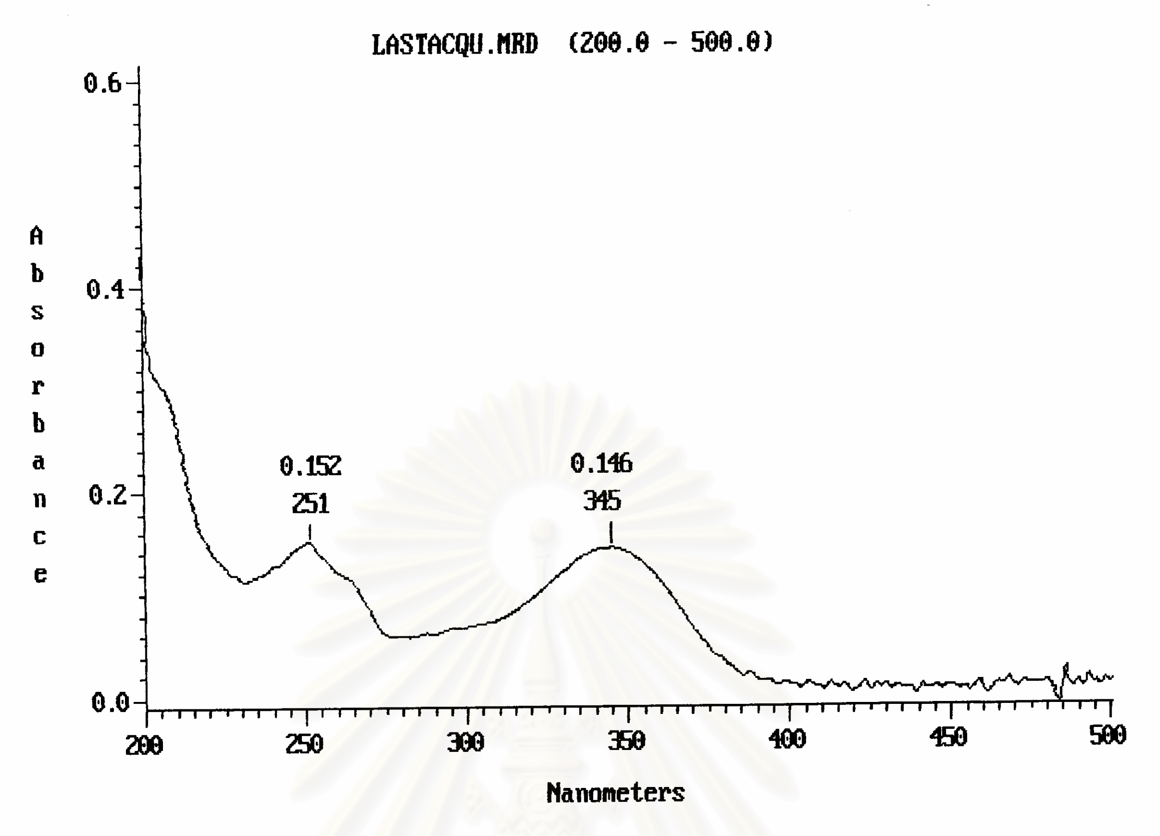

Figure 50 UV Spectrum of compound GT-6 (methanol)

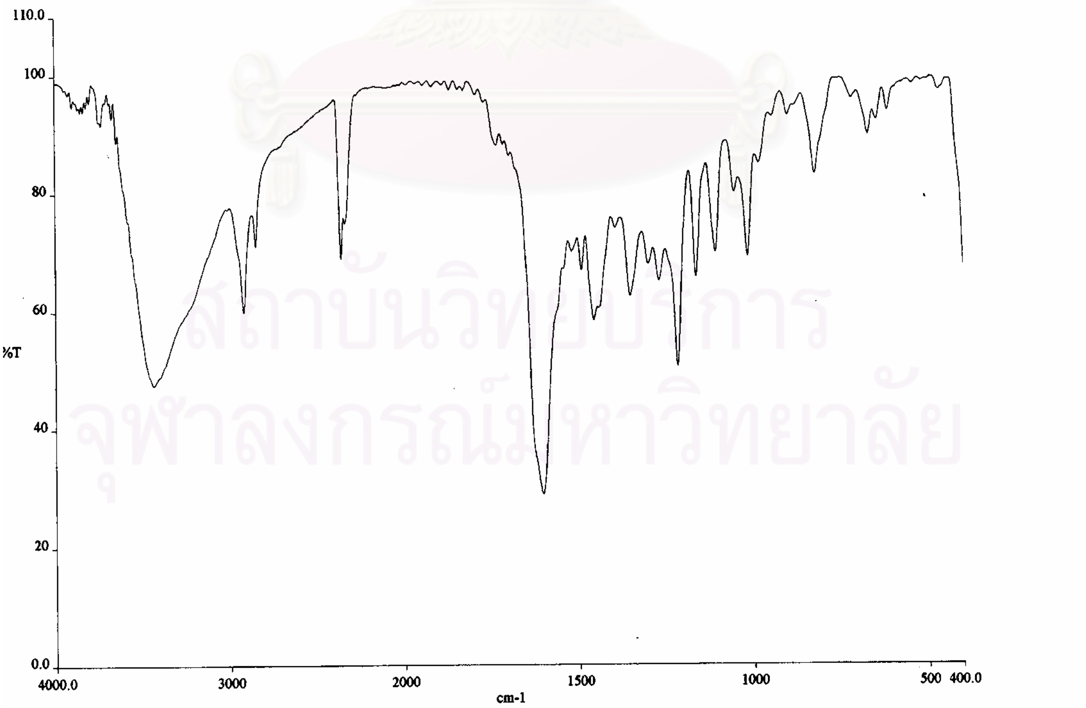

Figure 51 IR Spectrum of compound GT-6 (film)

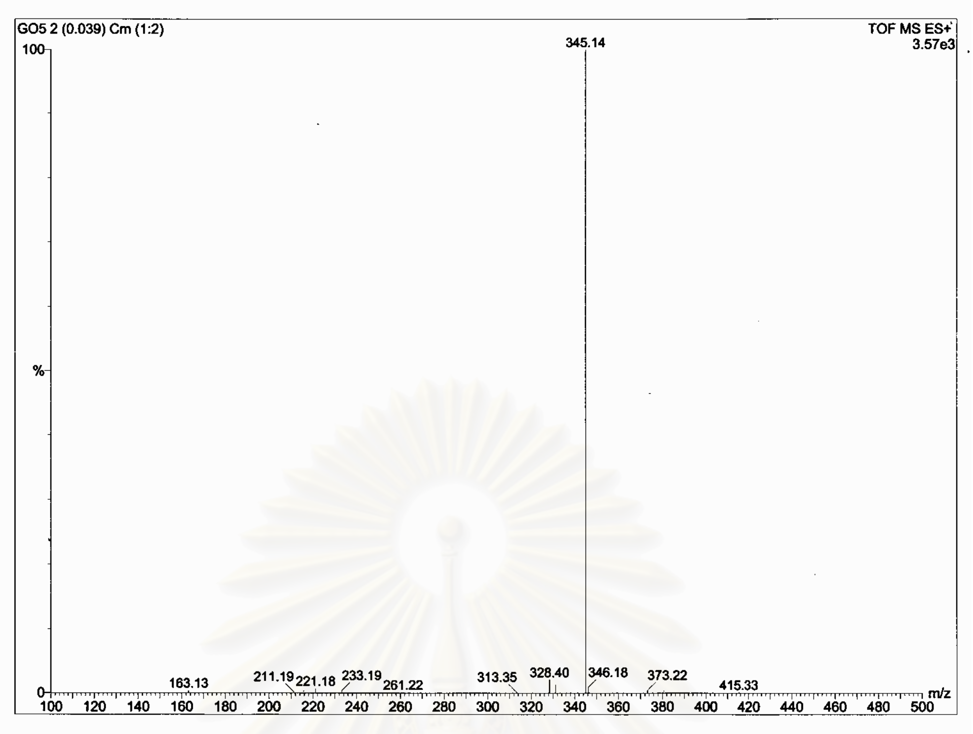

Figure 52 Mass spectrum of compound GT-6

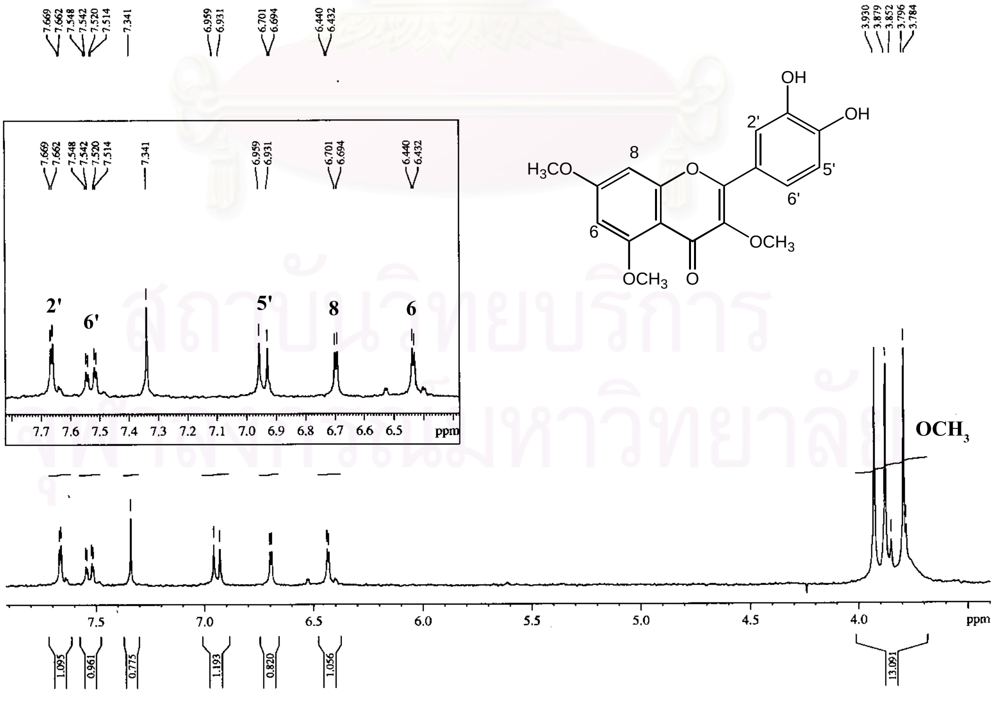

Figure 53 ^{1}H-NMR (300 MHz) Spectrum of compound GT-6 (acetone-d_6)

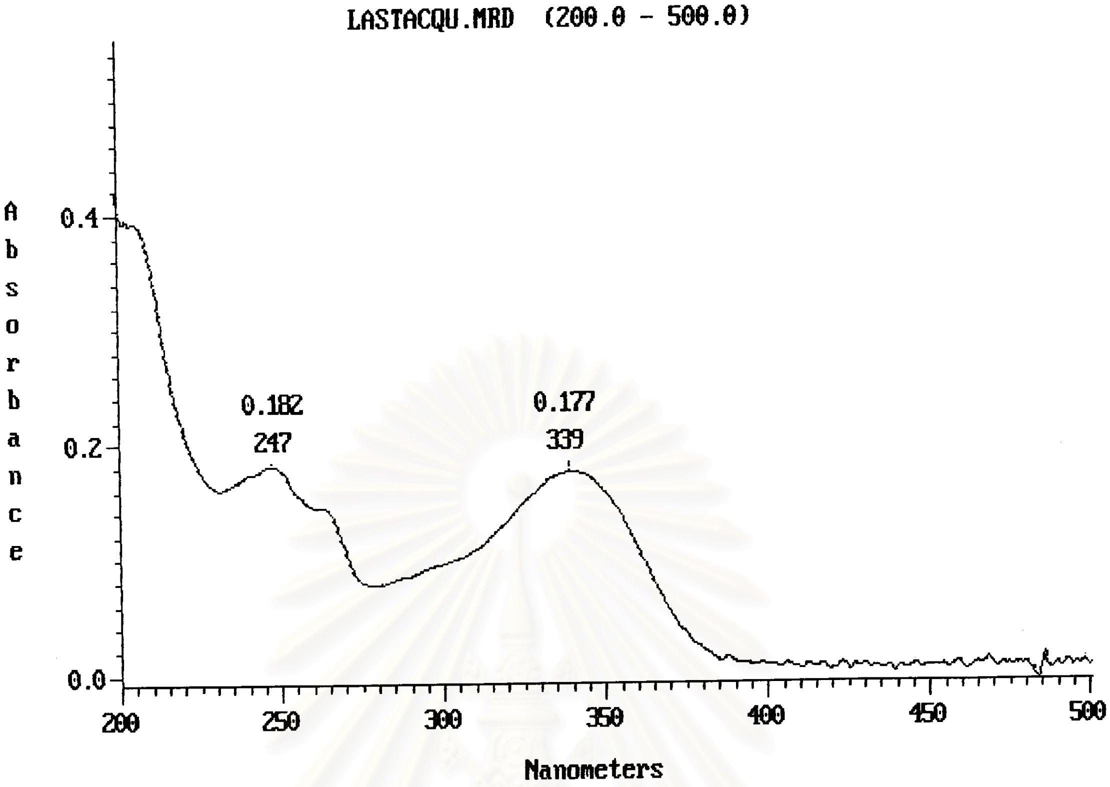

Figure 54 UV Spectrum of compound GT-7 (methanol)

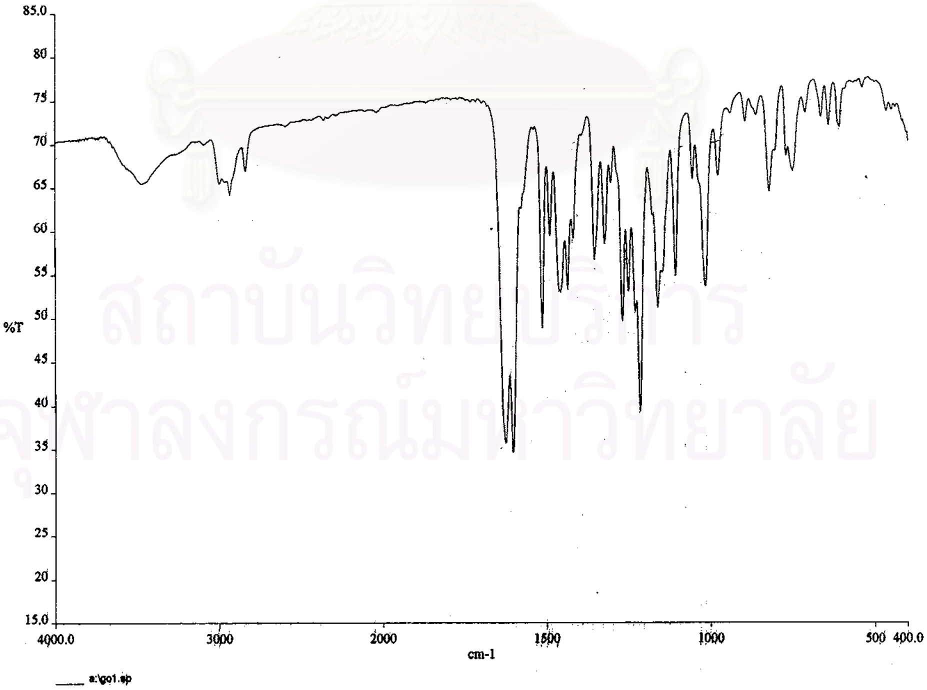

Figure 55 IR Spectrum of compound GT-7 (film)

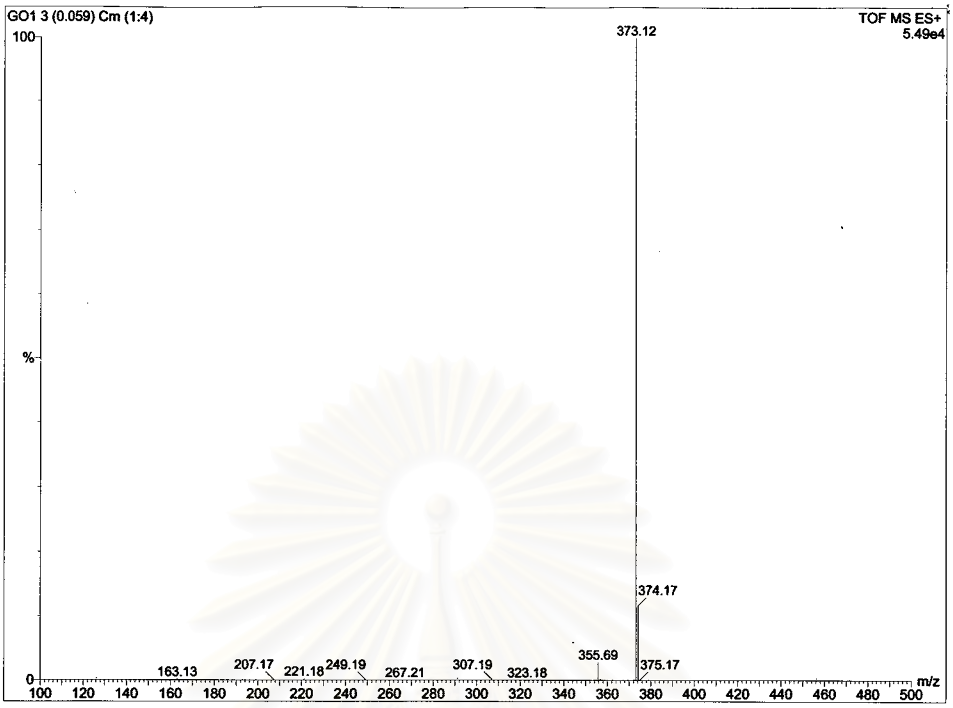

Figure 56 Mass spectrum of compound GT-7

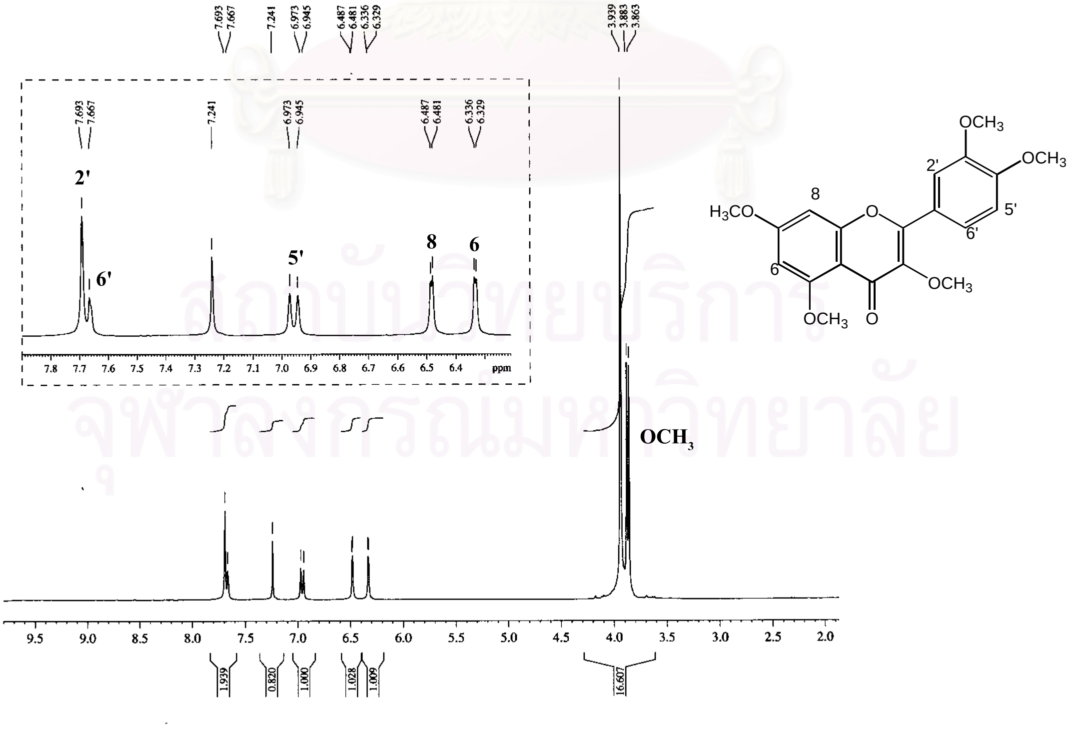

Figure 57 ^{1}H-NMR (300 MHz) Spectrum of compound GT-7 ($CDCl_3$)

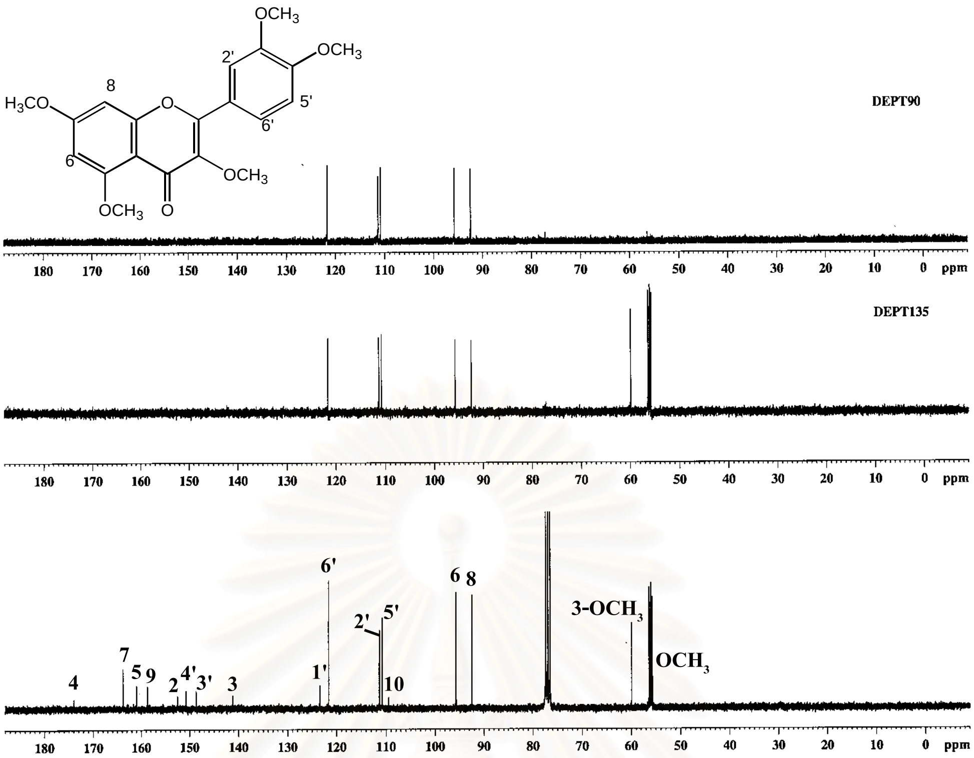

Figure 58 ^{13}C-NMR (75 MHz), DEPT 90 and DEPT 135 Spectra of compound GT-7 ($CDCl_3$)

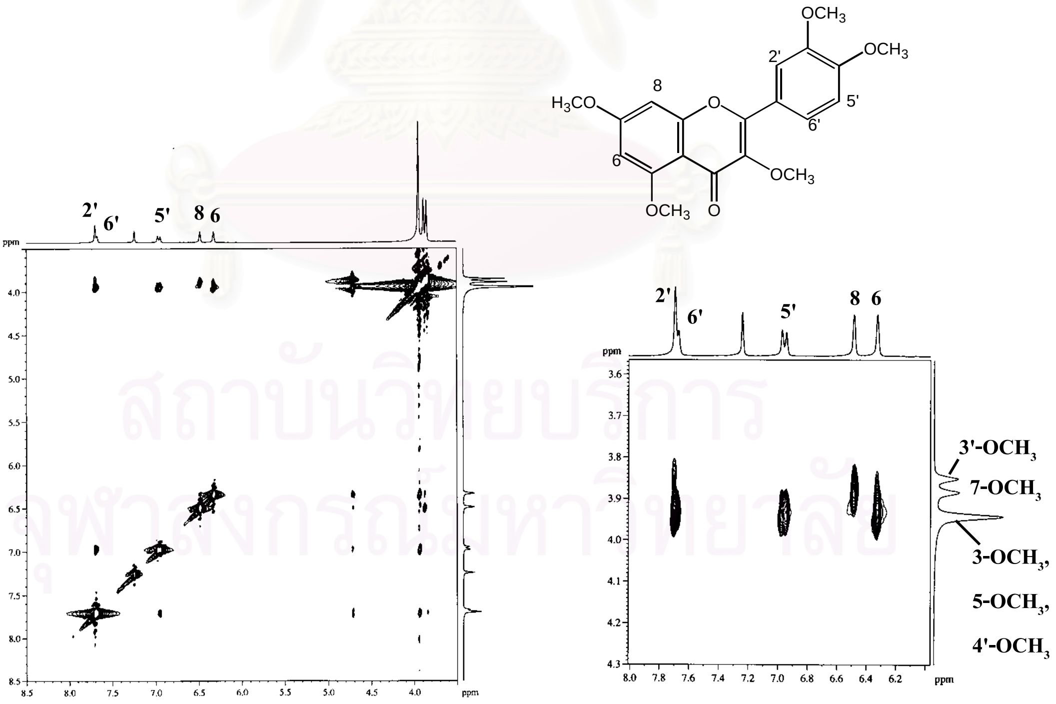

Figure 59 NOESY Spectrum of compound GT-7 ($CDCl_3$)

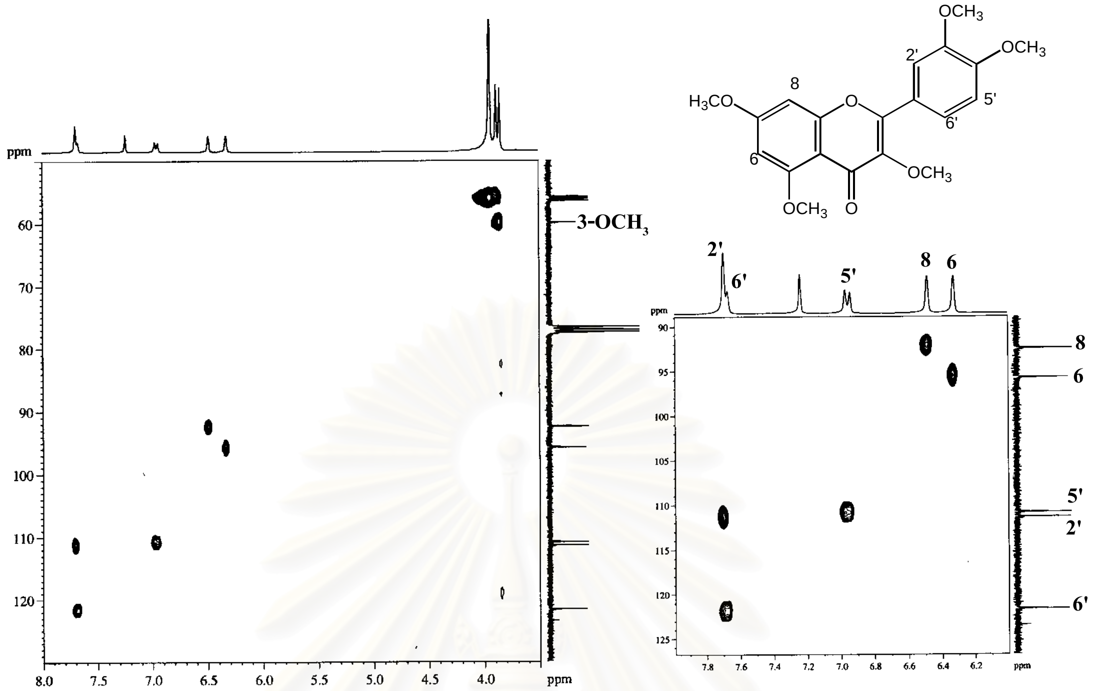

Figure 60 HMQC Spectrum of compound GT-7 ($CDCl_3$)

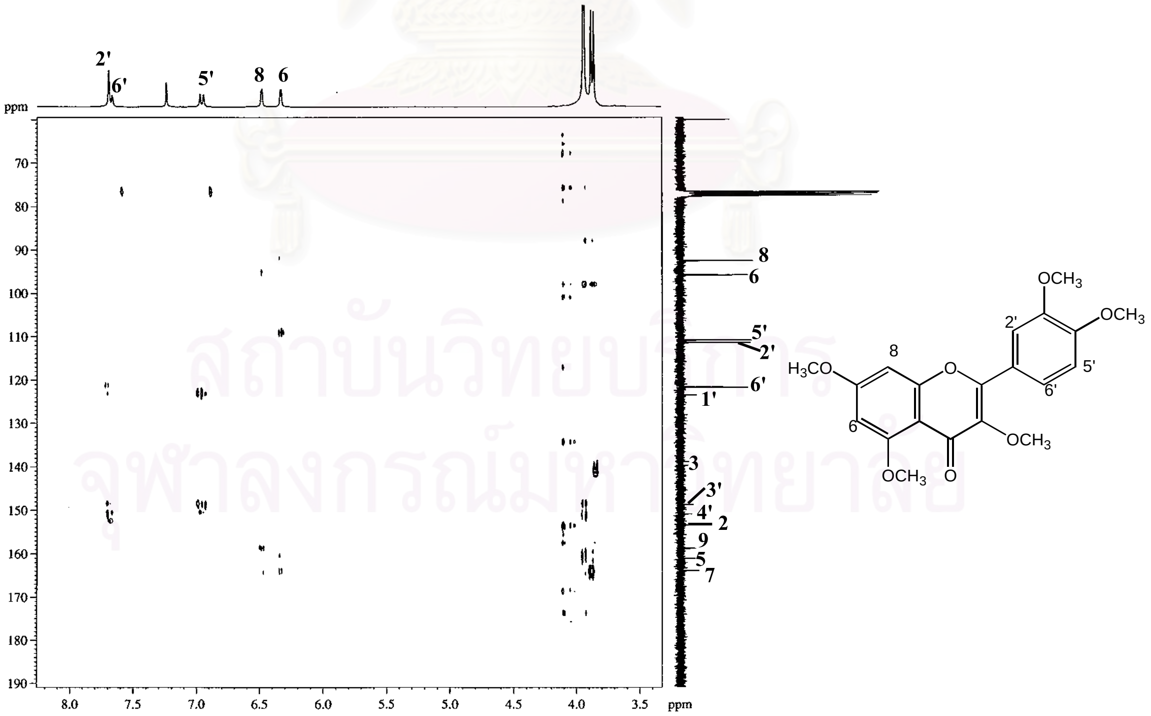

Figure 61 HMBC Spectrum of compound GT-7 ($CDCl_3$)

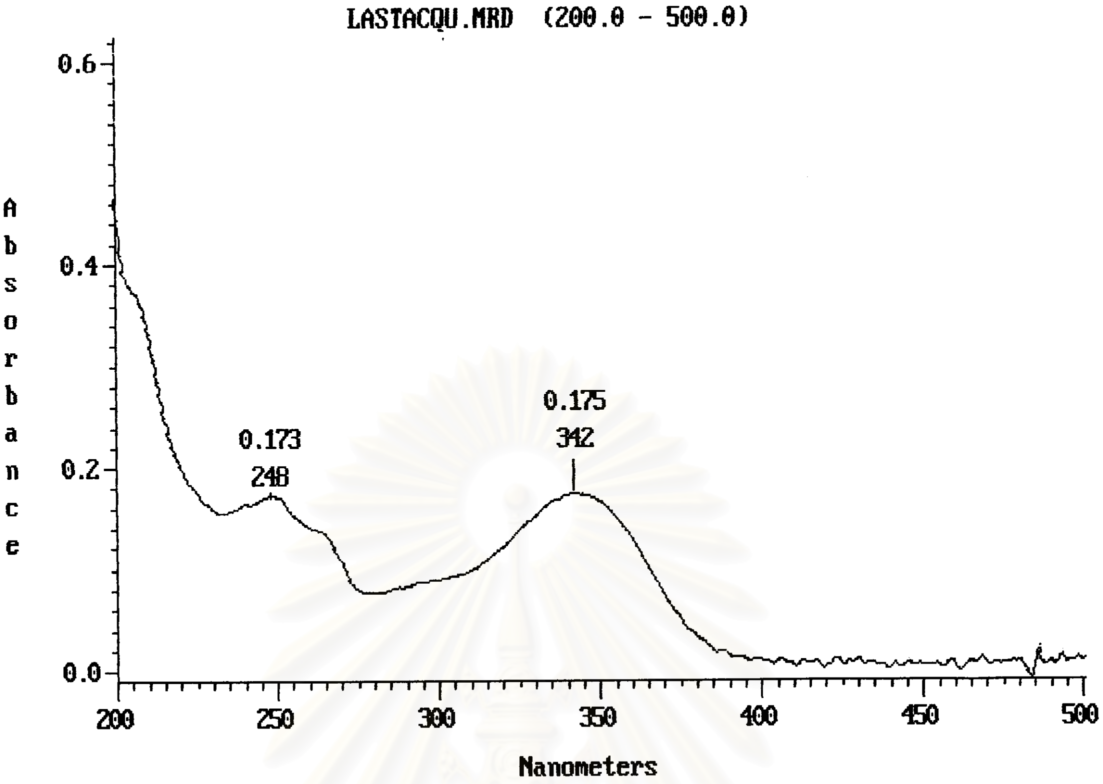

Figure 62 UV Spectrum of compound GT-8 (methanol)

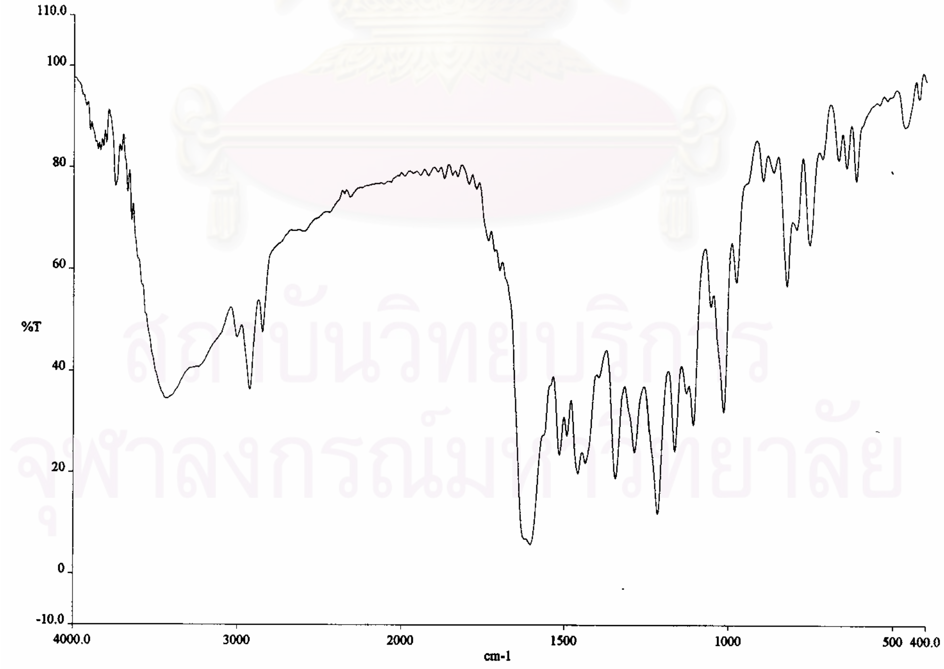

Figure 63 IR Spectrum of compound GT-8 (film)

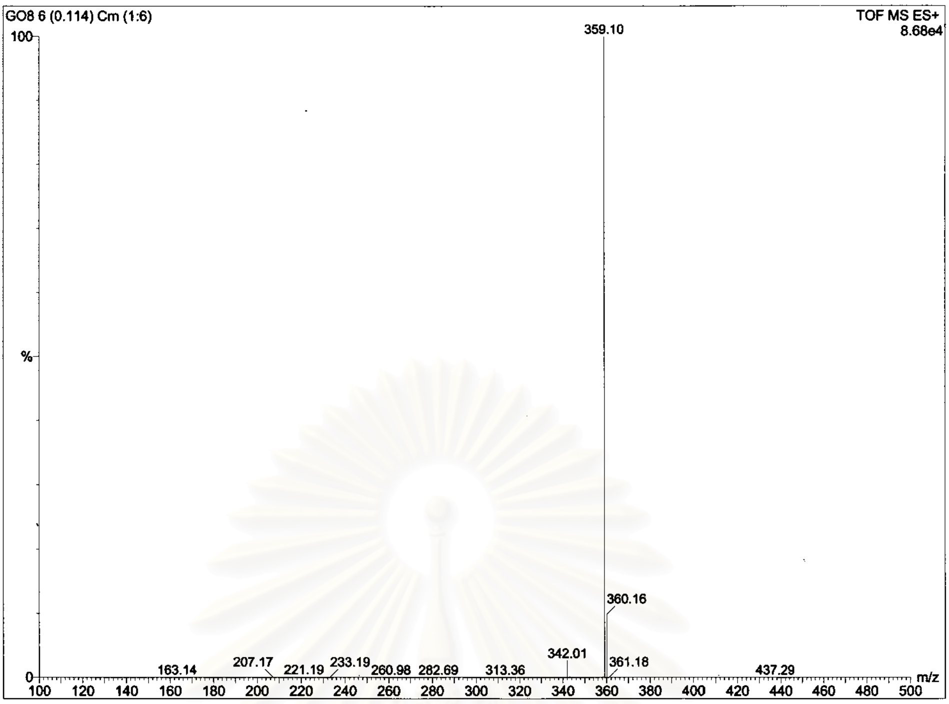

Figure 64 Mass spectrum of compound GT-8

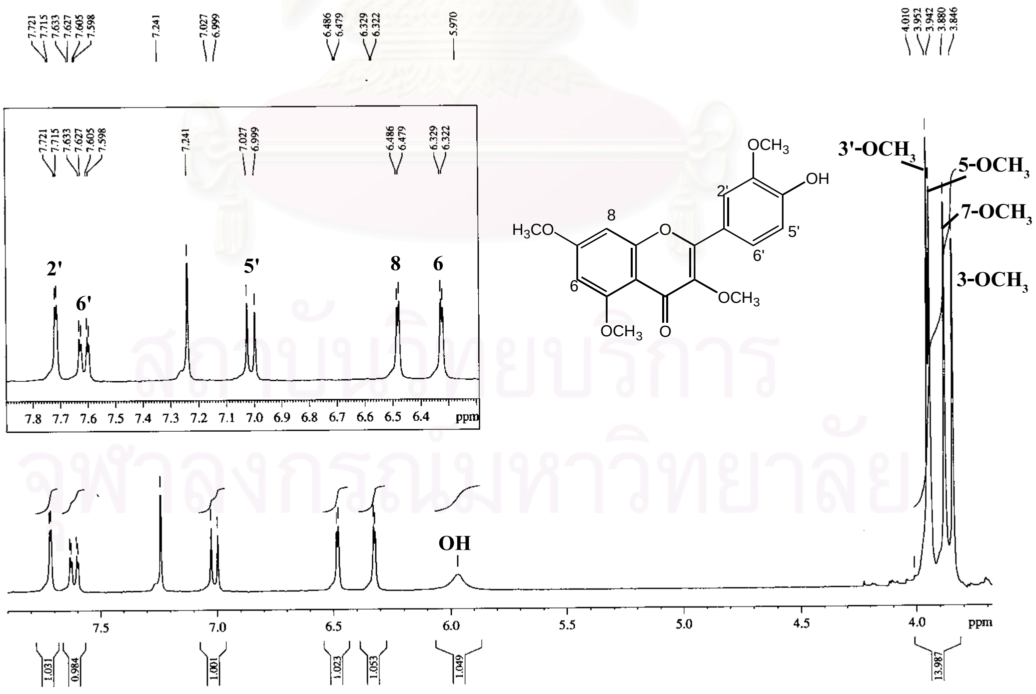

Figure 65 ^{1}H-NMR (300 MHz) Spectrum of compound GT-8 (CDCl$_3$)

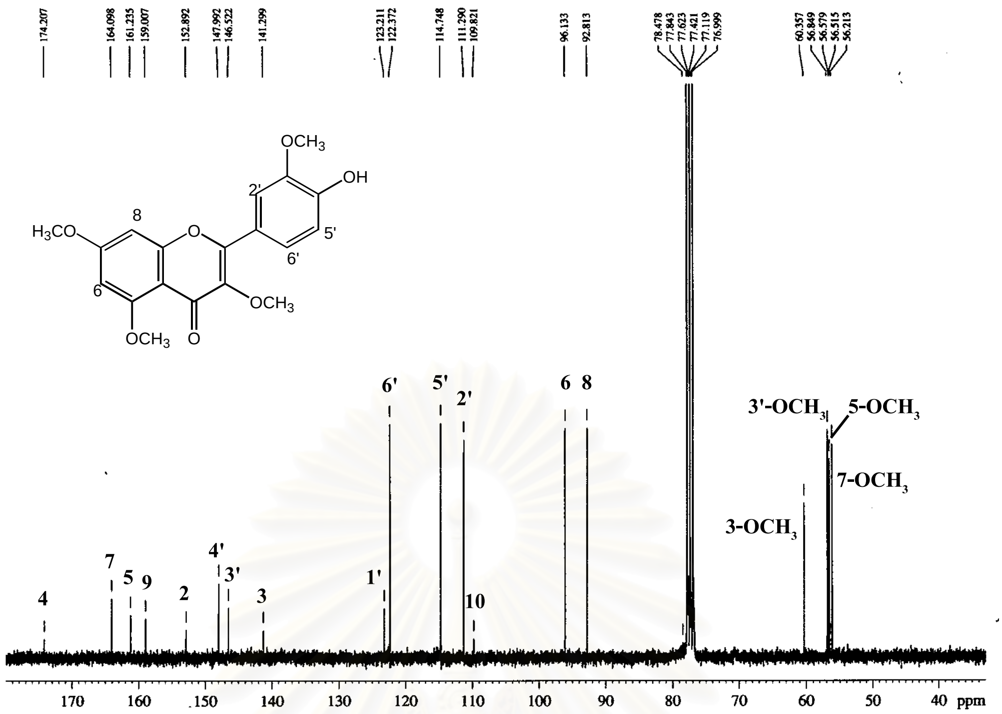

Figure 66 ^{13}C-NMR (75 MHz) Spectrum of compound GT-8 (CDCl$_3$)

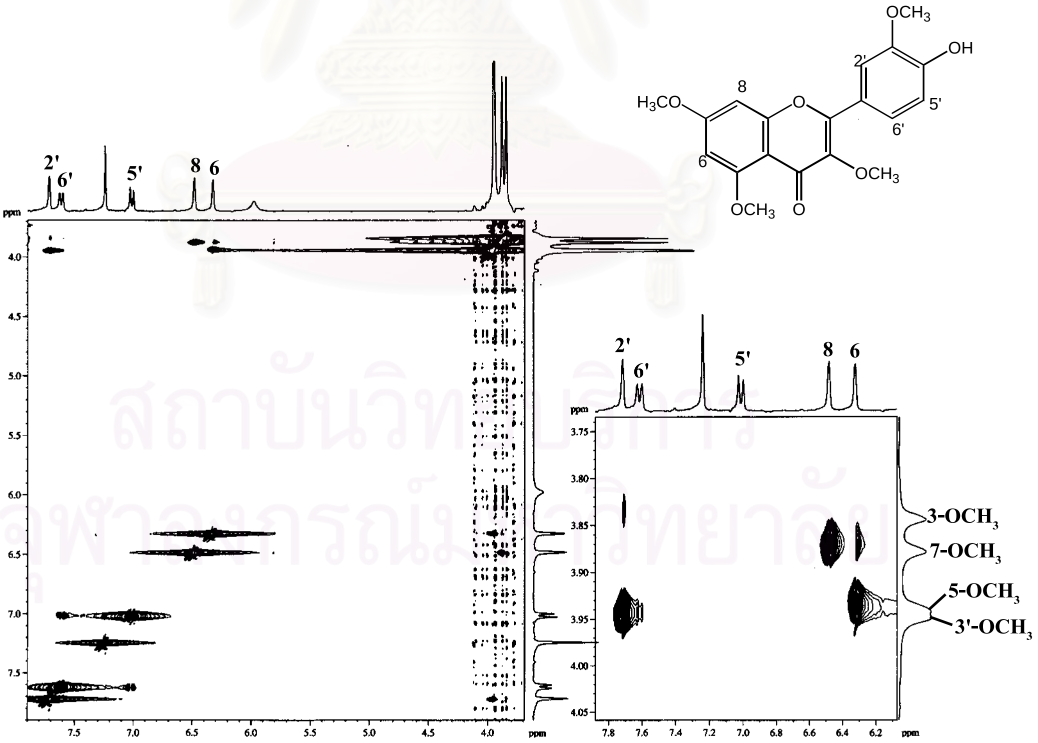

Figure 67 NOESY Spectrum of compound GT-8 (CDCl$_3$)

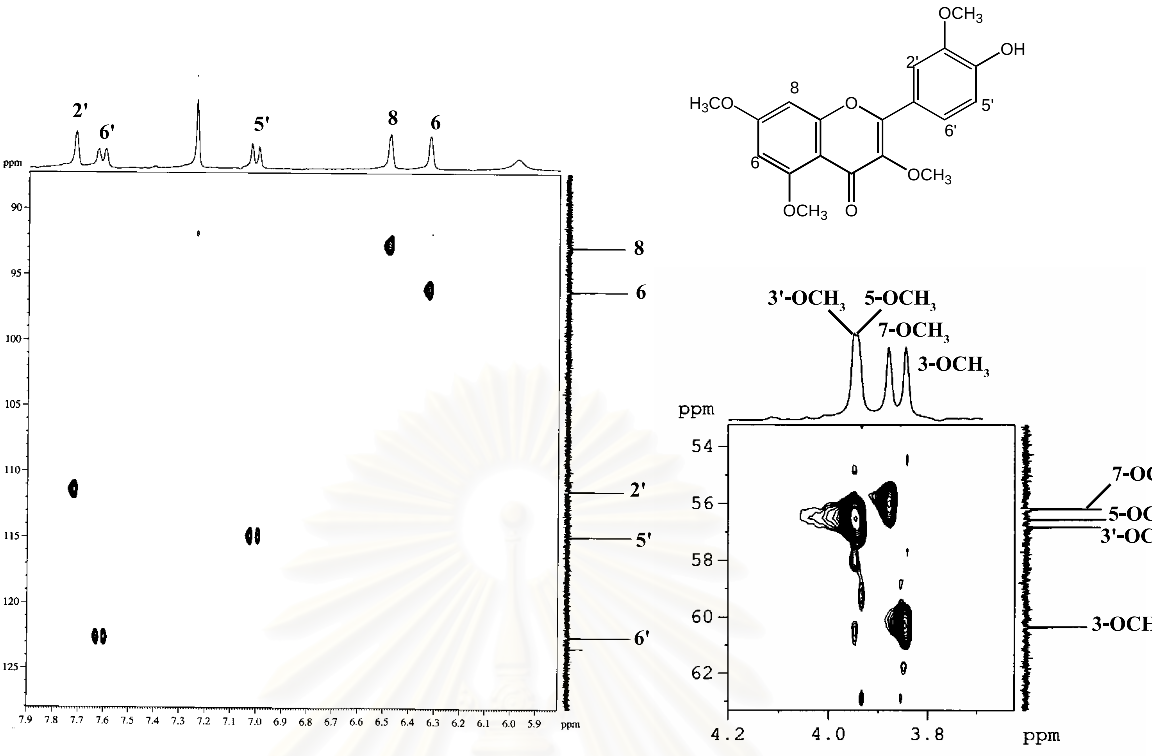

Figure 68 HMQC Spectrum of compound GT-8 ($CDCl_3$)

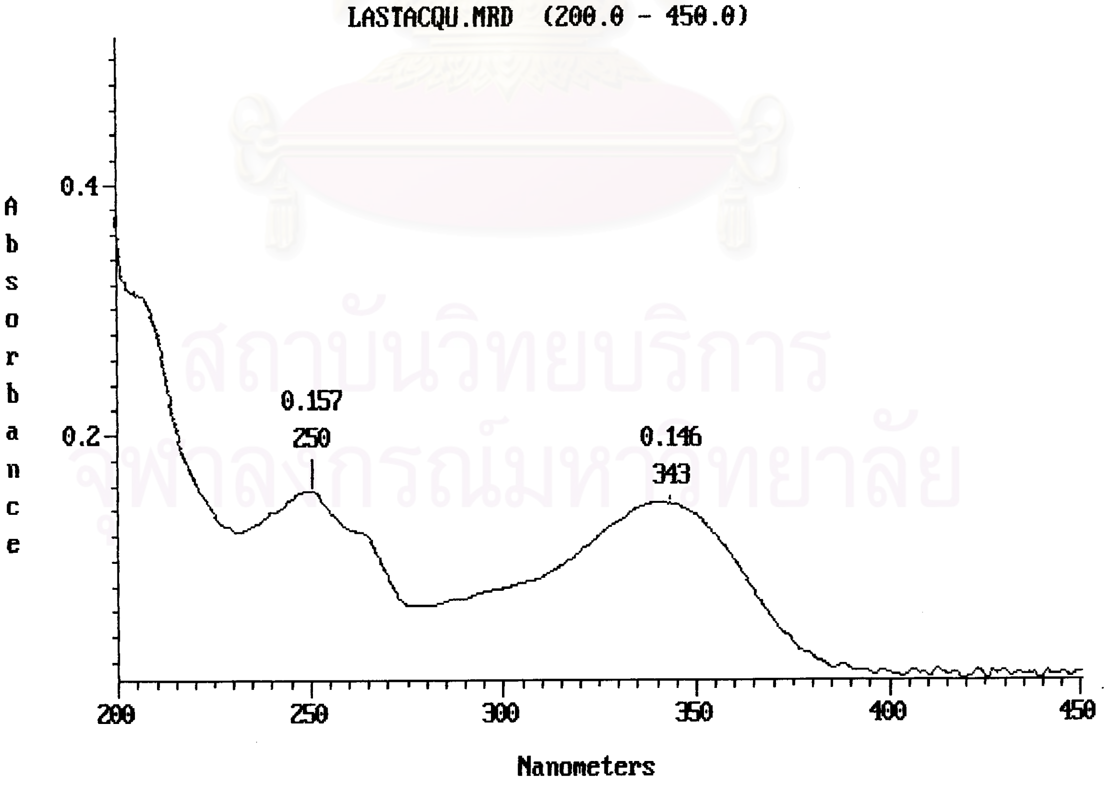

Figure 69 UV Spectrum of compound GT-9 (methanol)

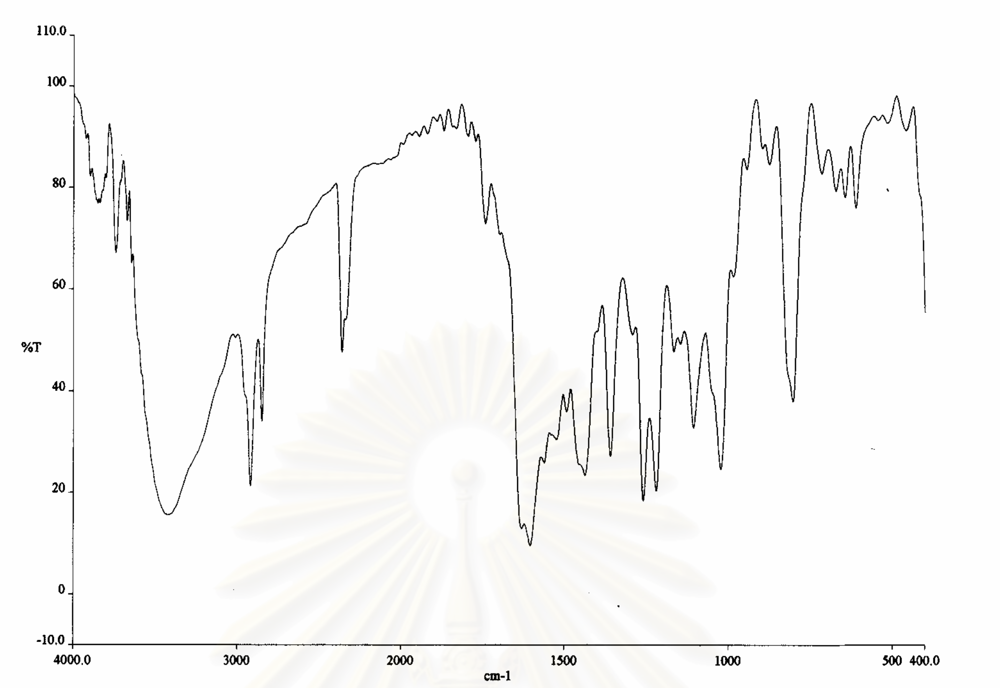

Figure 70 IR Spectrum of compound GT-9 (film)

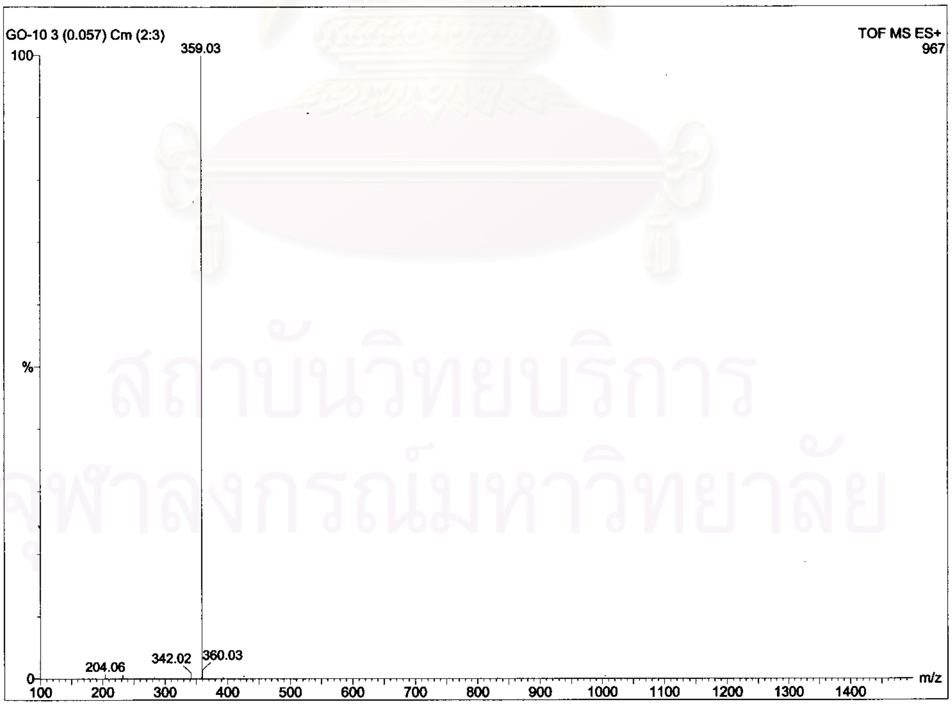

Figure 71 Mass spectrum of compound GT-9

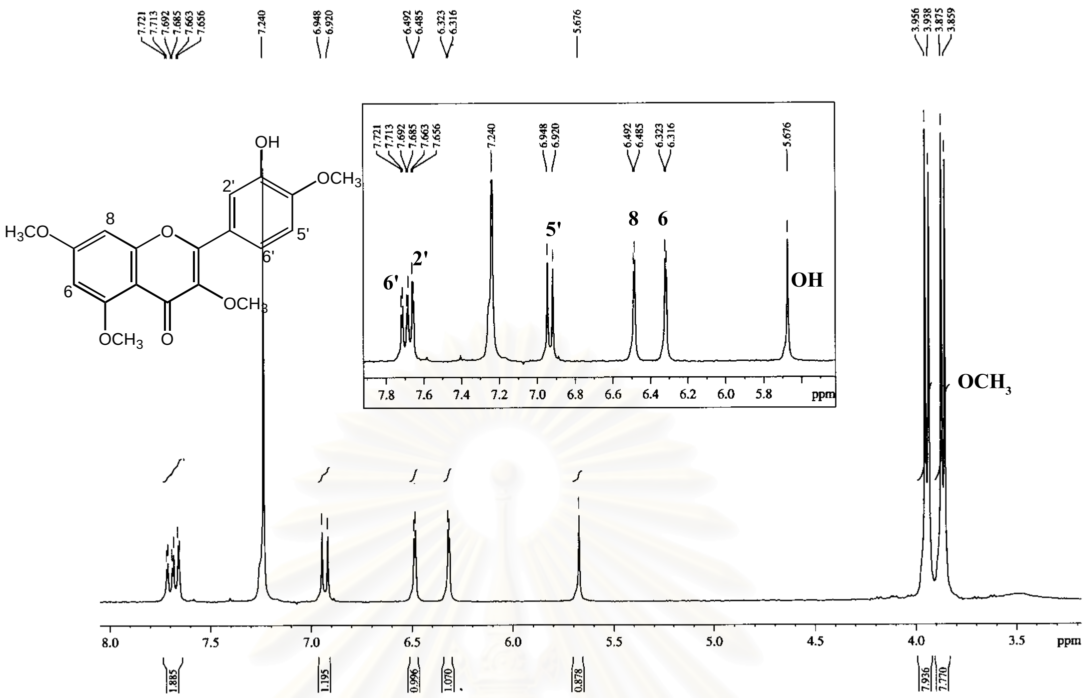

Figure 72 ^{1}H-NMR (300 MHz) Spectrum of compound GT-9 ($CDCl_3$)

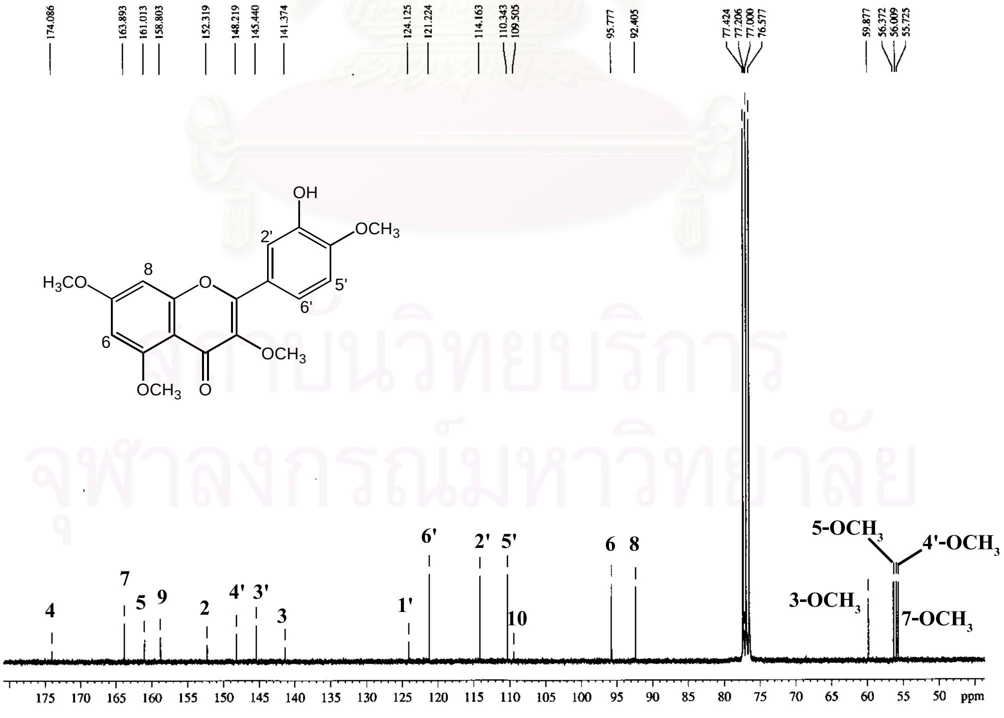

Figure 73 ^{13}C-NMR (75 MHz) Spectrum of compound GT-9 ($CDCl_3$)

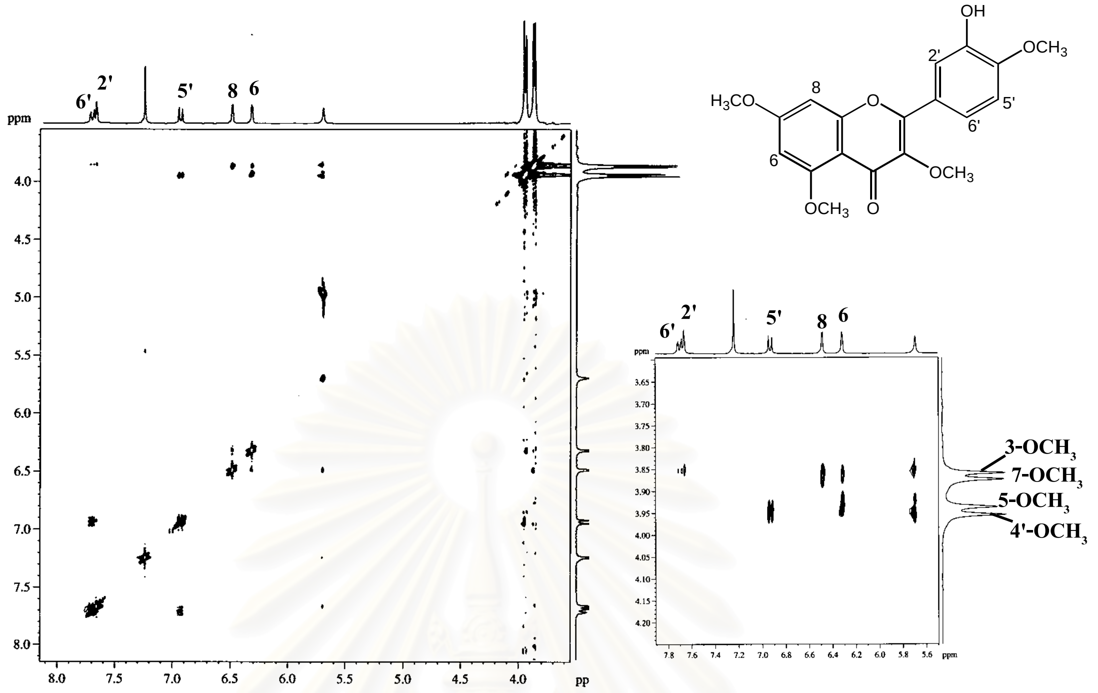

Figure 74 NOESY Spectrum of compound GT-9 ($CDCl_3$)

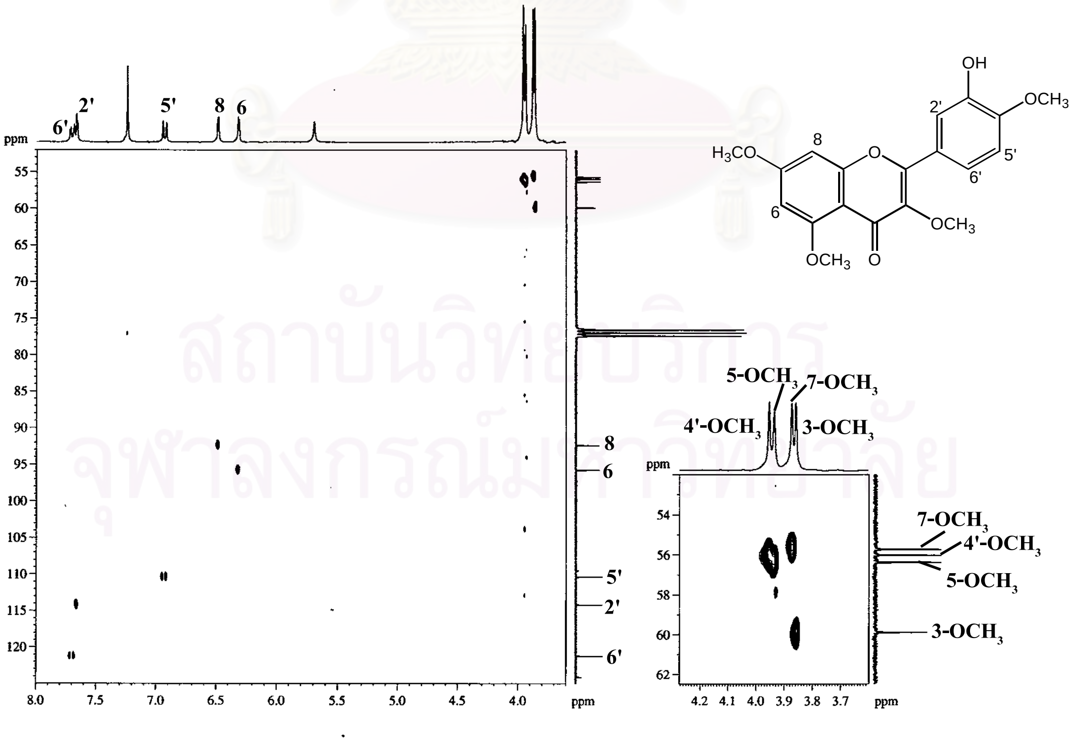

Figure 75 HMQC Spectrum of compound GT-9 ($CDCl_3$)

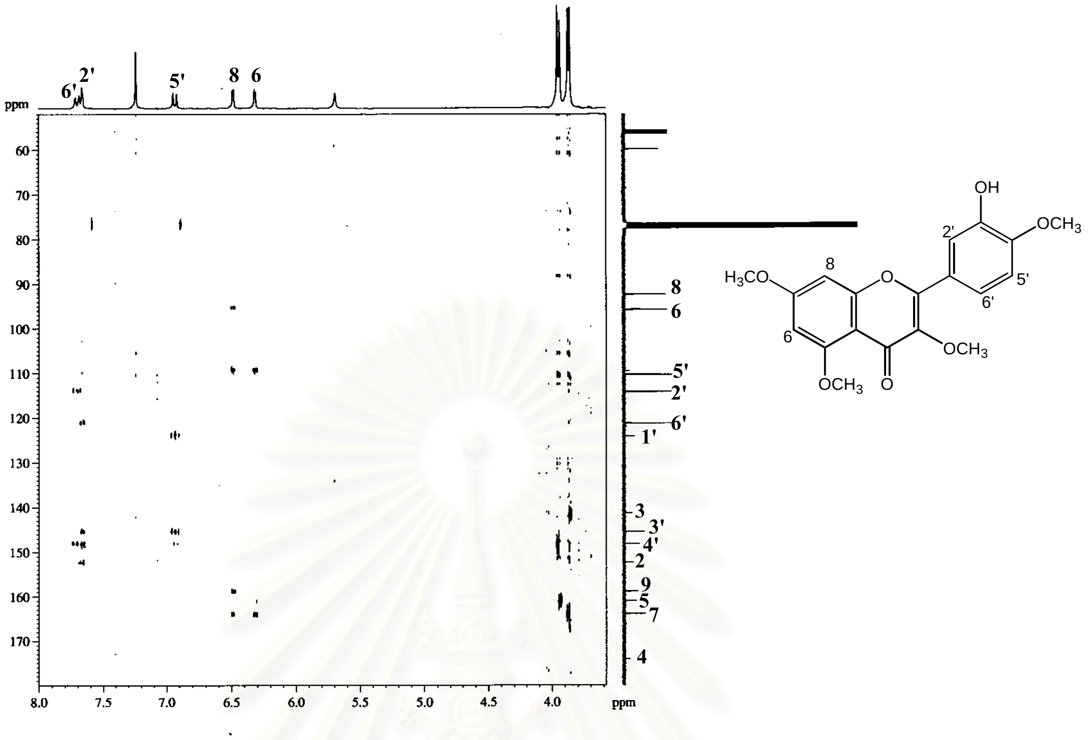

Figure 76 HMBC Spectrum of compound GT-9 ($CDCl_3$) [δ_H 3.6-8.0 ppm, δ_C 50-180 ppm]

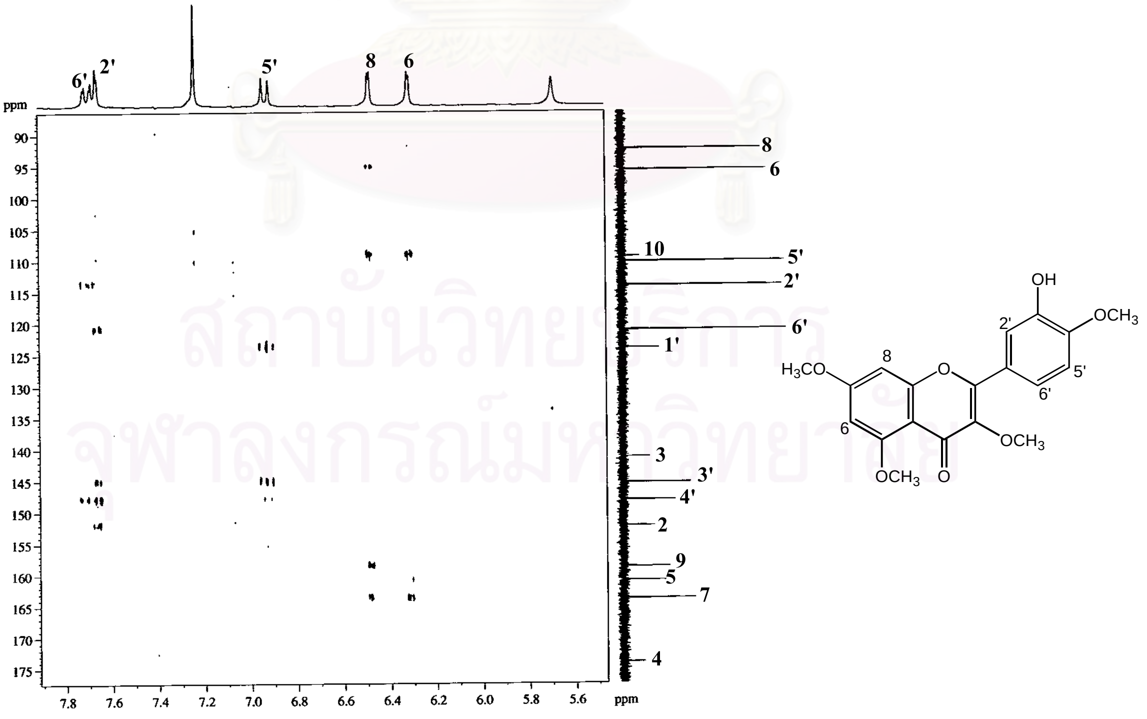

Figure 77 HMBC Spectrum of compound GT-9 ($CDCl_3$) [δ_H 5.5-7.9 ppm, δ_C 85-178 ppm]

VITA

Miss Chaweewan Klongsiriwet was born on April 4, 1976 in Yala, Thailand. She received her Bachelor's degree of Science in Pharmacy in 1997 from the Faculty of Pharmaceutical Sciences, Prince of Songkhla University, Thailand. She is presently working at Sirindhorn Public Health College Trang.

Poster Presentation

Klongsiriwet, C., Likhitwitayawuid, K., Wongseripipatana, S., and Jongbunprasert, V. Chemical constituents of *Goniothalamus tenuifolius* leaves. p. 80 The 19Th Annual Research Meeting in Pharmaceutical Sciences, December 4, 2002, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok.