BIFLAVONOIDS OF *GARCINIA FUSCA* AND *GARCINIA COWA*

A THESIS

BY

NAPAPORN CHAROENRAM

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

MASTER OF SCIENCE DEGREE IN CHEMISTRY

AT SRINAKHARINWIROT UNIVERSITY

JUNE 2014

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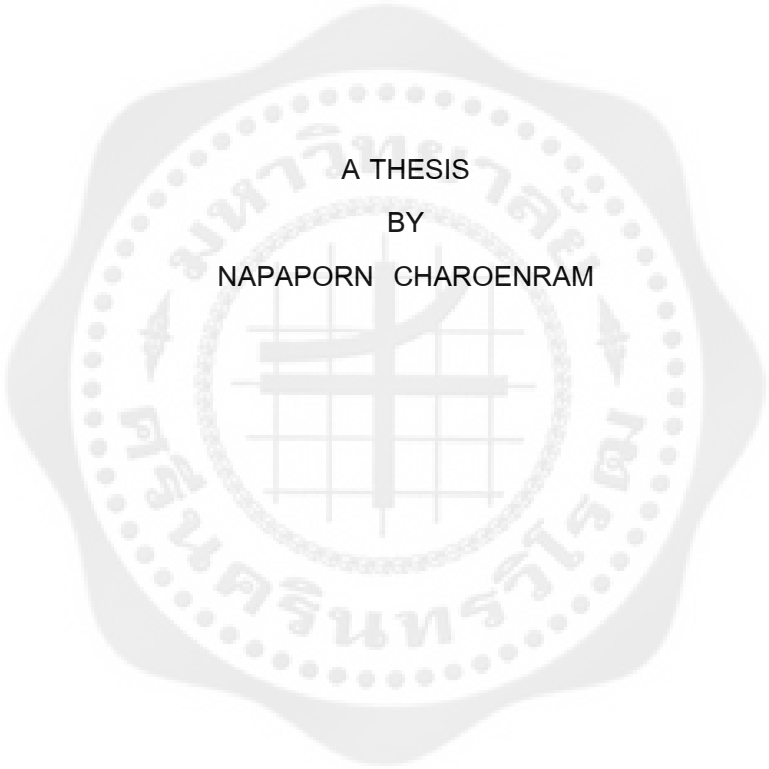

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AN ABSTRACT

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Advisor Committee: Assoc. Prof. Dr. Sunit Suksamrarn, Dr. Prasert Pattanaprateeb.

Garcinia fusca Pierre or “Madan-paa” in Thai (Clusiaceae) is distributed in the North East Thailand and *Garcinia cowa* is commonly known as “Cha muang” or Pru (Surin), which found distributed in Thailand. Chemical investigation of the root of *G. fusca* and *G. cowa* heartwood and bark, led to the isolation of five known biflavonoids named vokensiflavone (**48**), morelloflavone (**32**), fukugiside (**47**), GB-2 (**38**) and spicataside (**81**). This is the first report on isolation of spicataside (**81**) from *G. cowa* and the first time to describe on the antibacterial activity against *Helicobactor pylori* of biflavonoids. The structures of all isolated biflavonoids were elucidated by spectroscopic techniques, especially 1D- and 2D- NMR and MS including by comparison of their spectroscopic data with those reported in the literature. The stereochemistry at C-2 and C-3 of flavanone unit were provided by analysis of *J* coupling constant value between H-2 and H-3. The large coupling constant (12 Hz) of H-2 and H-3 for compounds A-E, in addition, no significant NOE enhancement was observed between both protons in their NOESY spectra, indicated that both hydrogens have a *trans*-diaxial arrangement. The sugar unit in biflavonoid glucosides (C and D) was determined by acid hydrolysis and the resulting sugar residue obtained was proved to be D-(+)-glucose by analysis of its optical rotation activity.

ไบฟลาโวนอยด์ของพืชมะดันป่าและชะมวง

บทคัดย่อ

ของ

นภาพร เจริญรัมย์

เสนอต่อบัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ เพื่อเป็นส่วนหนึ่งของการศึกษา
ตามหลักสูตรวิทยาศาสตรมหาบัณฑิต สาขาวิชาเคมี
มิถุนายน 2557

นภาพร เจริญรัมย์. (2557). *ไบฟลาโวนอยด์ของพืชมะดันป่าและชะมวง*. ปริญญานิพนธ์ วท.ม. (เคมี). กรุงเทพฯ: บัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ. อาจารย์ที่ปรึกษาปริญญานิพนธ์: รองศาสตราจารย์ ดร.สุนิตย์ สุขสำราญ, ดร. ประเสริฐ พัฒนาประทีป.

มะดันป่าเป็นพืชที่พบทางภาคตะวันออกเฉียงเหนือของประเทศไทย ส่วนชะมวงหรือผรู (สุรินทร์) เป็นพืชที่พบได้ทั่ว ๆไปของประเทศไทย จากการศึกษาองค์ประกอบทางเคมีจากรากพืชมะดันป่า แก่นต้นและเปลือกต้นของพืชชะมวง สามารถแยกสารไบฟลาโวนอยด์ได้ 5 ชนิด คือ vokensiflavone (**48**), morelloflavone (**32**), fukugiside (**47**), GB-2 (**38**) และ spicataside (**81**) รายงานนี้เป็นการรายงานครั้งแรกของการแยกสาร spicataside (**81**) จากต้นชะมวงและเป็นครั้งแรกที่มีการรายงานฤทธิ์ต้านเชื้อ *Helicobactor pylori* ของสารในกลุ่มไบฟลาโวนอยด์ ในการพิสูจน์โครงสร้างของสารบริสุทธิ์ใช้เทคนิคทางสเปกโทรสโกปี โดยเฉพาะอย่างยิ่ง 1D และ 2D นิวเคลียร์แมกเนติกเรโซแนนซ์สเปกโทรสโคปี (NMR) และแมสสเปกโทรเมตรี (MS) รวมทั้งเปรียบเทียบข้อมูลกับสารที่มีผู้รายงานไว้แล้ว สเตอริโอเคมีของคาร์บอนตำแหน่งที่ 2 และ 3 ของส่วนฟลาวาโนนพิสูจน์โดยการวิเคราะห์ค่า *J* coupling constant ซึ่งมีค่าใหญ่ (12 Hz) ร่วมกับการพบสัญญาณ NOE enhancement มีค่าน้อยของโปรตอน 2 และ 3 ในสเปกตรัม NOESY แสดงว่า โปรตอนทั้งสองมีการจัดตัวแบบ *trans* การพิสูจน์น้ำตาลของสารประกอบไบฟลาโวนอย์ C และ D ทำได้โดยการไฮโดรไลซิสด้วยกรด แล้วนำน้ำตาลที่ได้ไปศึกษาค่า optical rotation แล้วเปรียบเทียบกับค่า optical rotation กับสารมาตรฐานพบว่าเป็นน้ำตาล D-(+)-glucose

The thesis titled

"Biflavonoids of *Garcinia fusca* and *Garcinia cowa*"

by

Napaporn Charoenram

has been approved by the Graduate School as partial fulfillment of the requirements for the Master of Science Degree in Chemistry of Srinakharinwirot University.

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Napaporn Charoenram

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CHAPTER 1

INTRODUCTION

Background

Garcinia is a genus of plants belongs to the family Clusiaceae or Gutiferae (Negi; et al. 2008, 41: 1857-1861). There are about 450 species distributed in tropical and South Africa, Madagascar, tropical Asia, North East Australia, West Polynesia, tropical America and 20 species in China (Xiwen; et al. 2007, 13: 40-47). The fruit of most species in this genus are edible, among them, those of *Gaecinia mangostana* L. are famous. The seed yields more than 15% oil. The yellow resin of some species is used as a medicine. Species like *Gacinia hanburyi* J. D. Hooker provide medicinal resin and yellow dyes of the best quality. The timber of many species is used for building houses or making furniture (Xiwen; et al. 2007, 13: 40-47). In Thailand, there are 23 species of *Garcinia*s (Smitinand. 2001: 158-159).

Ethanopharmacological uses of *Garcinia* plants

The *Garcinia* species exhibit wide ranges of biological and pharmacological activities. Extracts of *Garcinia* species have been reported as major source of prenylated xanthones, benzophenones and biflavonoids (Babu; et al. 1988, 27: 3332-3335). The fruit hull of *G. mangostana* or “mangosteen” has been used in Thai folk medicine for healing skin infections and wounds, and for the relief of diarrhea (Mahabusarakam; et al. 1987:474-478). It is fairly widespread in India, Sri Lanka and Burma. In the Ayurvedic system of medicine, the fruit hull of *G. mangostana* finds wide application, mainly as an anti-inflammatory agent and in the treatment of diarrhea (Balasubramanian; et al. 1988: 1552-1554). The cytotoxicity of xanthones against three human cancer cell lines, epidermoid carcinoma of the mouth (KB), breast cancer (BC-1), and small cell lung cancer (NCI-H187) of *G. mangostana* young fruit (Suksamrarn; et al. 2006: 301-305) was described. *G. subelliptica* has been extensively cultivated as a windbreak in the Yaeyama islands of Japan. Its bark has been utilized as a source of a yellow colored dye (Fukuyama; et al. 1991: 3433-3436). Extracts of the leaf and flower of *G. livingstonei* are reported to display antibiotic properties (Diserens; et al. 1992: 313-316). The leaf and seed of *G. dulcis* have been used for traditional treatment of lymphatitis, parotitis, struma and other disease conditions (Iinuma; et al. 1996: 1195-1196). The root and leaf of *G. fusca* are used for relief

coughs and fever. Barks may be boiled in water to remedy for fever and skin disease, and the fruit has been used for making a refreshing drink. (Ito; et al. 2003: 200-205). The fruit and leaf of *G. cowa* are used for the improvement of blood circulation, as an expectorant for the treatment of coughs and indigestion, and as a laxative, while the root is used for fever relief and the bark has been used in Thai folk medicine as an antipyretic agent. Moreover, the biflavonoids of *G. cowa* twigs are reported to display antioxidant properties (Panthong; et al. 2009, 87: 1636-1640). The fruit of *G. xanthochymus* which contain biflavonoids was cytotoxic against the SW-480 colon cancer cell line (Muharni; et al. 2011, 11: 169-173). The biflavonoid of *G. kola* was active against a range of oral bacteria (Xu et al. 2013, 147: 497-502).

From the attractive biological activities of *Garcinia* species, especially biflavonoids, it is of interest to search for other bioactivity of biflavonoids from Thai *Garcinia* plants. Previously, only one study on phytochemicals of *G. fusca* stem bark has been reported (Ito; et al. 2003: 200-205). Moreover, no report was described on the antibacterial activity against *Helicobactor pylori* of the isolated biflavonoids. In addition, our preliminary results of TLC observation on the crude extracts of *G. fusca* and *G. cowa* showed the presence of biflavonoids, and these crude extracts exhibited anti *H. pyroli* activity. Therefore, the search for bioactive biflavonoids from *G. fusca* and *G. cowa* will be conducted.

Objectives of the study

1. To isolate and purify biflavonoids from the root of *G. fusca,* from the heartwood and from the stem bark of *G. cowa*.

2. To elucidate the chemical structures of the isolated biflavonoids.

3. To evaluate the antibacterial activity of the obtained biflavonoids against *Helicobactor pylori*.

CHAPTER 2

REVIEW OF LITERATURES

Botanical description of *Garcinia fusca* Pierre

G. fusca known in Thai as “Madan-paa” or Mak-Mong, is distributed in the North East of Thailand. Young leaf is eaten as vegetables either raw or in curry. Ripe fruit is edible but acidic, used to make a refreshing drink. *G. fusca* is similar to *G. subfalcata*, but the latter differs in having more numerous secondary leaf veins (in 28-32 pairs), staminodes united into 4 bundles, and stigma with papillae arranged in pairs (Xiwen; et al. 2007, 13: 40-47). *G. subfalcata* is an erect slow-growing tree about 7 m tall, about 15 cm in diameter with dark brown bark. A branch striate and twigs with broken rings. Petiole 0.4-1.2 cm. Leaf blade narrowly elliptic or elliptic-lanceolate, 3.5-8 × 0.8-2.5 cm. Female flowers solitary or in pairs, usually at apex of branchlet, sometimes axillary; pedicels about 2 mm. The fruit is globose, about 3 cm in diamiter, smooth, nearly sessile.

Figure 1 Pictures of *G. fusca*

Chemical constituents and their bioactivities of *G. fusca*

To date, only one study on the constituents of *G. fusca* reported, eight new xanthones, fuscaxanthones A-H (**1**-**8**), together with eight known xanthones, namely cowanin (**9**), cowanol (**10**)**,** cowaxanthone (**11**), rubraxanthone (**12**)**,** α-mangostin (**13**)**,** β-mangostin (**14**), norcowanin (**15**) and 7-*O*-methylgarcinone E (**16**) were isolated from the acetone extract of *G. fusca* stem bark collected in Thailand. The structures of xanthones **1**-**16** are shown in Figure 2-3.

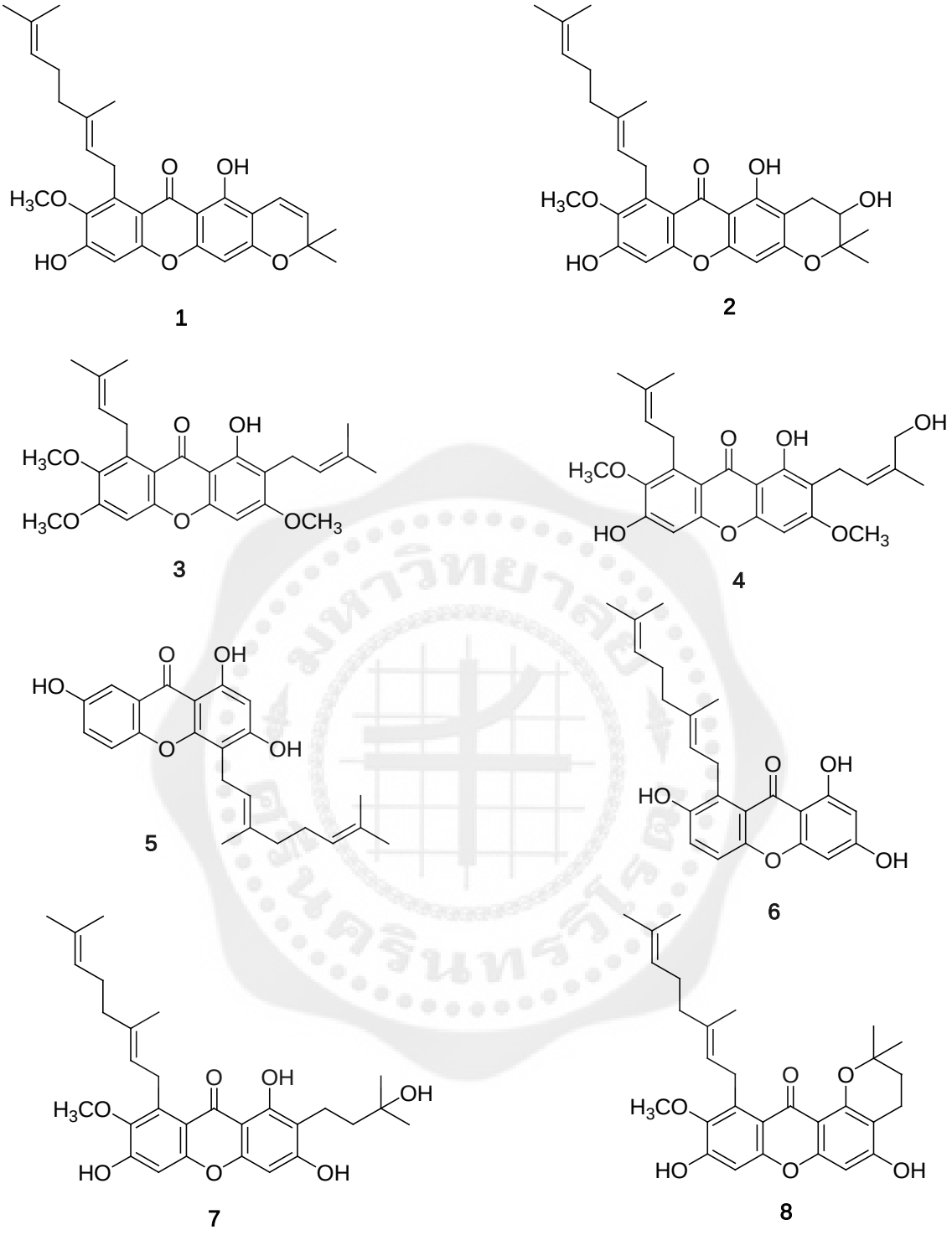


Figure 2 Structures of xanthones **1**-**8** from *G. fusca*

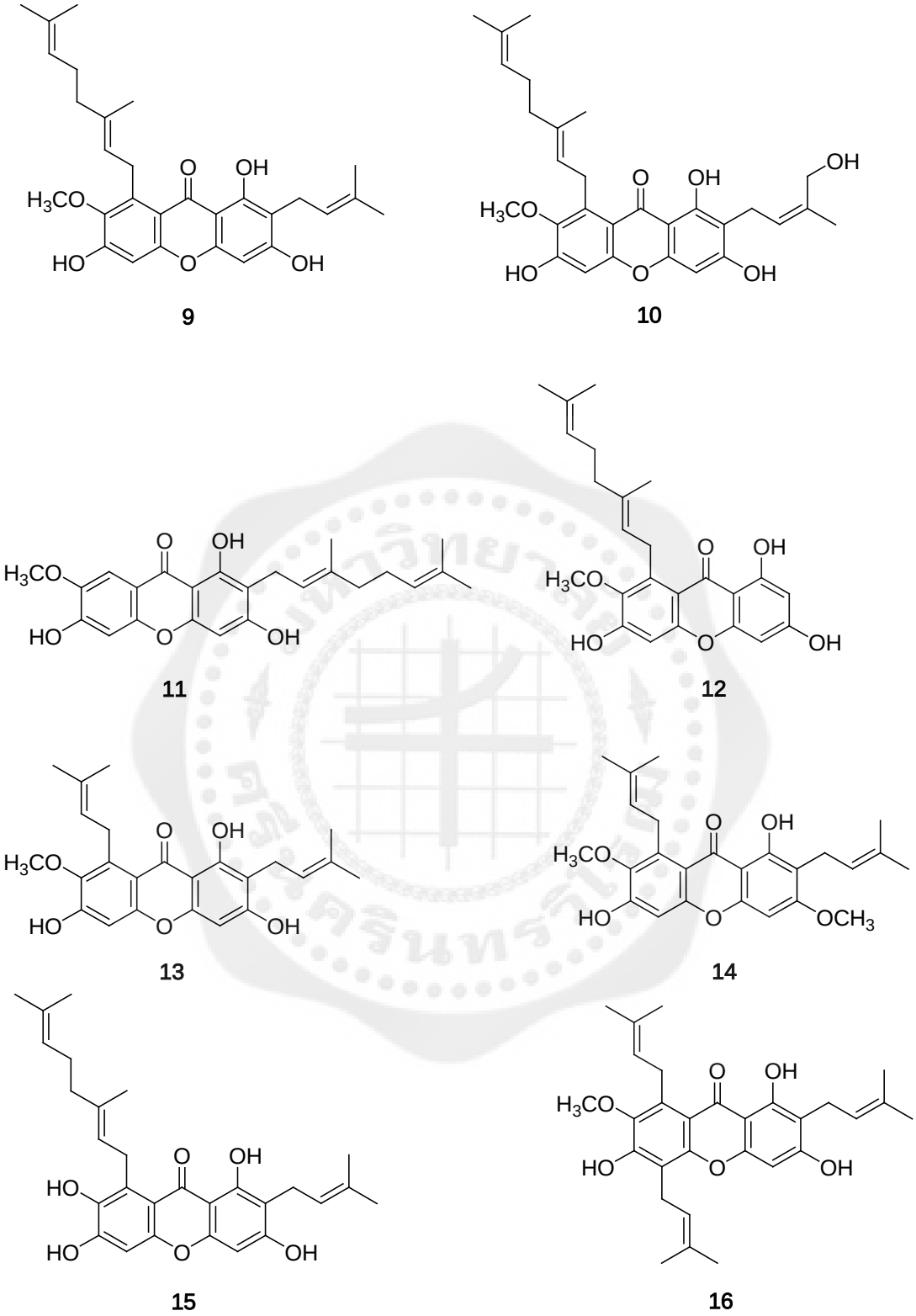


Figure 3 Structures of xanthones **9-16** from *G. fusca*

Fuscaxanthones A-H (**1**-**8**) and eight reported xanthones (**9**-**16**) played an important role in producing inhibitory effects on Epstein-Barr virus early antigen induction. 7-*O*-Methylgarcinone (**16**), having three prenyl side chains at C-2, C-5 and C-8 of the xanthone nucleus, exhibited the most potent inhibitory activity (IC_{50} 210, 100, 83.7, 40.8 and 16.2% inhibition of activation at 1000, 500, 100 mol ratio/TPA, respectively) (Ito; et al. 2003, 66: 200-205).

Botanical description of *Garcinia cowa* Roxb.

G. cowa is commonly known as “Cha muang” or Pru (Surin) and found distributed in Thailand. *G. cowa* is a small to medium-sized tree 15-30 m high. The trees are found scattered in lowland, undulating areas and peat swamp forests. The leaf is opposite, 7-15 cm long and 2-3.5 cm wide. The male flower is pink to red in color and is found in clusters in the leaf axils and also below the leafs. The flower has 4 sepals and 4 petals. The fruit is a subglobose berry, 3 cm in diameter and turns dull orange-yellow when ripe. The seed are embedded in the edible orange pulp. Both the young shoots and fruit are edible. Young leaf and shoot are used in many Thai soups (Poomipamorn; et al. 1997).

Figure 4 Pictures of *G. cowa*

Chemical constituents and their bioactivities of *G. cowa*

In 1997, Likhitwitayawui; et al. were the first group to isolate a new xanthone which was characterized as 7-*O*-methylgarcinone E (**16**) from the stem bark of *G. cowa* collected from the botanical garden of Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand (Likhitwitayawui; et al. 1997, 45: 1299-1301).

In 2005, Mahabusarakam; et al. have isolated six known xanthones, fucaxanthone A (**1**), cowanin (**9**), cowanol (**10**), cowaxanthone (**11**), 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-

methyl-2-butenyl)xanthone (**17**), mangostinone (**18**) and five new compounds, cowagarcinones A-E (**19-23**) from the latex of *G. cowa* which collected in Nakorn Sri Thammarat Province, Thailand. The crude latex and pure compounds were examined for the radical scavenging activity by the DPPH assay. The crude latex was found to be able to scavenge the DPPH radical with a significant result, IC_{50} 13.20 µg/mL. However, compounds **1**, **9**, **10**, **11**, **18**, **19** and **23** showed poor radical scavenging activity, the IC_{50} values being over 200 µM. (Mahabusarakam; et al. 2005, 66: 1148-1153). The structures of xanthones **17-23** are shown in Figure 5.

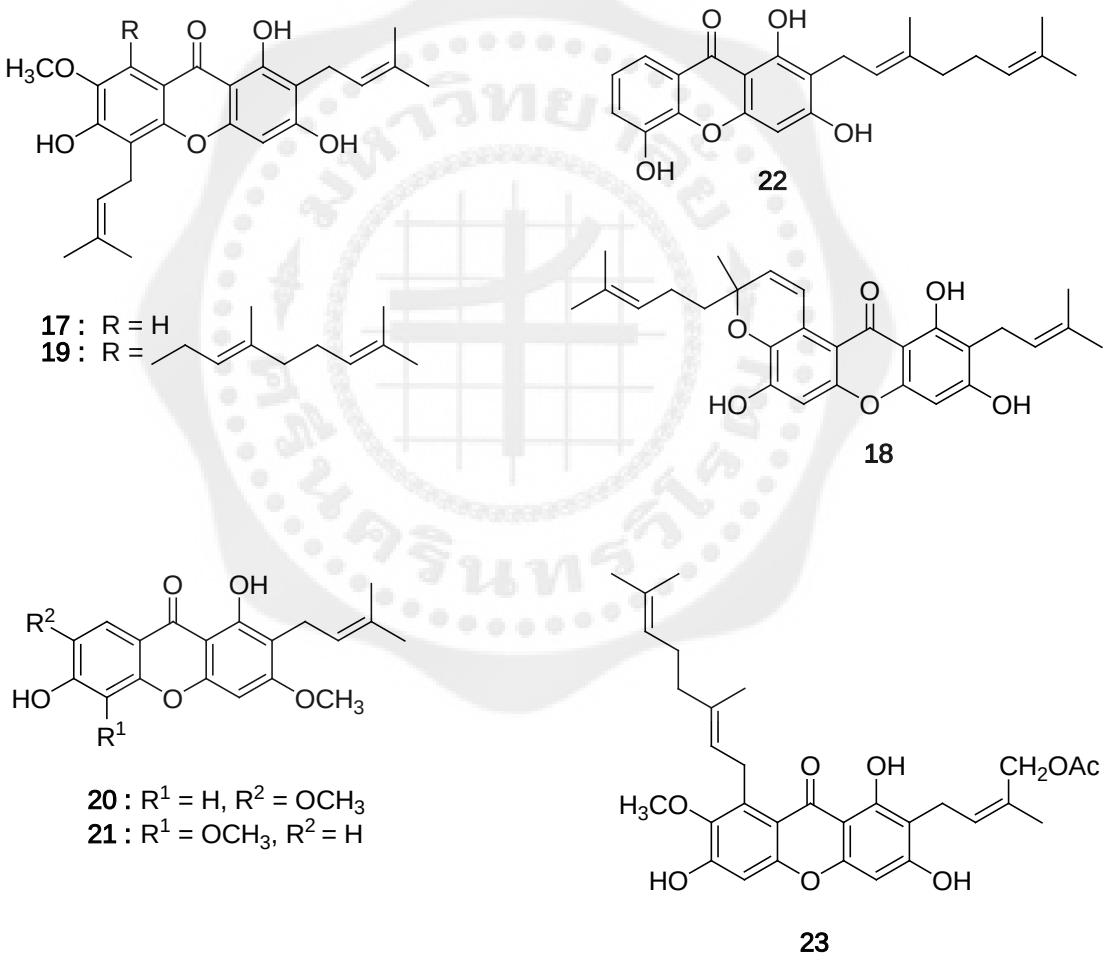


Figure 5 Structures of xanthones **17-23**

In 2006, Panthong; et al. isolated tetraoxygenated xanthones, cowaxanthones A-E (**24-28**), together with 10 previously reported tetraoxygenated xanthones, fuscaxanthone C (**3**), cowanin (**9**), cowanol (**1**0), cowaxanthone (**11**), α-mangostin (**13**), β-mangostin (**14**), 7-O-methylgarcinone E (**16**), 1,6-dihydroxy-3,7-dimethoxy-2-(3-methyl-2-butenyl)xanthone (**29**), mangostanin (**30**) and 6-*O*-methylmangostanin (**31**) from the hexane extract of the fruit of *G. cowa* which was collected from Sathingmoo district, Songkhla province, Thailand. Compound **30** showed the strongest inhibitory activity against *S. aureus*, both penicillin-sensitive strain ATCC 25923 and methicillinresistant strain MRSA SK1, with the same MIC value of 4 μg /mL. Compound **13** exhibited moderate activity against both strains of *S. aureus* with the same MIC value of 8 μg /mL (Panthong; et al. 2006, 67: 999-1004)**.** The structures of xanthones **24-31** are shown in Figure 6.

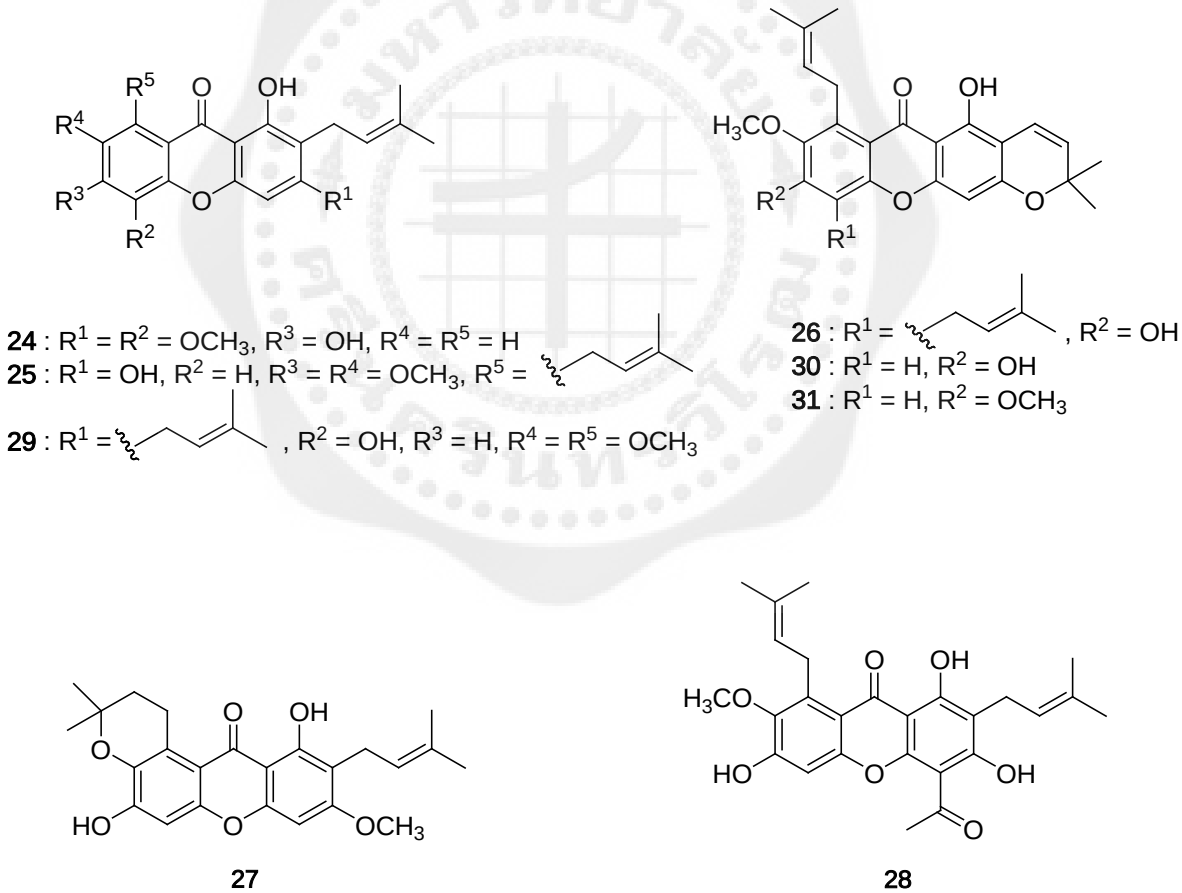


Figure 6 Structures of xanthones **24-31**

In 2006, Shen; et al. found six compounds, morelloflavone (**32**), amentoflavone (**33**), β-sitosterol (**34**), cirsiumaldehyde (**35**), *p*-coumaric acid (**36**) and daucosterol (**37**) which were isolated from the fruits of *G. cowa.* All compounds were isolated from the plant for the first time (Shen; et al. 2006, 41: 660-661). The structures of compounds **32-37** are shown in Figure 7.

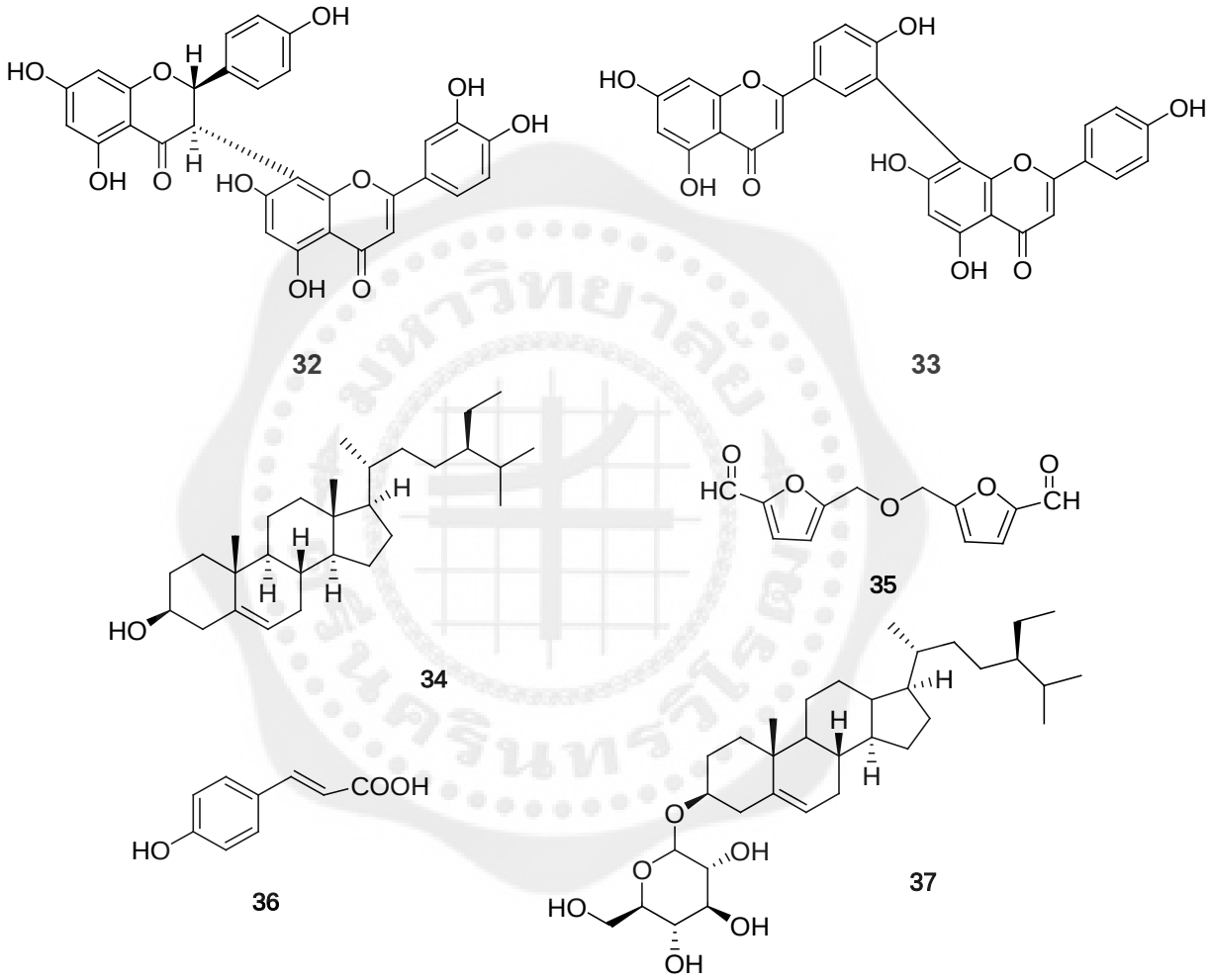


Figure 7 Structures of compounds **32-37**

In 2007, Shen; et al. found ten compounds as β-sitosterol (**34**), daucosterol (**37**) GB-2 (**38**), tetratriacontanoic acid (**39**), friedelin (**40**), palmitic acid (**41**), stigmasterol (**42**), 4-hydroxybenzoic acid (**43**), isovanillic acid (**44**) and kaempferol (**45**) from the branch of *G. cowa* (Shen; et al. 2007, 38: 993-994). The structures of compounds **38-45** are shown in Figure 8.

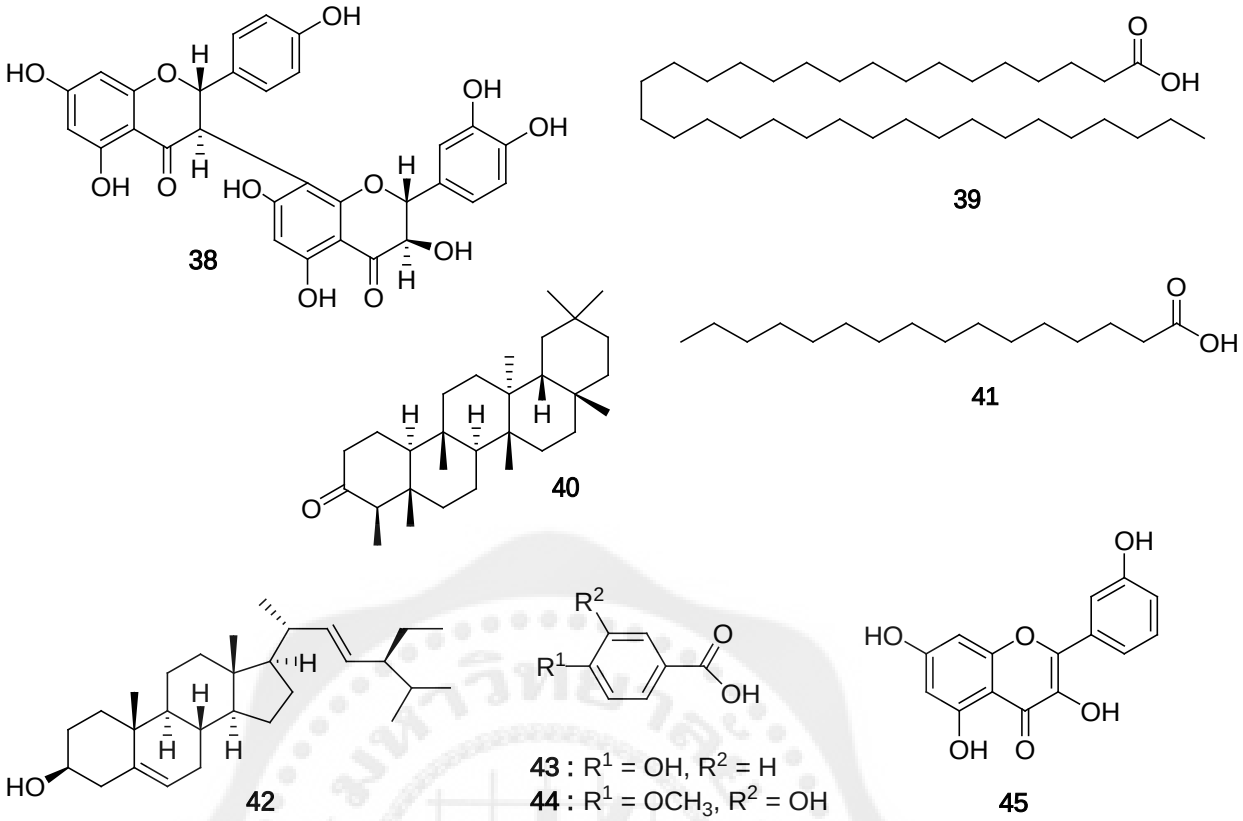


Figure 8 Structures of compounds **38-45**

In 2008, Negi; et al. coworker reported that the hexane and chloroform extracts from the fruit rind of *G. cowa* and *G. pedunculata* were studied for their antibacterial activities against some foodborne pathogens and spoilage bacteria such as *Bacillus cereus, Bacillus coagulans, Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli.* The MICs of the extracts were determined by the agar dilution method and have been ranging from 15 to 500 mg/mL and 300 to 1250 mg/mL for *G. cowa* and *G. pedunculata*, respectively. However, the hexane and chloroform extracts from the fruit rinds of *G. cowa* exhibited marked inhibitory effect against all the test organisms and were more effective than that of *G. pedunculata* extracts. The antibacterial activity of all the extracts has been pronounced against the tested Gram-positive bacteria than the tested Gram-negative bacteriam (Negi; et al. 2008, 41: 1857-1861).

A new tetraoxygenated xanthone, cowaxanthone F (**46**), as well as four known compounds, morelloflavone (**32**), fukugiside (**47**), volkensiflavone (**48**) and 1,6-dihydroxyxanthone (**49**) were isolated from the acetone extract of the twigs of *G. cowa* collected from Singhanakhon district, Songkhla province, Thailand (Panthong; et al. 2009, 87: 1636-1640). The structures of compounds **46**-**49** are shown in Figure 9.

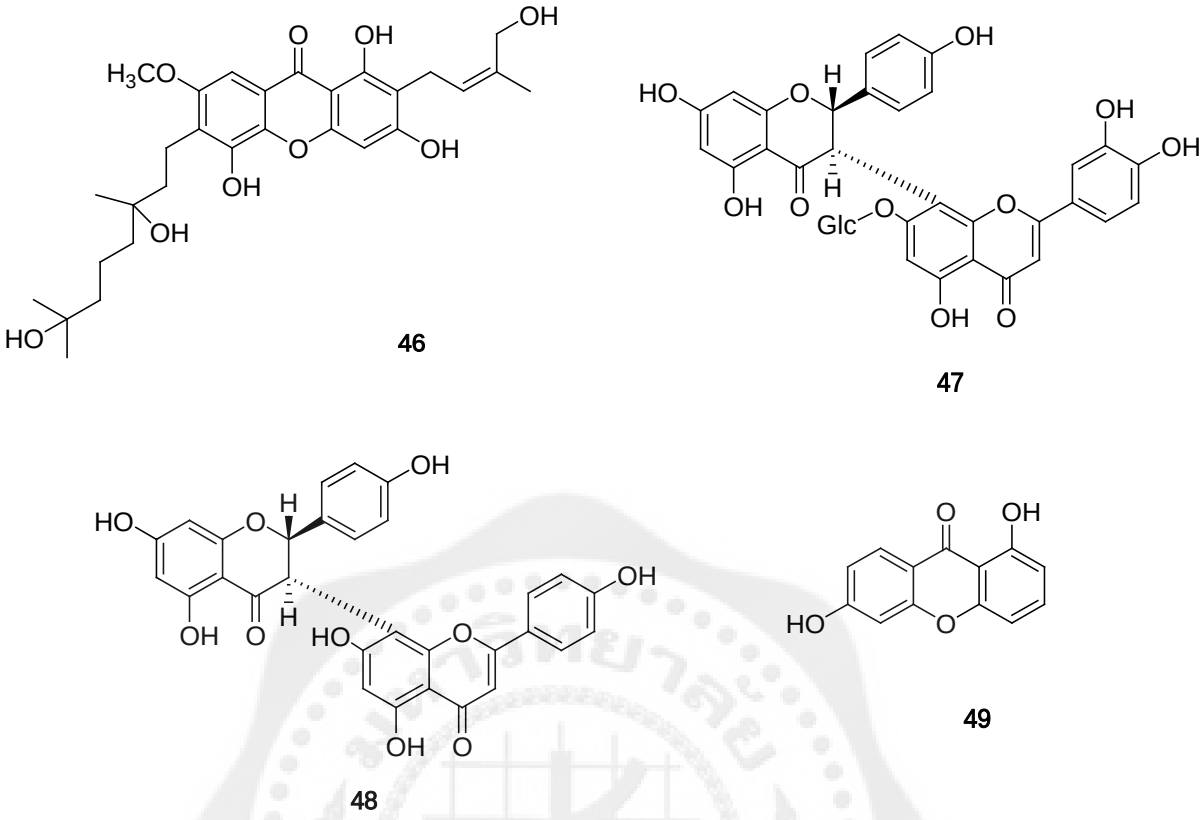
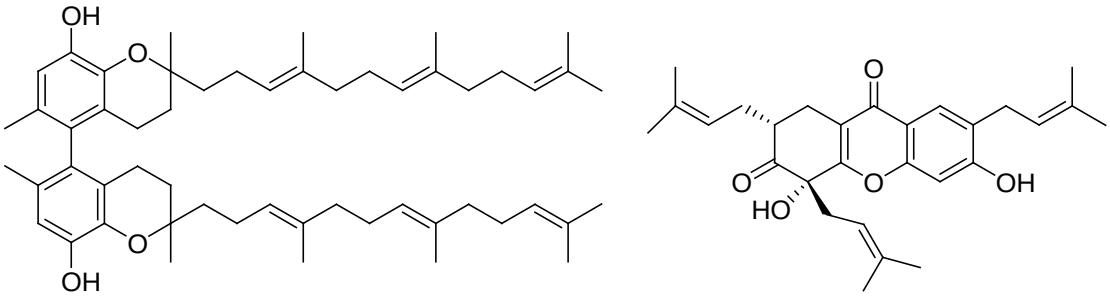


Figure 9 Structures of compounds **46**-**49**

In 2012, Siridechakorn; et al. have found two new compounds, garciniacowol (**50**) and garciniacowone (**51**) along with 15 known compounds, fuscaxanthone A (**1**), fuscaxanthone C (**3**), cowanin (**9**), cowanol (**10**), cowaxanthone (**11**), α-mangostin (**13**), β-mangostin (**14**), norcowanin (**15**), cowagarcinone B (**20**), cowagarcinone D (**22**), cowagarcinone E (**23**), cowaxanthone D (**27**), 6-*O*-methylmangostanin (**31**), parvifoliol F (**52**) and 1,7-dihydroxyxanthone (**53**) from the stem bark of *G. cowa*. Compounds **51** and **10** exhibited good antibacterial activity against MRSA SK1 with the same minimum inhibitory concentration (MIC) value of 2 µg/mL. Moreover, compound **33** also showed good antibacterial activity against *S. aureus* with an MIC value of 2 µg/mL (Siridechakorn; et al. 2012, 83: 1430-1434). The structures of compounds **50**-**53** are shown in Figure 10.



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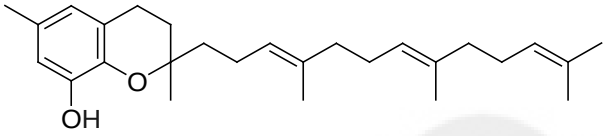

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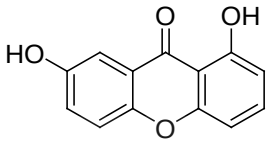
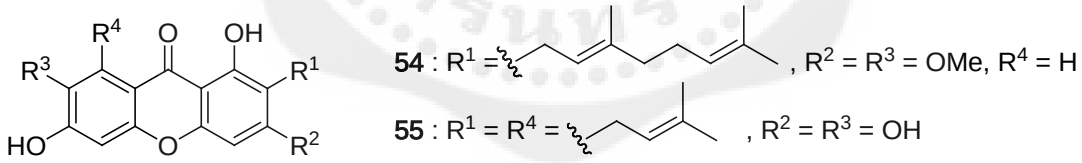


53

Figure 10 Structures of compounds **50**-**53**

In 2013, Na; et al. have found a new prenylated xanthone, 1,6-dihydroxy-3,7-dimethoxy-2-(3,7-dimethyloct-2,6-dienyl) xanthone (3-*O*-methylcowaxanthone) (**54**), together with four known xanthones, cowaxanthone (**11**), α-mangostin (**13**), 7-*O*-methylgarcinone (**16**) and γ-mangostin (**55**) from the latex of *G. cowa*. The cytotoxic activity of prenylated xanthone **54** against five human cancer cell lines, HL-60, SMMC-7721, A-549, MCF-7 and SW480, was evaluated, however it was inactive (IC_{50} > 40 μM) (Na; et al. 2013, 7: 220-224). The structures of xanthones **54**-**55** are shown in Figure 11.



54 : R^1 = , $R^2 = R^3$ = OMe, R^4 = H

55 : $R^1 = R^4$ = , $R^2 = R^3$ = OH

Figure 11 Structures of xanthones **54**-**55**

Ritthiwigrom; et al. reviewed chemical constituents and biological activities of *G. cowa.* Some of these compounds showed interesting pharmacological activities such as morelloflavone (**32**) and fukugiside (**47**) showed strong antioxidant activities. Cowanin (**9**), cowanol (**10**) and α-mangostin (**13**) were commonly found in all parts of *G. cowa* and they can be used as chemotaxonomic markers of this species (Ritthiwigrom; et al. 2013, 7: 212-231).

Biflavonoids

Basically, biflavonoids are flavonoid-flavonoid dimers with varied chemical structures (Kim; et al. 2008, 31: 265-273). Possible structures of biflavonoids could be formed from flavonol, flavone, flavanones, isoflavones, anthocyanin or dihydroflavonol. Basic structure of flavonoids are shown in Figure 13. In general each flavonoid is connected via a C-C or a C-O-C bond (Figure 12) (Lee; et al. 2008, 16: 732-738). There are many types of interflavonoyl link in biflavonoids, these are the 3-8'', 6-8'', 8-8'', 5'-8'', 3'-6'', 4'-6'' and 5'-4''' linkages as shown in Figure 14. However the majority is simply 3'-8'' and 3-8'' linked types (Ito; et al. 2013, 61: 551-558). The hydroxyl and methoxyl groups are substituted at various positions of the biflavonoid nucleus. Babu; et al. reported that the extracts of *Garcinia* species is a major source of several types of compounds including biflavonoids (Babu; et al. 1988, 27: 3332-3335).

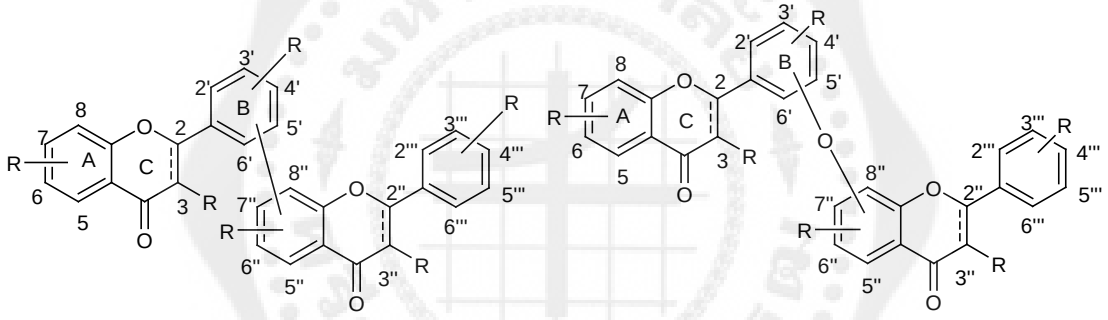


Figure 12 Structures of biflavonoids

Knowledge of the chemical characteristics of biflavonoids from this genus has accumulated since the 1970s and research into many species has shown that the majority of biflavonoids are simply 3'-8'' and 3-8'' linked types (Ito; et al. 2013, 61: 551-558). These biflavonoids have been shown various bioactivities such as peripheral vasodilation, stimulating RNA synthesis in rat hepatocyte suspensions, hypoglycemic effect, cytotoxicity against tissue-cultured cell of human mouth epidermoid carcinoma (KB), inhibition of the expression of the Epstein Barr virus (EBV) gene, inhibition of the interleukin-1β-induced expression of tissue factor on human monocytes, and inhibitory effects on lipid peroxidation, as well as anti-bradykinin, anti-spasmogenic, hepatoprotective, antimicrobial, antiviral activities (Lin; et al. 1999, 120-125), antibacterial (Xu; et al. 2013, 147: 497-502), against intracellular amastigotes and extracellular promastigotes of *L. (L.) amazonensis* (Gontijo; et al. 2012, 58: 613-623), (Table 1).

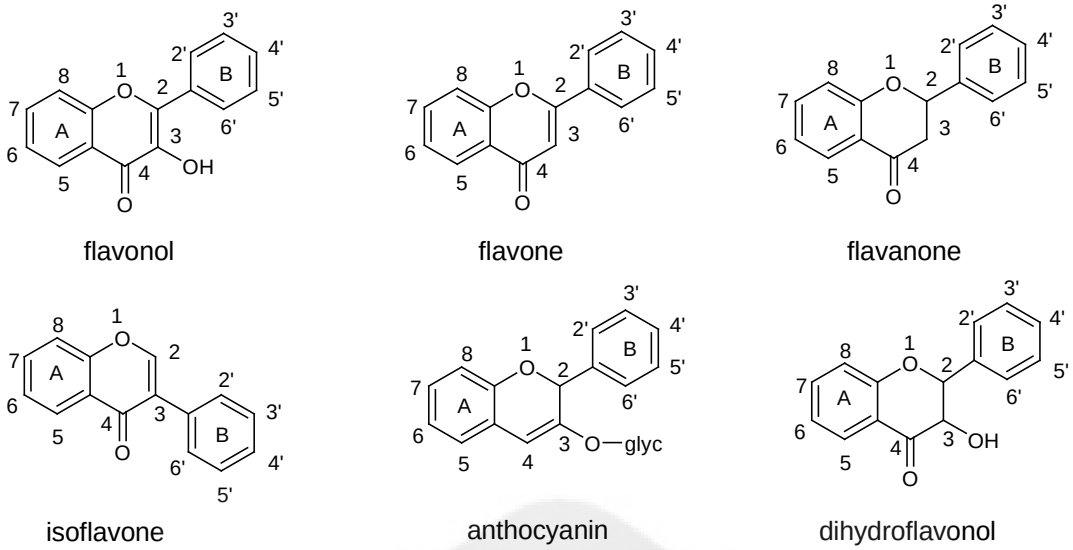
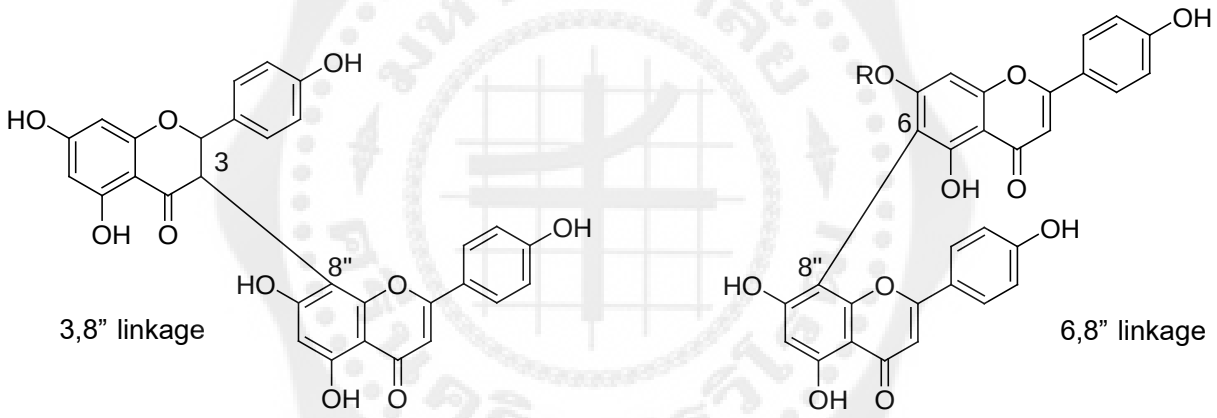


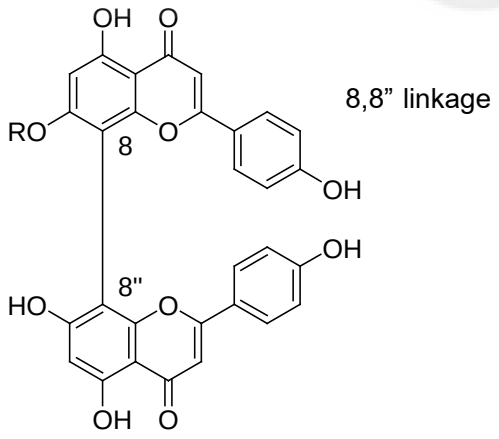
Figure 13 Basic structure of flavonoids



ex. vokensiflavone (**48**)

ex. agathisflavone (**56**) : R = H

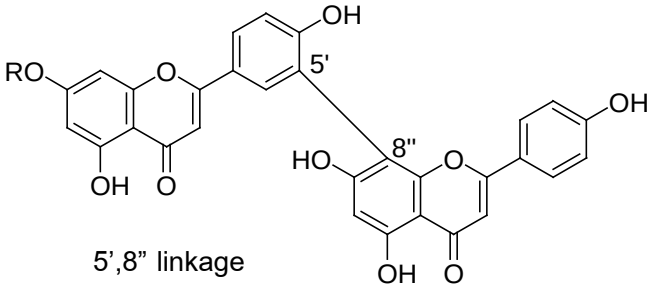
7-*O*-methyllagathisflavone (**57**) : R = CH_3



ex. cupressuflavone (**58**) : R = H

7-*O*-methylcupressuflavone (**59**) : R = CH_3

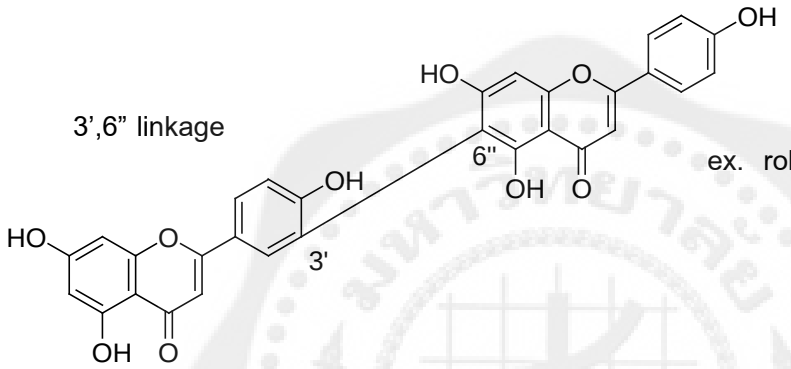
Figure 14 Types of interflavonyl link of biflavonoids



5',8'' linkage

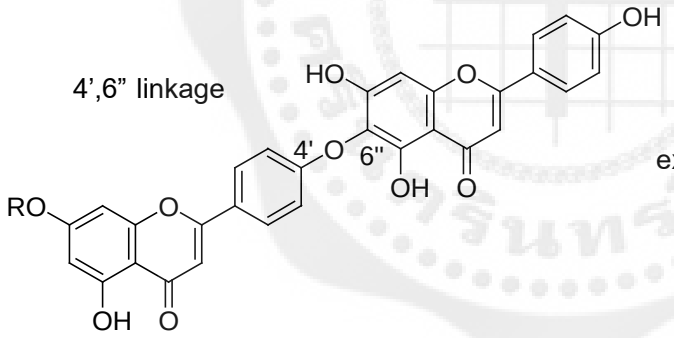
ex. amentoflavone (**33**) : R = H

sequoiaflavone (**60**) : R = CH_3



3',6'' linkage

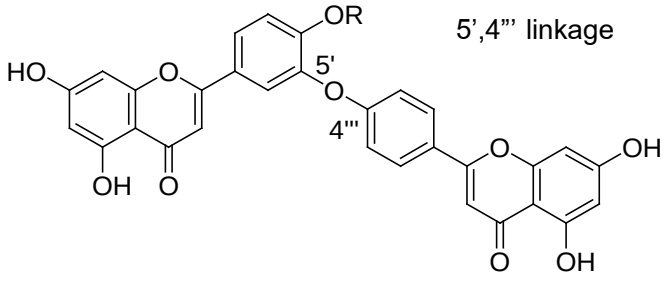
ex. robustaflavone (**61**)



4',6'' linkage

ex. hinokiflavone (**62**) : R = H

neocryptomerin (**63**) : R = CH_3



5',4''' linkage

ex. ochnaflavone (**64**) R = H

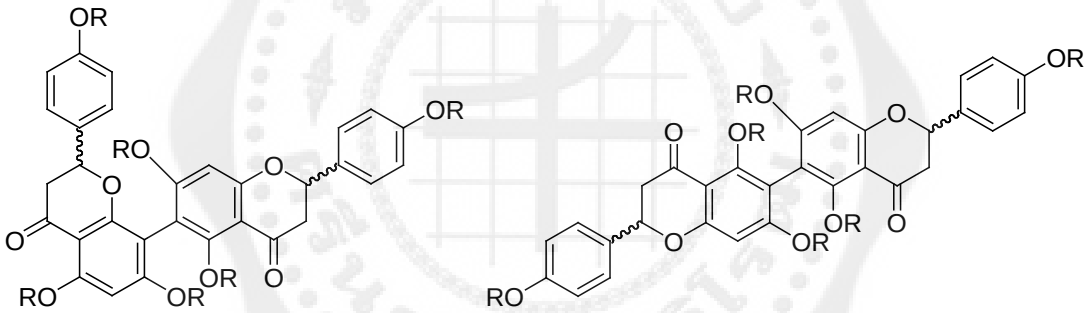
ocryptomerin 4',- methylether (**65**) : R = CH_3

Figure 14 (continued)

Biflavonoids of some *Garcinia* plants and their bioactivities.

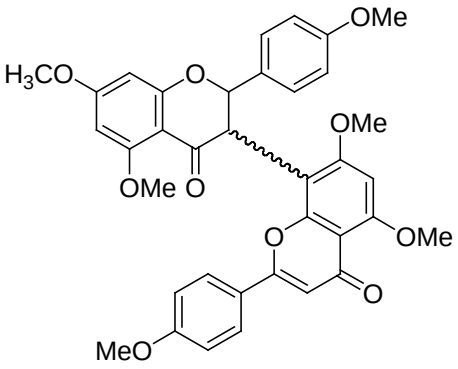
Morelloflavone (**32**) of *G. spicata* has shown anti-inflammatory activity in mice with potent inhibitory effects on TPA-induced edema (ID_{50} = 58.5 μg/ear) (Gil; et al. 1997, 53: 733-740), (Table 1).

In 1999, Lin; et al. found seven biflavonoids, amentoflavone (**33**), vokensiflavone (**48**), agathisflavone (**56**), robustaflavone (**61**), hinokiflavone (**62**), rhusflavanone (**66**), succedaneaflavanone (**67**) which were isolated from *Rhus succedanea* and *G. multiflora*, together with their methyl ether and acetates, vokensiflavone hexamethyl ether (**68**), ehusflavanone hexaacetate (**69**) and succedaneaflavanone hexaacetate (**70**). The structures of biflavonoids **66-70** are shown in Figure 15. Moreover, their antiviral activities have been also evaluated (Table 1). The result indicated that robustaflavone (**61**) exhibited strong inhibitory effect against influenza A and influenza B viruses with EC_{50} values of 2.0 μg/mL



66 : R = H
69 : R = $COCH_3$

67 : R = H
70 : R = $COCH_3$



68

Figure 15 Structures of biflavonoids **66-70**

and 0.2 μg/mL, respectively, and selectivity index values (SI) of 16 and 454, respectively. Amentoflavone (**33**) and robustaflavone (**61**) exhibited moderate anti-HSV-1 and anti-HSV-2 activities. Amentoflavone (**33**) showed its EC_{50} values of 17.9 μg/mL (HSV-1) and 48.0 μg/mL (HSV-2) and SI values of >5.6 (HSV-1) and >2.1 (HSV-2). Robustaflavone (**61**) showed its EC_{50} values of 8.6 μg/mL (HSV-1) and 8.5 μg/mL (HSV-2), and SI values of >11.6 (HSV-1) and >11.8 (HSV-2). Rhusflavanone (**66**) demonstrated inhibitory activities against influenza B, measles and HSV-2 viruses with SI values of 9.3, 8 and >6.4, respectively. Succedaneaflavanone (**67**) exhibited inhibitory activities against influenza B virus and VZV with SI values of 15 and <3.0, respectively (Lin; et al. 1999, 65: 120-125).

Morelloflavone (**32**), fukugiside (**47**) and volkensiflavone (**48**) which isolated from the acetone extract of the twigs of *G. cowa*, were tested for antioxidant activity against DPPH (diphenylpicrylhydrazyl), hydroxyl, and superoxide radicals. Morelloflavone (**32**), and fukugiside (**47**) exhibited high potency. In the DPPH assay both compounds (**32** and **47**) showed equal potency with an IC_{50} value of 18 mmol/L. Morelloflavone (**32**) and fukugiside (**47**) also exerted their inhibitory effects on the hydroxyl radical with IC_{50} values of 0.56 and 0.74 mmol/L, respectively. In the superoxide anion assay, fukugiside (**47**) (IC_{50} 0.89 mmol/L) was less effective than that of morelloflavone (**32**) (IC_{50} 0.27 mmol/L) (Table 1), (Panthong; et al. 2009, 87: 1636-1640).

The $CHCl_3$ and EtOAc partitions from *G. xanthochymus* fruits which contained biflavonoids, showed activity in the DPPH assay (IC_{50} = 32 and 105 μg/mL, respectively) and cytotoxicity against the SW-480 colon cancer cell line (IC_{50} = 15 and 50 μg/mL, respectively) (Muharni; et al. 2011, 11: 169-173).

Six new biflavonoids, garciniaflavones A-F (**71**-**76**) as well as the five known biflavonoids, (+)-morelloflavone (**32**), amentoflavone (**33**), (+)-fukugiside (**47**) podocarpusflavone A (**77**) and (+)-4'''-*O*-methylmorelloflavone (**78**) were isolated from the acetone extract of the leave of *G. subelliptica*. Only amentoflavone show strongly inhibited hypoxia-inducible factor-1 in human embryonic kidney 293 cells under hypoxic conditions (Table 1), (Ito; et al. 2013, 61: 551-558). The structures of biflavonoids **71**-**78** are shown in Figure 16.

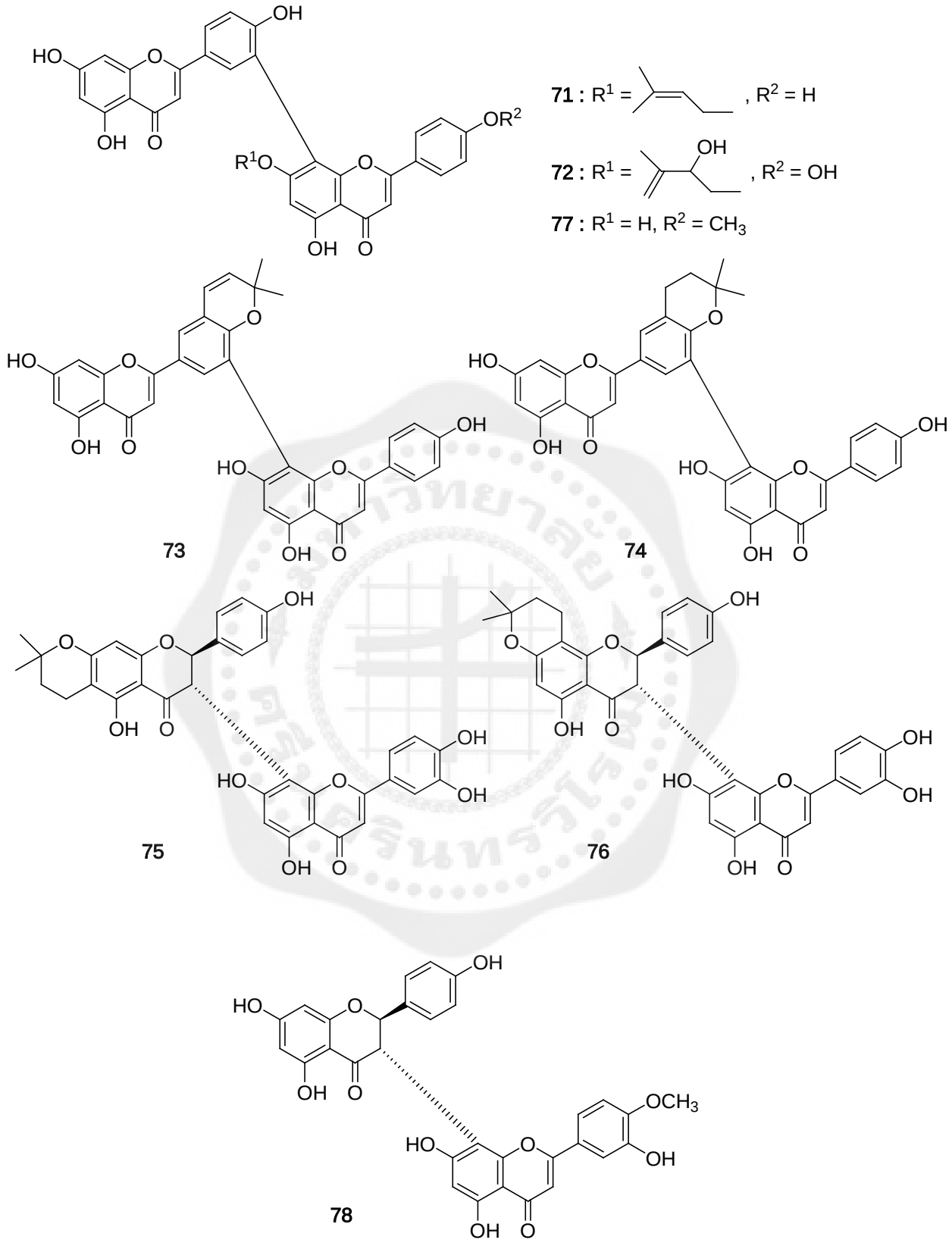


Figure 16 Structures of biflavonoids **71**-**78**

GB-1 (**79**) which was isolated from the MeOH extract of chewing stick *G. kola* was active against a range of oral bacteria including *Streptococcus mutans, Streptococcus mitis, Streptococcus downeii, Actinomyces naeslundii, Porphyromonas gingivalis and Prevotella intermedia* with MIC values of 32–64 μg/mL (Xu; et al. 2013, 147: 497-502), (Table 1).

TABLE 1 Bioactivities of some biflavonoids from some *Garcinia* plants

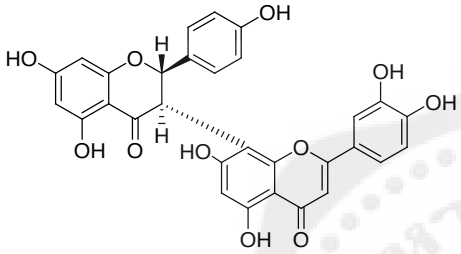
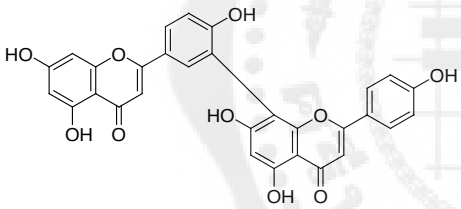
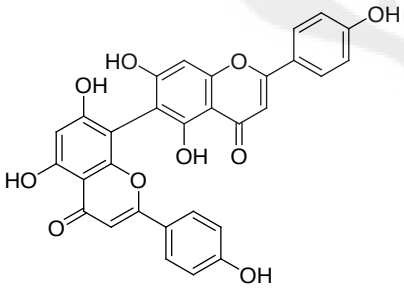
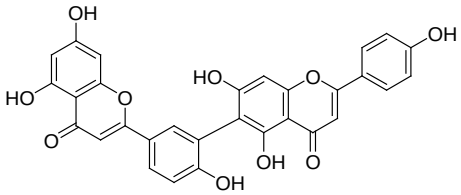
Compounds	Bioactivities	References
 morelloflavone (**32**)	Anti-inflammatory Antioxidant activity Inhibitor of HMG-CoA reductase	Gil; et al. 1997, 53: 733-740 Panthong; et al. 2009, 87: 1636-1640 Tuansulong; et al. 2011, 25: 424-428
 amentoflavone (**33**)	Antiviral activity Antibacterial activity	Lin; et al. 1999, 65: 120-125 Kaikabo; et al. 2009, 4: 1363-1366
 agathisflavone (**56**)	Antiviral activity	Lin; et al. 1999, 65: 120-125
 robustaflavone (**61**)	Antiviral activity	Lin; et al. 1999, 65: 120-125

TABLE 1 (continued)

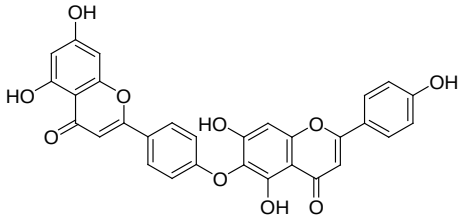
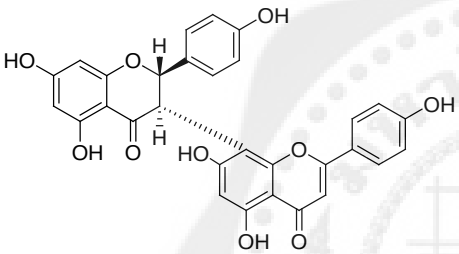
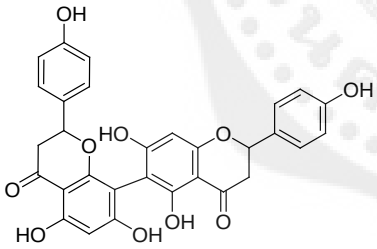
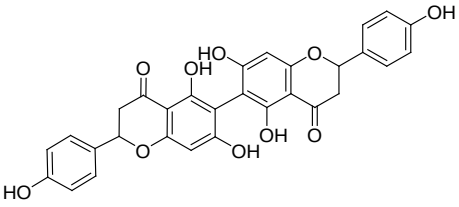
Compounds	Bioactivities	References
 hinokiflavone (**62**)	Antiviral activity	Lin; et al. 1999, 65: 120-125
 vokensiflavone (**48**)	Antiviral activity	Lin; et al. 1999, 65: 120-125
 rhusflavanone (**66**)	Antiviral activity	Lin; et al. 1999, 65: 120-125
 succedaneaflavanone (**67**)	Antiviral activity	Lin; et al. 1999, 65: 120-125

TABLE 1 (continued)

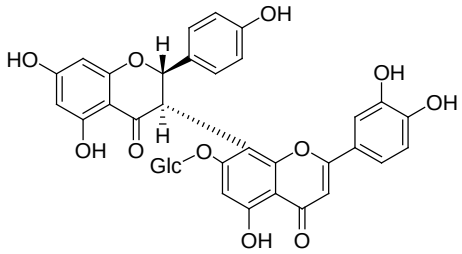
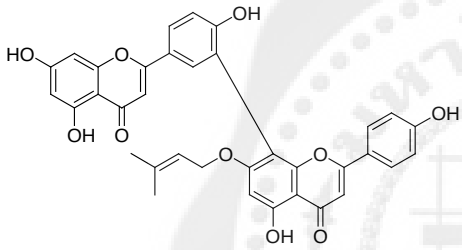
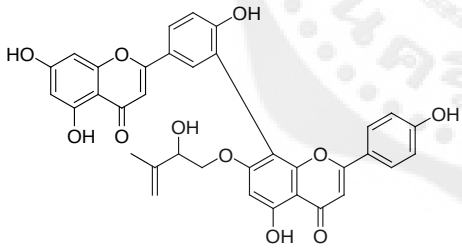
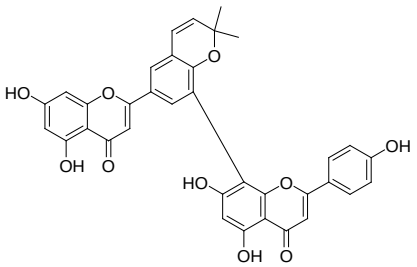
Compounds	Bioactivities	References
 fukugiside (**47**)	Antioxidant activity	Panthong; et al. 2009, 87: 1636-1640
 garciniaflavones A (**71**)	Inhibited hypoxia-inducible factor-1 in human embryonic kidney 293 cells	Ito; et al. 2013, 61: 551-558
 garciniaflavones B (**72**)	Inhibited hypoxia-inducible factor-1 in human embryonic kidney 293 cells	Ito; et al. 2013, 61: 551-558
 garciniaflavones C (**73**)	Inhibited hypoxia-inducible factor-1 in human embryonic kidney 293 cells	Ito; et al. 2013, 61: 551-558

TABLE 1 (continued)

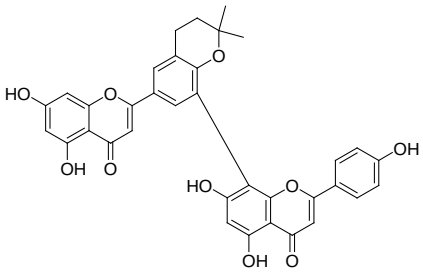
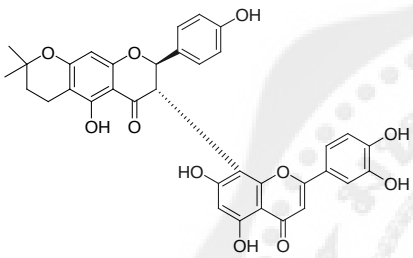
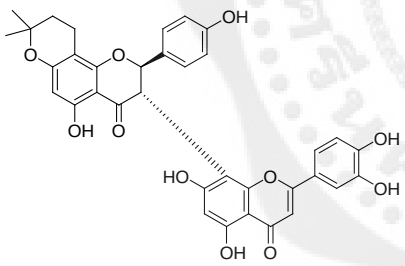
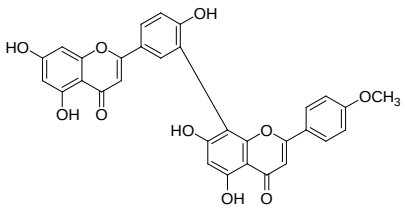
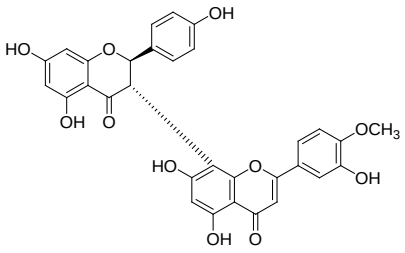
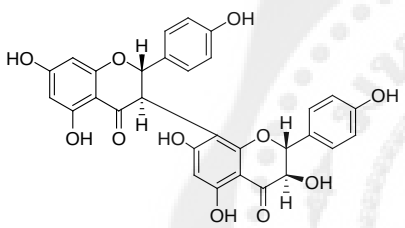
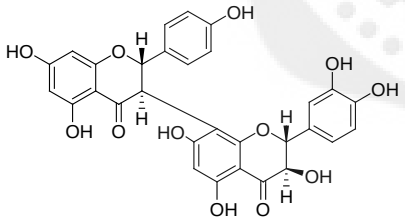
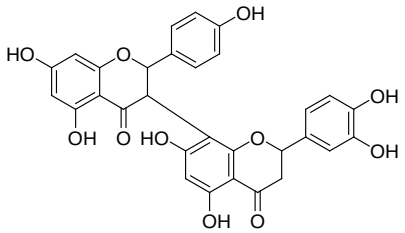
Compounds	Bioactivities	References
 garciniaflavones D (**74**)	Inhibited hypoxia-inducible factor-1 in human embryonic kidney 293 cells	Ito; et al. 2013, 61: 551-558
 garciniaflavones E (**75**)	Inhibited hypoxia-inducible factor-1 in human embryonic kidney 293 cells	Ito; et al. 2013, 61: 551-558
 garciniaflavones F (**76**)	Inhibited hypoxia-inducible factor-1 in human embryonic kidney 293 cells	Ito; et al. 2013, 61: 551-558
 podocarpusflavone (**77**)	Inhibited hypoxia-inducible factor-1 in human embryonic kidney 293 cells	Ito; et al. 2013, 61: 551-558

TABLE 1 (continued)

Compounds	Bioactivities	References
 (+)-4'''-*O*-methylmorelloflavone (**78**)	Inhibited hypoxia-inducible factor-1 in human embryonic kidney 293 cells	Ito; et al. 2013, 61: 551-558
 GB-1 (**79**)	Antibacterial activity	Xu; et al. 2013, 147: 497-502
	Antioxidant activity	Tebekeme; et al. 2009, 8: 7133-7137
	α-glucosidase inhibitory, aromatase inhibitory, and antiplasmodial activities	Antia; et al. 2010, 76: 276-277.
 GB-2 (**38**)	Antioxidant activity	Tebekeme; et al. 2009, 8: 7133-7137
 GB-2a (**80**)	Anti-inflammatory	Castardo; et al. 2008, 118: 405-411

CHAPTER 3

EXPERIMENTAL

Sources of plant materials

G. fusca

The root of *G. fusca* was collected from Buayai Subdistrict, Nampong District, Khon Kaen Province, Thailand, in July 2009. A voucher specimen (Jannarin Nontakham 001) has been deposited at the Chemistry Department of Srinakharinwirot University and was identified by Mr. James F. Maxwell, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

G. cowa

The air-dried heartwood and bark of *G. cowa* was collected in Tawang Subdistrict, Buached District, Surin Province, in April 2008. A voucher specimen (Kanlaya Lomchoey 001) was indentified by Prof. Dr. Pranom Chantaranothai and has been deposited at the herbarium of Department of Chemistry, Faculty of Science, KhonKhan University, Thailand.

General techniques

1. Thin-Layer chromatography (TLC)

Technique: One dimension, ascending

Adsorbent: Silica gel 60 GF_{254} precoated on aluminium plate (Merck 1.05554)

Layer Thickness: 1.25 mm

Plate size: 1 x 5 cm and 2 x 5 cm

Detection: 1) Spots on TLC were visualized under ultraviolet light at wavelengths of 254 and 365 nm.

2) Developing agent, Anisaldehyde-H_2SO_4 reagent (2.5% v/v in absoluted MeOH containing 3.4% v/v H_2SO_4 acid and 1.0% v/v glacial acetic acid). After heating of TLC plate at 100-110 ^{o}C for 10-30 seconds, the spots of organic compounds will give specific colors with this reagent.

2. Column chromatography (CC)

2.1 Liquid column chromatography

Adsorbent: 1) Silica gel 60 particle size < 0.063 mm (Merck 1.07729)

2. Silica gel 60 particle size 0.040-0.063 mm (Merck 1.09385)

Packing method: Slurry packing method for particle size < 0.063 mm and Dry vacuum packing method for particle size 0.040-0.063 nm.

Sample loading: The sample will be dissolved in a small volume of suitable organic solvent. The sample solution will be mixed with silica gel particle size 0.040-0.063 mm. Then evaporated under reduced pressure and added onto the top of column.

Elution: After loading of sample onto the column and appropriate solvent system will be used as a mobile phase in the isocratic or gradient systems.

2.2 Quick column chromatography

Adsorbent: Silica gel particle size 0.040-0.063 mm (Merck).

Packing method: Dry vacuum packing method

Sample loading: The sample was dissolved in a small volume of an appropriate solvent. The solution was mixed with silica gel particle size 0.040-0.063 mm. The sample was evaporated under reduced pressure and added onto the top of column.

Elution: After loading of sample onto the column, an appropriate solvent system was used as a mobile phase in the gradient systems.

2.3 Size-Exclusion gel column chromatography

Adsorbent: Sephadex LH-20

Packing method: Slurry packing method

Sample loading: The sample was dissolved in a small volume of MeOH and added onto the top of column.

Elution: The column was eluted with MeOH.

Physical Properties

1. Optical rotation: Optical rotation activity was recorded in MeOH or $CHCl_3$ on a JASCO-1020 digital polarimeter.

2. Melting point: Melting point was measured on Griffin melting point apparatus in degree Celsius of temperature.

Spectroscopy

1. Infrared (IR) Absorption Spectra

IR spectra were measured on Perkin Elmer FT-IR spectrum BX spectrometer by using potassium bromide (KBr) disc.

2. Ultraviolet (UV) Absorption Spectra

UV spectra were obtained on a Shimadzu UV-2401 PC spectrophotometer.

3. Mass Spectra

Electrospray ionization mass spectra (ESIMS) were measured on Finnigan LC-Q mass spectrometer.

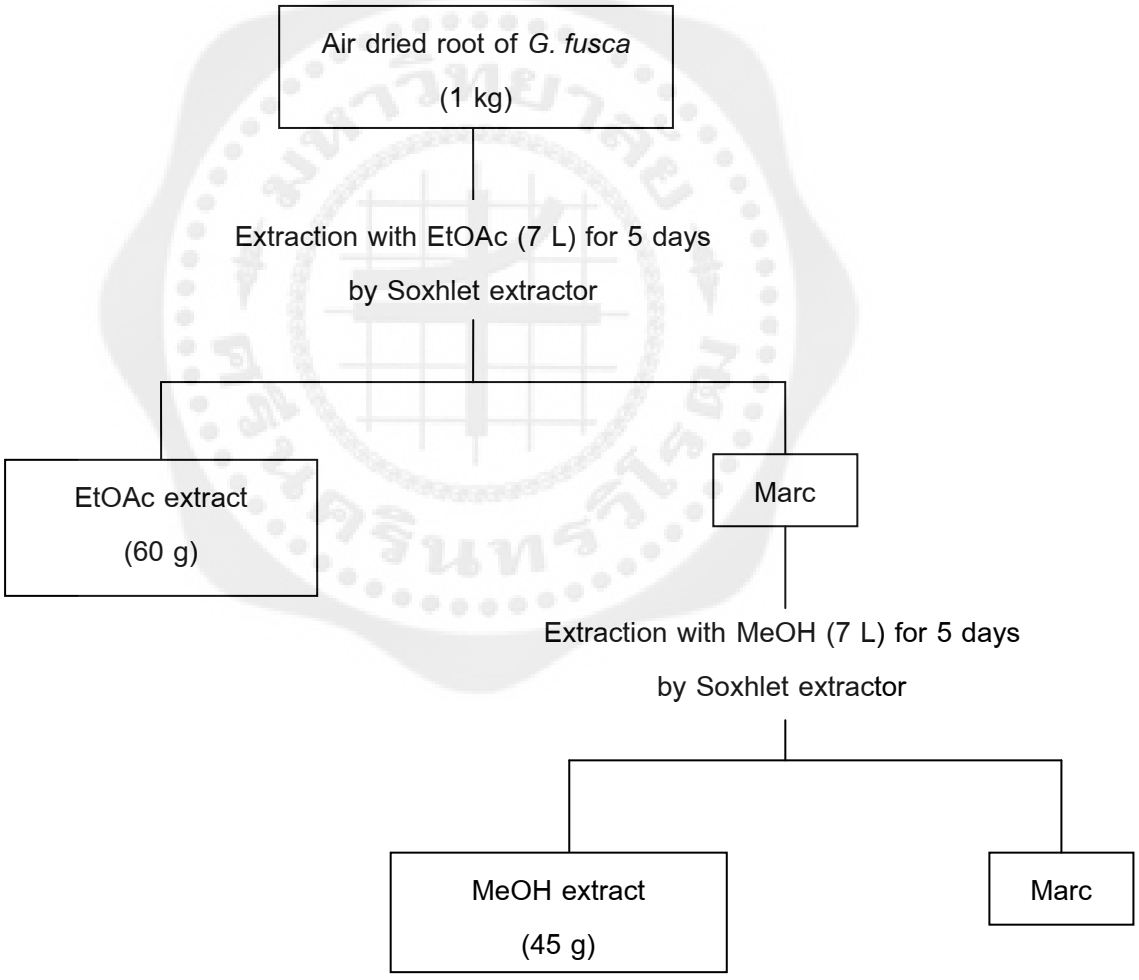
4. Nuclear Magnetic Resonance (NMR) Spectra

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were determined on a Bruker Avance 300 FT-NMR spectrometer.

Extraction and isolation

1. Extraction of the air dried root of *G. fusca*

The air dried root of *G. fusca* (1 kg) was extracted with EtOAc (7 L) and MeOH (7 L) for each 5 days by using Soxhlet extractor, respectively. Evaporation of the filtrate under reduced pressure at about 40 $^{\circ}$C gave an EtOAc and MeOH extracts as brown gum (60 g) and dark brown gum (45 g), respectively. A typical intense green and orange coloration with anisaldehyde-H_2SO_4 reagent for EtOAc extract indicated the presence of xanthones and biflavonoids, respectively. However, MeOH extract showed only orange color in same test. The extraction procedure is shown in Scheme 1.



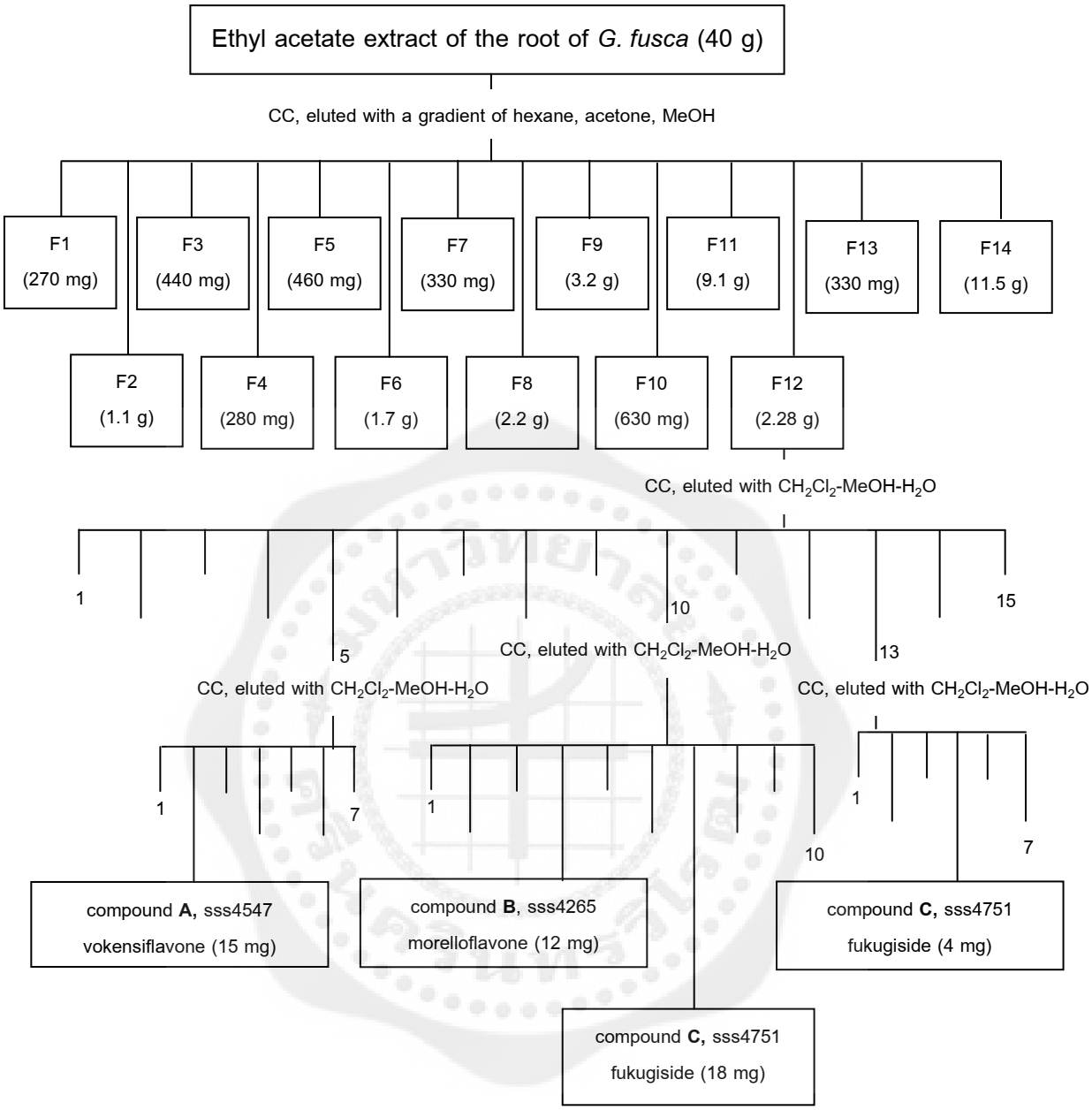
Scheme 1 Extraction procedure for the root of *G. fusca*

2. Isolation of compounds from the ethyl acetate extract of the root of *G. fusca*

The EtOAc extract (40 g) was fractionated by quick column chromatography (ϕ 10 × 15 cm) eluting with a gradient of *n*-hexane-acetone (95:5-5:95, v/v), acetone-MeOH (95:5-0:100, v/v) and MeOH to afford 14 main fractions (Fr.1-14).

2.1 Isolation of compound A (vokensiflavone, sss4594) compound B (morelloflavone, sss4595) and compound C (fukugiside, sss4751)

Fraction 12 (500 mg) were chromatographed on a silica gel column (finer than 0.063 mm, 35 g) CH_2Cl_2-H_2O-MeOH (99.5:0.2:0.5) as eluting solvent (0.5% increment of MeOH, each 200 mL) to give fifteen subfractions. Subfraction 5 (30 mg) was rechromatographed using CH_2Cl_2-H_2O-MeOH (99.5:0.2:0.5) as eluting solvent, with increasing amount of the more polar solvent to provide seven subfractions (fr.5.1-5.7). Subfraction 5.2 gave compound **A** (vokensiflavone, sss4594) as an orange solid (15.0 mg). Subfraction 10 (45 mg) was rechromatographed using CH_2Cl_2-H_2O-MeOH (99:0.2:1) as eluting solvent, with increasing amount of the more polar solvent to provide ten subfractions (fr.10.1-10.10). Fraction 10.4 (12 mg) gave compound **B** (morelloflavone, sss4595) as an orange solid and fraction 10.7 gave compound **C** (fukugiside, sss4751) as an orange solid (18 mg). Subfraction 13 (34 mg) was rechromatographed using CH_2Cl_2-H_2O-MeOH (99:0.2:1) as eluting solvent, with increasing amount of the more polar solvent to provide seven subfractions (fr.13.1-13.7). Fraction 13.4 (4 mg) gave compound **C** (fukugiside, sss4751) as an orange solid (see Scheme 2).



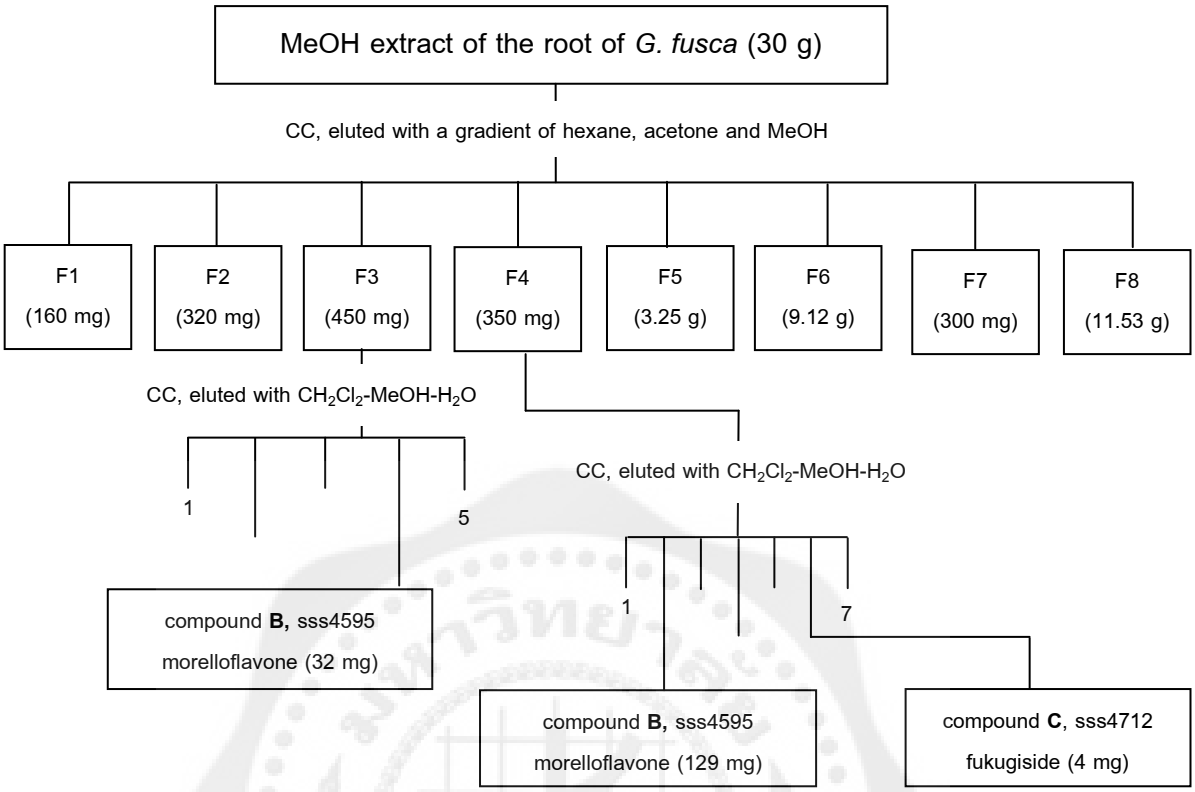
Scheme 2 Isolation of compounds from EtOAc extract of *G. fusca* root

3. Isolation of compounds from the MeOH extract of the root of *G. fusca*

A portion of the MeOH extract (30 g) was fractionated by quick column chromatography (ϕ 10 × 15 cm) eluting with a gradient of *n*-hexane-acetone (80:20-0:100, v/v), acetone-MeOH (95:5-0:100, v/v) and MeOH to afford 8 main fractions (Fr.1-8). The eluates were examined by TLC and eight combined fractions were obtained.

3.1 Isolation of compound B (morelloflavone, sss4595) and compound C (fukugiside, sss4712)

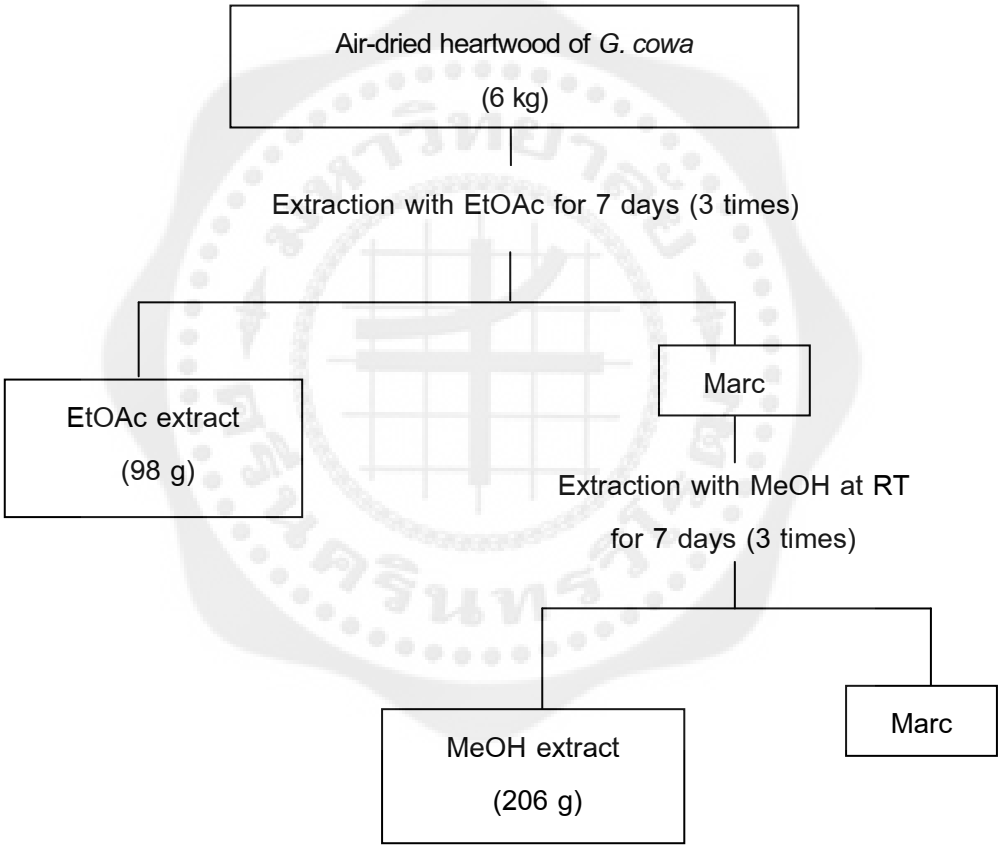
A portion of fraction 3 (300 mg) was purified by CC, using CH_2Cl_2-H_2O-MeOH (95:0.2:5) as eluting solvent to give compound **B** (morelloflavone, sss4595, 32 mg) as an orange solid. Another portion of Fraction 4 (500 mg) was purified by CC, using CH_2Cl_2-H_2O- MeOH (95:0.2:5) as eluting solvent to give compound **B** (morelloflavone, sss4595, 129 mg) as an orange solid and compound **C** (fukugiside, sss4712, 4 mg) as an orange solid (see Scheme 3).



Scheme 3 Isolation of compounds MeOH extract of *G. fusca* root

4. Extraction of the heartwood of *G. cowa*

The air dried heartwood of *G. cowa* (6 kg) was cut into small pieces, which was extracted with EtOAc (27 L) and followed by with MeOH (22 L) at room temperature for 3 times and each time for 7 days. The EtOAc extract was concentrated to give a brownish residue (98 g) and the MeOH extract was concentrated to give a very dark brown residue (206 g). A typical intense orange coloration with anisaldehyde-H_2SO_4 reagent for both of EtOAc and MeOH extract indicated for the presence of biflavonoids. The extraction procedure is shown in Scheme 4.



Scheme 4 Extraction procedure of the heartwood of *G. cowa*

5. Isolation of compounds from the ethyl acetate extract of the heartwood of *G. cowa*

The EtOAc extract (46 g) was fractionated by quick column chromatography (ϕ 10 × 15 cm) eluting with a gradient of *n*-hexane-acetone (98:2-0:100, v/v), acetone-MeOH (50:50, v/v) and MeOH to afford 13 main fractions (Fr.1-13) (Scheme 5).

5.1 Isolation of compound A (vokensiflavone, sss4594) and B (morelloflavone, sss4595)

A portion of fraction 10 (F10A, 338.0 mg) was chromatographed on a silica gel column (finer than 0.063 mm, 35 g) using CH_2Cl_2-H_2O-MeOH (99.5:0.2:0.5) as eluting solvent (0.5% increment of MeOH, each 200 mL) to give 23 subfractions. An orange solid of subfraction 13 was proved to be compound **A** *(*vokensiflavone, sss4594, 8 mg). Subfraction 16 afforded compound **B** (morelloflavone, sss4595, 49 mg) as yellow solid (see Scheme 5).

5.2 Isolation of compounds C (fukugiside, sss5225)

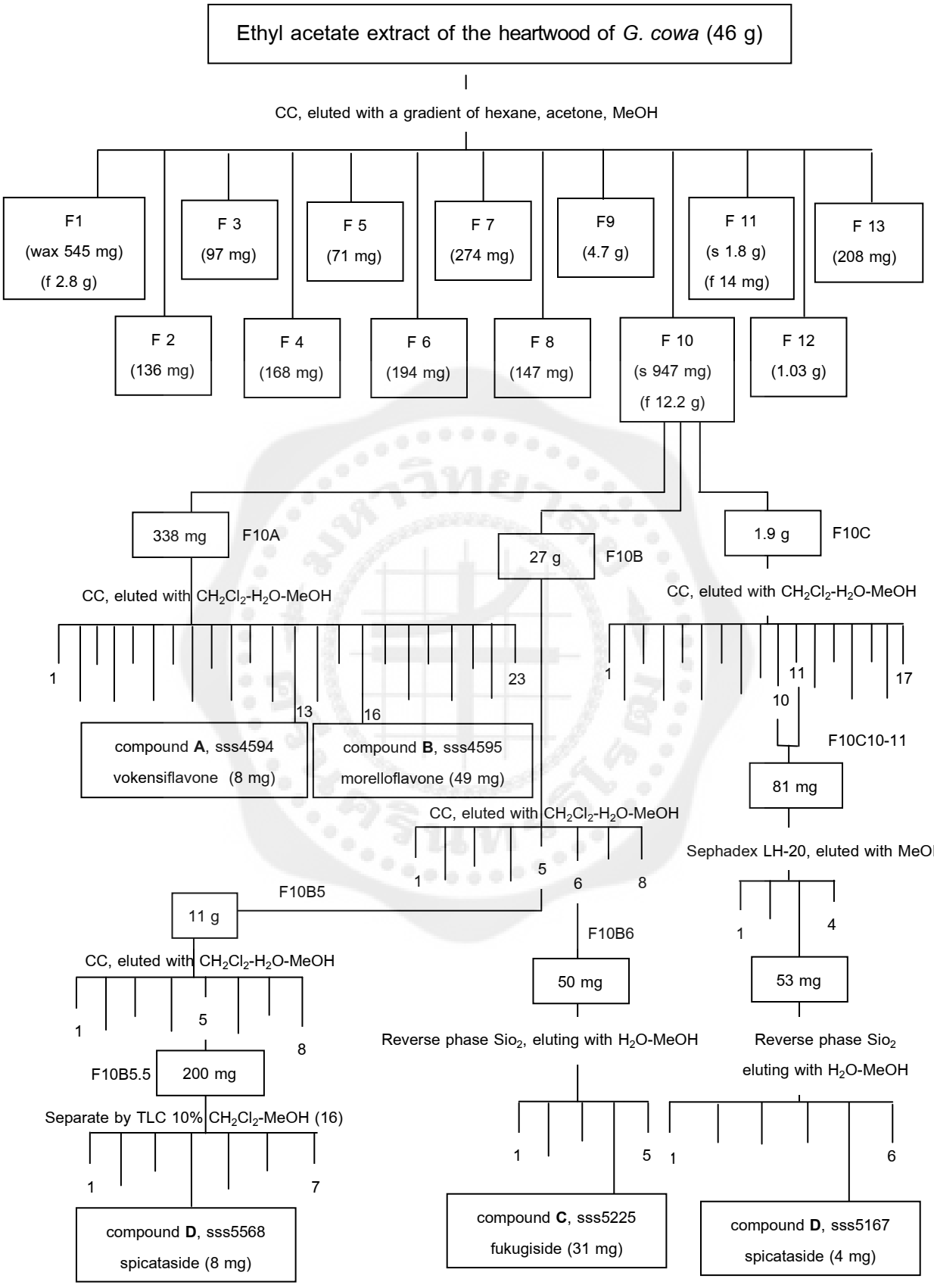
Another portion of fraction 10 (F10B, 27 g) was chromatographed over silica gel (finer than 0.063 mm, 70 g) and using CH_2Cl_2-H_2O-MeOH (97:0.2:3) as eluting solvent (1% increment of MeOH, each 200 mL) to give eight subfractions. Subfraction 6 (F10B6, 50 mg) was further purified by reversed phase SiO_2 column, eluting with H_2O-MeOH, to afford compound **C** (fukugiside, sss5225, 31 mg) as orange solid from subfration 4 (see Scheme 5).

5.3 Isolation of compounds D (spicataside, sss5167)

Subfraction F10B5 (11 g) was rechromatographed over silica gel (finer than 0.063 mm, 70 g) with CH_2Cl_2-H_2O-MeOH (98:0.2:2) as eluting solvent (1% increment of MeOH, each 300 mL) to give 8 subfractions. Subfraction 5 (F10B5.5, 200.0 mg) was further purified by TLC, eluting with 10% CH_2Cl_2-MeOH (20 elutions) to afford compound **D** (spicataside, sss5167, 12 mg) as an orange solid (see Scheme 5).

Another portion of fraction 10 (F10C, 1.9 g) was chromatographed over silica gel (finer than 0.063 mm, 70 g) using CH_2Cl_2-H_2O-MeOH (98:0.2:2) as eluting solvent (1% increment of MeOH, each 300 mL) to give 17 subfractions. Subfractions F10C10 (5 mg) and F10C11 (76 mg) were combined (81.6 mg) and was further purified by Sephadex LH-20, eluting with MeOH to give four main subfractions (40 mL per fraction). Subfraction 3 (53

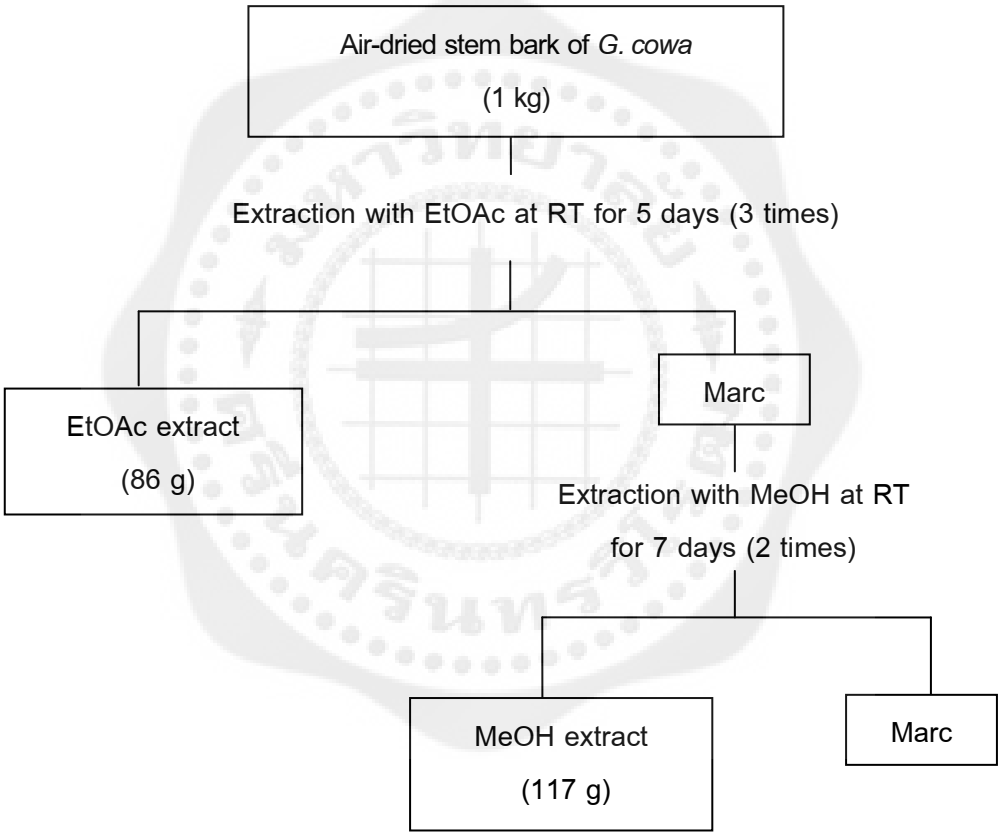
mg) was further purified by reversed phase SiO_2 column, eluting with H_2O-MeOH, to afford compound **D** (spicataside, sss5167, 4.0 mg) as orange solid (see Scheme 5).



Scheme 5 Isolation of compounds from EtOAc extract of *G. cowa* heartwood

6. Extraction of the stem bark of *G. cowa*

The air dried bark of *G. cowa* (1 kg) was milled into small pieces, which was extracted with EtOAc (5 L) at room temperature 3 times and each time for 5 days and then with MeOH (5 L) at room temperature 2 times and each time for a week. The EtOAc extract was concentrated to give a brownish residue (86.0 g) and the MeOH extract was concentrated to give a very dark brown residue (117.0 g). A typical intense orange coloration with anisaldehyde-H_2SO_4 reagent for EtOAc and MeOH extract indicated for the presence of biflavonoids. The isolation procedure is shown in Scheme 6.



Scheme 6 Extraction procedure of the stem bark of *G. cowa*

7. Isolation of compounds from the ethyl acetate extract of the bark of *G. cowa*

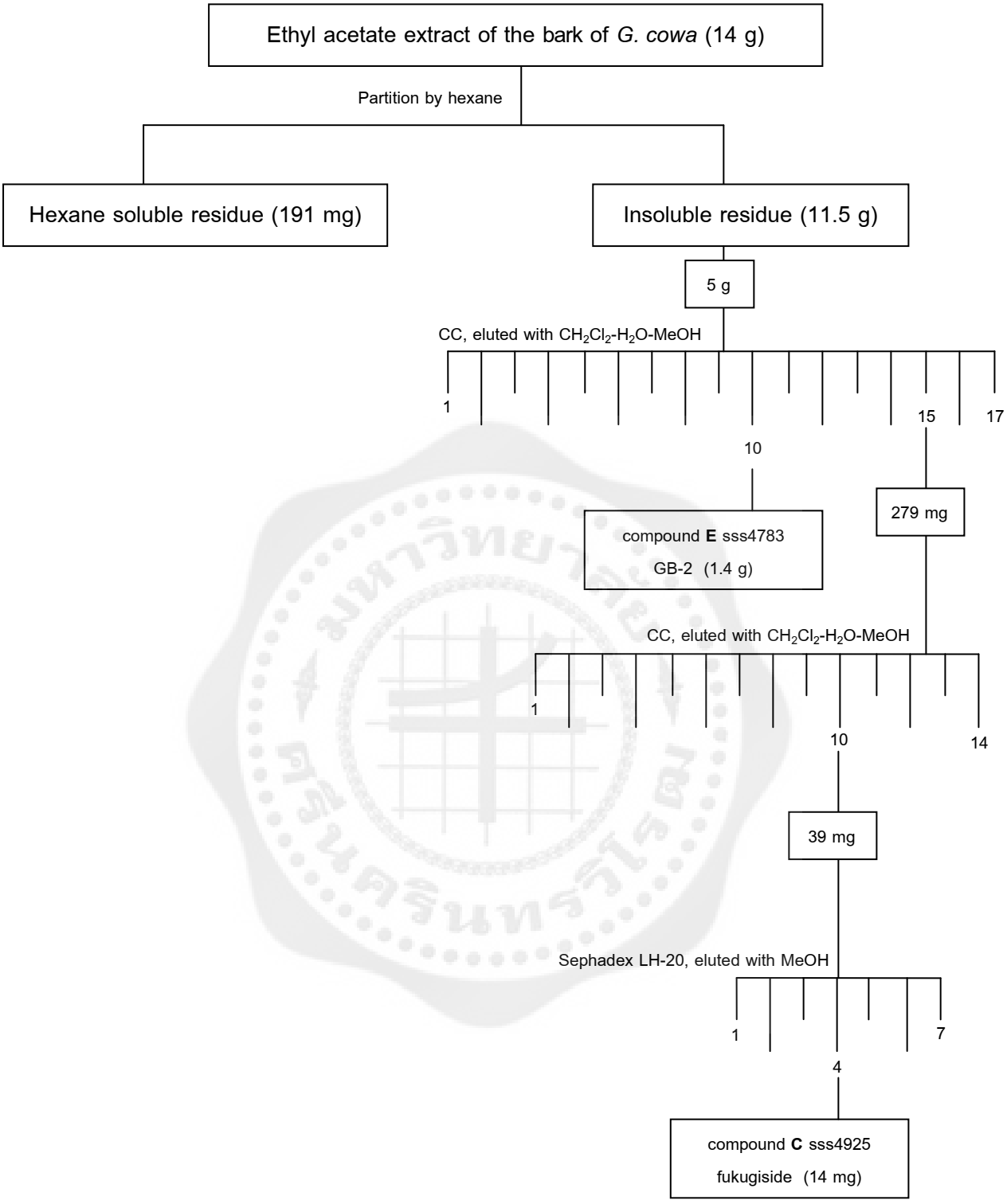
The EtOAc extract (14.0 g) was partition by hexane (120 mL) to give a hexane soluble fraction (191 mg) and a hexane insoluble residue (11.5 g)

7.1 Isolation of compound E (GB-2, sss4783)

A portion of the hexane insoluble residue (5 g) was chromatographed on a silica gel column (finer than 0.063 mm, 65 g) using CH_2Cl_2-H_2O-MeOH (99.5:0.2:0.5) as eluting solvent (1 % increment of MeOH, each 200 mL) to give 17 subfractions (8 mL per fraction). Solid of subfraction 10 was proved to be compound **E** (GB-2, sss4783, 1.4 g) as orange solid (see Scheme 7).

7.2 Isolation of compound C (fukugiside, sss4925)

From subfraction 15 (279 mg) was chromatographed over silica gel (finer than 0.063 mm, 70 g) using CH_2Cl_2-H_2O-MeOH (95:0.2:5) as eluting solvent (1% increment of MeOH, each 300 mL) to give 14 subfractions (8 mL per fraction). Subfraction 10 (39 mg) was further purified by Sephadex LH-20 column, eluting with MeOH to afford compound **C** (fukugiside, sss4925, 14 mg) as brown solid (see Scheme 7).

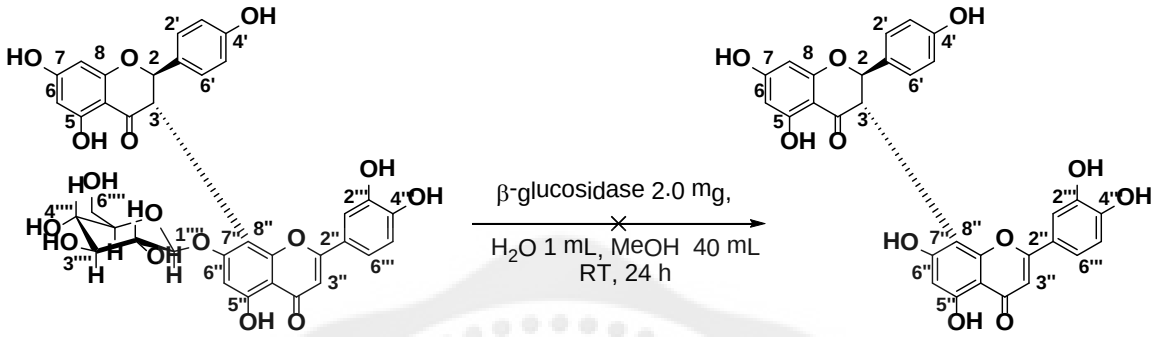


Scheme 7 Isolation of compounds from the EtOAc extract of *G. cowa* bark

8. Determination of sugar unit in biflavonoid glycosides

Determination of a sugar unit in biflavoniod glycosides was performed by using well-known hydrolysis reaction methods.

8.1 Attemp to hydrolysis by using β-glucosidase

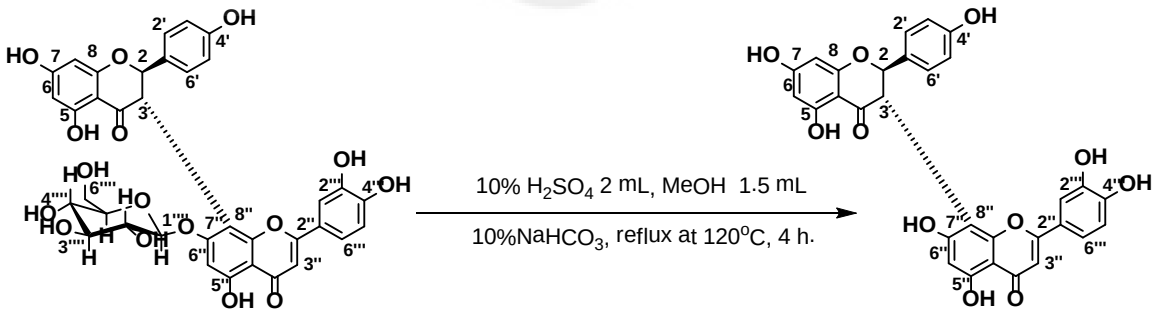


fukugiside

Fukugiside (compound **C**, 15 mg, 0.0207 mmol) was dissolved in a mixture of H_2O (1 mL) and MeOH (10 mL), then 2 mg of β-glucosidase was added to solution and the resulting reaction mixture was stirred at RT for 24 hours. TLC chromatogram showed only spot for fukugiside but no other spot was observed.

PBS buffer saline (2 mL) was then added to the mixture and the mixture was stirred at RT for 50 hours. By comparison with the authentic morelloflavone (**32**) however, the product was not observed on TLC chromatogram.

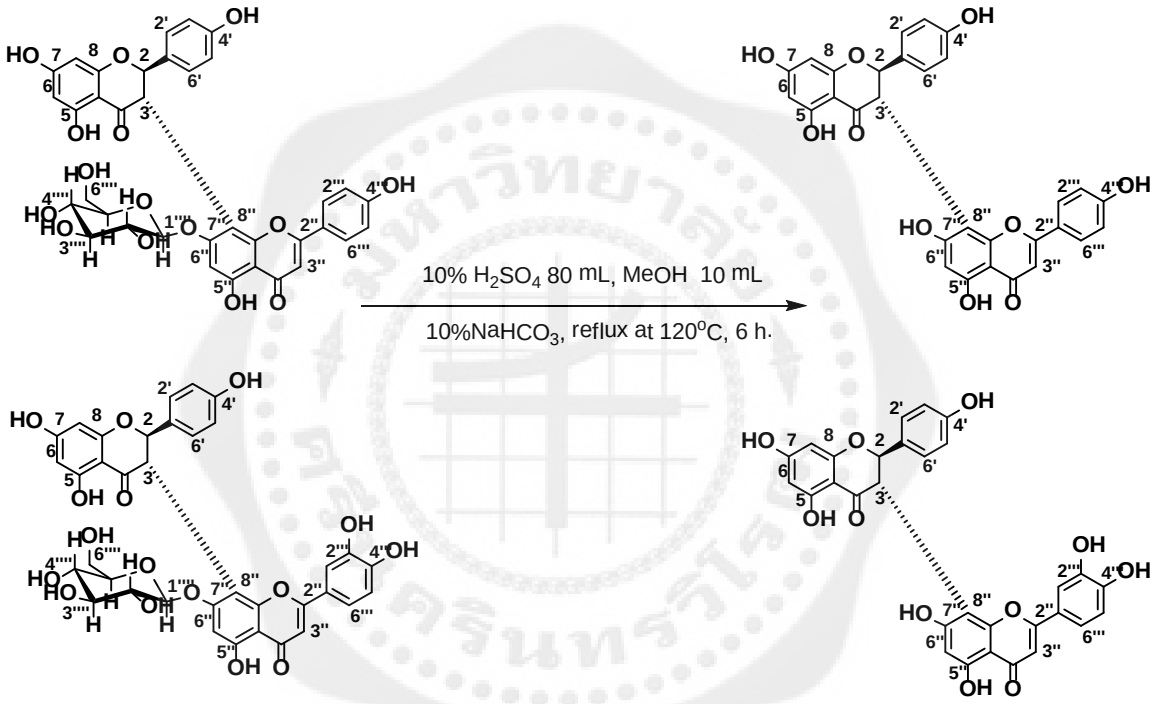
8.2 H_2SO_4 Acid hydrolysis



fukugiside

Fukugiside (5 mg, 0.007 mmol) was hydrolyzed via reflux at 120°C in 10% H_2SO_4 (1.5 mL) for 4 h, followed by 1.5 mL of MeOH was added to solution. After stirring for 4 hours, the aqueous layer was then neutralized with sodium hydrogen carbonate ($NaHCO_3$) followed by extraction with ethyl acetate (5 x 1 mL). The aqueous layer was air-dried to an insoluble precipitate. The ethyl acetate layer was evaporated to dryness to yield a yellow amorphous solid (2 mg). this solid showed the same R_f value as that of morelloflavone (**32**) on TLC chromatogram comparison.

8.3 Hydrolysis of a mixture of biflavonoid by using 10% H_2SO_4



A mixture of biflavonoid which obtain from F10B5.5 fraction in Scheme 5 (230 mg) was dissolved in 10 mL of MeOH, and 10% H_2SO_4 (10 ml) refluxed at 120°C for 8 h. TLC showed no starting material. The aqueous layer was then neutralized with sodium hydrogen carbonate ($NaHCO_3$) followed by extraction with ethyl acetate (3 x 50 mL). The aqueous layer was dried to obtain a colorless solid. The ethyl acetate fraction was evaporated to dryness and was further chromatographed over silica gel (finer than 0.063 mm, 5.0 g) with CH_2Cl_2-H_2O-MeOH (97:0.2:3) as eluting solvent (0.5 % increment of MeOH, each 50 mL) to give compound **C** (2 mg) and compound **D** (3 mg)

9. Bioassays

9.1 Bacterial strains and preparation

The clinical isolate of *Helicobacter pylori* HP40 was obtained from King Chulalongkorn Memorial Hospital kindly provided by Dr. Thanitta Chatsuwan, Chulalongkorn University, Thailand. *H. pylori* DMST 20165 was obtained from the Department of Medical Science, Ministry of Public Health, Thailand and *H. pylori* ATCC 43504 was used as the reference strain. Bacterial strains were grown on Columbia Blood agar Base (CB) (Hi-Media, India) supplemented with 7 % horse serum (GIBCO Invitrogen, England) and 7% sheep blood (Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University, Thailand.) and incubated at 37^{O}C for 2-4 days under microaerobic conditions (5% O_2 ,10% CO_2 ,85%N_2) generated by using a micro-aerobic GasPak (Mitsubishi, Japan) in an anaerobic box (Mitsubishi, Japan). To prepare inocula, tested bacteria were prepared in Brain Heart Infusion (BHI) broth (Hi-Media, India) supplemented with 10 % horse serum by adjusting to 0.5 McFarland standard that had 1.5 x 10^8 colony forming units (CFU)/mL.

9.2 Minimum inhibitory concentration (MIC) assay

A broth micro dilution method (Wangchuk; et al. 2011: 730-742) carried out in a 96-well sterilized micro plate was used to determine the MICs of the pure compounds. MICs were determined as the lowest concentration that produces complete growth inhibition of the tested bacteria. Test samples were dissolved in dimethyl sulfoxide (DMSO, Merck). Serial two fold dilutions of the test samples were mixed with BHI broth in 96-well microtiter plates (Corning, USA) in an initial concentration of 250 μg/mL. The suspension of each tested bacteria (1.5 x 10^8 CFU/mL) was added in each well in equal volume of test sample (50 μL). The microtiter plates were incubated at 37^{O}C for 4 days under microaerobic conditions. MICs were recorded by reading the lowest concentration that inhibited visible growth. The test was performed in triplicates. Amoxicillin, clarithromycin and metronidazole (Government Pharmaceutical Organization, Thailand) were used as positive control drugs.

Physical and spectroscopic data of the isolated compounds A-E

1. Compound A (vokensiflavone (48), sss4594)

Yellow solid 15 mg, soluble in acetone or MeOH

mp : 222-223 °C (d) [lit. (±) vokensiflavone: 290-293 °C (d) (Konoshima; Ikeshiro; & Miyahara. 1970: 4203-4206) and vokensiflavone: 220-221 °C (d) (Nontakham. 2011: 34-75)]

R_f : 0.42 (10% MeOH-CH_2Cl_2), an orange coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{25.8}$: +126.4 (c = 0.18, MeOH) [lit. (+) vokensiflavone: $[\alpha]_D^{25.8}$ = +142.0 (c = 0.1, MeOH) (Nontakham. 2011: 34-75) and (+) vokensiflavone-7-sulfate: $[\alpha]_D^{25}$ = +113 (c = 1.32, MeOH) (Li; et al. 2002: 8709-8717)]

IR ν_{max}^{KBr} cm^{-1} : 3184, 2920, 2681, 1634, 1506, 1455, 1362, 1270, 1084, 969, 833 [lit :IR ν_{max}^{nujol} cm^{-1} : 3100 (hydroxyl groups), 1640 and 1610 (conjugated γ-pyrone), 1570 and 1510 (benzene), (Konoshima; Ikeshiro; & Miyahara. 1970: 4203-4206]

UV λ_{max}^{MeOH} nm (log ε) : 342(4.5), 325(4.6), 289(4.8), 221(5.0), [lit. λ_{max}^{MeOH} 330, 289, 275, 225(shoulder), (Konoshima; et al. 1970: 4203-4206)]

ESMS (-ve) *m/z* (% rel. intensity) : 539 $[M-H]^-$ (100) for $C_{30}H_{20}O_{10}$ - H

^{1}H NMR : δ ppm, in $CDCl_3$+ DMSO-d_6; Table 3, Figure 26

^{13}C NMR : δ ppm, in $CDCl_3$+ DMSO-d_6; Table 3, Figure 27

2. Compound B (morelloflavone (32), sss4595)

Yellow solid 173 mg, soluble in acetone and MeOH

mp : 230-232 °C (d) [lit. (±) morelloflavone: 298-299 °C (d) and (+) morelloflavone: 244-245 °C (d) (Konoshima; & Ikeshiro. 1969: 121-124)
(+) morelloflavone: 280 °C (Li; et al. 2002: 8709-8717) (+) morelloflavone: 230-232 °C (Nontakham. 2011: 34-75)

R_f : 0.27 (10% MeOH-CH_2Cl_2), an orange coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{25.8}$: +177.6 ° (c = 0.20, MeOH) [lit. (±) morelloflavone: $[\alpha]_D^{29}$ = 0 (solvent not reported) (+) morelloflavone: $[\alpha]_D^{29}$ = +170

°(MeOH) (Konoshima; & Ikeshiro. 1969; 121-124)]

(+) morelloflavone: $[\alpha]_D^{25}$ = +188 °(c= 0.1, MeOH) (Li; et al. 2002: 8709-8717)

(+) morelloflavone: $[\alpha]_D^{25}$ = +161.6 °(c= 0.2, MeOH) (Nontakham. 2011: 34-75)

IR ν_{max}^{KBr} cm^{-1} : 3218, 2943, 2688, 1645, 1609, 1516, 1456, 1425, 1368, 1261, 1167, 1111, 1088, 1050, 1012, 967, 839 [lit :IR ν_{max}^{nujol} cm^{-1} : 3250 (hydroxyl groups), 1645 (conjugated γ-pyrone), 1600 and 1570 (benzene), (Konoshima; & Ikeshiro. 1969: 121-124)]

UV λ_{max}^{MeOH} nm (log ε): 332(4.2), 292(48, shoulder), 223(5.0, shoulder) [lit. 345 (4.13), 288(4.35), 275(4.33), 224(4.57, shoulder) nm, (Konoshima; & Ikeshiro. 1969; 121-124)]

ESMS (-ve) *m/z* (% rel. intensity) : 555 $[M-H]^-$ (100) for $C_{30}H_{20}O_{11}$ - H

^{1}H NMR : δ ppm, in $CDCl_3$+ DMSO-d_6; Table 5, Figure 28

^{13}C NMR : δ ppm, in $CDCl_3$+ DMSO-d_6; Table 5, Figure 29

3. Compound C (fukugiside (47), sss4751)

Yellow solid 80 mg, soluble in acetone or MeOH

mp : 242-243°C (d) [lit. (+) fukugiside: 242-243°C (d) (Konoshima; et al. 1970: 1717-1720)

(+) fukugiside: 240-242°C (d) (Chen; et al. 1975: 818-820)

R_f : 0.38 (10% MeOH-CH_2Cl_2), an orange coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{25.8}$: +150 (c = 0.2804, MeOH) [lit., (+) fukugiside: $[\alpha]_D^{15}$ = + 116 (c = 0.31, MeOH) (Konoshima; et al: 1970; 1717-1720)

(+) fukugiside: $[\alpha]_D^{20.0}$ = +24.68 (c = 0.1, MeOH) (Chen: et al: 1975; 818-820)

IR ν_{max}^{KBr} cm^{-1} : 3395, 2927, 2363, 1651, 1519, 1439, 1368, 1262, 1079, 874, 837 [lit :IR ν_{max}^{KBr} cm^{-1} : 3300, 1070 (hydroxyl groups), 1645 and 1603 (conjugated γ-pyrone), 1520 (benzene) (Chen: et al: 1975; 818-820)

UV λ_{max}^{MeOH} nm (log ε) : 346(4.2), 330(4.2, shoulder), 289(4.4), 226(4.6, shoulder) [lit. 344(4.39), 289(4.47), 275(4.45), 256(4.24, shoulder), 224(4.71) (Chen: et al: 1975; 818-820)

ESMS (-ve) *m/z* (% rel. intensity) : 717 $[M-H]^-$ (100) for $C_{36}H_{30}O_{16}$ - H

^{1}H NMR : δ ppm, in $CDCl_3$+ DMSO-d_6; Table 7, Figure 30

^{13}C NMR : δ ppm, in $CDCl_3$+ DMSO-d_6; Table 7, Figure 31

4. Compound D (spicataside (81), sss5167)

Yellow solid 12 mg, soluble in acetone or MeOH

mp : 233-234°C (d) [lit. 232-233°C (d) (Konoshima; et al. 1970: 4203-4206)
235-238°C (Chen; et al. 1975: 818-820)

R_f : 0.25 (15% MeOH-CH_2Cl_2), an orange coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{27.3}$: +14.8 (c = 0.20, MeOH) [lit. not reported]

IR ν_{max}^{KBr} cm^{-1} : 3243, 2901, 1652, 1514, 1453, 1365, 1262, 1164, 1066, 833 [lit : ν_{max}^{nujol} cm^{-1} : 3300 (hydroxyl groups), 1640 (conjugated γ-pyrone), 1600 and 1560 (benzene rings) (Konoshima; et al. 1970: 1717-1720]
ν_{max}^{KBr} cm^{-1} : 3350 (hydroxyl groups), 1643 (conjugated γ-pyrone), 1605, 1580 and 1520 (benzene rings), 1070 and 1040 (OH) (Chen: et al: 1975: 818-820)]

UV λ_{max}^{MeOH} nm (log ε) : 325(4.2), 320(4.2), 288(4.4), 287(4.4), 221(4.6, shoulder) [lit. λ_{max}^{EtOH} 330, 292, 275, 225(shoulder), (Konoshima; et al. 1970: 1717-1720); 330(shoulder, 4.10), 290(4.33), 280(4.32), 216(4.60), (Chen; et al. 1975; 818-820)]

ESMS (-ve) *m/z* (% rel. intensity) : 701 $[M-H]^-$ (100) for $C_{36}H_{30}O_{15}$ - H

^{1}H NMR : δ ppm, in $CDCl_3$+ DMSO-d_6(1:1); Table 9, Figure 32

^{13}C NMR : δ ppm, in $CDCl_3$+ DMSO-d_6(1:1); Table 9, Figure 33

5. Compound E (GB-2 (38), sss4783)

Yellow solid 1 g, soluble in acetone and MeOH

mp : 217-220°C (d) [lit. 220 °C (d) (Jackson; et al. 1971: 3791-3804)

R_f : 0.40 (10% MeOH-CH_2Cl_2), an orange coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{27.3}$: +18.4 (c = 0.31, MeOH) [lit. not reported]

IR ν_{max}^{KBr} cm^{-1} : 3253, 2882, 2356, 1651, 1519,1456, 1360, 1276, 1182, 1085, 999, 832 [lit. : IR ν_{max}^{nujol} cm^{-1} : 3300 (OH) and 1650 (C=O) (Jackson; et al. 1971: 3791-3804]

UV λ_{max}^{MeOH} nm (log ε) : 332(4.0), 292(4.6, shoulder), 225(4.8, shoulder) [lit. 329 and 292 (Jackson; et al. 1971: 3791-3804)]

ESMS (-ve) *m/z* (% rel. intensity) : 573 $[M-H]^-$ (100) for $C_{30}H_{22}O_{12}$ - H

^{1}H NMR : δ ppm, in $CDCl_3$+ DMSO-d_6; Table 11, Figure 34

^{13}C NMR : δ ppm, in $CDCl_3$+ DMSO-d_6; Table 11, Figure 35

CHAPTER 4

RESULTS AND DISCUSSION

The EtOAc and MeOH extracts of root of *G. fusca* and the EtOAc of heartwood and bark of *G. cowa* were investigated by column chromatographic methods to give five known biflavonoid compounds, **A**-**E** (Table 2). The structures of these compounds were determined mainly based on their NMR and MS data analysis, and by comparison with previously reported data.

Table 2 Compounds isolated from the root of *G. fusca* and the heartwood and stem bark of *G. cowa*

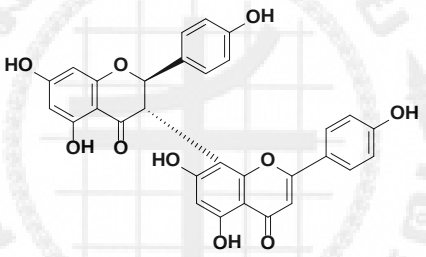
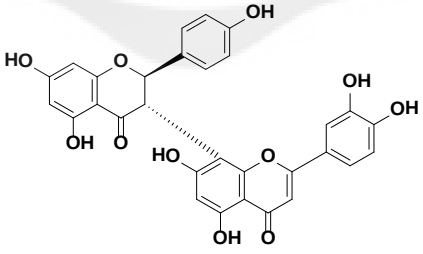
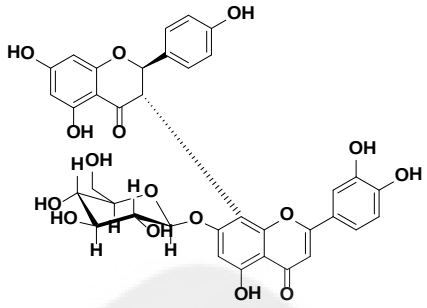
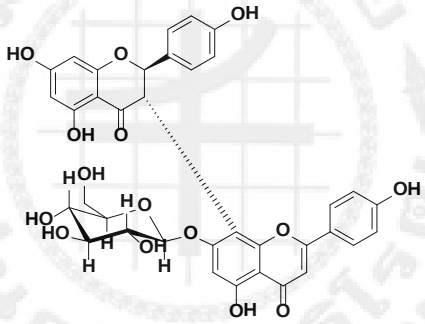
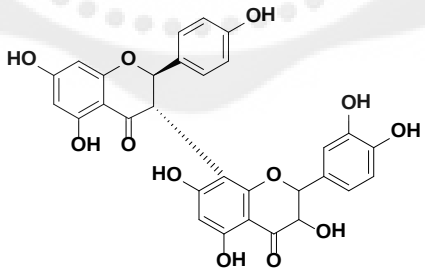
Compounds	Structures	References
A (vokensiflavone (**48**), sss4594, 15 mg obtained from the root of *G. fusca* 8 mg obtained from the heartwood of *G. cowa*)		Chen; et al. 1957: 300-303. Nontakham. 2011: 34-75.
B (morelloflavone (**32**) or fukugitin, sss4595, 173 mg obtained from the root of *G. fusca* 449 mg obtained from the heartwood of *G. cowa*)		Terashima; et al. 2008: 407-413. Nontakham. 2011: 34-75.

Table 2 (Continued)

Compounds	Structures	References
C (fukugiside (**47**) sss4952, 21 mg obtained from the root of *G. Fusca* 45 mg obtained from the heartwood and bark of *G. cowa*)		Chen; et al. 1975: 818-820. Elfita; et al. 2009: 907-912.
D (spicataside (**81**) sss5167, 17 mg obtained from the heartwood of *G. cowa*)		Chen; et al. 1975: 818-820. Konoshima; et al: 1970; 4203-4206.
E (GB-2 (**38**) sss4783, 1 g obtained from the bark of *G. cowa*)		Jackson; et al. 1971: 3791. Duddeck. 1978: 1369-1373.

1. Structural determination of compounds A-E

1.1 Compound **A** (vokensiflavone (**48**), sss4594)

Compound **A** was obtained as a yellow solid from the EtOAc and MeOH soluble fractions of *G. fusca* root and the EtOAc soluble fraction of *G. cowa* heartwood and bark. Compound **A** gave an orange coloration with anisaldehyde-H_2SO_4 reagent. On the basis of its ESIMS ($[M-H]^-$ at *m/z* 539), a molecular formula of compound **A** was established as $C_{30}H_{20}O_{10}$ with the support of ^{13}C NMR data. Its IR absorption spectrum showed the presence of hydroxy groups at 3184 cm^{-1}, conjugated γ-pyrone at 1634 cm^{-1} and benzene ring at 1506 cm^{-1}. The UV absorption maxima in MeOH at 221 (shoulder), 289, 325 and 342 nm were found to belong to a biflavonoid skeleton. The ^1H, ^{13}C NMR and Dept spectra (Table 3, Figure 26 and 27) indicated for the presence of a biflavonoid skeleton in compound **A**. The ^1H and ^{13}C NMR spectra of compound **A** showed signals forming respective pairs (approximate relative ratio; 2:1). Its ^{13}C NMR spectrum showed major signals for 14 methines and 16 quaternary carbons and 6 major doublets in its ^1H NMR data. The H-2 and H-3 methine doublets exhibited at δ_H 5.86 (*J* = 12.0 Hz) and 4.76 (*J* = 12.0 Hz), respectively. The two aromatic singlet protons of flavanone part (ring A) appeared at δ_H 6.06 and 6.08 and two doublet protons (ring B) appeared at δ_H 7.09 and 6.51 with *J* coupling constant of 8.4 Hz. Two doublets aromatic *ortho* coupled protons at δ_H 7.61(*J* = 8.7 Hz, H-2'''), 6.97 (*J* = 8.6 Hz, H-3''') were assigned for flavone moiety (ring E). The phenolic hydroxyl protons also appered at 8.70, 9.76, 10.17 and 10.61. Furthermore, the three singlet signals of chelated OH apperares at δ_H 12.29, 12.85 and 12.94.

Connections among rings A, B and C of flavanone subgroup were provided by analysis of its HMBC and NOESY spectra (Table 4 and Figures 17 and 18). The NOESY correlations were observed for methine proton at δ_H 5.86 (H-2) to aromatic protons H-2' and H-6' (δ_H 7.09) together with HMBC correlations of H-2 to C-1' (δ_C 128.2), C-2' (δ_C 128.2) and C-6' (δ_C 128.2), in HMBC spectra, indicated that ring B was connected to ring C. The HMBC correlation of 5-OH (δ_H 12.29), H-6 (δ_H 6.06) and H-8 (δ_H 6.08) to C-4a (δ_C 101.9) confirmed that ring A connected to ring C.

Connections among rings D, E and F of flavone subsgroup were provided by analysis of its HMBC and NOESY spectra. The correlations of H-2''' (δ_H 7.61), H-6''' (δ_H 7.61) to C-4''' (δ_C 161.1) and C-2'' (δ_C 163.5), H-3''' (δ_H 6.97), H-5''' (δ_H 6.97) to C-4'''

(δ_C 161.1) and C-1''' (δ_C 121.7), in HMBC spectra together with NOESY correlations of H-6''' to H-3'' (δ_H 6.36) indicated that ring E connected to ring F. The HMBC correlations were observed for 5''-OH at δ_H 12.94, H-6'' at δ_H 6.34 and H-3'' at δ_H 6.36 to C-4a'' (δ_C 103.3) and NOE correlations of 5''-OH to H-6'' confirmed that ring D connected to ring F.

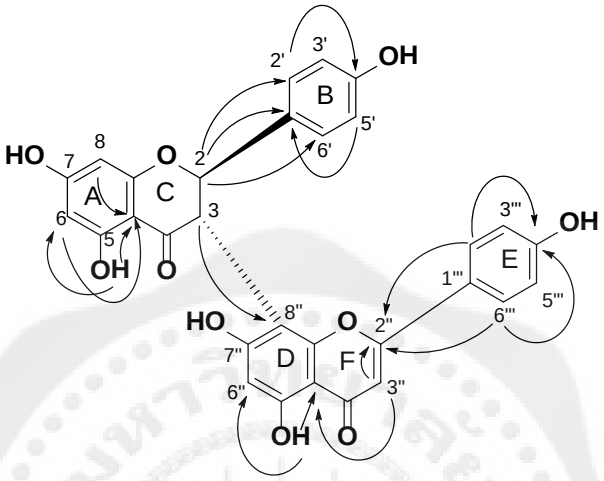


Figure 17 Selected HMBC correlations for compound **A**

Connections among flavanone and flavone subsgroups were provided by analysis of its HMBC and NOESY spectra. The HMBC correlations were observed for methine proton at δ_H 4.76 (H-3) to C-8'' (δ_C 99.9) and NOESY correlations of H-2''' to H-3, indicating that flavone unit was attached to flavanone moiety. The result indicates that of the structure of compound **A** was proposed to be vokensiflavone (**48**).

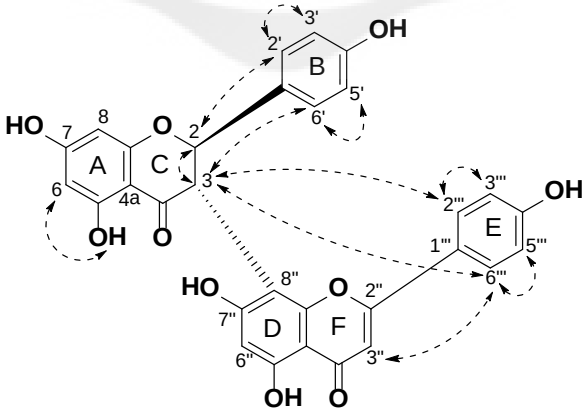


Figure 18 Selected NOESY correlations for compound **A**

The stereochemistry at C-2 and C-3 of flavanone unit in conpound **A** were provided by analysis of its *J* coupling constant value. The large coupling constants (*J* = 12.0 Hz) of C-2 and C-3 protons in ring C of compound **A**, in addition, no significant NOE enhancement was observed between both protons, indicated that both hydrogens have a *trans*-diaxial arrangement.

Compound **A** gave m.p. at 222-223°C (d) and dextrorotatory optical rotation, $[\alpha]_D^{25.8}$ = +126.4. Comparison these physical data with that of (+) vokensiflavone: $[\alpha]_D^{25.8}$ = +142.0 (c = 0.1, MeOH), m.p. 220-221 °C (d) (Nontakham. 2011: 34-75.) and (+) vokensiflavone-7-sulfate, $[\alpha]_D^{25}$ = +113 (c = 1.32, MeOH) (Li; et al. 2002: 8709-8717), therefore, the structure of **A** was assigned to be (+) vokensiflavone. (+) Vokensiflavone-7-sulfate was previously assigned as *2R,3S*-configurations by its CD spectrum analysis (positive Cotton effect at near 340 and 290 nm) (Li; et al. 2002: 8709-8717). This led to conclude that compound **A** also has the same *2R,3S*-configurations.

Vokensiflavone (**48**) was found in *Garcinia* plants such as *G. livingstonei* (Yang; et al. 2010: 4749-55), *G. spicataside* (Konoshima; Ikeshiro; & Miyahara. 1970: 1203-1206).

Table 3 Comparison of ^{1}H and ^{13}C NMR data of compound **A** (in $CDCl_3$ + DMSO-d_6) with vokensiflavone (**48**) (in DMSO-d_6)

Position	δ_H (*mult.*, *J* in Hz)			δ_C		
	vokensiflavone (**48**)[a]	compound **A**		vokensiflavon(**48**)[a]	compound **A**	
		major	minor		major	minor
2	5.80 (*d*, *J* = 12.0, 1H)	5.86 (*d*, *J* = 12.0, 1H)	5.62 (*d*, *J* = 12.3, 1H)	81.4	80.9	81.9
3	4.90 (*d*, *J* = 12.0, 1H)	4.76 (*d*, *J* = 12.0, 1H)	4.99 (*d*, *J* = 12.3, 1H)	48.2	49.2	47.9
4	-	-	-	196.6	196.2	196.6
4a	-	-	-	101.7	101.9	-
5	-	-	-	163.7	163.0	-
6	6.22 (*br s*, 1H)	6.06 (*br* s, 1H)	-	96.4	96.5	96.6
7	-	-	-	166.6	166.4	166.6
8	6.30 (*d*, *J* = 2, 1H)	6.08 (*br* s, 1H)		95.3	95.4	95.6
8a	-	-	-	163.8	162.4	-
1'	-	-	-	128.1	128.2	-
2'	7.13 (*d*, *J* = 9, 1H)	7.09 (*d*, *J* = 8.4, 1H)	7.06 (*d*, *J* = 8.8, 1H)	128.1	128.2	-
3'	6.63 (*d*, *J* = 9, 1H)	6.51 (*d*, *J* = 8.4, 1H)	6.75 (*d*, *J* = 8.8, 1H)	114.6	114.7	-
4'	-	-	-	162.2	157.0	-
5'	6.63 (*d*, *J* = 9, 1H)	6.51 (*d*, *J* = 8.4, 1H)	6.75 (*d*, *J* = 8.8, 1H)	114.6	114.7	-
6'	7.13 (*d*, *J* = 9, 1H)	7.09 (*d*, *J* = 8.4, 1H)	7.06 (*d*, *J* = 8.8, 1H)	128.1	128.2	-
2''	-	-	-	163.7	163.5	-
3''	6.50 (*s*, 1H)	6.36 (*s*, 1H)	6.17 (*s*, 1H)	102.8	102.8	-
4''	-	-	-	181.6	182.1	-
4a''	-	-	-	103.6	103.3	-
5''	-	-	-	160.4	160.7	-
6''	6.22 (*s*, 1H)	6.34 (*s*, 1H)	6.13 (*s*, 1H)	98.5	99.1	98.6
7''	-	-	-	162.8	164.0	-
8''	-	-	-	100.6	99.9	-
8a''	-	-	-	155.3	155.5	-
1'''	-	-	-	121.3	121.7	-
2'''	7.70 (*d*, *J* = 9, 1H)	7.61 (*d*, *J* = 8.6, 1H)	7.50 (*d*, *J* = 8.2, 1H)	128.1	127.6	-
3'''	6.68 (*d*, *J* = 9, 1H)	6.97 (*d*, *J* = 8.6, 1H)	6.75 (*d*, *J* = 8.2, 1H)	115.9	115.9	-
4'''	-	-	-	161.0	161.1	-
5'''	6.87 (*d*, *J* = 9, 1H)	6.97 (*d*, *J* = 8.6, 1H)	6.75 (*d*, *J* = 8.2, 1H)	115.9	115.9	-
6'''	7.70 (*d*, *J* = 9, 1H)	7.61 (*d*, *J* = 8.6, 1H)	7.50 (*d*, *J* = 8.2, 1H)	128.1	127.7	-
5-OH	12.40 (*s*)	12.29 (*s*, 1H)	-	-	-	-
5''-OH	13.27 (*d*)	12.94 (*s*, 1H)	12.85 (*s*, 1H)	-	-	-
OH	-	8.70 (*s*, 1H)	8.88 (*s*, 1H)	-	-	-
		9.76 (*s*, 1H)	9.68 (*s*, 1H)	-	-	-
		10.17 (*br* s, 1H)	10.30 (*br* s, 1H)	-	-	-
		10.61 (*br* s, 1H)		-	-	-

[a] Chen; et al. 1975: 300-303.

Table 4 ^{1}H and ^{13}C NMR and 2D NMR data of compound **A** (in $CDCl_3$ + DMSO-d_6)

Position	δ_H (*mult.*, *J* in Hz)		δ_C		HMBC correlations	NOESY correlations
	major	minor	major	minor		
2	5.86 (*d*, *J* = 12.0, 1H)	5.62 (*d*, *J* = 12.3, 1H)	81.0	82.0	C-1', C-2', C-6'	H-2', H-6'
3	4.76 (*d*, *J* = 12.0, 1H)	4.99 (*d*, *J* = 12.3, 1H)	49.2	48.0	C8''	H-2', H-6', H-2'''
4	-	-	196.3	196.7	-	-
4a	-	-	102.0	-	-	-
5	-	-	163.7	-	-	-
6	6.06 (*br* s, 1H)	-	96.6	96.7	C-4a,C-5, C-7	5-OH
7	-	-	166.6	166.9	-	-
8	6.08 (*br* s, 1H)	-	95.6	95.7	C-7	-
8a	-	-	163.1	-	-	-
1'	-	-	128.6	-	-	-
2'	7.09 (*d*, *J* = 8.4, 1H)	7.06 (*d*, *J* = 8.8, 1H)	128.4	-	C-4'	H-3
3'	6.51 (*d*, *J* = 8.4, 1H)	6.75 (*d*, *J* = 8.8, 1H)	114.9	-	C-1', C-2', C-4'	H-2'
4'	-	--	161.2	-	-	-
5'	6.51 (*d*, *J* = 8.4, 1H)	6.75 (*d*, *J* = 8.8, 1H)	114.9	-	C-1', C-4', C-6'	H-6'
6'	7.09 (*d*, *J* = 8.4, 1H)	7.06 (*d*, *J* = 8.8, 1H)	128.4	-	C-4'	-
2''	-	-	162.6	-	-	-
3''	6.36 (*s*, 1H)	6.17 (*s*, 1H)	102.9	-	C-2'', C-4a''	H-6'''
4''	-	-	182.2	-	-	-
4a''	-	-	104.0	-	-	-
5''	-	-	161.0	-	-	-
6''	6.34 (*s*, 1H)	6.13 (*s*, 1H)	99.2	98.7	C-4a'', C-5'', C-8''	-
7''	-	-	164.2	-	-	-
8''	-	-	100.1	-	-	-
8a''	-	-	155.3	-	-	-
1'''	-	-	121.7	-	-	-
2'''	7.61 (*d*, *J* = 8.6, 1H)	7.50 (*d*, *J* = 8.2, 1H)	127.7	-	C-2'', C-4'''	H-3'''
3'''	6.97 (*d*, *J* = 8.6, 1H)	6.75 (*d*, *J* = 8.2, 1H)	116.0	-	C-1''', C-4'''	-
4'''	-	-	161.6	-	-	-
5'''	6.97 (*d*, *J* = 8.6, 1H)	6.75 (*d*, *J* = 8.2, 1H)	116.0	-	C-1''', C-4'''	-
6'''	7.61 (*d*, *J* = 8.6, 1H)	7.50 (*d*, *J* = 8.2, 1H)	127.9	-	C-2'', C-4'''	H-5'''
5-OH	12.29 (*s*, 1H)	-	-	-	C-4a, C-6	-
5''-OH	12.94 (*s*, 1H)	12.85 (*s*, 1H)	-	-	-	-
OH	8.70 (*s*, 1H)	8.88 (*s*, 1H)	-	-	-	-
	9.76 (*s*, 1H)	9.68 (*s*, 1H)	-	-	C-4a'', C-5'', C-6''	-
	10.17 (*br* s, 1H)	10.30 (*br* s, 1H)	-	-	-	-
	10.61 (*br* s, 1H)		-	-	-	-

1.2 Compound **B** (morelloflavone (**32**), sss4595)

Compound **B** was obtained as a yellow solid from the EtOAc and MeOH soluble fractions of *G. fusca* root and the EtOAc soluble fraction of *G. cowa* and gave an orange coloration with anisaldehyde-H_2SO_4 reagent same as that of compound **A** (vokensiflavone). On the basis of its ESIMS ($[M-H]^-$ at *m/z* 555), a molecular formula of compound **B** was established as $C_{30}H_{20}O_{10}$ with the support of ^{13}C NMR data. Its IR absorption spectrum showed the presence of hydroxy groups at 3218 cm^{-1}, conjugated γ-pyrone at 1645 cm^{-1} and benzene ring at 1609, 1516 and 1456 cm^{-1}. The UV absorption maxima in MeOH at 223 (shoulder), 292 and 332 nm were found to belong to a biflavonoid skeleton. The ^1H and ^{13}C NMR spectra of compound **B** showed signals forming respective pairs (relative ratio; 4:1), the major compound of which revealed six aromatic protons doublets, one aromatic proton singlet and the H-2 and H-3 methine protons exhibited at δ_H 5.71 (*d*, *J* = 12.0 Hz), 4.89 (*d*, *J* = 12.0 Hz), respectively. The aromatic protons of flavanone part appeared at δ_H 7.15 (*d, J* = 8.3 Hz), 6.39 (*d*, *J* = 8.3 Hz,), 6.39 (*d*, *J* = 8.3 Hz) and 7.15 (*d*, *J* = 8.3 Hz) and two protons showed at δ_H 5.97 (*br s*) and 5.97 (*br s*), for flavone moiety. Furthermore, the two singlet signals of chelated OH at δ_H 12.25 and 13.07 as singlet and four signals of phenolic OH showed δ_H 8.70, 8.88, 10.16 and 10.59 as broad singlet. The ^{13}C NMR and DEPT spectra (Table 5, Figure 28 and 29) displayed 30 major signals attributable to thirteen methines and seventeen quaternary carbons which were assigned from the ^1H-^1H COSY and ^1H-^{13}C HMQC spectra of compound **B** and comparison with the reported values.

Connections among ring A, B and C of flavanone subgroups were provided by analysis of its HMBC and NOESY spectra (Table 6 and Figures 19 and 20). The NOESY correlations were observed for methine proton at δ_H 4.89 (H-3) to H-6' (δ_H 7.15) and H-2' (δ_H 7.15) of aromatic proton together with HMBC correlations of H-2 to C-2' (δ_C 128.6) and C-6' (δ_C 128.6), and the correlations of H-3 to C-2 (δ_C 81.0) in HMBC spectra, indicating that ring B was connected to ring C. The HMBC correlation of 5-OH (δ_H 12.25) to C-4a (δ_C 101.6), H-6 and H-8 to C-4 (δ_C 196.3) and of 5-OH to C-7 (δ_C 163.6) and C-8 (δ_C 96.3) confirmed that ring A connected to ring C.

Connections among ring D, E and F of flavone subgroups were provided by analysis of its HMBC and NOESY spectra. The correlations of H-3'' (δ_H 6.58) to C-1''' (δ_C

121.1) and C-4'' (δ_C 181.7), of H-6''' (δ_H 7.43) to C-2'' (δ_C 163.8) and C-4''' (δ_C 149.8) in HMBC spectra together with NOE correlations of H-2''' (δ_H 7.42) to H-3'' indicated that ring E connected to ring F. The HMBC correlations were observed for 5''-OH at δ_H 13.07 *s* to C-4a'' (δ_C 103.2) and C-6'' (δ_C 98.7) that ring D attached to ring F.

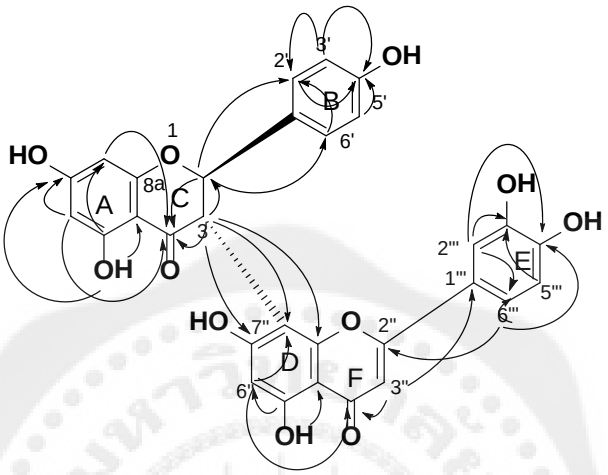


Figure 19 Selected HMBC correlations for compound **B**

Connections among flavanone and flavone subgroups were provided by analysis of its HMBC and NOESY spectra. The HMBC correlations were observed for methine proton at δ_H 4.89 (H-3) to C-8'' (δ_C 100.6) and C-8a'' (δ_C 155.3) and NOESY correlations of H-2''' to H-3 confirmed that flavone unit was attached to flavanone moiety. On the basis of these data coupled with the compairison of its NMR data with that of morelloflavone (**32**), Table 5, the structure of compound **B** was proposed to be morelloflavone (**32**) (*synonyms* fukugetin).

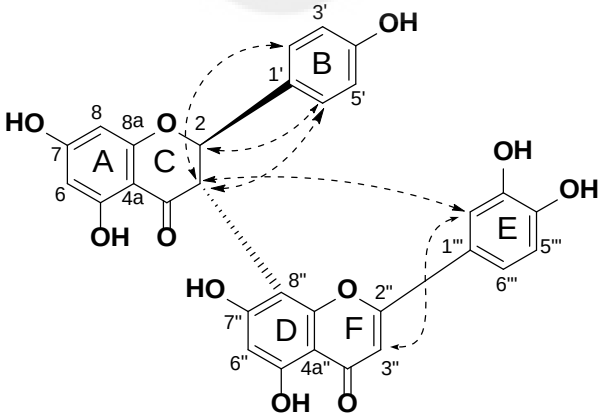


Figure 20 Selected NOESY correlations for compound **B**

The stereochemistry at C-2 and C-3 of flavanone unit in conpound **B** were provided by analysis of its *J* coupling constant value and the large coupling constants (*J* = 12.0 Hz) of C-2 and C-3 protons in ring C of compound **B**, in addition, no significant NOE enhancement was observed between both protons, indicated that both hydrogens have a *trans*-diaxial arrangement.

Compairison of the melting points (230-232 °C, d) and dextrorotatory optical rotation ($[\alpha]_D^{25.8}$ = +177.6 °) of compound **B** with the reported value of (+) morelloflavone ($[\alpha]_D^{25.8}$ = +161.6 °, m.p. 230-232 °C, d) (Nontakham. 2011: 34-75.), (±) morelloflavone ($[\alpha]_D^{25}$ = 0 °, m.p. 298-299 °C, d) and (+) morelloflavone ($[\alpha]_D^{25}$ = +188 ° (Li; et al. 2002: 8709-8717), m.p. 244-245 °C (d) (Konoshima; et al. 1969; 121-124)), therefore, the structure of **B** was assigned to be (+) morelloflavone. The stereochemistry of (+) morelloflavone was previously assigned as *2R,3S*-configurations by its CD spectrum analysis (positive Cotton effect at near 340 and 290 nm) (Li; et al. 2002: 8709-8717). This led to conclude that compound **B** also has the same *2R,3S*-configurations as shown in Figure 20.

Morelloflavone (**32**) (Terashima; et al. 2008: 407-13) was found in *Garcinia* plants such as *G. dulcis* (Roxb.) (Hutadilok; et al. 2007: 655-662) Kurz., *G. livingstonei* (Yang; et al. 2010: 4749-4755), *G. Morella* (Karanjgaokar; et al. 1967: 3195-3198), *G. spicata* Hook. F. (Konoshima; & Ikeshiro. 1969: 121-124). *G. xanthochymus* Hook. f. (Konoshima; Ikeshiro; & Miyahara. 1970: 1203-1206) and *G. multiflora* Cham. (Konoshima; Ikeshiro; & Miyahara. 1970: 1203-1206). It was exhibited DPPH and SW-480 colon cancer cells cytoxicity (Baggett; et al. 2005: 354-360) and strong antioxidation (Hutadilok; et al. 2007: 655-662).

Table 5 Comparison of ^{1}H and ^{13}C NMR data of compound **B** (in $CDCl_3$ + DMSO-d_6) with morelloflavone (**32**) (in DMSO-d_6)

Position	δ_H (*mult.*, *J* in Hz)			δ_C	
	morelloflavone(**32**)[a]	**B**		Morelloflavone (**32**)[a]	**B**
		major	minor		
2	5.71 (*d*, *J* = 12.0,	5.71 (*d*, *J* = 12.0, 1H)	5.59 (*d*, *J* = 12.3, 1H)	81.0	81.0
3	4.89 (*d*, *J* = 12.0, 1H)	4.89 (*d*, *J* = 12.0, 1H)	4.99 (*d*, *J* = 12.3, 1H)	48.4	48.9
4	-	-	-	196.3	196.
4a	-	-	-	101.6	101.
5	-	-	-	61.8	161.
6	5.97 (*br s*, 1H)	5.97 (*br s*, 1H)	5.99 (*br s*, 1H)	95.4	95.4
7	-	-	-	163.6	163.
8	5.97 (*br s*, 1H)	5.97 (*br s*, 1H)	6.02 (*br s*, 1H)	96.3	96.4
8a	-	-	-	166.6	166.
1’	-	-	-	128.2	128.
2’	7.15 (*d*, *J* = 8.3, 1H)	7.15 (*d*, *J* = 8.3, 1H)	7.09 (*d*, *J* = 7.7, 1H)	128.6	128.
3’	6.39 (*d*, *J* = 8.3, 1H)	6.39 (*d*, *J* = 8.3, 1H)	6.61 (*d*, *J* = 7.7, 1H)	114.5	114.
4’	-	-	-	157.4	157.
5’	6.39 (*d*, *J* = 8.3, 1H)	6.39 (*d*, *J* = 8.3, 1H)	6.61 (*d*, *J* = 7.7, 1H)	114.5	114.
6’	7.15 (*d*, *J* = 8.3, 1H)	7.15 (*d*, *J* = 8.3, 1H)	7.09 (*d*, *J* = 7.7, 1H)	128.6	127.
2’’	-	-	-	163.8	
3’’	6.58 (*s*, 1H)	6.58 (*s*, 1H)	6.61 (*s*, 1H)	102.3	102.
4’’	-	-	-	181.7	182.
4a’’	-	-	-	103.2	103.
5’’	-	-	-	160.6	161.
6’’	6.23 (*s*, 1H)	6.23 (*s*, 1H)	6.06 (*br s*, 1H)	98.7	99.0
7’’	-	-	-	162.9	163.
8’’	-	-	-	100.6	100.
8a’’	-	-	-	155.3	155.
1’’’	-	-	-	121.1	122.
2’’’	7.42 (*br s*, 1H)	7.42 (*br s*, 1H)	7.25 (*br s*, 1H)	113.4	113.
3’’’	-	-	-	145.7	144.
4’’’	-	-	-	49.8	148.
5’’’	6.91 (*d*, *J* = 8.1, 1H)	6.91 (*d*, *J* = 8.1, 1H)	6.50 (*d*, *J* = 8.4, 1H)	116.2	115.
6’’’	6.97 (*d*, *J* = 8.0, 1H)	7.43 (br *d*, *J* = 8.0, 1H)	6.97 (*br d*, *J* = 8.0, 1H)	119.4	118.
5-OH	12.25 (*s*, 1H)	12.25 (*s*, 1H)	12.14 (*s*, 1H)	-	-
5’’-OH	13.07 (*s*, 1H)	13.07 (*s*, 1H)	12.97 (*s*, 1H)	-	-
OH	-	8.70 (*br s*, 1H)	-	-	-
		8.88 (*br s*, 1H)	-	-	-
		10.16 (*br s*, 1H)	-	-	-
		10.59 (*br s*, 1H)	-	-	-
			-	-	-

[a] Kenji, T.; et al. 2008: 407-413.

Table 6 ^{1}H, ^{13}C NMR and 2D NMR data of compound **B** (in DMSO-d_6)

Position	δ_H (*mult.*, *J* in Hz)		δ_C	HMBC	NOESY
	major	minor		correlations	correlations
2	5.71 (*d*, *J* = 12.0, 1H)	5.59 (*d*, *J* = 12.3, 1H)	81.0	C-2'	H-2', H-6'
3	4.89 (*d*, *J* = 12.0, 1H)	4.99 (*d*, *J* = 12.3, 1H)	48.9	C-2, C-4, C-7'', C-8'', C-8a''	H-2', H-6', H-2'''
4	-	-	196.4	-	-
4a	-	-	101.9	-	-
5	-	-	161.3	-	-
6	5.97 (*br s*, 1H)	5.99 (*br s*, 1H)	95.4	C-4, C-7	-
7	-	-	163.5	-	-
8	5.97 (*br s*, 1H)	6.02 (*br s*, 1H)	96.4	C-4	-
8a	-	-	166.3	-	-
1'	-	-	128.4	-	-
2'	7.15 (*d*, *J* = 8.3, 1H)	7.09 (*d*, *J* = 7.7, 1H)	128.2	C-4'	H-3
3'	6.39 (*d*, *J* = 8.3, 1H)	6.61 (*d*, *J* = 7.7, 1H)	114.7	C-2', C-4'	-
4'	-	-	157.0	-	-
5'	6.39 (*d*, *J* = 8.3, 1H)	6.61 (*d*, *J* = 7.7, 1H)	114.7	C-4'	-
6'	7.15 (*d*, *J* = 8.3, 1H)	7.09 (*d*, *J* = 7.7, 1H)	127.8	C-2'''	-
2''	-	-	164.0	-	-
3''	6.58 (*s*, 1H)	6.61 (*s*, 1H)	102.8	C-4'', C-1'''	H-2'''
4''	-	-	182.0	-	-
4a''	-	-	103.9	-	-
5''	-	-	161.0	-	-
6''	6.23 (*s*, 1H)	6.06 (*br s*, 1H)	99.0	C-4'', C-7'', C-8''	-
7''	-	-	163.0	-	-
8''	-	-	100.0	-	-
8a''	-	-	155.5	-	-
1'''	-	-	122.3	-	-
2'''	7.42 (*br s*, 1H)	7.25 (*br s*, 1H)	113.0	C-3''', C-4''', C-6'''	H-3, H-3''
3'''	-	-	144.9	-	-
4'''	-	-	148.6	-	-
5'''	6.91 (*d*, *J* = 8.1, 1H)	6.50 (*d*, *J* = 8.4, 1H)	115.4	-	-
6'''	7.43 (br *d*, *J* = 8.0, 1H)	6.97 (*br d*, *J* = 8.0, 1H)	118.7	C-2'', C-4'''	-
5-OH	12.25 (*s*, 1H)	12.14 (*s*, 1H)	-	C-4a, C-7	-
5''-OH	13.07 (*s*, 1H)	12.97 (*s*, 1H)	-	-	-
OH	8.70 (*br s*, 1H)	-	-	-	-
	8.88 (*br s*, 1H)	-	-	C-4a'', C-6''	-
	10.16 (*br s*, 1H)	-	-	-	-
	10.59 (*br s*, 1H)	-	-	-	-
		-	-	-	-

1.3 Compound **C** (fukugiside (**47**), sss4751)

Compound **C** was obtained as a yellow amorphous solid from the EtOAc and MeOH soluble fractions of *G. fusca* root and the EtOAc soluble fraction of *G. cowa* heartwood and bark. It gave an orange coloration with anisaldehyde-H_2SO_4 reagent as same as that of compound **B** (morelloflavone). On the basis of its ESIMS ($[M-H]^-$ at *m/z* 717), a molecular formula of compound **C** was established as $C_{36}H_{30}O_{16}$ with the support of ^{13}C NMR data. Its IR absorption spectrum showed the presence of hydroxy groups at 3395 cm^{-1}, conjugated γ-pyrone at 1651 cm^{-1} and benzene ring at 1602, 1519 and 1439 cm^{-1}. The UV absorption maxima in MeOH at 226, 289, 330 and 346 nm were found to belong to a biflavonoid skeleton. The NMR data of compound **C** was very similar to that compound **B** (morelloflavone) except additional the five protons of a glucose at about δ_H 3.3-3.9 (*m*, 5H).

The ^1H NMR spectrum of compound **C** also showed the two groups of signals for biflavonoid (approximate relative ratio; 3:1), that revealed nine doublets (that include *br d*) and seven singlets (that include *br s*) protons of aromatic rings. The methine protons H-2 and H-3 displayed at δ_H 5.80 (*d*, *J* = 12.3 Hz) and 5.28 (*d*, *J* = 12.3 Hz), respectively. The two aromatic protons of flavone part appeared at δ_H 5.89 (*br s*) and 5.97 (*br s*). The three signals at δ_H 6.50 (*s*), 6.60 (*d*, *J* = 8.3 Hz), 7.36 (*br s*) and 7.15 (*d*, *J* = 8.3 Hz), were described as flavanone moiety. Moreover, the two singlet protons of chelated OH at δ_H 13.01 and 12.83 and two broad singlet signals of phenolic OH also showed at δ_H 12.05 and 11.99. Importantly, ^{13}C NMR of compound **C** exhibited signals for a glucose unit at δ_C 60-80 and showed an anomeric proton at δ_H 5.16 (*d*, *J* = 7.4 Hz) in its ^1H NMR. The ^{13}C NMR and DEPT spectra (Table 7, Figure 30 and 31) displayed 36 major signals attributable to eighteen methines, seventeen quaternary and one methylene carbons which were assigned from the ^1H-^1H COSY and ^1H-^{13}C HMQC spectra of compound **C** and comparison with the reported values.

The analysis of ^1H-^{13}C HMBC and ^1H-^1H NOESY corelations of compound **C** described the connections between flavanone and flavone subgroups as well as attachment of glucose moiety to flavanone subunit (Table 8 and Figures 21).

The HMBC correlations of H-3 (δ_H 5.28) to δ_C at 102.8 (C-4a) togetherwith (δ_C 103.6) H-8 (δ_H 5.97) to C-4a and C-8a (δ_C 167.9) indicated that ring A attached to ring C.

The connection between ring B and C of flavone unit was confirmed by HMBC correlations of H-2' (δ_H 7.15) and H-6' (δ_H 7.15), to C-2 (δ_C 83.4) as well as HMBC correlations of H-2 (δ_H 5.80) to C-1' (δ_C 129.7).

Connections among ring D, E and F of flavanone subgroups were confirmed by analysis of its HMBC spectrum. The correlations of H-3'' (δ_H 6.40) to C-2'' (δ_C 165.6) and C-4a'' (δ_C 106.4), of H-6'' (δ_H 6.60) to C-4a'' (δ_C 106.4) and C-8a'' (δ_C 155.9) in HMBC spectra indicated that ring D connected to ring F. The HMBC correlations were observed for H-3'' (δ_H 6.40) to C-2'' (δ_C 165.6) and C-1''' (δ_C 123.1), for H-2''' (δ_H 7.36) to C-2'' (δ_C 165.6) that revealed ring E attached to ring F.

The NOE enhancements were observed for methine proton at δ_H 5.97 (H-6''') to H-3 (δ_H 5.28) and H-2 (δ_H 5.80) of aromatic proton together with HMBC correlations of H-3 to C-7" (δ_C 162.0), C-8" (δ_C 103.6) and C-8a'' (δ_C 155.9) confirmed the conection between ring C of flavone and ring D flavanone subgroups.

Moreover, the attachment of a glucosyl group to the biflavonoid moiety was also described by HMBC and NOESY correlations. The correlations of the anomeric proton H-1"" (δ_H 5.16) to C-7" (δ_C 162.0) in HMBC spectrum and NOE effects of H-1"" (δ_H 5.16) to H-6'' (δ_H 6.60), of H-2 (δ_H 5.80) and H-3 (δ_H 5.28) to H-6''' (δ_H 7.24).

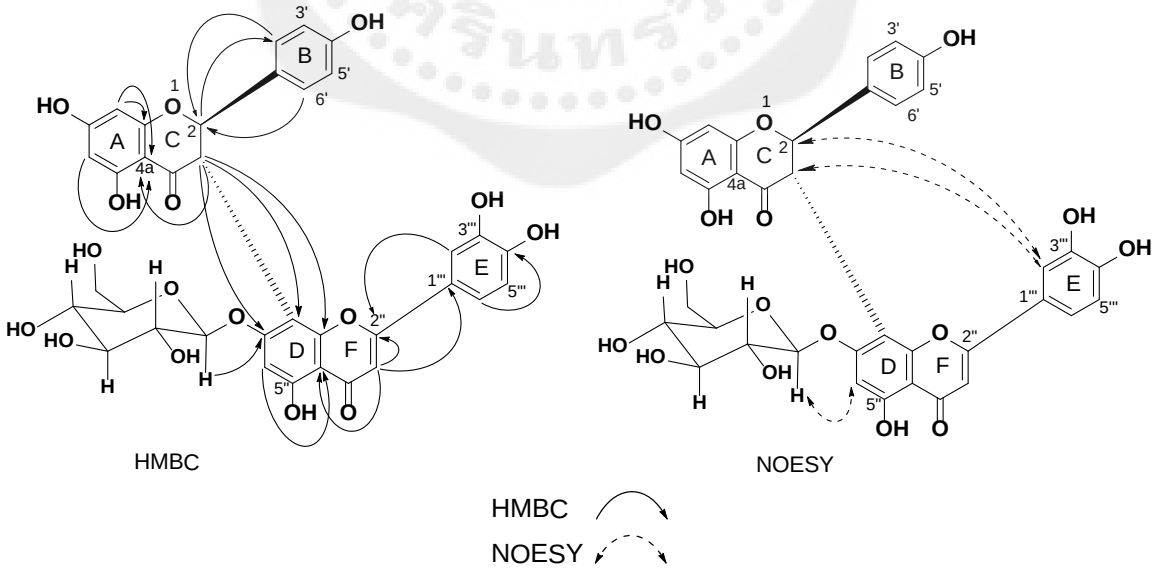


Figure 21 Selected HMBC and NOESY correlations for compound **C**

The stereochemistry at C-2 and C-3 of flavone unit in conpound **C** were provided as same as morelloflavone by analysis of its *J* coupling constant value and the large coupling constants (*J* = 12.3 Hz) of H-2 and H-3 protons in ring C of compound **C**, in addition, no significant NOE enhancement was observed between both protons, indicated that both hydrogens have a *trans*-diaxial arrangement.

Hydrolysis of compound **C** (fukugiside (**47**)) with 10% H_2SO_4 afforded, along with a sugar unit, a yellow amorphous compound. In TLC comparisons in several solvent systems between the obtained yellow solid with the authentic morelloflavone (**32**), both compounds showed identical spots and R_f values. The obtained sugar unit was proved to be D-(+)-glucose by analysis of its optical rotation activity value $[\alpha]_D^{27.8}$ +13.8 ° (c = 0.20, H_2O). From these evidences, compound **C** was suggested to be fukugiside (**47**) or morelloflavone glucoside.

Compairison of the melting points (242-243°C (d)) and dextrorotatory optical rotation ($[\alpha]_D^{25.9}$ = +150.0 °) of compound **C** with the reported value of its related analogue (+) morelloflavone ($[\alpha]_D^{25.8}$ = +161.6 °, m.p. 230-232 °C, d) (Nontakham. 2011: 34-75.), (±) morelloflavone ($[\alpha]_D^{25}$ = 0 °, m.p. 298-299 °C, d) and (+) morelloflavone ($[\alpha]_D^{25}$ = +188 ° (Li; et al. 2002: 8709-8717), m.p. 244-245 °C (d) (Konoshima; et al. 1969; 121-124)), therefore, the structure of **C** was assigned to be (+) fukugiside. The stereochemistry of (+) fukugiside was related to previously assigned of (+) morelloflavone as *2R,3S*-configurations as described above. This led to conclude that compound C also has the same *2R,3S*-configurations as shown in Figure 21.

Fukugiside (**47**) was found in *Garcinia* plants such as *G. spicata* (Konoshima; et al. 1970: 1717-1720), *G. multiflora* (Chen; et al. 1975: 818-820), *G. cymosa* (Elfita; et al. 2009: 907-912).

Table 7 Comparison of ^{1}H and ^{13}C NMR data of compound **C** with fukugiside (**47**) (in acetone-d_6)

Position	δ_H (*mult.*, *J* in Hz)			δ_C		
	Fukugiside (**47**)[a]	compound **C**		Fukugiside (**47**)[a]	compound **C**	
		major	minor		major	minor
2	5.74 (*d*, *J* = 11.6, 1H)	5.80 (*d*, *J* = 12.3, 1H)	5.75 (*d*, *J* = 11.9, 1H)	82.4	83.4	82.2
3	4.83	5.28 (*d*, *J* = 12.3, 1H)	4.93 (*d*, *J* = 11.9, 1H)	51.01	50.0	48.6
4	-	-	-	197.64	197.87	196.7
4a	-	-	-	103.32	102.8	-
5	-	-	-	164.82	164.3	-
6	5.98 (*br s*, 1H)	5.89 (*s*, 1H)	5.87 (*s*, 1H)	96.51	96.1	95.9
7	-	-	-	165.72	165.4	-
8	5.93 (*br s*, 1H)	5.97 (*s*, 1H)	5.87 (*s*, 1H)	97.70	97.1	-
8a	-	-	-	168.33	167.9	167.4
1’	-	-	-	130.39	129.7	128.5
2’	7.10 (*d*, *J* = 8.1, 1H)	7.15 (*d*, *J* = 7.1, 1H)	7.15 (*d*, *J* = 7.1, 1H)	129.40	130.2	129.3
3’	6.35 (*d*, *J* = 8.1, 1H)	6.55 (*br d*, *J* = 7.1, 1H)	6.37 (*d*, *J* = 7.1, 1H)	115.46	115.8	115.3
4’	-	-	-	158.46	158.8	158.3
5’	6.35 (*d*, *J* = 8.1, 1H)	6.55 (*br d*, *J* = 7.1, 1H)	6.37 (*d*, *J* = 7.1, 1H)	115.46	115.8	115.3
6’	7.10 (*d*, *J* = 8.1, 1H)	7.15 (*d*, *J* = 7.1, 1H)	7.15 (*d*, *J* = 7.1, 1H)	129.40	130.2	129.3
2’’	-	-	-	166.10	165.6	-
3’’	6.38 (*s*, 1H)	6.50 (*s*, 1H)	6.40 (*br s*, 1H)	103.58	104.3	103.6
4’’	-	-	-	183.90	183.23	-
4a’’	-	-	-	106.47	106.4	106.0
5’’	-	-	-	162.71	162.4	-
6’’	6.62 (*s*, 1H)	6.60 (*s*, 1H)	6.34 (*br s*, 1H)	99.51	100.1	99.2
7’’	-	-	-	161.64	162.0	161.3
8’’	-	-	-	104.13	103.6	-
8a’’	-	-	-	156.65	155.9	155.1
1’’’	-	-	-	123.05	123.1	122.9
2’’’	7.31 (*br s*, 1H)	7.36 (*br s*, 1H)	7.24 (*br s*, 1H)	114.38	114.2	114.0
3’’’	6.68 (1H, *d*, *J* = 9)	7.61 (1H, *d*, *J* = 8.6)	6.96 (1H, *d*, *J* = 8.5)	146.72	146.8	146.5
4’’’	-	-	-	151.13	150.5	-
5’’’	6.87 (*d*, *J* = 8.3, 1H)	6.89 (*d*, *J* = 8.2, 1H)	6.56 (br *d*, *J* = 8.4, 1H)	116.87	116.5	116.1
6’’’	7.26 (*d*, *J* = 8.3, 1H)	7.04 (*br d*, *J* = 8.2, 1H)	7.40 (*br d*, *J* = 8.4, 1H)	120.84	120.4	119.6
1’’’’	5.15 (*d*, *J* = 7.5, 1H)	5.16 (*d*, *J* = 7.4, 1H)	4.73 (*br d*, *J* = 6.1, 1H)	101.52	101.4	101.0
2’’’’	3.3-3.9 (*m*, 1H)	-	-	75.19	74.7	74.3
3’’’’	3.3-3.9 (*m*, 1H)	-	-	78.32	77.7	77.5
4’’’’	3.3-3.9 (*m*, 1H)	-	-	71.10	70.9	-
5’’’’	3.3-3.9 (*m*, 1H)	-	-	78.52	78.0	77.8
6’’’’	3.3-3.9 (*m*, 1H)	-	-	62.47	62.3	62.2
5-OH	-	12.83 (*s*, 1H)	11.99 (*s*, 1H)	-	-	-
5’’-OH	-	13.01 (*s*, 1H)	12.83 (*s*, 1H)	-	-	-

[a] Elfita; et al. 2009: 907-912.

Table 8 ^{1}H and ^{13}C NMR and 2D NMR data of compound **C** (in Acetone-d_6)

Position	δ_H (*mult.*, *J* in Hz)		δ_C		HMBC correlations	NOESY correlations
	major	minor	major	minor		
2	5.80 (*d*, *J* = 12.3, 1H)	5.75 (*d*, *J* = 11.9, 1H)	83.4	82.2	C-2'	H-2'''
3	5.28 (*d*, *J* = 12.3, 1H)	4.93 (*d*, *J* = 11.9, 1H)	50.0	48.6	C-4a, C-7'', C-8'', C-8a''	H-2'''
4	-	-	197.87	196.7	-	-
4a	-	-	102.8	-	-	-
5	-	-	164.3	-	-	-
6	5.89 (*s*, 1H)	5.87 (*s*, 1H)	96.1	95.9	C-4a	-
7	-	-	165.4	-	-	-
8	5.97 (*s*, 1H)	5.87 (*s*, 1H)	97.1	-	C-4a, C-8a	-
8a	-	-	167.9	167.4	-	-
1'	-	-	129.7	128.5	-	-
2'	7.15 (*d*, *J* = 7.1, 1H)	7.15 (*d*, *J* = 7.1, 1H)	130.2	129.3	C-2	-
3'	6.55 (*br d*, *J* = 7.1, 1H)	6.37 (*d*, *J* = 7.1, 1H)	115.8	115.3	-	-
4'	-	-	158.8	158.3	-	-
5'	6.55 (*br d*, *J* = 7.1, 1H)	6.37 (*d*, *J* = 7.1, 1H)	115.8	115.3	-	-
6'	7.15 (*d*, *J* = 7.1, 1H)	7.15 (*d*, *J* = 7.1, 1H)	130.2	129.3	C-2	-
2''	-	-	165.6	-	-	-
3''	6.50 (*s*, 1H)	6.40 (*br s*, 1H)	104.3	103.6	C-2'', C-4a'', C-1'''	-
4''	-	-	183.23	-	-	-
4a''	-	-	106.4	106.0	-	-
5''	-	-	162.4	-	-	-
6''	6.60 (*s*, 1H)	6.34 (*br s*, 1H)	100.1	99.2	C-4a''	H-1''''
7''	-	-	162.0	161.3	-	-
8''	-	-	103.6	-	-	-
8a''	-	-	155.9	155.1	-	-
1'''	-	-	123.1	122.9	-	-
2'''	7.36 (*br s*, 1H)	7.24 (*br s*, 1H)	114.2	114.0	C-2''	H-2, H-3
3'''	7.61 (1H, *d*, *J* = 8.6)	6.96 (1H, *d*, *J* = 8.5)	146.8	146.5	-	-
4'''	-	-	150.5	-	-	-
5'''	6.89 (*d*, *J* = 8.2, 1H)	6.56 (br *d*, *J* = 8.5, 1H)	116.5	116.1	-	-
6'''	7.04 (*br d*, *J* = 8.2, 1H)	7.40 (*br d*, *J* = 8.5, 1H)	120.4	119.6	C-4'''	-
1''''	5.16 (*d*, *J* = 7.4, 1H)	4.73 (*br d*, *J* = 6.1, 1H)	101.4	101.0	C-7''	H-6''
2''''	-	-	74.7	74.3	-	-
3''''	-	-	77.7	77.5	-	-
4''''	-	-	70.9	-	-	-
5''''	-	-	78.0	77.8	-	-
6''''	-	-	62.3	62.2	-	-
5-OH	12.83 (*s*, 1H)	11.99 (*s*, 1H)	-	-	-	-
5''-OH	13.01 (*s*, 1H)	12.83 (*s*, 1H)	-	-	-	-

1.4 Compound **D** (spicataside (**81**), sss5167)

Compound **D** was obtained as a yellow solid from the EtOAc soluble fraction of *G. cowa* heartwood. Compound **D** gave an orange coloration with anisaldehyde-H_2SO_4 reagent. On the basis of its ESIMS ($[M-H]^-$ at *m/z* 701), a molecular formula of compound **D** was established as $C_{36}H_{30}O_{15}$ with the support of ^{13}C NMR data. Its IR absorption spectrum showed the presence of hydroxy groups at 3243 cm^{-1}, conjugated γ-pyrone at 1652 cm^{-1} and benzene ring at 1514 cm^{-1}. The UV absorption maxima in MeOH at 221 (shoulder), 287, 288, 320 and 325 nm were found to belong to a biflavonoid skeleton. The NMR data of compound **D** was very similar to that of compound **A** (vokensiflavone) except for additional carbon signals of a glucose unit at about δ_C 60.5-77.0.

The ^1H NMR spectrum of compound **D** (in $CDCl_3$ + DMSO-d_6 ; 1:1) also showed signals forming respective pairs with approximate of relative ratio 3:1. The methine protons H-2 and H-3 displayed at δ_H 5.62 (*d*, *J* = 12.4 Hz) and 5.31 (*d*, *J* = 12.4 Hz), respectively. The two aromatic protons of flavone part appeared at δ_H 5.88 (*br s*) and 5.88 (*br s*). The two signals at δ_H 6.53 (*d*, *J* = 8.4 Hz) and 7.14 (*d*, *J* = 8.4 Hz), were described as flavanone moiety, in addition with the two singlet protons of chelated OH at δ_H 12.83 and 12.07. ^{13}C NMR spectrum of compounds **D** exhibited signals for a glucose unit at δ_C 60.5-77.0 and an anomeric proton at δ_H 4.69 (*d*, *J* = 7.0 Hz) in its ^1H NMR data. The ^{13}C NMR and DEPT spectra (Table 9, Figure 32 and 33) displayed 36 major signals attributable to nineteen methines, sixteen quaternary and one methylene carbons which were assigned from the ^1H-^1H COSY and ^1H-^{13}C HMQC spectra of compound **D**

The analysis of ^1H-^{13}C HMBC and ^1H-^1H NOESY corelations of compound **D** described the connections between flavanone and flavone subgroups as well as attachment of a glucose moiety to flavanone subunit (Table 10 and Figures 22).

The HMBC correlations of H-2' (δ_H 7.14) and H-6' (δ_H 7.14) to δ_C at 82.1 (C-2) indicated that ring B attached to ring C.

Connections among ring D, E and F of flavanone subgroups were confirmed by analysis of its HMBC spectrum. The correlations of H-3'' (δ_H 6.57) to C-1''' (δ_C 120.8) in HMBC spectra indicated that ring F connected to ring E.

Connections between ring C of flavone subgroup and ring D flavanone subgroup were confirmed by analysis of its HMBC spectrum. The correlations of H-3 (δ_H 5.31) to C-7'' (δ_C 160.7) and C-8'' (δ_C 102.4).

The NOE enhancements were observed for methine proton at δ_H 7.14 (H-6') and δ_H 7.14 (H-6') to H-3 (δ_H 5.31) confirmed the conection between ring B and ring C.

Moreover, the attachment of a glucosyl group to biflavonoid moiety was also deduced by its NOESY correlations. The correlation of the anomeric proton H-1'''' (δ_H 4.69) to H-6'' (δ_H 6.43) in its NOESY spectrum comfirmed the glucose moiety attached to ring D at C-7''.

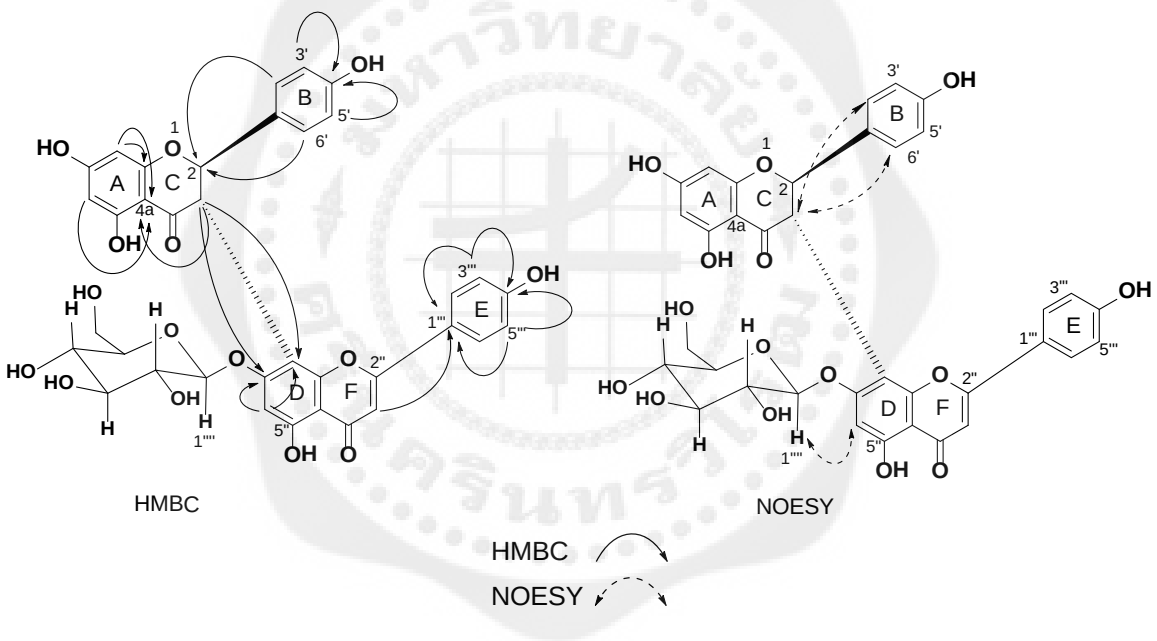


Figure 22 Selected HMBC and NOESY correlations for compound **D**

Compound **D** gave a molecular ion peak at *m/z* 701 in its ESIMS data (negative mode) which was 17 mass unit less than of compound **C** (*m/z* 717). The NMR data of compound **D** was very similar to that of compound **A** (vokensiflavone) except for additional carbon signals of a glucose unit at about δ_C 60.5-77.0. From these data, the structure of compound **D** was propose to be vokensiflavone glucoside.

The stereochemistry at C-2 and C-3 of flavanone unit were provided by analysis of *J* coupling constant value between H-2 and H-3. The large coupling constants (12 Hz) of H-

2 and H-3. In addition, no significant NOE enhancement was observed between both protons in their NOESY spectra, indicated that both hydrogens have a *trans*-diaxial arrangement.

Compairison of the melting points (233-234°C (d)) and dextrorotatory optical rotation ($[\alpha]_D^{27.3}$ = +14.8 °) of compound **D** with the reported value of spicataside (m.p. 232-233 °C, d) (Konoshima; et al. 1970: 4203-4206), 235-238°C (Chen; et al. 1975: 818-820), therefore, the structure of **D** was assigned to be spicataside.

Spicataside (**81**) was found in *Garcinia* plants such as *G. spicata* (Konoshima; et al. 1970: 1717-1720), *G. multiflora* (Chen; et al. 1975: 818-820).

Table 9 ^{1}H and ^{13}C NMR data of compound **D** (in $CDCl_3$+DMSO-d_6; 1:1)

Position	δ_H (mult., J in Hz)		δ_C
	major	minor	major
2	5.62 (*d*, *J* = 12.4, 1H)	5.75 (*d*, *J* = 12.2, 1H)	82.1
3	5.31 (*d*, *J* = 12.4, 1H)	-	46.9
4	-	-	196.2
4a	-	-	-
5	-	-	162.8
6	5.88 (*br s*, 1H)	-	95.5
7	-	-	-
8	5.88 (*br s*, 1H)	-	-
8a	-	-	153.6
1’	-	-	129.2
2’	7.14 (*d*, *J* = 8.4, 1H)	7.08 (*d*, *J* = 8.4, 1H)	129.2
3’	6.53 (*d*, *J* = 8.4, 1H)	-	114.3
4’	-	-	-
5’	6.53 (*d*, *J* = 8.4, 1H)	-	114.3
6’	7.14 (*d*, *J* = 8.4, 1H)	7.08 (*d*, *J* = 8.4, 1H)	129.2
2’’	-	-	163.8
3’’	6.57 (*s*, 1H)	-	103.1
4’’	-	-	181.8
4a’’	-	-	-
5’’	-	-	-
6’’	6.60 (*s*, 1H)	-	98.7
7’’	-	-	160.7
8’’	-	-	102.4
8a’’	-	-	-
1’’’	-	-	120.8
2’’’	7.57 (*d*, *J* = 8.4, 1H)	7.79 (*d*, *J* = 7.3, 1H)	128.0
3’’’	6.65 (*d*, *J* = 8.4, 1H)	-	115.8
4’’’	-	-	-
5’’’	6.65 (*d*, *J* = 8.4, 1H)	-	115.8
6’’’	7.57 (*d*, *J* = 8.4, 1H)	7.79 (*d*, *J* = 7.3, 1H)	128.0
1’’’’	4.69 (*d*, *J* = 7.0, 1H)	5.18 (*d*, *J* = 7.3, 1H)	101.2
2’’’’	-	-	73.0
3’’’’	-	-	76.2
4’’’’	-	-	69.5
5’’’’	-	-	77.0
6’’’’	-	-	60.5
5-OH	12.07 (*s*, 1H)	-	-
5’’-OH	12.83 (*s*, 1H)	-	-

Table 10 ^{1}H and ^{13}C NMR and 2D NMR data of compound **D** (in $CDCl_3$+DMSO-d_6)

Position	δ_H (mult., J in Hz)		δ_C	HMBC correlations	NOESY correlations
	major	minor	major		
2	5.62 (*d*, *J* = 12.4, 1H)	5.75 (*d*, *J* = 12.2, 1H)	82.1	-	
3	5.31 (*d*, *J* = 12.4, 1H)	4.86 (*d*, *J* = 12.2, 1H)	46.9	C-7'', C-8''	H-2', H-6'
4	-	-	196.2	-	-
4a	-	-	-	-	-
5	-	-	162.8	-	-
6	5.88 (*br s*, 1H)	-	95.5	-	-
7	-	-	-	-	-
8	5.88 (*br s*, 1H)	-	-	-	-
8a	-	-	153.6	-	-
1'	-	-	129.2	-	-
2'	7.14 (*d*, *J* = 8.4, 1H)	7.08 (*d*, *J* = 8.4, 1H)	129.2	C-2	H-3
3'	6.53 (*d*, *J* = 8.4, 1H)	-	114.3	C-4'	-
4'	-	-	-	-	-
5'	6.53 (*d*, *J* = 8.4, 1H)	-	114.3	C-4'	-
6'	7.14 (*d*, *J* = 8.4, 1H)	7.08 (*d*, *J* = 8.4, 1H)	129.2	C-2	-
2''	-	-	163.8	-	-
3''	6.57 (*s*, 1H)	-	103.1	C-1'''	-
4''	-	-	181.8	-	-
4a''	-	-	-	-	-
5''	-	-	-	-	-
6''	6.60 (*s*, 1H)	-	98.7	C-7'', C-8''	H-1''''
7''	-	-	160.7	-	-
8''	-	-	102.4	-	-
8a''	-	-	-	-	-
1'''	-	-	120.8	-	-
2'''	7.57 (*d*, *J* = 8.4, 1H)	7.79 (*d*, *J* = 7.3, 1H)	128.0	-	-
3'''	6.65 (*d*, *J* = 8.4, 1H)	-	115.8	C-1''', C-4'''	-
4'''	-	-	-	-	-
5'''	6.65 (*d*, *J* = 8.4, 1H)	-	115.8	C-1''', C-4'''	-
6'''	7.57 (*d*, *J* = 8.4, 1H)	7.79 (*d*, *J* = 7.3, 1H)	128.0	-	-
1''''	4.69 (*d*, *J* = 7.0, 1H)	5.18 (*d*, *J* = 7.3, 1H)	101.2	-	H-6''
2''''	-	-	73.0	-	-
3''''	-	-	76.2	-	-
4''''	-	-	69.5	-	-
5''''	-	-	77.0	-	-
6''''	-	-	60.5	-	-
5-OH	12.07 (*s*, 1H)	12.13 (*s*, 1H)	-	-	-
5''-OH	12.83 (*s*, 1H)	13.04 (*s*, 1H)	-	-	-

1.5 Compound **E** (GB-2 (**38**), sss4783)

Compound **E** was obtained as a yellow solid, and gave an orange coloration with anisaldehyde reagent same as that of compound **B** which was indicated that **E** was also a biflavonoid. On the basis of its ESIMS ($[M-H]^-$ at *m/z* 573), a molecular formula of compound **E** was established as $C_{30}H_{22}O_{12}$ which was 18 mass units more than that of compound **B**. This indicated that **E** has one hydroxyl group more than that of compound **B**. Its IR spectrum exhibited absorption bands for hydroxyl (3253 cm^{-1}), chelated γ-pyrone (1651 cm^{-1}) and aromatic ring (1519 cm^{-1}) and showed UV absorption maxima in MeOH at 292, 332 and 358 nm which were very similar to those of compound **B** (morelloflavone). The ^{1}H, ^{13}C NMR and Dept spectra (Table 11, Figure 34 and 35) of compound **E** also showed signals forming respective pairs (approximate relative ratio; 3:2) indicated for the presence of a biflavonoid skeleton as for compounds **A**-**C**. The ^{13}C and dept spectra comprised of major signals for 14 methines and 16 quaternary carbons. The NMR data also supported that compound **E** has one hydroxyl group more than that of compound **B**. Compound **E** revealed 7 proton doublets, five aromatic protons doublets, one aromatic singlet and the H-2 and H-3 methine protons exhibited at δ_H 4.54 (*d*, *J* = 11.8 Hz), 5.68 (*d*, *J* = 11.8 Hz), respectively. The aromatic protons of flavanone part appeared at δ_H 5.95 (*d,* *J* = 3.9 Hz), 7.12 (*d*, *J* = 7.9 Hz), and 6.82 (*d*, *J* = 7.9 Hz), and two protons showed at δ_H 4.07 (*d*, *J* = 11.0 Hz) and 4.82 (*d*, *J* = 11.0 Hz), for dihydroflavonol moiety. Furthermore, the two singlet signals of chelated OH at δ_H 11.59 and 12.28 and four signals of phenolic OH showed δ_H 10.78, 10.15, 9.01, 8.25 as four broad singlets. The ^{13}C NMR and DEPT spectra (Table 17, Figure 19) displayed 30 major signals attributable to fourteen methines and sixteen quaternary carbons which were assigned from the ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC spectra of compound **E** and comparison with the reported values.

Connections among rings A, B and C of flavanone subgroup were provided by analysis of its HMBC and NOESY spectra (Table 20 and Figures 21 and 22). The NOESY correlations were observed for methine proton at δ_H 4.54 (H-2) to H-2' (δ_H 7.12), δ_H 5.68 (H-3) to H-2' (δ_H 7.12) and H-6' (δ_H 7.12) of aromatic protons together with HMBC correlations of H-2 to C-1' (δ_C 128.5), C-2' (δ_C 128.2) and C-6' (δ_C 128.2), and the correlations of H-3 to C-2 (δ_C 80.9) in HMBC spectra, indicated that ring B was connected

to ring C. The HMBC correlation of H-6 and H-8 to C-4a (δ_C 101.4) and H-8 to C-8a (δ_C 165.9) confirmed that ring A connected to ring C.

Connections among rings D, E and F of dihydroflavonol subsgroup were provided by analysis of its HMBC and NOESY spectra. The correlations of H-2''' (δ_H 6.89) to C-3''' (δ_C 144.1) and C-6''' (δ_C 119.7) in HMBC spectra together with NOESY correlations of H-2''' to H-3'' (δ_H 4.82) and H-2'' (δ_H 4.07), H-6''' to H-3'' (δ_H 4.82) and H-2'' (δ_H 4.07) indicated that ring E connected to ring F. The HMBC correlations were observed for H-6'' at δ_H 6.06 *s* to C-4a'' confirmed that ring D connected to ring F.

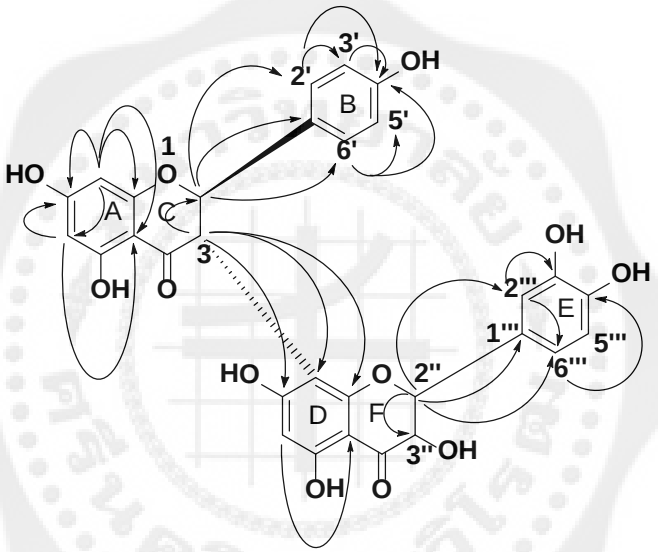


Figure 23 Selected HMBC correlations for compound **E**

Connections among flavanone and dihydroflavonol subsgroups were provided by analysis of its HMBC and NOESY spectra. The HMBC correlations were observed for methine proton at δ_H 5.68 (H-3) to C-8'' (δ_C 100.9), C-8a'' (δ_C 165.1) and C-7'' (δ_C 160.1) indicating that flavone unit was attached to dihydroflavonol moiety. The result indicates that of the structure of compound **E** was proposed to be GB-2 (**38**).

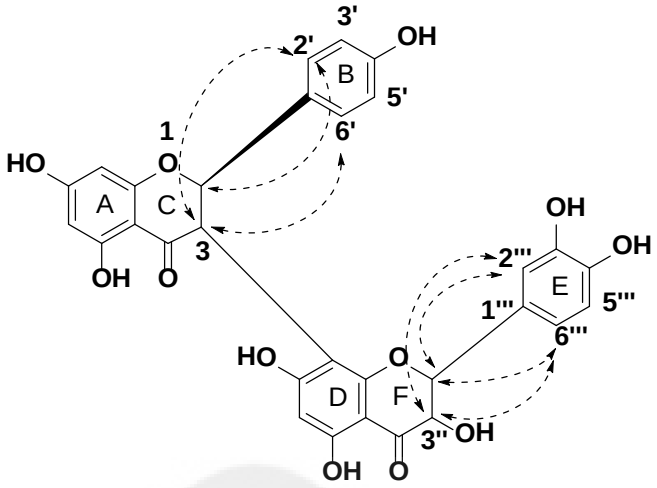


Figure 24 Selected NOESY correlations for compound **E**

The stereochemistry at C-2 and C-3 of flavanone unit in conpound **E** were provided by analysis of its *J* coupling constant value. The large coupling constants (*J* = 11.8 Hz) of C-2 and C-3 protons in ring C of compound **E**, in addition, no significant NOE enhancement was observed between both protons, indicated that both hydrogens have a *trans*-diaxial arrangement.

Compound **E** gave m.p. at 220-221°C (d) and dextrorotatory optical rotation, $[\alpha]_D^{27.3}$ = +18.4. Not previous reported of dextrorotatory optical rotation. Therefore, the structure of **E** was assigned to be (+) GB-2.

GB-2 (**38**) was found in *Garcinia* plants such as *G. livingstonei* (Yang; et al. 2010: 4749-55), *G. xanthochymus* (Baggett; et al. 2005: 354-360), *G. spicata* Hook. F. (Konoshima; & Ikeshiro. 1969: 121-124) and *G. xanthochymus* Hook. f. (Konoshima; Ikeshiro; & Miyahara. 1970: 1203-1206). It was reported to exhibit significantant DPPH and SW-480 colon cancer cells cytoxicity (Baggett; et al. 2005: 354-360).

Table 11 Comparison of ^{1}H and ^{13}C NMR data of compound **E** with GB-2 (**38**) (in DMSO-d_6)

Position	δ_H (*mult.*, *J* in Hz)				δ_C		
	GB-2[a] DMSO-d_6	**E** acetone-d_6	**E** $CDCl_3$ + DMSO-d_6 major	**E** $CDCl_3$ + DMSO-d_6 minor	GB-2[b] DMSO-d_6	**E** acetone-d_6	**E** $CDCl_3$+ DMSO-d_6
2	5.50 (*d*, *J* = 12.0, 1H)	5.74 (*d*, *J* = 11.9, 1H)	5.68 (*d*, *J* = 11.8, 1H)	5.44 (*d*, *J* = 11.9, 1H)	81.5	82.5	80.9
3	4.49 (*d*, *J* = 12.0, 1H)	4.59 (*d*, *J* = 11.9, 1H)	4.54 (*d*, *J* = 11.8, 1H)	4.73 (*d*, *J* = 11.9, 1H)	47.4	48.6	47.5
4	-	-	-	-	196.2	197.6	196.1
4a	-	-	-	-	101.1	102.5	101.4
5	-	-	-	-	160.2	163.7	162.3
6	5.82 (*s*, 1H)	5.97 (*d*, *J* = 1.8, 1H)	5.95 (*d*, *J* = 3.9, 1H)	-	94.9	95.7	94.9
7	-	-	-	-	161.9	165.1	164.4
8	5.76 (*d*, 1H)	5.97 (*d*, *J* = 1.8, 1H)	5.95 (*d*, *J* = 3.9, 1H)	-	95.7	96.9	96.0
8a	-	-	-	-	162.6	167.3	165.9
1'	-	-	-	-	128.0	129.7	128.5
2'	7.01 (*d*, 2H)	7.18 (*d*, *J* = 8.4, 1H)	7.12 (*d*, *J* = 7.9, 1H)	-	128.6	129.7	128.2
3'	-	-	6.82 (*d*, *J* = 8.2, 1H)	-	114.7	115.7	114.6
4'	-	-	-	-	157.4	158.6	156.8
5'	-	-	6.82 (*d*, *J* = 8.2, 1H)	-	114.7	115.7	114.6
6'	7.01 (*d*, 2H)	7.18 (*d*, *J* = 8.4, 1H)	7.12 (*d*, *J* = 7.9, 1H)	-	128.6	129.7	128.2
2''	4.84 (*d*, *J* = 12.0, 1H)	4.89 (*d*, *J* = 11.4, 1H)	4.82 (*d*, *J* = 12.0, 1H)	4.88 (*d*, *J* = 12.2, 1H)	82.9	84.3	82,6
3''	4.01 (*d*, *J* = 12.0, 1H)	4.14 (*d*, *J* = 10.4, 1H)	4.07 (*d*, *J* = 11.0, 1H)	4.31 (*d*, *J* = 11.0, 1H)	72.0	73.1	71.5
4''	-	-	-	-	197.0	198.0	196.5
4a''	-	-	-	-	101.3	102.4	99.4
5''	-	-	-	-	163.4	164.0	162.6
6''	5.81 (*d*, 1H)	6.03 (*s*, 1H)	6.06 (*s*, 1H)	-	96.0	96.9	96.1
7''	-	-	-	-	164.7	161.5	160.1
8''	-	-	-	-	100.0	102.4	100.9
8a''	-	-	-	-	166.1	165.8	165.1
1'''	-	-	-	-	128.1	129.4	127.5
2'''	6.80-6.50 (5H)	7.0-6.6 (5H)	6.89 (*s*, 1H)	-	115.1	115.6	114.6
3'''	-	-	-	-	144.6	145.7	144.1
4'''	-	-	-	-	145.5	146.5	145.3
5'''	6.80-6.50 (5H)	7.0-6.6 (5H)	-	-	115.3	115.6	114.3
6'''	6.80-6.50 (5H)	7.0-6.6 (5H)	-	-	118.4	120.4	119.7
5-OH	-	11.69 (*s*, 1H)	11.59 (*s*, 1H)	-	-	-	-
7-OH	-	-	-	-	-	-	-
4'-OH	-	-	-	-	-	-	-
5''-OH	-	12.23 (*s*, 1H)	12.28 (*s*, 1H)	-	-	-	-
7''-OH	-	-	-	-	-	-	-
3'''-OH	-	-	-	-	-	-	-
4'''-OH	-	-	-	-	-	-	-

[a] Jackson; et al. 1971: 3791-3804

[b] Kabangu; et al. 1986: 275-277

Table 11 (continued)

Position	δ_H (*mult.*, *J* in Hz)			δ_C	
	GB-2[a]	**E** (DMSO-d_6)		GB-2[b]	**E**
	DMSO-d_6	major	minor	DMSO-d_6	DMSO-d_6
2	5.50 (*d*, *J* = 12.0, 1H)	5.65 (*d*, *J* = 11.8, 1H)	5.33 (*d*, *J* = 11.6, 1H)	81.5	82.9
3	4.49 (*d*, *J* = 12.0, 1H)	4.45 (*d*, *J* = 11.8, 1H)	4.64 (*d*, *J* = 11.6, 1H)	47.4	47.2
4	-	-	-	196.2	196.5
4a	-	-	-	101.1	99.8
5	-	-	-	160.2	160.2
6	5.82 (*s*, 1H)	5.87 (*d*, *J* = 2.0, 1H)	-	94.9	95.0
7	-	-	-	161.9	162.2
8	5.76 (*d*, 1H)	5.87 (*d*, *J* = 2.0, 1H)	-	95.7	96.1
8a	-	-	-	162.6	162.8
1'	-	-	-	128.0	128.9
2'	7.01 (*d*, 2H)	7.09 (*br d*, *J* = 7.05, 1H)	-	128.6	128.9
3'	-	6.50-6.80 (5H)	-	114.7	114.8
4'	-	-	-	157.4	157.8
5'	-	6.50-6.80 (5H)	-	114.7	114.8
6'	7.01 (*d*, 2H)	7.09 (*br d*, *J* = 7.05, 1H)	-	128.6	128.9
2''	4.84 (*d*, *J* = 12.0, 1H)	4.85 (*d*, *J* = 11.4, 1H)	4.97 (*d*, *J* = 10.6, 1H)	82.9	82.9
3''	4.01 (*d*, *J* = 12.0, 1H)	3.95 (*br d*, 1H)	4.18 (*br d*, 1H)	72.0	72.0
4''	-	-	-	197.0	197.5
4a''	-	-	-	101.3	101.3
5''	-	-	-	163.4	163.6
6''	5.81 (*d*, 1H)	5.95 (*s*, 1H)	-	96.0	96.1
7''	-	-	-	164.7	164.5
8''	-	-	-	100.0	101.1
8a''	-	-	-	166.1	166.4
1'''	-	-	-	128.1	127.9
2'''	6.50-6.80 (5H)	6.50-6.80 (5H)	-	115.1	114.8
3'''	-	-	-	144.6	144.9
4'''	-	-	-	145.5	145.9
5'''	6.50-6.80 (5H)	6.50-6.80 (5H)	-	115.3	115.3
6'''	6.50-6.80 (5H)	6.50-6.80 (5H)	-	118.4	119.0
5-OH	-	11.81 (*s*, 1H)	-	-	
7-OH	-	-	-	-	
4'-OH	-	-	-	-	
5''-OH	-	12.10 (*s*, 1H)	-	-	
7''-OH	-	-	-	-	
3'''-OH	-	-	-	-	
4'''-OH	-	-	-	-	

[a] Jackson; et al. 1971: 3791-3804

[b] Kabangu; et al. 1986: 275-277

Table 12 ^{1}H, ^{13}C NMR and 2D NMR data of compound **E** (in $CDCl_3$+DMSO-d_6)

Position	δ_H (*mult.*, *J* in Hz)		δ_C	HMBC correlations	NOESY correlations
2	5.68 (*d*, *J* = 11.8, 1H)	5.44 (*d*, *J* = 11.9, 1H)	80.9	C-3, C-1', C-2', C-6'	H-2'
3	4.54 (*d*, *J* = 11.8, 1H)	4.73 (*d*, *J* = 11.9, 1H)	47.5	C-2, 'C-8'', C-8a''	H-2', H-6'
4	-	-	196.1	-	-
4a	-	-	101.4	-	-
5	-	-	162.3	-	-
6	5.95 (*d*, *J* = 3.9, 1H)	-	94.9	C-4a, C-7, C-8	-
7	-	-	164.4	-	-
8	5.95 (*d*, *J* = 3.9, 1H)	-	96.0	C-4a, C-6, C-7, C-8a	-
8a	-	-	165.9	-	-
1'	-	-	128.5	-	-
2'	7.12 (*d*, *J* = 7.9, 1H)	-	128.2	C-2, C-3', C-4'	H-2, H-3
3'	6.82 (*d*, *J* = 7.9, 1H)	-	114.6	C-2', C-4',	-
4'	-	-	156.8	-	-
5'	6.82 (*d*, *J* = 8.2, 1H)	-	114.6	C6'	-
6'	7.12 (*d*, *J* = 7.9, 1H)	-	128.2	C-2, C-4', C-5'	H-3
2''	4.82 (*d*, *J* = 11.0, 1H)	4.88 (*d*, *J* = 11.0, 1H)	82,6	C-3'', C-1''', C-2''', C-6'''	H-2''', H-6'''
3''	4.07 (*d*, *J* = 11.0, 1H)	4.31 (*d*, *J* = 11.0, 1H)	71.5	C-2''	H-2''', H-6'''
4''	-	-	196.5	-	-
4a''	-	-	99.4	-	-
5''	-	-	162.6	-	-
6''	6.06 (*s*, 1H)	-	96.1	C--4a''	-
7''	-	-	160.1	-	-
8''	-	-	100.9	-	-
8a''	-	-	165.1	-	-
1'''	-	-	127.5	-	-
2'''	6.89 (*s*, 1H)	-	114.6	C-2'', C-3''', C-6'''	H-3'', H-1'''
3'''	-	-	144.1	-	-
4'''	-	-	145.3	-	-
5'''	-	-	114.3	-	-
6'''	-	-	119.7	C-2'', C-2''', C-4'''	H-2'', H-3''
5-OH	11.59 (*s*, 1H)	-	-	-	-
7-OH	-	-	-	-	-
4'-OH	-	-	-	-	-
5''-OH	12.28 (*s*, 1H)	-	-	-	-
7''-OH	-	-	-	-	-
3'''-OH	-	-	-	-	-
4'''-OH	-	-	-	-	-

2. Antibacterial activity of compounds A-C against *Helicobactor pylori*

In this work, the *in vitro* antibacterial effect of three isolated biflavonoids **A-C** was evaluated against metronidazole-resistant reference *H. pylori* ATCC43504, reference *H. pylori* DMST20165 and metronidazole-resistant clinical isolate *H. pylori* HP40. The results (Table 13) revealed that compound **C**, displayed most efficacious against the reference *H. pylori* DMST with MIC 10.8 μM compared with that of the control metronidazole (MIC 11.1 μM). The biflavonoid glucoside, compound **C** (MIC 87.0 μM) also exhibited more potency against *H. pylori* ATCC43504 than that of compound **A** (MIC 115.7 μM) and compound **B** (MIC 112.3 mM), though none of the compound assayed was as effective as the control amoxicillin (MIC 0.3 μM) and clarithromycin (41.8 μM). It should be noted that the glucose unit substitution at C-7'' in flavone moiety played important role for strong inhibitory activity against the *H. pylori* DMST strain as observed in compound C when compared with no sugar substitution in compound **A** and compound **B**. However, compound **C** (MIC 174.0 μM) was less active than that of compound **A** (MIC 115.7 μM) and compound **B** (MIC 112.3 μM) against *H. pylori* HP40.

Table 13 MIC values (μM) of compounds **A-C** against *H. pylori* ATCC43504, *H. pylori* DMST20165 and *H. pylori* HP40

Compound	**MIC**		
	ATCC43504	DMST20165	HP40
A	115.7	14.4	115.7
B	112.3	14.0	112.3
C	87.0	10.8	174.0
Amoxicillin[a]	0.3	0.6	42.7
Metronidazole[a]	-	11.1	-
Clarithromycin[a]	41.8	-	0.6

[a]Positive control

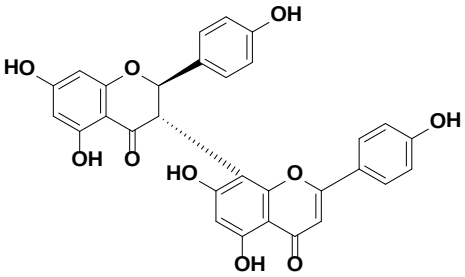
CHAPTER 5

CONCLUSION

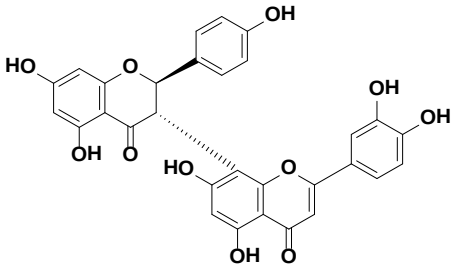
Investigation of the chemical constituents of the EtOAc and MeOH extract of root of *G. fusca* led to the isolation of three known biflavonoids **A**-**C** named, vokensiflavone (**48**), morelloflavone (**32**) and fukugiside (**47**) (Figure 25). The structures of known biflavonoids were elucidated by spectroscopic techniques and by comparison of spectroscopic data with those of reported values and including chromatographic comparison with authentic samples in several solvent systems.

Investigation of the chemical constituents of the EtOAc of heartwood and stem bark of *G. cowa* led to the isolation of five known biflavonoids **A**-**E**, morelloflavone (**32**), GB-2 (**38**), fukugiside (**47**), vokensiflavone (**48**) and spicataside (**81**) (Figure 25). The structures of all compounds were elucidated by spectroscopic techniques, especially 1D-and 2D-NMR and MS including by comparison of their spectroscopic data with those reported in the literature. The stereochemistry at C-2 and C-3 of flavanone unit were provided by analysis of *J* coupling constant value between H-2 and H-3. The large coupling constants (12 Hz) of H-2 and H-3 of compounds **A**-**E**, in addition with no significant NOE enhancement was observed between both protons in their NOESY spectra, indicated that both hydrogens have a *trans*-diaxial arrangement. The sugar unit in biflavonoid glucosides (**C** and **D**) was determined by acid hydrolysis and the resulting sugar residue obtained was proved to be D-(+)-glucose by analysis of its optical rotation activity.

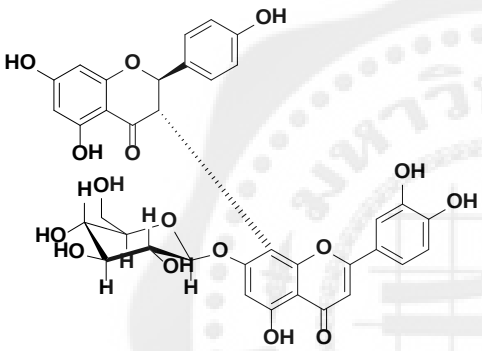
The three isolated biflavonoids **A**-**C**, morelloflavone (**32**), fukugiside (**47**) and vokensiflavone (**48**) were evaluated for antibacterial activity against *Helicobacter pylori*. Compound **C**, morelloflavone glucoside, exhibited stronger inhibitory activity at MIC 10.8 μM against *H. pylori* DMST reference strain than that of the control metronidazole (MIC 11.1 μM).



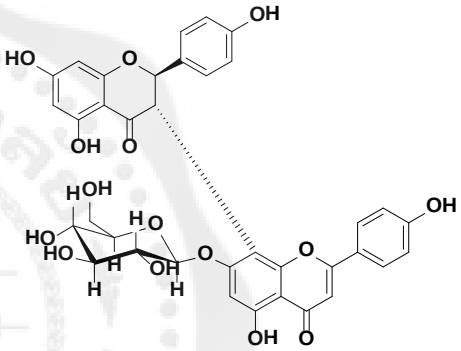
vokensiflavone (**A**)



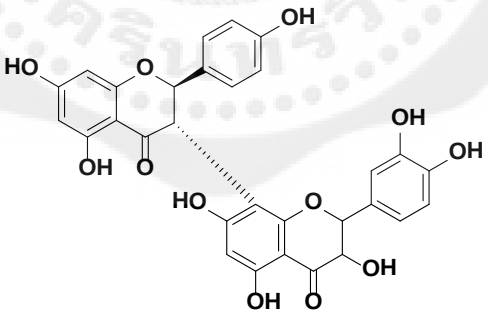
morelloflavone (**B**)



fukugiside (**C**)



spicataside (**D**)



GB-2 (**E**)

Figure 25 Biflavonoids from *G. fusca* and *G. cowa*

BIBLIOGRAPHY

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Antia, B. S.; Pansanit, A.; Ekpa, O. D.; Ekpe, U. J.; Mahidol, C.; & Kittakoop, P. (2010). α-glucosidase inhibitory, aromatase inhibitory, and antiplasmodial activities of a biflavonoid GB1 from *Garcinia kola* stem bark. *Planta Med*. 76(3): 276-277.

Babu, V.; Alia, S. M.; Sultana, S.; & Ilyas, M. (1988). A biflavonoid from *Garcinia nervosa*. *Phytochemistry 27*: 3332-3335.

Balasubramanian, K.; & Rajagopalan, K. (1988). Novel xanthones from *Gacinia mangostana*, structures of BR-xanthone-A and BR-xanthone-B. *Phytochemistry*. 27(5): 1552-1554.

Castardo, J. C.; Prudente, A. S.; Ferreira, J.; Guimaraes, C. L.; Delle M. F.; Cechinel F. V.; Otuki, M. F.; & Cabrini, D. A. (2008). Anti-inflammatory effects of hydroalcoholic extract and two biflavonoids from *Garcinia gardneriana* leaves in mouse paw oedema. *J. Ethnopharm*. 118(3): 405-411.

Chen, F. C.; Lin, Y. M.; Hung, J. C. (1975). New biflavone glucoside from Garcinia multiflora. *Phytochemistry.* 14: 818-820.

Chen, F. C.; Lin, Y. M.; Hung, J. C. (1975). Phenolic compounds from the heartwood of *Garcinia multiflora*. *Phytochemistry.* 14: 300-303.

Diserens, I. S.; Rogers, C.; Sordat, B.; & Hostettmann, K. (1992). Prenylated xanthones from *Gacinia livingstonei*. *Phytochemistry.* 31(1): 313-316.

Duddeck, H.; Snatzke, G.; Yemul, S. S.; (1978). ^{13}C NMR and CD of some 3,8″-biflavanoids from *Garcinia*species and of related flavanones. *Phytochemistry* 17: 1369-1373.

Elfita, E.; Muharni, M.; Madyawati, L.; Darwati, D.; Ari, W.; Supriyatna, S.; Husein H., B.; Dachriyanus, D.; Paul, C.; Louis, M. (2009). Antiplasmodial and other constituents from four Indonesian Garcinia spp. *Phytochemistry* 70: 907-912.

Gil, B.; Sanz, M. J.; Terencio, M. C.; Gunasegaran, R.; Paya, M.; & Alcaraz, M. J. (1997). Morelloflavone, a novel biflavonoid inhibitor of human secretory phospholipase A_2 with anti-inflammatory activity. *Biochem. Pharm*. 53: 733-740.

Gontijo, V. S.; Judice, W. A.S.; Codonho, B.; Pereira, I. O.; Assis, D. M.; Januario, J. P.; Caroselli, E. E.; Juliano, M. A.; Dosatti, A. C.; Marques, M. J.; Junior, C. V.; & Santos, M. H. (2012). Leishmanicidal, antiproteolytic and antioxidant evaluation of natural bioflavonoids isolated from *Garcinia brasiliensis* and their semisynthetic derivatives. *Eur. J. Med. Chem*. 58: 613-623.

Iinuma, M.; Tosa, H.; Ito, T.; Tanaka, T.; & Madulid, D. A. (1996). Two xanthones from root of *Cratoxylum Formosanum*. Phytochemistry 42(4): 1195-1198.

Ito, C.; Itoigawa, M.; Miyamoto, Y.; Rao, S. K.; Takayasu, J.; Okuda, Y.; Mukainaka, T.; Tokuda, H.; Nishino, H.; Furukawa, H. (1999). A new biflavonoid from *Calophyllum panciflorum* with antitumor-promoting activity. *J. Nat. Prod.* 62: 1668-1671.

Ito, C.; Itoigawa, M.; Takakura, T.; Ruangrungsi, N.; Enjo, F.; Tokuda, H.; Nishino, H.; & Furukawa, H. (2003). Chemical constituents of *Garcinia fusca*, Structure elucidation of eight new xanthones and their cancer chemopreventive activity. *J. Nat. Prod.* 66: 200-205.

Ito, T.; Yokota, R.; Watarai, T.; Mori, K.; Oyama, M.; Nagasawa, H.; Matsuda, H.; & Iinuma, M. (2013). Isolation of six isoprenylated biflavonoids from the Leaves of *Garcinia subelliptica. Chem. Pharm. Bull.* 61(5): 551-558.

Jackson, B.; Locksley, H. D.; Scheinmann, F. (1971). Extractives from Guttiferae. Part XXII. The isolation and structure of four novel bioflavonoids from the heartwoods of *Garcinia buchananii* Baker and G. eugeniifolia Wall. *J. Chem. Soc.* 3791-3804.

Kabangu, K.; Galeffi, C.; Aonzo, E.; Nicoletti, M.; & Messana, I. (1987). A New Biflavanone from the Bark of *Garcinia kola*. *Planta Med.* 53(3): 275-277.

Kaikabo, A. A.; Samuel, B. B.; & Eloff, J. N. (2009). Isolation and activity of two antibacterial biflavonoids from leaf extracts of *Garcinia livingstonei* (*Clusiaceae*). *Nat. Prod. Commun*. 4(10): 1363-1366.

Konoshima, Y.; Ikeshiro, A.; Nishinaga, T.; Matsuura, T.; Kubota, H.; & Sakamoto. (1969). The constitution of flavonoids from *Garcinia spicata hook. f. Tetrahedron Lett.* 10(2): 121-124.

Konoshima, M.; Ikeshiro, I.; Miyahara, S. (1970). The constitution of bioflavonoids from *Garcinia* plants. *Tetrahedron Lett.* 11(48): 4203-4206.

Kim, H. P.; Park, H.; Son, K. H.; Chang, H. W.; & Kang, S. S. (2008). Biochemical pharmacology of biflavonoids: Implications for anti-inflammatory action. *Pharmacal Research*. 31(3): 265-273.

Lee, C. W.; Choi, H. J.; Kim, H. S.; Kim, D. H.; Chang, I. S.; Moon, H. T.; Lee, S. Y.; Ohb, W. K.; & Woob, E. R. (2008). Biflavonoids isolated from *Selaginella tamariscina* regulate the expression of matrix metalloproteinase in human skin fibroblasts. *Bioorg. Med. Chem.* 16: 732-738.

Li, X.C.; Joshi, A.S.; Tan, B.; ElSohly, H.N.; Walker, L.A.; Zjawiony, J.K.; Ferreira, D. (2002). Absolute configuration, conformation, and chiral properties of flavanone-(3⟶8")-flavone biflavonoids from *Rheedia acuminata*. *Tetrahedron* 58, 8709-8717.

Likhitwitayawuid, K.; Phadungcharoen, T.; Mahidol, C.; & Ruchirawatt, S. (1997). 7-O-Methylgarcinone E from *garcinia cowa*. *Phytochemistry*. 45(6): 1299-1301.

Lin, Y. M.; Flavin, M. T.; Schure, R.; Chen, F. C.; Sidwell, R.; Barnard, D. L.; Huffman, J. H.; & Kern, E. R. (1999). Antiviral activities of biflavonoids. *Planta Med*. 65: 120-125.

Mahabusarakam, W.; Chairerk, P.; & Taylor, W.C. (2005). Xanthones from *Garcinia cowa* Roxb. Latex. *Phytochemistry* 66: 1148-1153.

Mahabusarakam, W.; Wiriyachtra, P.; and Taylor, W. (1987). Chemical constituents of *Garcinia mangostana*. *J. Nat. Prod.* 50: 474-478.

Muharni; Elfita; & Amanda. (2011). Biflavonoid compound from the stem bark of gamboge (*Garcinia xanthochymus*). *Indo. J. Chem.* 11(2): 169-173.

Na, Z.; Song, Q.; & Hu, H. (2013). A new prenylated xanthone from latex of *Garcinia cowa* Roxb. *Rec. Nat. Prod*. 7(3): 220-224.

Negi, P. S.; Jayaprakasha, G. K.; & Jena, B. S. (2008). Antibacterial activity of the extracts from the fruit rinds of *Garcinia cowa* and *Garcinia pedunculata* against food borne pathogens and spoilage bacteria. *LWT - Food Science and Technology*. 41: 1857-1861.

Nontakham J. (2011). Chemical constituents of *garcinia fusca* pierre. Dissertation, M.Sc. (Chemistry). Bankok: Graduate school Srinakharinwirot University.

Panthong, K.; Hutadilok-Towatana, N.; & Panthong, A. (2009). Cowaxanthone F, a new tetraoxygenated xanthone, and other anti-inflammatory and antioxidant compounds from *Garcinia cowa*. *Can. J. Chem*. 87(11): 1636-1640.

Panthong, K.; Pongcharoen, W.; Phongpaichit, S.; & Taylor, W. C. (2006). Tetraoxygenated xanthones from the fruits of *Garcinia cowa*. *Phytochemistry* 67: 999-1004.

Pumipamorn, S.; & Kumkong, A. (1997). Edible multipurpose tree species. Faung Fa printing, Bangkok. 486.

Ritthiwigrom, T.; Laphookhieo, S.; & Pyne, S. G. (2013). Chemical constituents and biological activities of Garcinia cowa Roxb. *Mj. Int. J. Sci. Tech*. 7(2): 212-231.

Shen, J.; & Yang, J. S. (2006). Chemical constituents from fruit of *Garcinia cowa. Zhongguo Yaoxue Zazhi* 41: 660-661.

Shen, J.; & Yang, J. S. (2007). Chemical constituents of branch of *Garcinia cowa* Roxb. *Zhongcaoyao*. 38: 993-994.

Siridechakorn, I.; Phakhodee, W.; Ritthiwigrom, T.; Promgool, T.; Deachathai, S.; Cheenpracha, S.; Prawat, U.; & Laphookhieo, S. (2012). Antibacterial dihydrobenzopyran and xanthone derivatives from *Garcinia cowa* stem barks. *Fitoterapia* 83: 1430-1434.

Smitinand, T. (2001). Thai plant names. Rev. ed. Bangkok: The forest herbarium, Royal Forest Department. 158-159.

Suksamrarn, S.; Komutiban, O.; Ratananukul, P.; Chimnoi, N.; Lartpornmatulee, N.; & Suksamrarn, A. (2006). Cytotoxic prenylated xanthones from the young fruit of *Garcinia mangostana. Chem. Pharm. Bull.* 54(3): 301-305.

Tebekeme, O. (2009). Chromatographic characterization, in vitro antioxidant and free radical scavenging activities of *Garcinia kola* seeds. *Afr. J. Biotechnol*. 8(24): 7133-7137.

Terashima, K.; Ishida, T.; Furukawa, T.; Takaya, Y.; Niwa, M. (2008). Constituents of green and ripened fruit of *Garcinia subelliptica*. *Heterocycles* 75(2): 407-413.

Tuansulong, K.; Nongporn, H.; Mahabusarakam, W.; Pinkaew, D.; & Fujise, K. (2011). Morelloflavone from *Garcinia dulcis* as a novel biflavonoid inhibitor of HMG-CoA reductase. *Phytotherapy Research.* 25(3): 424-428.

Xiwen, L.; Jie, L.; & Stevens, P. F. (2007). *Garcinia Linnaeus. Flora of China.* 13: 40-47.

Xu, H.; Mughal, S.; Taiwo, O.; & Lee, S. F. (2013). Isolation and characterization of an antibacterial biflavonoid from an African chewing stick *Garcinia kola* Heckel (*Clusiaceae*). *J. Ethnopharm*. 147: 497-502.

Yang, H.; Figueroa, M.; To, S.; Baggett, S.; Jiang, B.; Basile, M. J.; Weinstein, I. B.; Kennelly, E. J. (2010). Benzophenones and Biflavonoids from *Garcinia livingstonei* Fruits. *J. Agric. Food Chem.* 58: 4749-4755.

APPENDIX

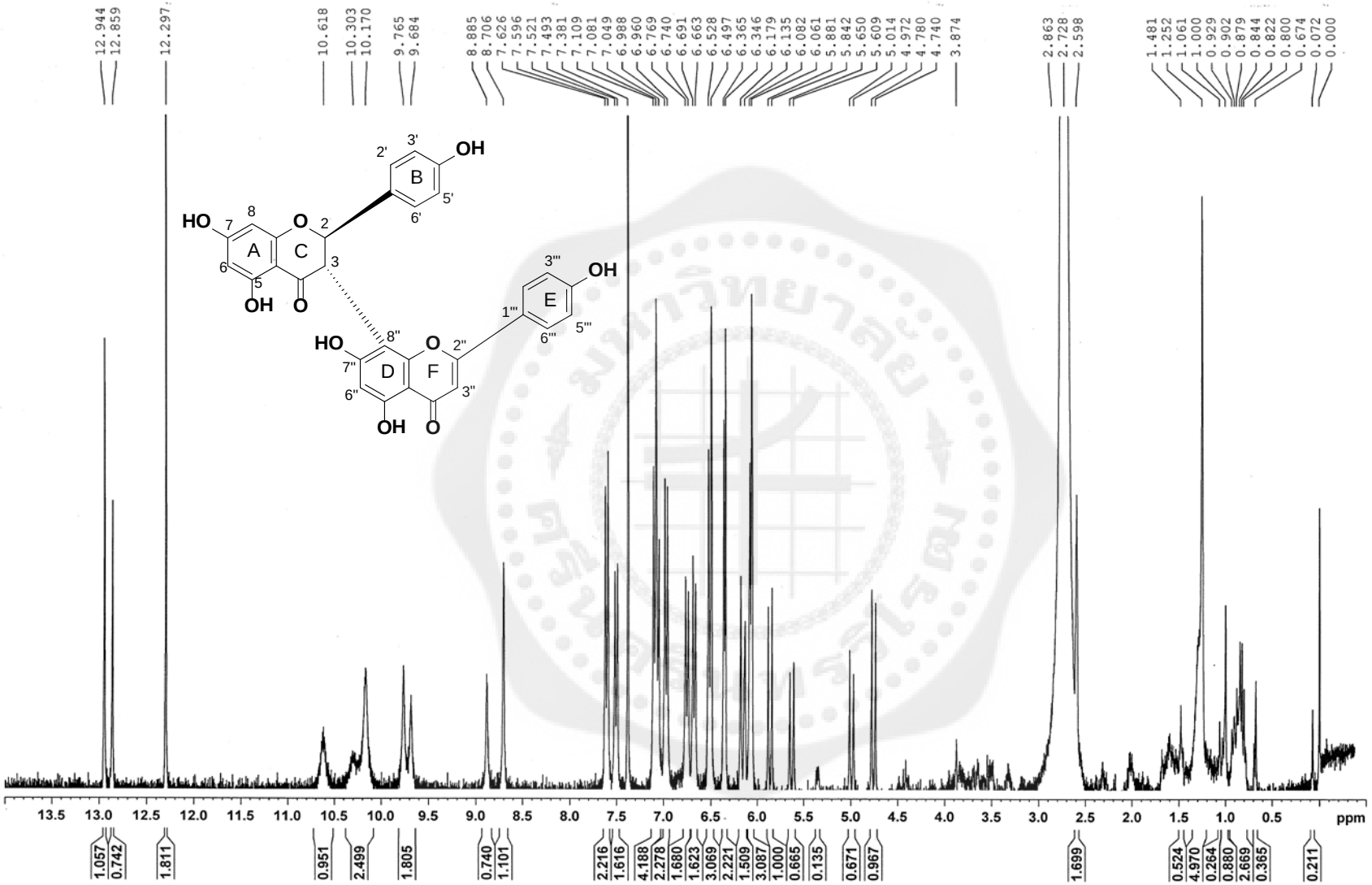

Figure 26 ^{1}H NMR of compound **A** (vokensiflavone (**48**), sss4594) in $CDCl_3$ + DMSO-d_6

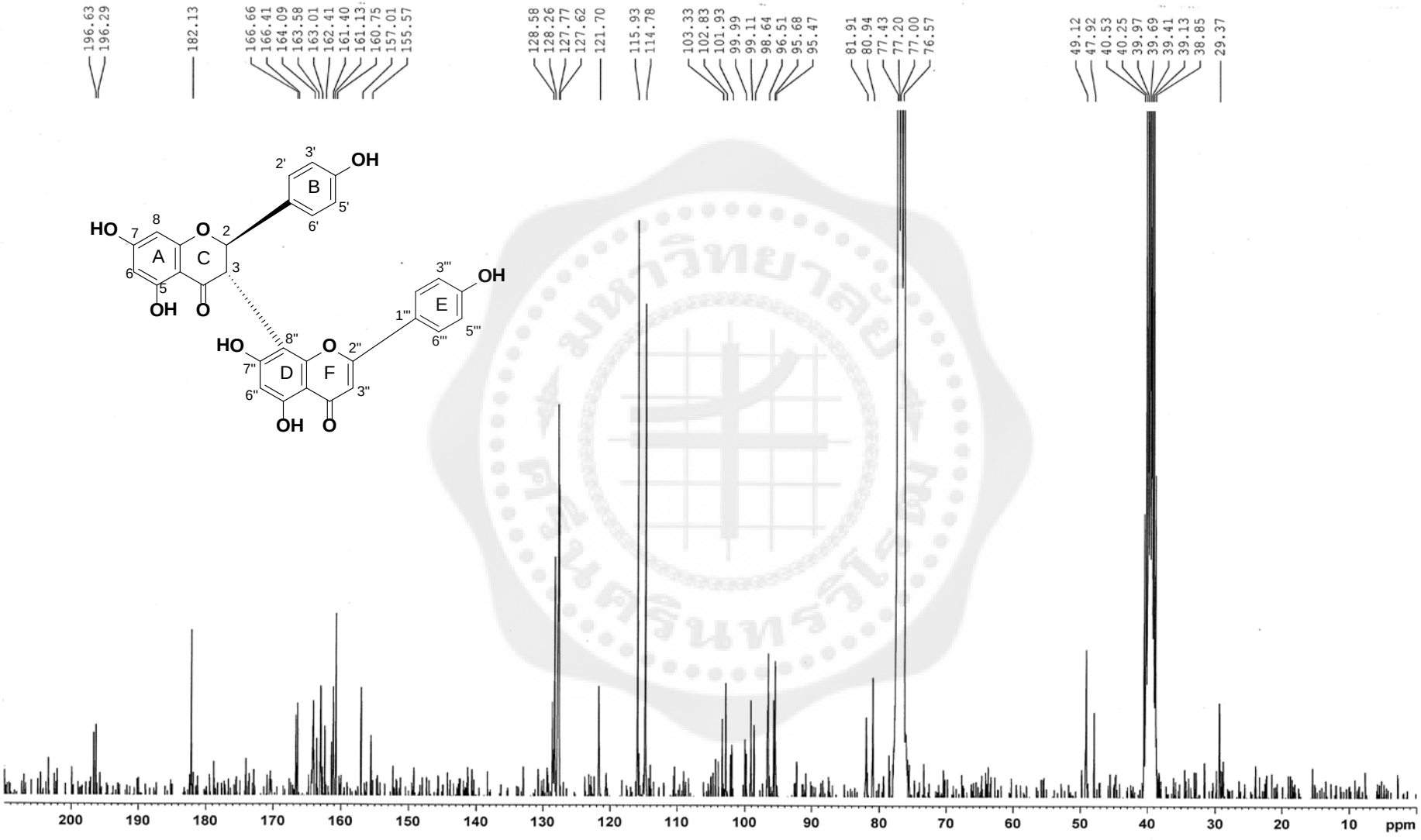

Figure 27 ^{13}C NMR of compound **A** (vokensiflavone (**48**), sss4594) in $CDCl_3$ + DMSO-d_6

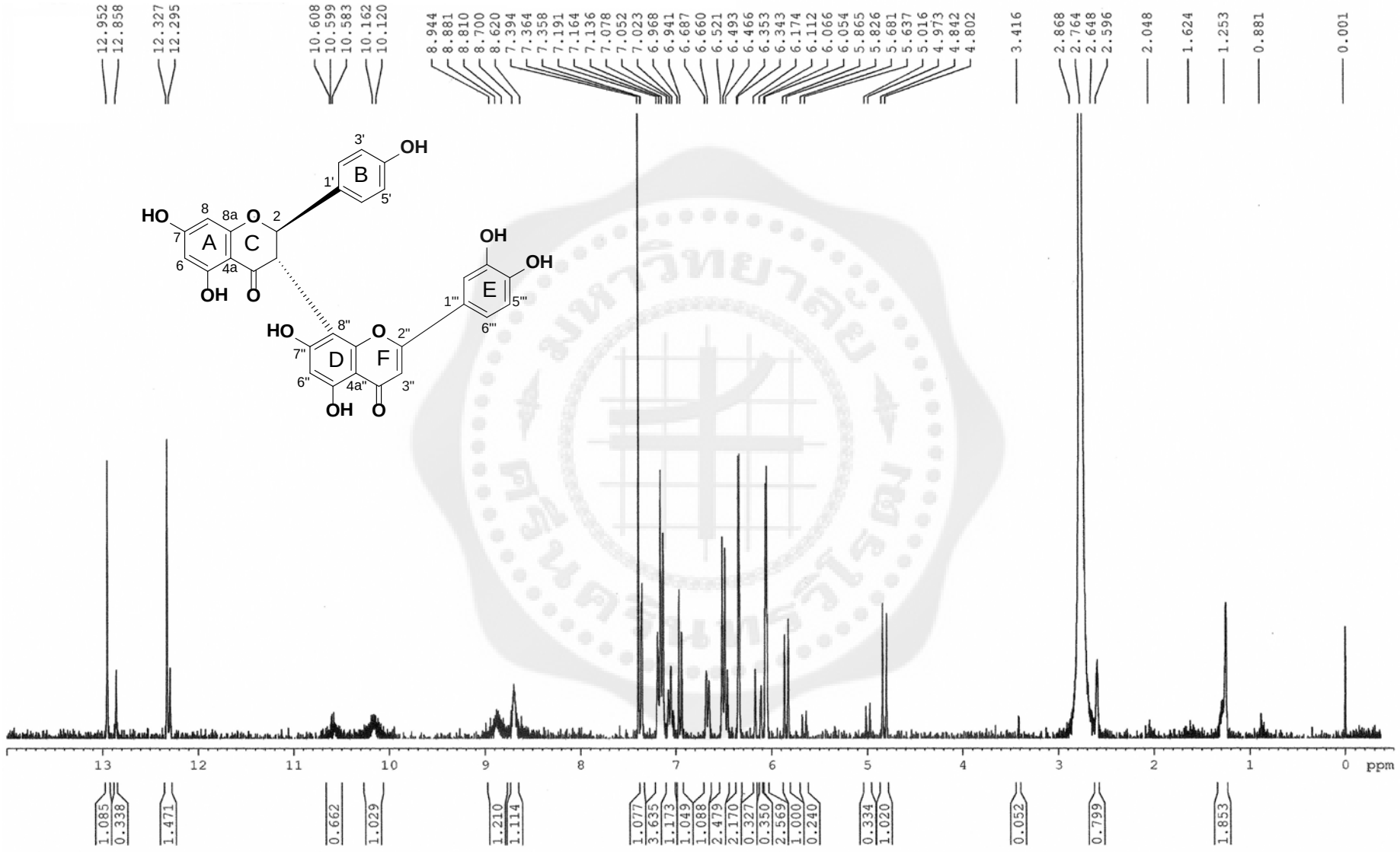

Figure 28 ^{1}H NMR of compound **B** (morelloflavone (**32**), sss4595) in $CDCl_3$ + DMSO-d_6)

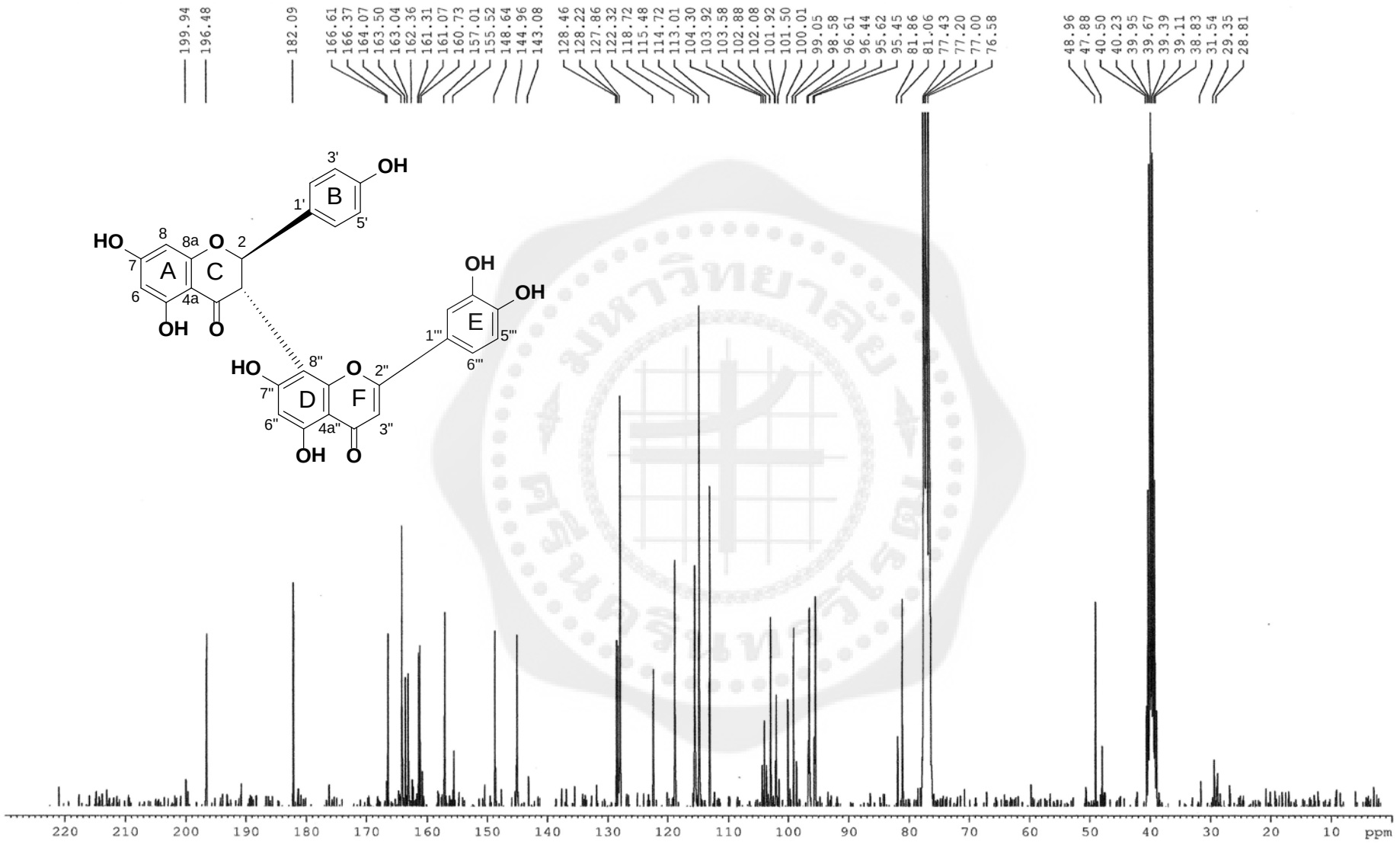

Figure 29 ^{13}C NMR of compound **B** (morelloflavone (**32**), sss4595) in $CDCl_3$ + DMSO-d_6

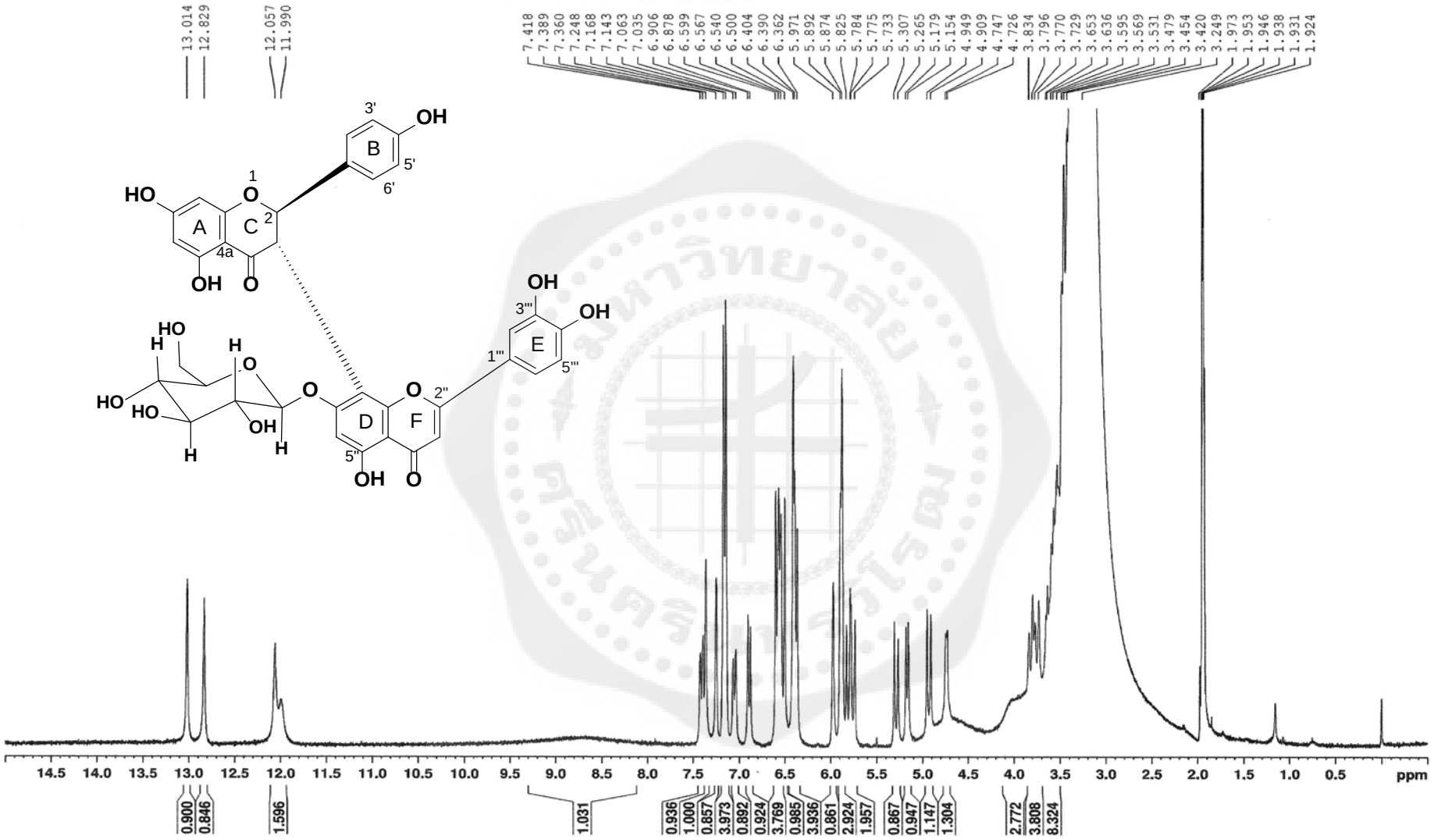

Figure 30 ^{1}H NMR of compound **C** (fukugiside (**47**), sss4751) in Acetone-d_6

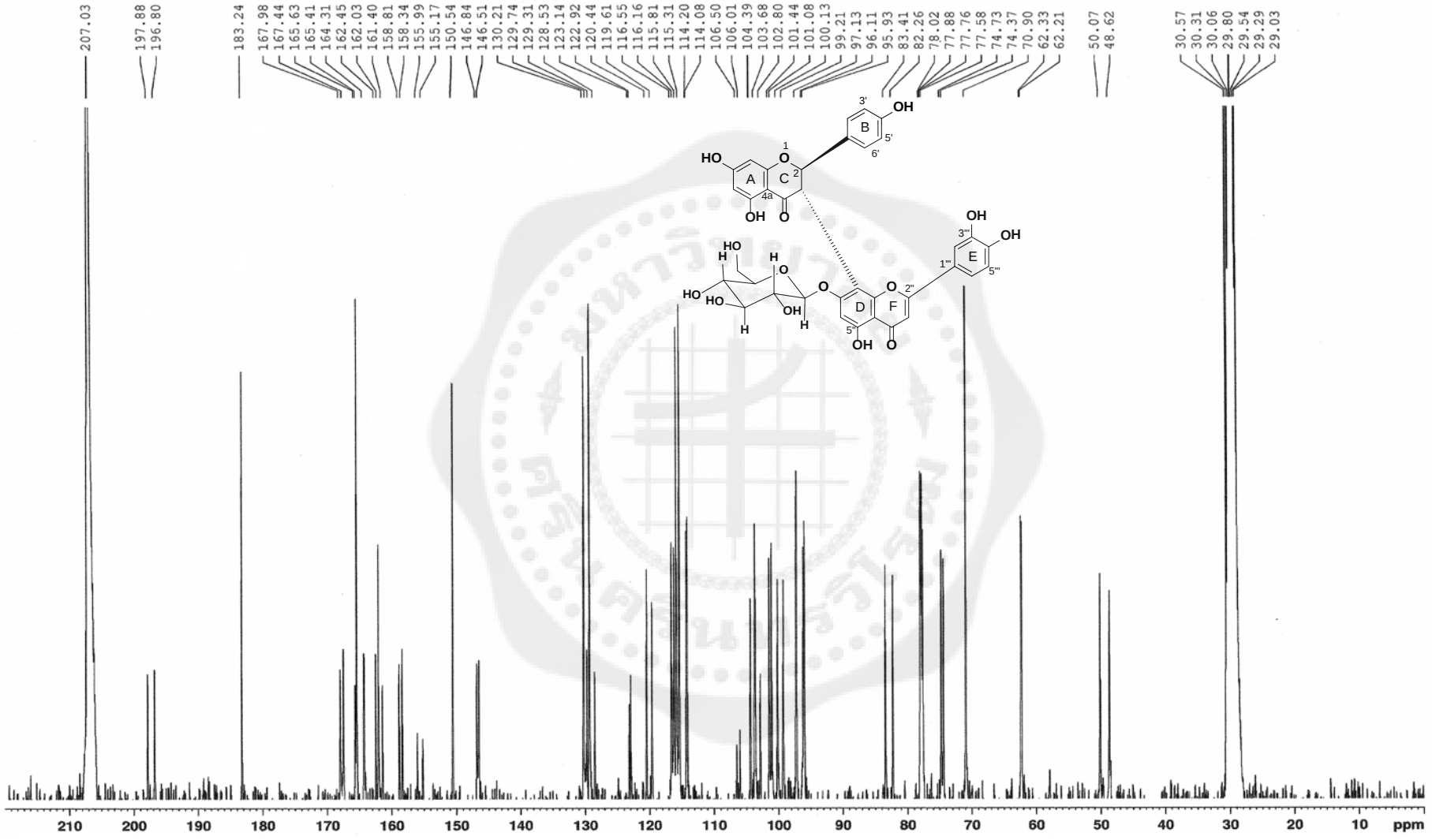

Figure 31 ^{13}C NMR of compound **C** (fukugiside (**47**), sss4751) in Acetone-d_6

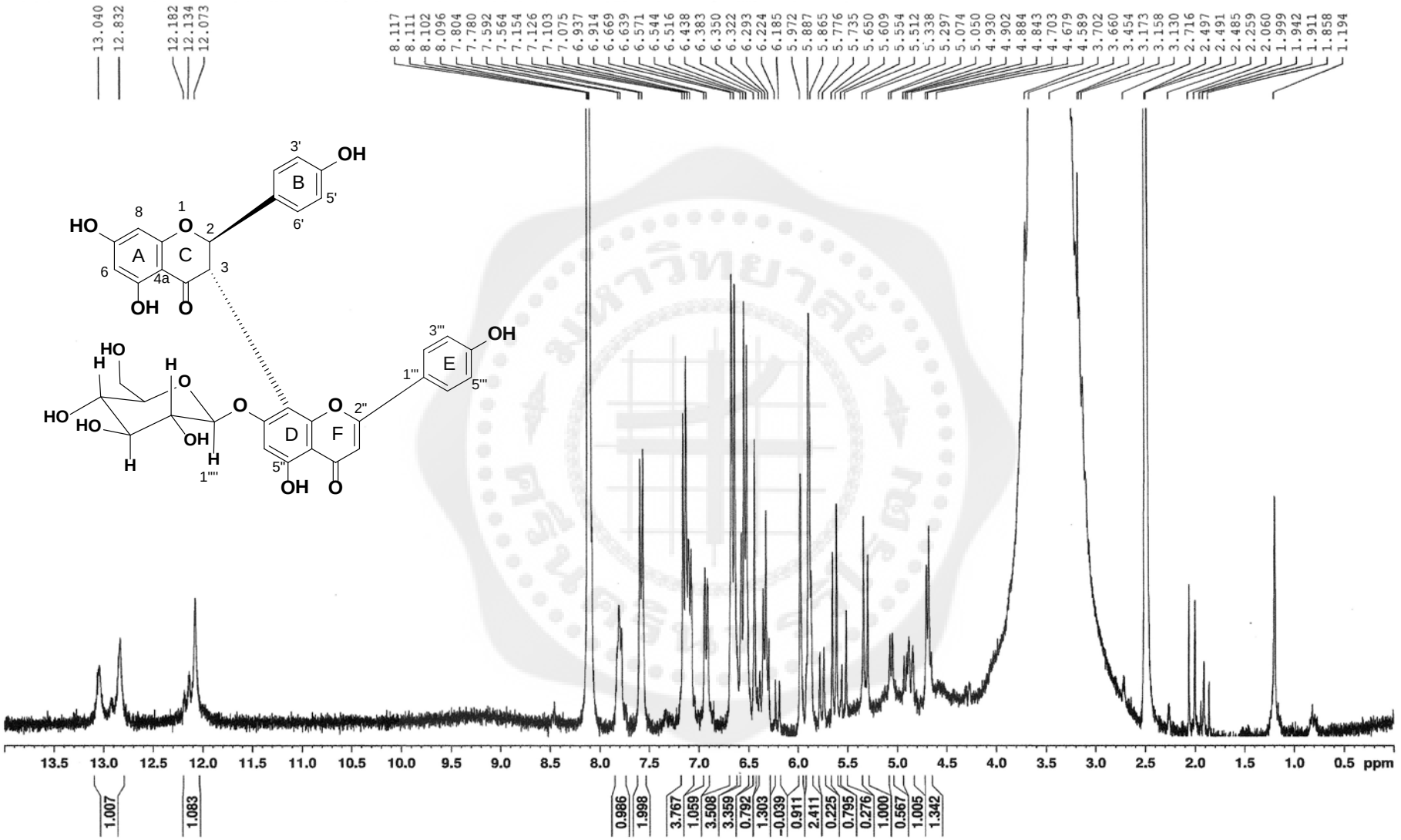

Figure 32 ^{1}H NMR of compound **D** (spicataside (**81**), sss5167) in $CDCl_3$ + DMSO-d_6

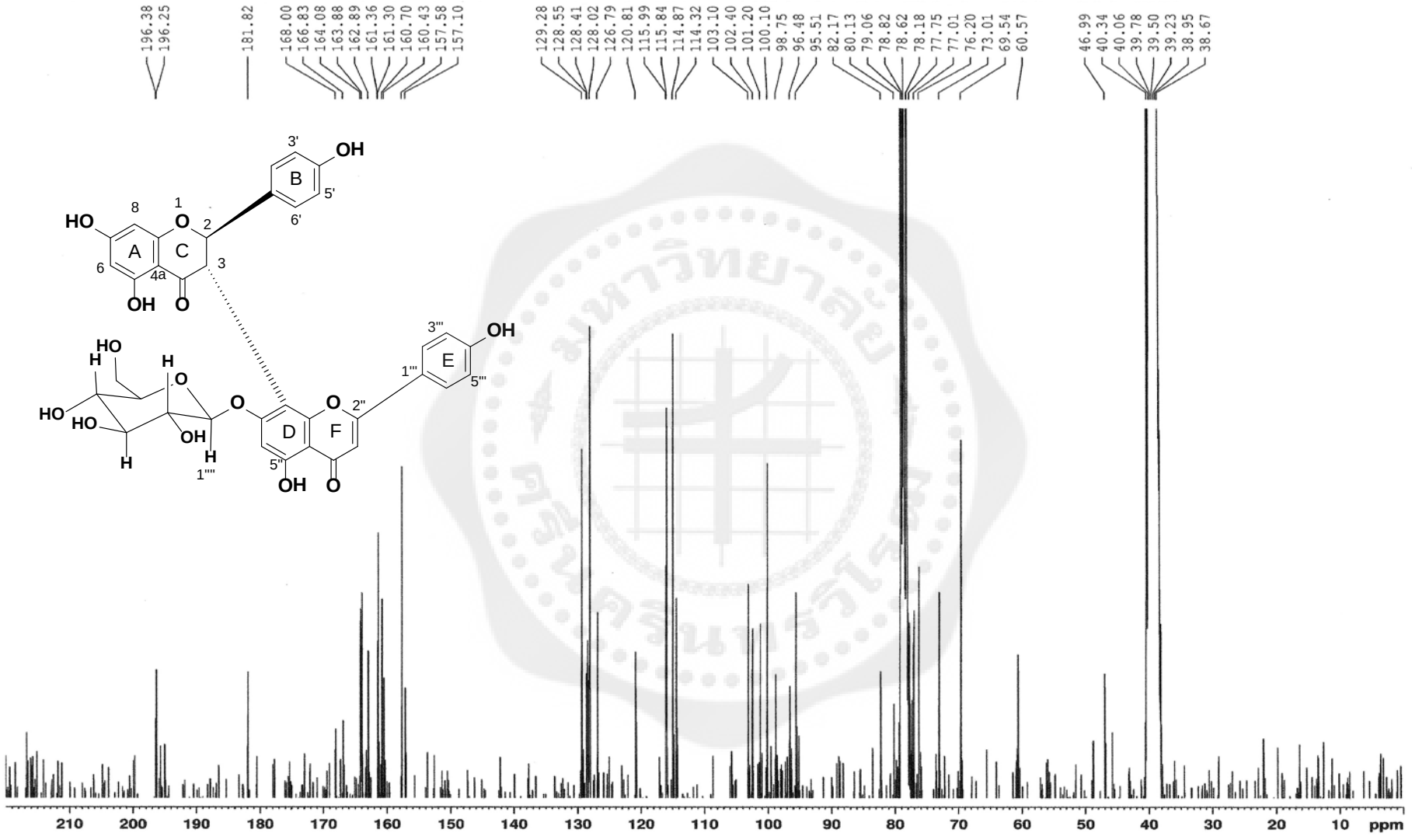

Figure 33 ^{13}C NMR of compound **D** (spicataside (**81**), sss5167) in $CDCl_3$ + DMSO-d_6 (1:1)

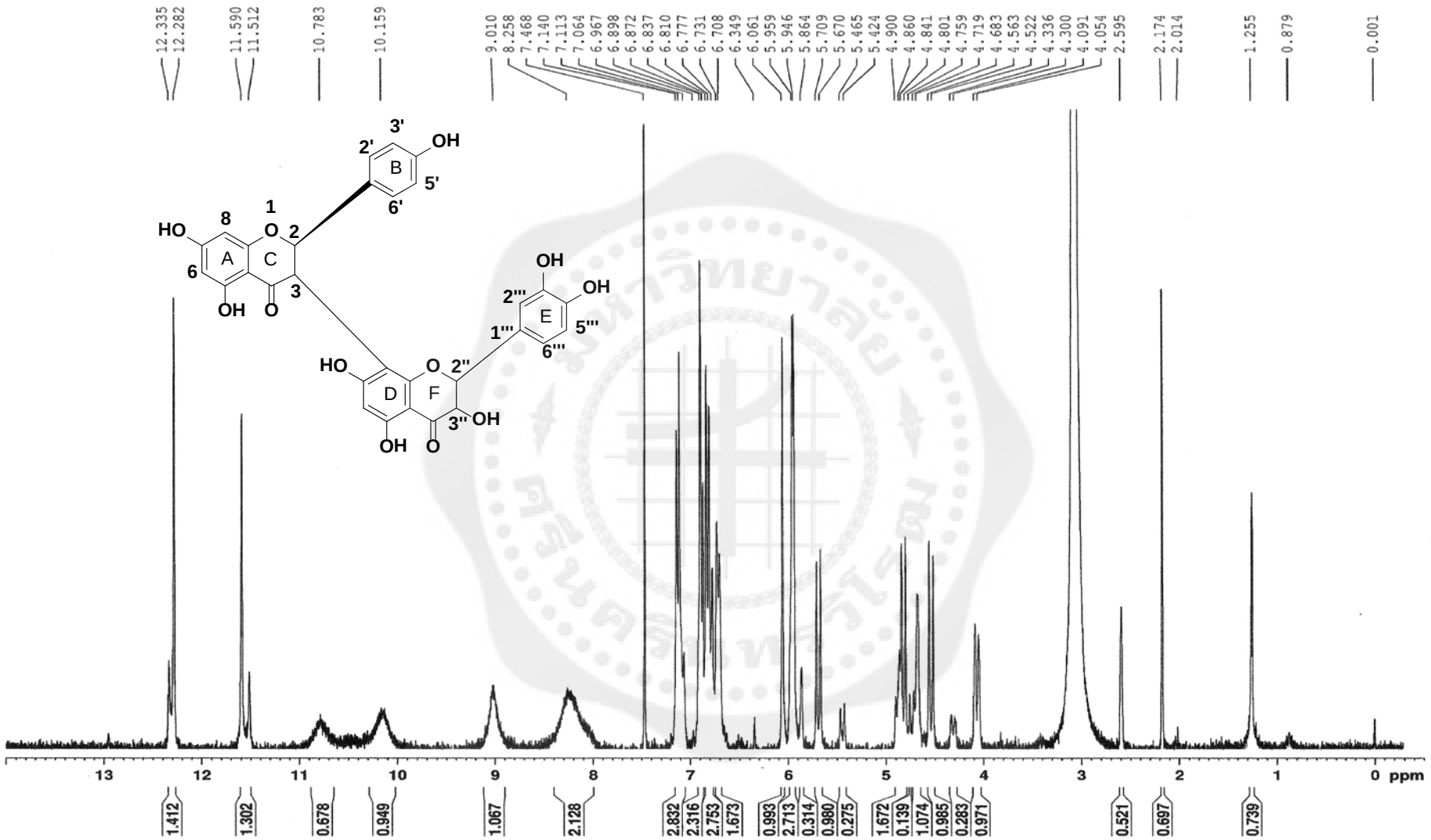

Figure 34 ^{1}H NMR of compound **E** (GB-2 (**38**), sss4783) in $CDCl_3$ + DMSO-d_6

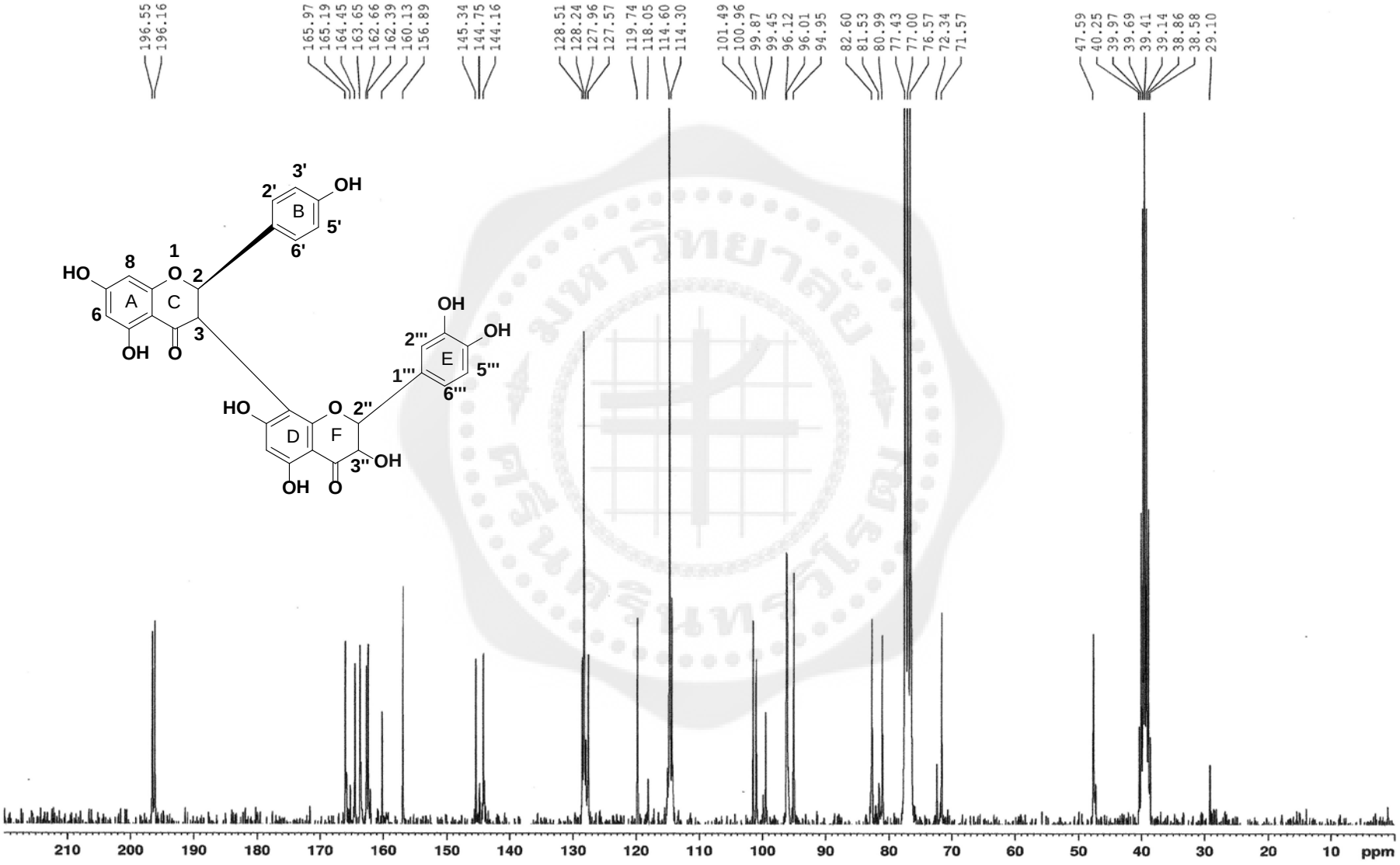

Figure 35 ^{13}C NMR of compound **E** (GB-2 (**38**), sss4783) in $CDCl_3$ + DMSO-d_6

GLOSSARY

LIST OF ABBREVIATIONS AND SYMBOLS

$[\alpha]_D^{25.8}$	Specific rotation at 25.8° C and sodium D line
$[\alpha]_D^{27.3}$	Specific rotation at 27.3 ° C and sodium D line
δ	Chemical shift (for NMR data)
ε	Molar absorptivity
μL	Microliter
μM	Micromolar
λ_{max}	Wavelength at maximal absorption
ν_{max}	Wave number at maximal absorption
$[M-H]^-$	Deprotonated molecular ion
^{13}C NMR	13-Carbon Nuclear Magnetic Resonance Spectroscopy
^{1}H NMR	Proton Nuclear Magnetic Resonance Spectroscopy
^{1}H-^{1}H COSY	Homonuclear (Proton-Proton) Correlation Spectroscopy
br d	Broad doublet (for NMR data)
br s	Broad singlet (for NMR data)
br t	Broad triplet (for NMR data)
calcd	Calculated
C_6H_6	Benzene
CC	Column chromatography
$CDCl_3$	Deuterated chloroform
CH_2Cl_2	Dichloromethane
$CHCl_3$	Chloroform
cm	Centimeter
cm^{-1}	Reciprocal centimeter (unit of wave number)
d	Doublet (for NMR data)
DEPT	Distortionless Enhancement by Polarization Transfer
dq	Doublet of quartets (for NMR data)
dt	Doublet of triplets (for NMR data)
EIMS	Electron Impact Ionization Mass Spectrometry
ESIMS	Electrospray Ionization Mass Spectrometry

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

EtOAc	Ethyl acetate
g	Gram
Glc	Glucoside
h	Hour
H_2O	Water
HMBC	^{1}H-Detected Heteronuclear Multiple Bond Coherence
HMQC	^{1}H-Detected Heteronuclear Multiple Quantum Coherence
HRTOFMS	High Resolution Time of Flight Mass Spectrometry
Hz	Hertz
IC_{50}	50% Inhibitory Concentration
IR	Infrared
J	Coupling constant
KBr	Potassium bromide
kg	Kilogram
L	Liter
m	Multiplet (for NMR data)
MeOH	Methanol
mg	Milligram
MIC	Minimum Inhibitory Concentration
mL	Milliliter
mm	Millimeter
NMR	Nuclear Magnetic Resonance Spectroscopy
NOESY	Nuclear Overhauser Effect Spectroscopy
°C	Degree Celsius
QCC	Quick column chromatography
Rha	Rhamnoside
s	Singlet (for NMR data)
sh	shoulder

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

TLC	Thin Layer Chromatography
UV	Ultraviolet

CURRICULUM VITAE

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