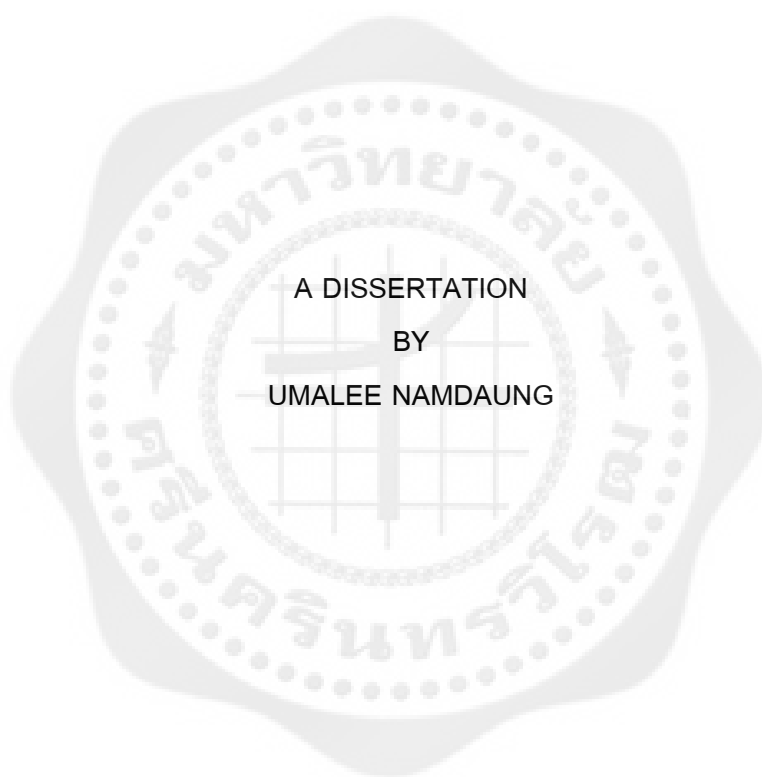


ANTICHOLINESTERASE PHENOLIC COMPOUNDS FROM THE ROOT BARKS OF
ARTOCARPUS LAKOOCHA



Presented in Partial Fulfillment of the Requirement for the
Doctor of Philosophy Degree in Applied Chemistry
at Srinakharinwirot University

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AChE and BuChE inhibitory activities of the active EtOAc and MeOH extracts from *Artocarpus lakoocha* root barks led to the purification of 2-arylbenzofurans as major components and other phenolic compounds. Two new 2-arylbenzofurans, named lakoochins C and D, together with six known 2-arylbenzofurans, artolakoochol, 4-hydroxyartolakoochol, cycloartolakoochol, lakoochins A and B and albafuran B, as well as a stilbenoid oxyresveratrol and a flavonoid (+)-catechin, were isolated. The structures of all constituents were determined based on spectroscopic data analyses (NMR, MS, UV and IR) and comparison of their spectral data with those published in the literature.

From the AChE and BuChE inhibitions of all isolated substances except for albafuran B, artolakoochol displayed the highest AChE inhibition (IC_{50} 0.87 μ M), comparing to standard drug galantamine (IC_{50} 1.45 μ M). Lakoochin B was found to be the most potent BuChE inhibitor (IC_{50} 0.10 μ M) and also more active than that of the control galatamine (IC_{50} 3.72 μ M). It was found that the inhibition was generally reduced when the hydroxyl group on arylbenzofuran was replaced by the methyl ether function. In order to better understand the structure-activity relationships (SAR), some methylated derivatives of artolakoochol and lakoochin B were then synthesized and tested. All newly derivatives were found AChE inactive. For BuChE inhibition, the artolakoochol mono-methyl ethers exhibited stronger action than that of the starting compound and control, whereas the lakoochin B derivatives slightly reduced the inhibition as compared to that of their parent.

สารประกอบฟีนอลิกที่มีฤทธิ์ยับยั้งเอนไซม์โคสไลน์เอสเทอร์เรสจากเปลือกรากมะหาด



บทคัดย่อ
ของ
อุมาลี นามดวง

เสนอต่อบัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ เพื่อเป็นส่วนหนึ่งของการศึกษา
ตามหลักสูตรปรัชญาดุษฎีบัณฑิต สาขาเคมีประยุกต์
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มหาวิทยาลัยศรีนครินทรวิโรฒ. คณะกรรมการควบคุม: รองศาสตราจารย์ ดร. สุนิตย์
สุขสำราญ และดร. ประเสริฐ พัฒนาประทีป

การแสดงฤทธิ์ยับยั้งการทำงานของเอนไซม์อะเซทิลโคลีนเอสเทอเรสและบิวทิลโคลีน
เอสเทอเรสของสารสกัดชั้น EtOAc และ MeOH จากเปลือกรากมะหาด นำไปสู่การแยก
สารประกอบหลัก 2-arylbenzofurans และสารประกอบฟีนอลิกชนิดอื่น โดยได้พบสารประกอบ 2-
arylbenzofurans ชนิดใหม่ 2 ชนิดคือ lakoochin C และ lakoochin D ร่วมกับพบสารประกอบ 2-
arylbenzofurans ที่มีรายไว้แล้ว 6 ชนิดคือ artolakoochol, 4-hydroxyartolakoochol,
cycloartolakoochol, lakoochin A, lakoochin B และ albufuran B และพบสาร stilbinoid
oxyresveratrol และ ฟลาโวนอยด์ (+)-catechin การพิสูจน์โครงสร้างของสารประกอบได้จากการ
วิเคราะห์ข้อมูลสเปกโทรสโกปี (โดยใช้เทคนิค NMR, MS, UV และ IR) รวมทั้งการเปรียบเทียบ
ข้อมูลกับที่มีรายงานไว้แล้ว

จากการศึกษาฤทธิ์ยับยั้งการทำงานของเอนไซม์อะเซทิลโคลีนเอสเทอเรสและบิวทิล
โคลีนเอสเทอเรสของสารประกอบที่แยกได้ทั้งหมดยกเว้น albufuran B พบว่า artolakoochol
แสดงฤทธิ์ยับยั้งการทำงานของเอนไซม์อะเซทิลโคลีนเอสเทอเรสดีที่สุด (ที่ IC_{50} เท่ากับ $0.87 \mu M$)
เมื่อเทียบกับสารมาตรฐาน galatamine (IC_{50} เท่ากับ $1.45 \mu M$) ในขณะที่ lakoochin B แสดงฤทธิ์
ยับยั้งการทำงานของเอนไซม์บิวทิลโคลีนเอสเทอเรสดีที่สุด (ที่ IC_{50} เท่ากับ $0.10 \mu M$) เมื่อเทียบกับ
สารมาตรฐาน galatamine (IC_{50} เท่ากับ $3.72 \mu M$) โดยทั่วไปพบว่าฤทธิ์ยับยั้งเอนไซม์ทั้งสองชนิด
ลดลงหรือไม่แสดงฤทธิ์เมื่อหมู่ hydroxyl บน arylbenzofuran ถูกแทนที่ด้วยหมู่ methyl ether
เพื่อศึกษาความสัมพันธ์ของโครงสร้างและการออกฤทธิ์ ดังนั้นจึงได้สังเคราะห์ และทดสอบฤทธิ์
อนุพันธ์ methyl ether บางชนิดของ artolakoochol และ lakoochin B ซึ่งพบว่าสารอนุพันธ์ชนิด
ใหม่ทั้งหมดไม่แสดงฤทธิ์ยับยั้งเอนไซม์อะเซทิลโคลีนเอสเทอเรส สำหรับฤทธิ์ยับยั้งการทำงานของ
เอนไซม์บิวทิลโคลีนเอสเทอเรส พบว่าสารอนุพันธ์ mono-methyl ของ artolakoochol แสดง
ฤทธิ์ดีกว่าสารตั้งต้น และสารควบคุม galantamine ในขณะที่สารอนุพันธ์ของ lakoochin B แสดง
ฤทธิ์ลดลงเล็กน้อยเมื่อเทียบกับสารตั้งต้น lakoochin B

The dissertation titled

“Anticholinesterase phenolic compounds from the root barks of *Artocarpus lakoocha*”

By

Umalee Namdaung

has been approved by the Graduate School as partial fulfillment of the requirements for the

Doctor of Philosophy Degree in Applied Chemistry of Srinakharinwirot University.

..... Acting Dean of Graduate School

(Assoc. Prof. Dr. Parin Chaivisuthangkura)

July 2016

Dissertation Committee

Oral Defense Committee

..... Major-advisor

..... Chair

(Assoc. Prof. Dr. Sunit Suksamrarn)

(Prof. Dr. Apichart Suksamrarn)

..... Co-advisor

..... Committee

(Dr. Prasert Pattanapruteeb)

(Assoc. Prof. Dr. Sunit Suksamrarn)

..... Committee

(Dr. Prasert Pattanapruteeb)

..... Committee

(Assoc. Prof. Dr. Siritron Samosorn)

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Umalee Namdaung

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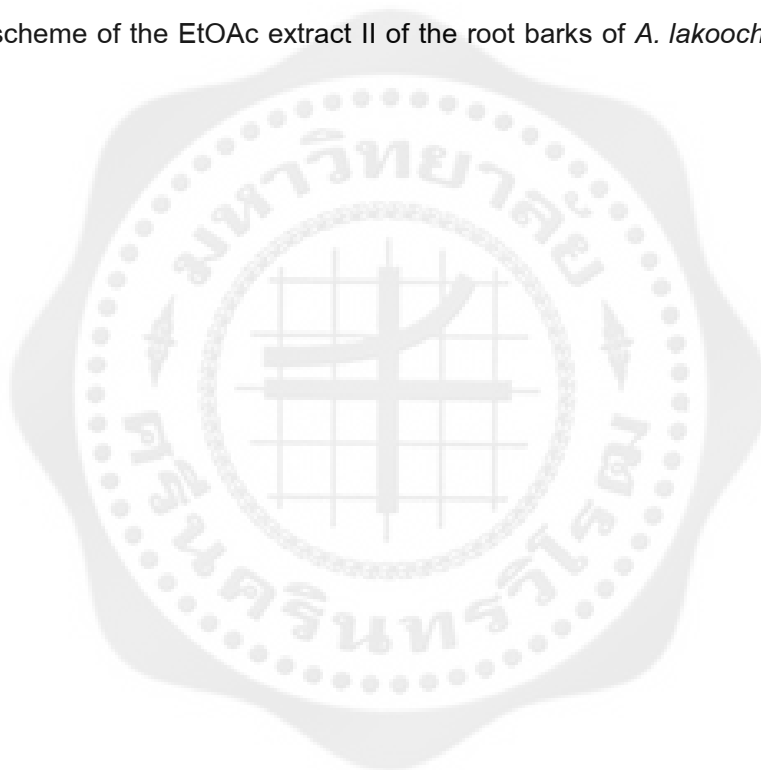
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CHAPTER 1

INTRODUCTION

Background

The family Moraceae of the order Urticales comprises of 60 genera and over 1400 species worldwide throughout the tropical and subtropical regions of Asia (Jagtap; et al. 2010: 142-166). The genus *Artocarpus*, one of the most important genera, has about 50 species and is indigenous to Ceylon, India, Pakistan, Burma, Thailand, Indo-China, South-China, Malaysia and Solomon island (Kochummen. 1978: 119-133). *Artocarpus* species, including *Artocarpus heterophyllus* (jackfruit), *Artocarpus altilis* (breadfruit) and *Artocarpus chempeden* (Chempedak) are economically importance as sources of edible fruits, fairly good timber and folk medicines (Jagtap; et al. 2010: 142-166). *Artocarpus* plants are evergreen or monoecious trees. Leaves spirally arranged (subg. *Artocarpus*) or distichous (subg. *Pseudojaca*); lamina pinnately veined or sometimes subtriplinerved, entire or pinnately divided, sometimes down to the midrib; stipules free, amplexicaul (subg. *Artocarpus*) or lateral (subg. *Pseudojaca*). Inflorescences unisexual, solitary or in paris in the leaf axils or on short-shoots on the older wood, pendunculate (or subsessile), bracteate or ebracteate. Staminate inflorescences spicate, ellipsoid to cylindrical to clavate to subglobose-capitate; flowers numerous, free; perianth tubular, subentire or 2-4-lobate to – partite; stamen 1. Pistillate inflorescences (sub)globose to ellipsoid-capitate, flowers numerous, with the upper parts of the perianth (or all of it) fused with adjacent perianths and/or interfloral bracts; perianth tubular with a narrow aperture; ovary free, stigma 1 or 2 of equal or unequal length. Infructescences consisting of a solid outer layer with a smooth areolate or with various protuberances, a soft, more or less fleshly middle layer containing the fruits, and a solid central part; fruit free, with a chartaceous to coriaceous or to crustaceous pericarp or endocarp; seed without endosperm, embryo straight, longitudinally to obliquely aligned, cotyledons equal or unequal, radical minute (Berg; et al. 2011, 480-497).

According to Berg 2011, only 14 species are found in Thailand (Berg; et al. 2011, 480-497) as follows:

1. *A. altilis* (Parkinson) Fosberg or *A. communis* J.R. & G.Forst. or *A. incisa* L.f. (Sake, Khanun sampalo: Central)
2. *A. altissimus* J.J. Sm. (Sanai: Surat Thani)

3. *A. chama* Buch.-Ham. (Khanun pan: Surat thani; Khanun pa: Chachoengsao)
4. *A. elasticus* Blume (Ka ok, Ka-o: Peninsular; Tue-ka: Malay-Yala; O: Trang, Ranong)
5. *A. gomezianus* Wall. ex Trécul (Ta pang, Tam-pang: Malay-Peninsular; Hat nun: Northern; I po: Trang)
6. *A. heterophyllus* Lam. or *A. integrifolius* L.f. (Khanun: General; Kh-nu: Chong-Chanthaburi; Kha-noe: Khmer; Si-khue, Pa-noi: Karen-Mae Hong Son; Na-yuai-sa: Karen-Kanchanaburi; Na-ko: Malay-Pattani; Nen: Chaobon-Nakhon Ratchasima; Manun: Northern, Peninsular; Lang: Shan-Northern; Makmi: Northeastern; Mak lang: Shan-Mae Hong Son; Jack fruit tree)
7. *A. integer* (Thunb.) Merr. (Champada: General; Champado: Peninsular; Champedak)
8. *A. kemando* Miq. (Khunun pa: Narathiwat; Yatu: Malay-Narathiwat)
9. *A. lakoocha* Roxb. or *A. lakucha* Roxb. (Mahat: Peninsular; Mahat bai yai: Trang; Hat: General; Ka-yae, ta-pae, ta-paeng: Malay-Narathiwat)
10. *A. lanceifolius* Roxb. (Khunun pa: Peninsular; Nang-ka pi-to, Nang-ka pi-pit: Malay-Peninsular; Nang-ka pi-pae: Malay-Narathiwat)
11. *A. nitidus* Trécul subsp. *lingnanensis* Jarrett or *A. parva* Gagnep. (Mahat khoi: Surat Thani)
12. *A. rigidus* Blume subsp. *rigidus* (Khunun pa: Peninsular)
13. *A. teijsmannii* Miq.
14. *A. thailandicus* C. C Berg (Mahat)

Ethnopharmacological uses of *Artocarpus* plants

A number of extracts from aerial and underground parts of *Artocarpus* species have traditionally been employed as folk medicine for relief of ailments such as diarrhea, diabetes, malarial fever, tapeworm infection and asthma (Jagtap; et al. 2010: 142-166). Many reports on various *Artocarpus* species and their ethnopharmacological studies will be described.

A decoction of *A. altilis* leaves have been used against liver cirrhosis, hypertension and diabetes (Wang; et al. 2006: 1052-1055), and its roots have been applied to treat

headache, beriberi, vomiting, parulis and edema (Fu; et al. 2014: doi: 10.1155/2014/724314). In addition, a decoction of *A. camansi* Blanco leaves has been also used to treat diabetes and baths of people with rheumatism in Philippines (Salonga; & Hisaka. 2014: 493-497).

The seeds of *A. chempeden* have been utilized as a remedy for diarrhea and its roots against malaria fever in Indonesian folk medicine (Heyne. 1987).

In the western part of Java, some parts of *A. elasticus* (barks, latex and young leaves) have been used to treat female contraception, dysentery and tuberculosis, respectively (Heyne. 1987).

The pulps and seeds of *A. heterophyllus* are employed as a cooling tonic and pectorial. Its latex mixed with vinegar promotes healing of abscesses, snakebite and glandular swellings, while the leaves and stem barks have been used for the treatment of anemia, asthma, dermatitis, diarrhea, cough and as an expectorant (Balbach; & Boarim. 1992). Moreover, the jackfruit roots are employed for healing skin disorders and asthma, and its extract is used to relief of fever and diarrhea (ICUC, 2003). In rural Northeastern Thailand's forest tradition, monks used the heartwoods of jackfruit, called as gaen-kanun, for dyeing their robes and the advantage of the dye is to provide protection from fungal infections and skin diseases (Salguero. 2003: 119). While local people in Cambodia use the wood as a nervous sedative and for relief of convulsions. Furthermore, the pith is taken as abortifacient (Rao; Mukhopadhyay; & Radhakrishnan. 2010: 1264-1266).

The unripe fruits of *A. hirsutus* Lam. are used for constipation and flatulence. Its barks are utilized to treat small pimples and cracks on the skin, and its dried leaves have been used for the treatment of bubose and hydrocele (Dibinlal; Sathish; & Senthil. 2010: 22-27).

In Thai folklore medicine, the dried aqueous extract of *A. lakoocha* heartwoods, a brown powder which called as Puag-Haad, is used as antihelmintic drug for the eradication of tapeworms (Charoenlarp; Radomyos; & Harinasuta. 1981; Salguero. 2003). Additionally, the durable heartwoods of *A. lakoocha* and *A. heterophyllus* have been also used for furniture and construction (Puntumchai; et al. 2004: 485-456). The *A. lakoocha* woods and roots also provide a lavish color dye (Joshee; et al. 2002). The fresh lakocha fruits are commonly eaten and edible fruit pulps are employed as a tonic for the liver whereas the raw fruits and male flowers spikes are applied in pickles and chutney.

In Vietnam, the barks and leaves of *A. tonkinensis* are commonly used as traditional medicine to treat backache and joint disorders (Dang; et al. 2009: 110-118).

Local people in China use some parts of *A. nanchuanensis* such as fruits, barks and roots for the treatment of constipation and dermatosis (Ren; et al. 2013: 98-100). Meanwhile, *A. nitidus* is used as a Chinese medicine for the treatment of gastritis and rheumatism (Ti; et al. 2011: 662-665).

Previous phytochemical investigations of *Artocarpus* species have revealed the presence of flavonoids, 2-arylbenzofurans, stilbenoids and triterpenoids. Many of these constituents have shown several biological properties, including antibacterial, antimalarial, antituberculosis, antioxidant, antidiabetic, anti-inflammatory, anthelmintic and antityrosinase properties (Jagtap; et al. 2010: 142-166).

From the attractive biological properties of *Artocarpus* species, it motivated us to search for other bioactivities from Thai *Artocarpus* plants. Interestingly, our preliminary screening of TLC observation from some parts (root barks, heartroots, twig stems, twig barks and leaves) of the crude extracts of *A. lakoocha*, it was found that the root bark extracts gave some more obvious major spots than the remaining extracts. Furthermore, the root bark extracts containing 2-arylbenzofurans as a major component, exerted the strongest anti-acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities. To date, few reports have revealed in the anti-cholinesterase (ChE) activity of the natural 2-arylbenzofurans (Liu; et al. 2011: 2081-2088). This investigation was thus search for the active 2-arylbenzofuran (s) which responsible for the observed activity from *A. lakoocha*. Moreover, some active compounds will be further selected for methylation in order to investigate for the structure-activity relationship (SAR).

Objectives of the study

1. To isolate and purify phenolic compounds from the root barks of *A. lakoocha*
2. To elucidate the chemical structures of the isolated compounds from these plant species
3. To evaluate the anticholinesterase effect of the isolated compounds

CHAPTER 2

LITERATURE REVIEW

Artocarpus species are indigenous and are one of the most important genera of Moraceae overall tropical and subtropical regions in Asia. There are 14 species growing in Thailand, especially five species, *A. chama*, *A. communis*, *A. heterophyllus*, *A. lakoocha* and *A. nitidus* were also found in China (Forster; et al. 2003: 30-34). Additional nine species, *A. gongshanensis*, *A. hypargyreus*, *A. nanchuanensis*, *A. nigrifolius*, *A. petelotii*, *A. pithecogallus*, *A. styracifolius*, *A. tonkinensis* and *A. xanthocarpus*, were distributed in China. Among these, *A. nanchuanensis* is a rare species (Jagtap; & Bapat. 2010: 142-166). Moreover, 13 species, including *A. altilis*, *A. bracteata*, *A. champeden*, *A. fretessi*, *A. glaucus*, *A. gomezianus*, *A. kemando*, *A. lanceifolius*, *A. maingayi*, *A. rotunda*, *A. reticulatus*, *A. scortechinii* and *A. teysmanii*, grow in Indonesia (Hakim; et al. 2006: 161-184). Among these, *A. bracteata* and *A. reticulatus* are rare species whereas *A. altilis*, *A. glaucus*, *A. gomezianus*, *A. lanceifolius*, *A. kemando* and *A. teysmanii* also are found in Malaysia (Kochummen. 1978: 119-133). There are further 12 species in Malaysia, *A. anisophyllus*, *A. dadah*, *A. elasticus*, *A. fulvicortex*, *A. heterophyllus*, *A. hispidus*, *A. integer*, *A. lowii*, *A. maingayi*, *A. nitidus*, *A. rigidus* and *A. scortechinii*, especially *A. fulvicortex* and *A. lowii* are rare species (Jamil; et al. 2012: 1587-1588).

2.1 Chemical constituents and biological activities of *Artocarpus* species except *A. lakoocha*

Artocarpus species are noted as an abundant source of prenylated phenolic compounds, including prenylated flavonoids, prenylated 2-arylbenzofurans and prenylated stilbenes. They are found in several parts of this genus, including roots, root barks, heartwood, stem barks, twigs and leaves. In the genus, flavonoids present as a major components whereas 2-arylbenzofurans and stilbenes seem to be minor components (Hakim; et al. 2006: 161-184).

Boonchoo has intensively reviewed on chemical constituents of *Artocarpus* plants during the year 1956-2002. An updated information from 2002 to 2014 will be followed (Boonchoo. 2002:1-411).

A new prenylated stilbene, artoindonesianin N (**1**) and a new prenylated 2-arylbenzofuran, artoindonesianin O (**2**), along with a known stilbene, oxyresveratrol (**3**) were investigated on the CH_2Cl_2 extract of the tree barks of *A. gomezianus* by Hakim in 2002 (Hakim; et al. 2002: 597-603) (Figure 1).

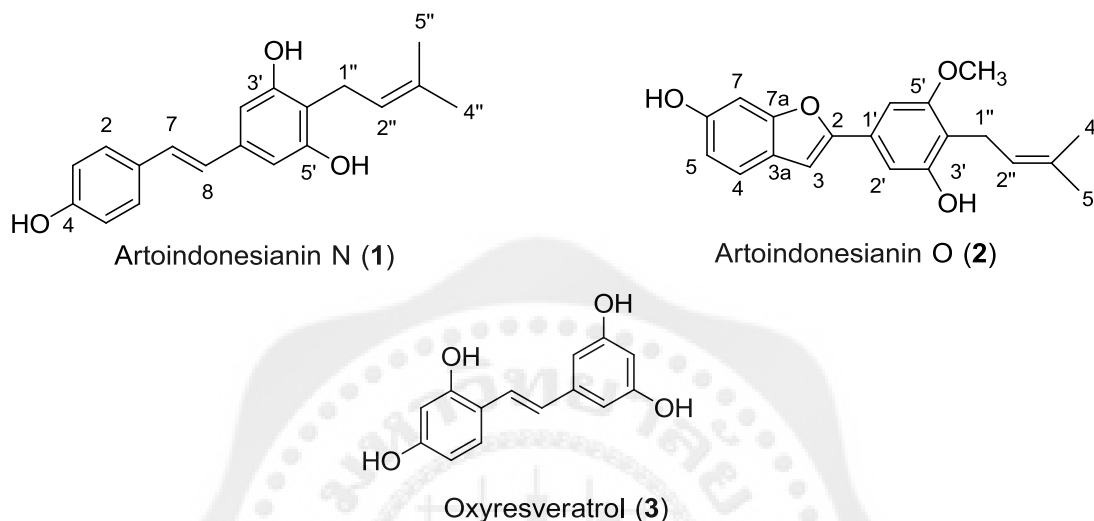


Figure 1 Structures of artoindonesianins N and O (**1** and **2**) and oxyresveratrol (**3**)

In 2002, Patil et al. obtained a $\text{MeOH}/\text{CH}_2\text{Cl}_2$ extract from the bud covers of *A. altilis*. The extract which exhibited cathepsin K inhibitory activity was then isolated to give two novel compounds, cycloaltilisins 6 and 7 (**4** and **5**), and three known compounds, AC-3-1 (**6**), AC-3-3 (**7**) and AC-5-1 (**8**) (Figure 2). All compounds were examined for cathepsin K inhibitory activity. Compound **4** showed the highest activity with IC_{50} value of 0.08 $\mu\text{g}/\text{mL}$, followed by compounds **8** and **5** with IC_{50} of values of 0.06 and 0.39 $\mu\text{g}/\text{mL}$, respectively (Patil; et al. 2002: 624-627).

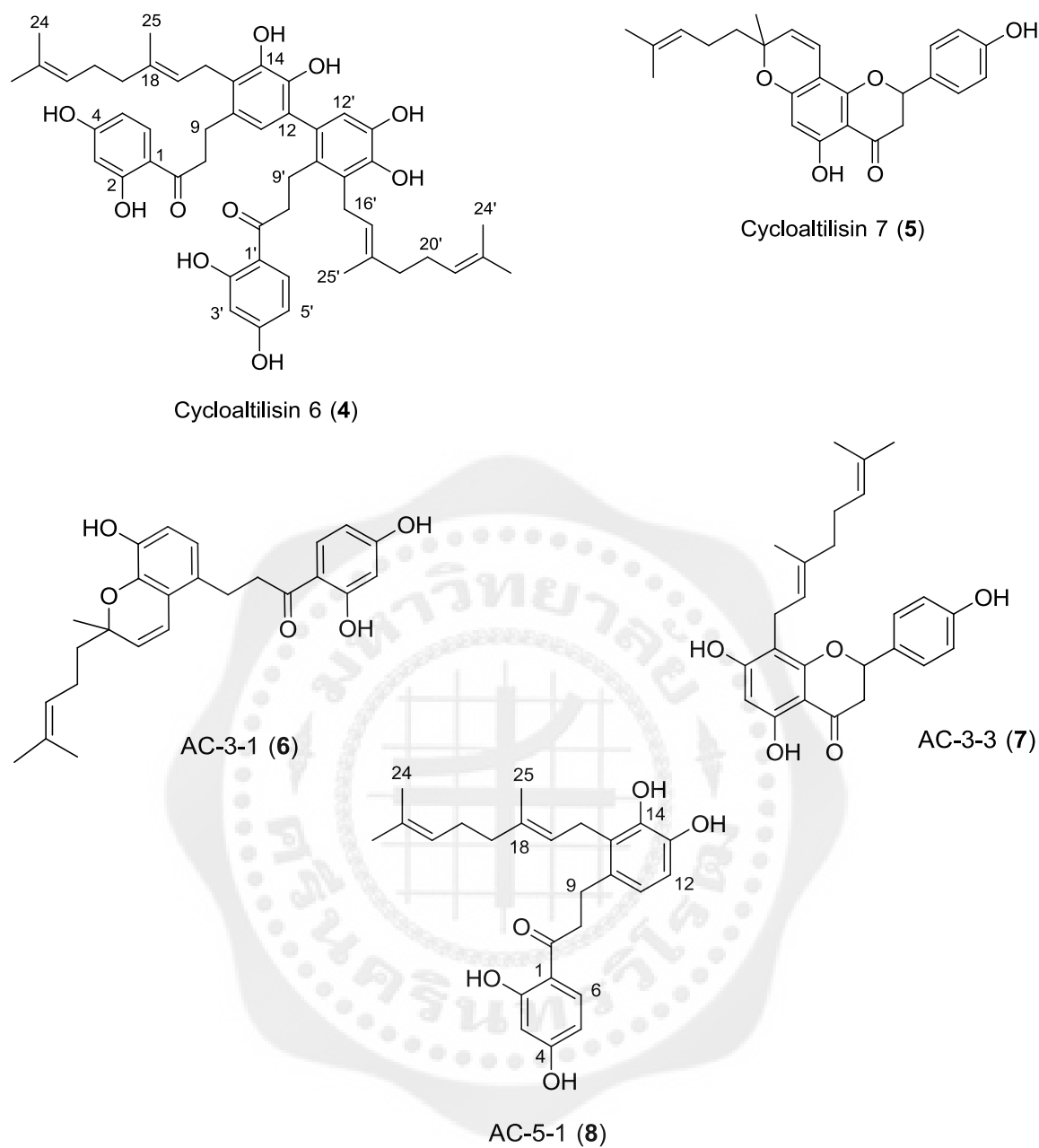


Figure 2 Structures of compounds 4-8

In 2002, Syah et al. found four new isoprenylated flavones, artoindonesianins Q-T (9-12) (Figure 3), from the CHCl_3 extract of the dried heartwoods of *A. champeden* (Syah; et al. 2002: 949-953).

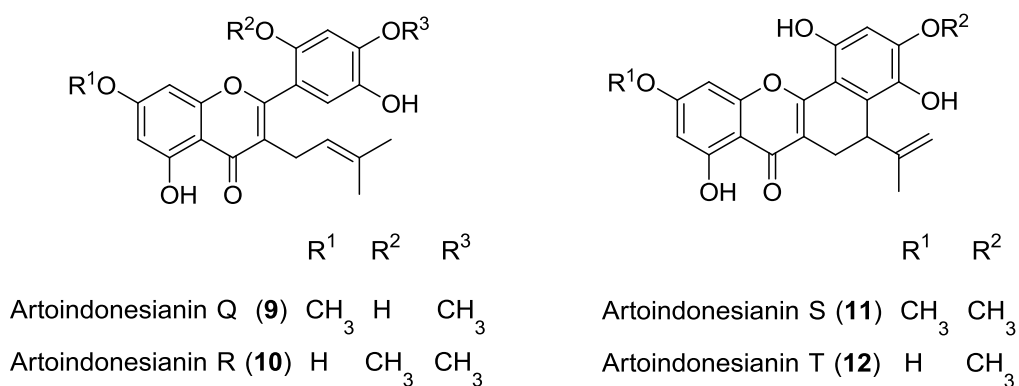


Figure 3 Structures of artoindonesianins Q-T (**9-12**)

In 2003, Chan et al purified five new prenylflavonoids, artocommunols CA-CE (**13-17**) (Figure 4), from the CHCl₃ extract of the heartroots of *A. communis*, together with a known compound, cyclomorusin (**18**) (Chan; Ko; & Lin. 2003: 427-430).

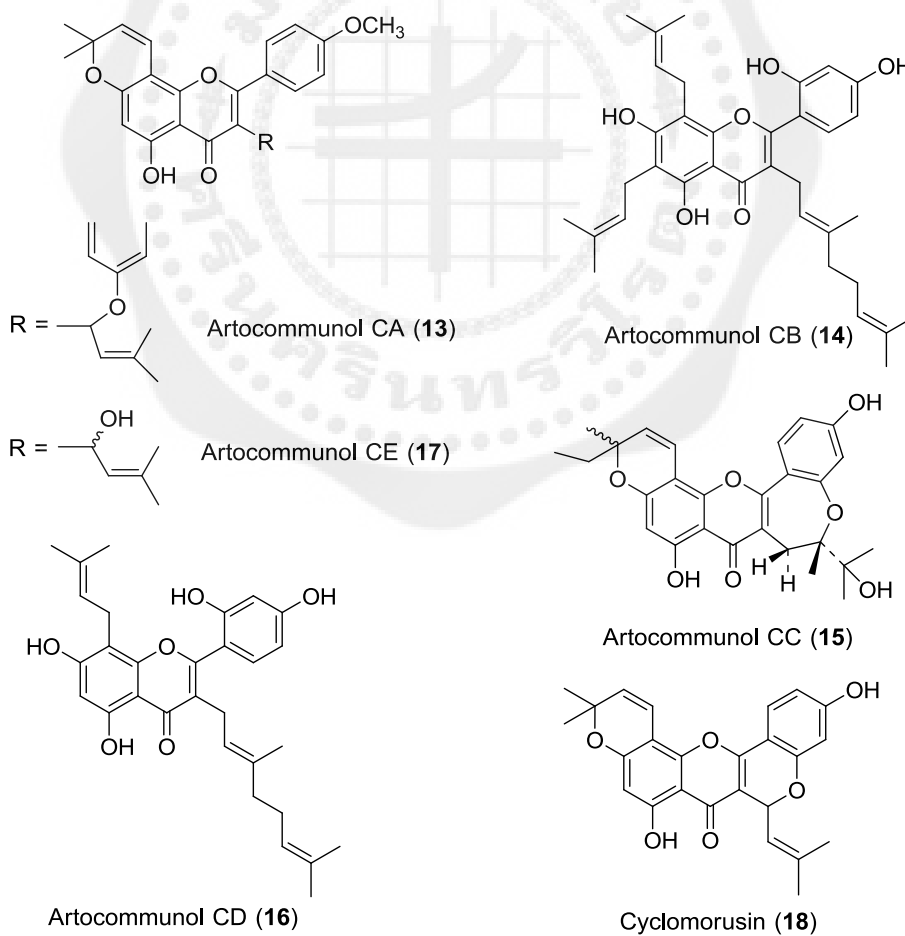


Figure 4 Structures of artocommunols CA-CE (**13-17**) and cyclomorusin (**18**)

In 2003, Lu et al. reported on the isolation and structure determination of a novel phenolic compound, artocarpol I (**19**), and a new biphenylpropanoid, artocarpol J (**20**) from the CHCl_3 extract of the root barks of *A. rigida*. Compound **20** were further acetylated to yield artocarpol J peracetate (**21**) (Figure 5). Compounds **19** and **21** showed highly anti-inflammatory activity on superoxide anion formation stimulated with formyl-Met-Leu-Phe (fMLP)/cytochalasin B (CB) and PMA from rat neutrophils with IC_{50} values of 7.9 and 9.0 $\mu\text{g/mL}$, respectively (Lu; & Lin. 2003: 2566-2572).

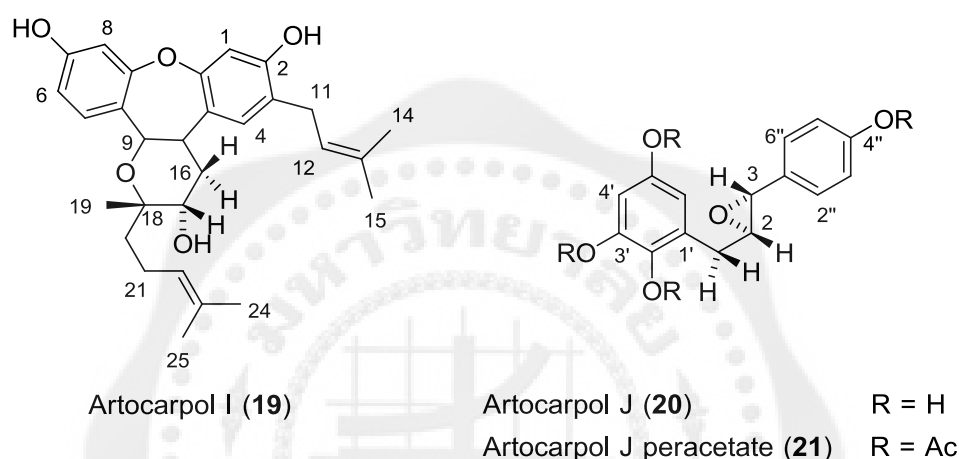


Figure 5 Structures of compounds **19-21**

In 2003, Seo et al. isolated of four known flavonoids, artonins E and O (**22** and **23**), artobiloxanthone (**24**) and cycloartobiloxanthone (**25**) (Figure 6), from the stem barks of *A. kemando*. All of them were examined for DNA strand-scission and human oral epidermoid carcinoma (KB) cytotoxicity assays. Compounds **22** and **24** exerted significant DNA strand-scission activity with 93% and 84% relaxation at 2.5 $\mu\text{g/mL}$, respectively, whereas the rest were inactive. For KB cytotoxicity, all compounds showed moderate activity with IC_{50} values ranging of 0.5-3.5 $\mu\text{g/mL}$ (Seo; et al. 2003: 124-127).

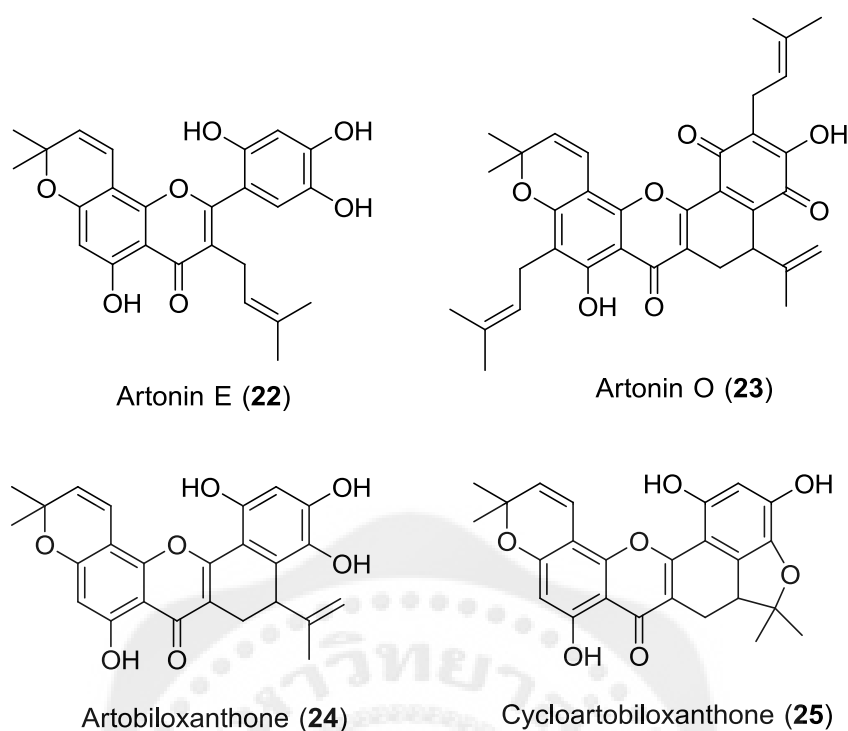
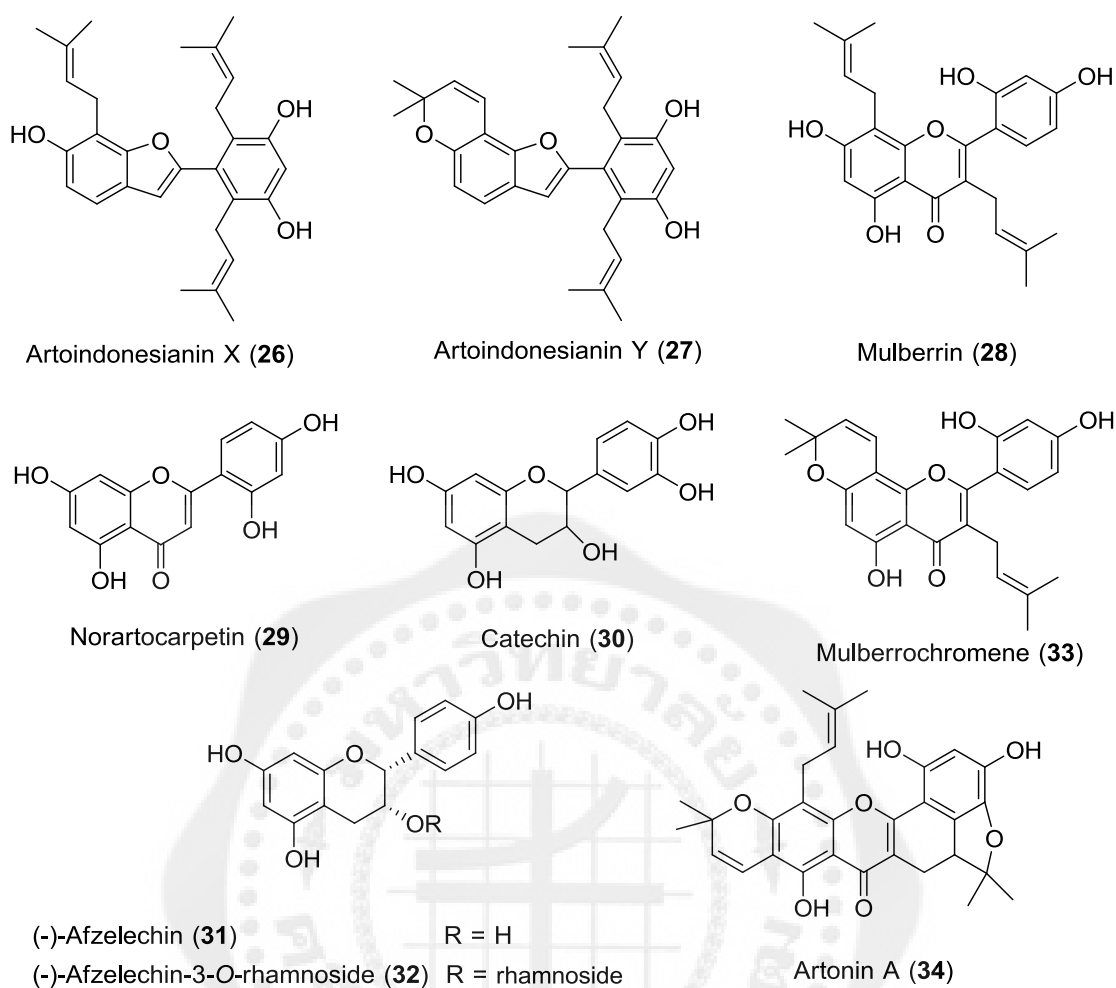


Figure 6 Structures of the flavonoids **22-25**

In 2003, Soekamto; et al. found two new prenylated 2-arylbenzofurans, artoindonesianins X and Y (**26** and **27**) from the CH_2Cl_2 fraction of the root barks of *A. gomezianus*. Furthermore, seven known flavonoids, including mulberrin (**28**), norartocarpetin (**29**), ($\pm$)-catechin (**30**), (–)-afzelechin (**31**) and (–)-afzelechin-3-O-rhamnoside (**32**), were found from the EtOAc fraction, while mulberrochromene (**33**) and artonin A (**34**) were purified from CH_2Cl_2 fraction of the tree barks of the same plant species (Figure 7). The isolated compounds were tested for the brine shrimp (*Artemia salina*) bioassay. From the results, compounds **26-28**, **33** and **34** were found to possess moderate activity with LC_{50} values of 78.7, 294.1, 77.4, 67.8 and 100.6 $\mu\text{g/ml}$, respectively, while compounds **30-32** were inactive ($\text{LC}_{50} > 500 \mu\text{g/ml}$) (Soekamto; et al. 2003: 831-834).

Figure 7 Structures of compounds **26-34**

The *n*-butanol fraction of the leaves of *A. nobilis* exhibited antifungal activity led to the isolation of two new chalcones, 2',4',4-trihydroxy-3'-[2-hydroxy-7-methyl-3-methylene-6-octaenyl]chalcone (**35**) and 2',3,4,4'-tetrahydroxy-3'-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl]chalcone (**36**), along with three known chalcones, 2',4',4-trihydroxy-3'-geranylchalcone (**37**), 2',4',4-trihydroxy-3'-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl]chalcone (**38**) and 2',3,4,4'-tetrahydroxy-3'-geranylchalcone (**39**) (Figure 8). All of them were evaluated for antifungal activity against *Cladosporium cladosporioides* and antioxidant activity using TLC bio-autography method. Antifungal activity on TLC bio-autography method appeared as white zones at **35** (5 µg/spot), **36** (15 µg/spot), **37** (5 µg/spot), **38** (5 µg/spot) and **39** (2 µg/spot). Moreover, all compounds showed strong activity against DPPH radical as off-white spots in purple background at the level of 1 µg/spot (Jayasinghe; et al. 2004: 1287-1290).

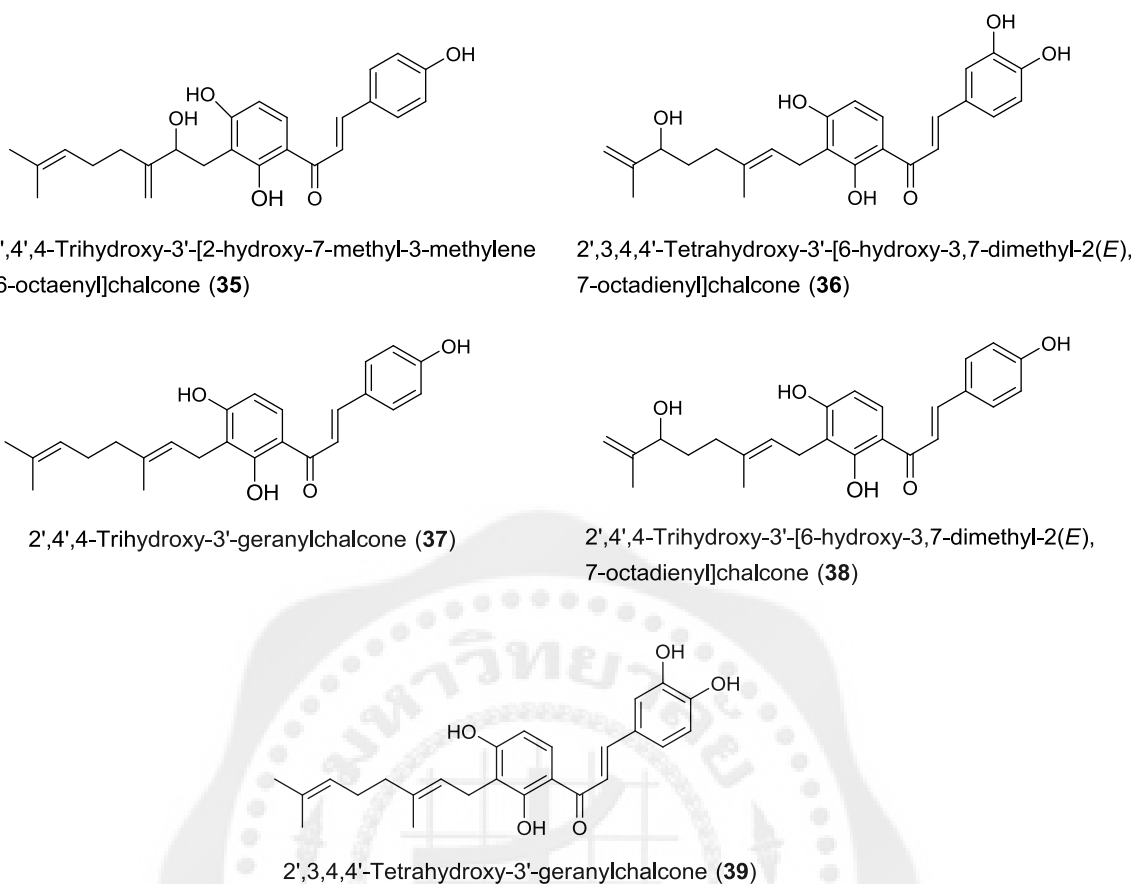


Figure 8 Structures of chalcones **35-39**

In 2004, Syah et al. isolated two new prenylated flavones, artoindonesianins U and V (**40** and **41**), together with three known prenylated flavones, 5'-hydroxycudraflavone A (**42**), cyclocommunin (**43**) and artonin B (**44**) (Figure 9), from the CHCl_3 extract of *A. champeden* heartwoods. Furthermore, compounds **40** and **41** displayed strong cytotoxic activity against P-388 leukemia tumor cells with IC_{50} values of 2.0 and 0.5 $\mu\text{g/mL}$, respectively (Syah; et al. 2004: 134-140).

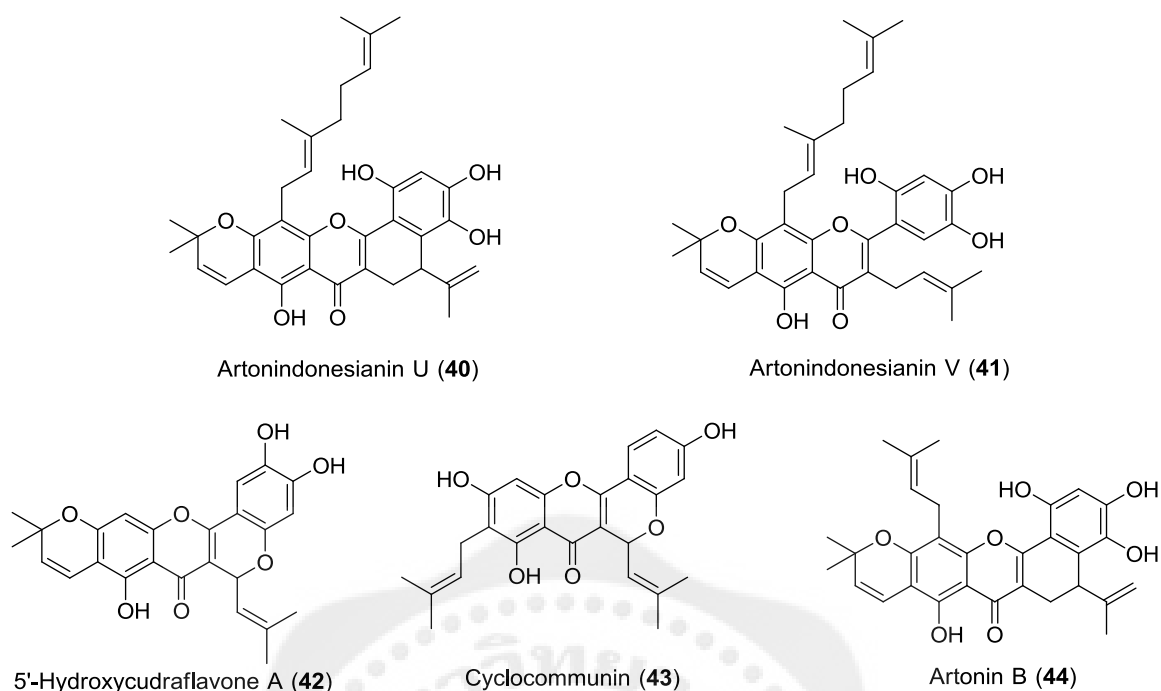


Figure 9 Structures of prenylated flavones **40-44**

In 2004, Wang et al. obtained four extracts, petroleum ether (PE), CHCl_3 , EtOAc and *n*-BuOH extracts, from the dried roots of *A. chama*. All extracts were screened for *in vitro* cytotoxicities against A549 and MCF-7 cell lines. The CHCl_3 extract exhibited the most potent activities. It was thus purified to furnish five new isoprenylated flavones, artochamins A-E (**45-49**) and eight known flavones, artonin E (**22**), cycloartobiloxanthone (**25**), artonin A (**34**), artocarpin (**50**), cycloartocarpin A (**51**), cudraflavone A (**52**), artonin U (**53**) and 3',4',5,7-tetrahydroxy-8-(methylbut-2-enyl)flavone (**54**) (Figure 10). Compounds **22**, **25**, **34**, **47**, **50** and **51** were selected to examine for cytotoxicities against various human tumor cell lines as shown in Table 1 (Wang; et al. 2004:757-761).

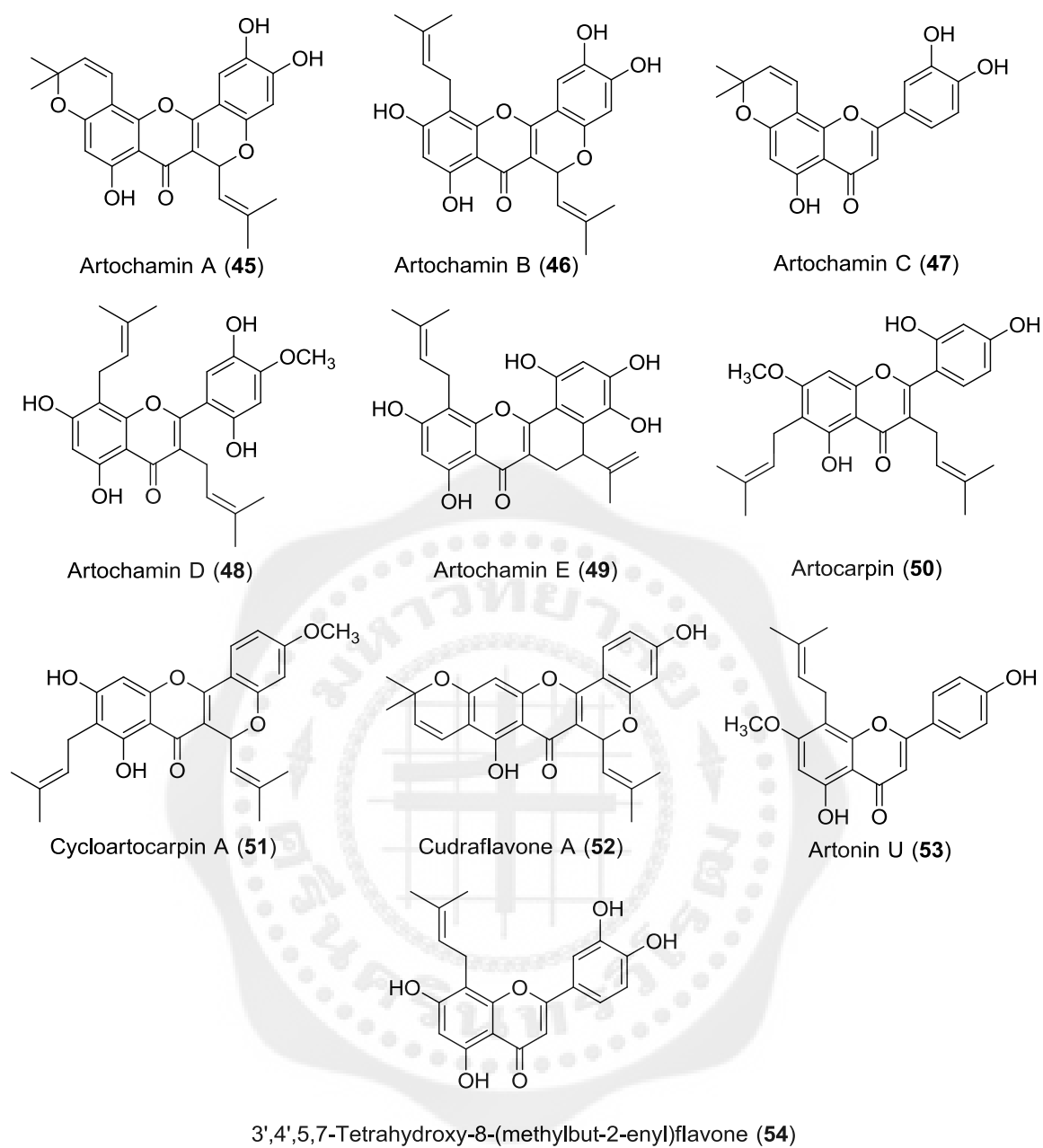
Figure 10 Structures of flavones **45-54**

Table 1 Cytotoxicities of compounds **22**, **25**, **34**, **47**, **50** and **51** against human tumor cell lines

Compound	Cytotoxicities (ED ₅₀ , µg/mL)										
	A549	MCF-7	1A9 ^a	HCT-8 ^b	CAKI-1 ^c	SK-MEL-2 ^d	U-87-MG ^e	PC-3 ^f	MDA-MB-231 ^g	KB ^h	KB-VIN ⁱ
22	8.5	2.2	<1.25	3.3	9.7	4.3	6.4	6.9	3.0	6.5	>10
25	7.2	7.7	j	j	j	j	j	j	j	j	j
34	6.1	6.7	j	j	j	j	j	j	j	j	j
47	3.0	2.2	2.0	2.3	5.3	2.3	7.2	4.2	6.2	3.4	3.4
50	3.3	3.3	3.4	3.8	4.9	5.4	3.7	4.1	3.8	3.2	3.6
51	6.6	6.0	j	j	j	j	j	j	j	j	j

^a ovarian carcinoma; ^b ileocecal carcinoma; ^c kidney carcinoma; ^d melanoma; ^e glioblastoma; ^f prostate cancer; ^g breast adenocarcinoma; ^h epidermoid carcinoma of the nasopharynx; ⁱ its subclone; ^j not tested

In 2005, Chen et al. purified three new prenylated 2-arylbenzofurans, artopetelins A-C (**55-57**) from the EtOH extract of the root barks of *A. petelotii* (figure 11) (Chen; & Hou. 2005: 2554-2560).

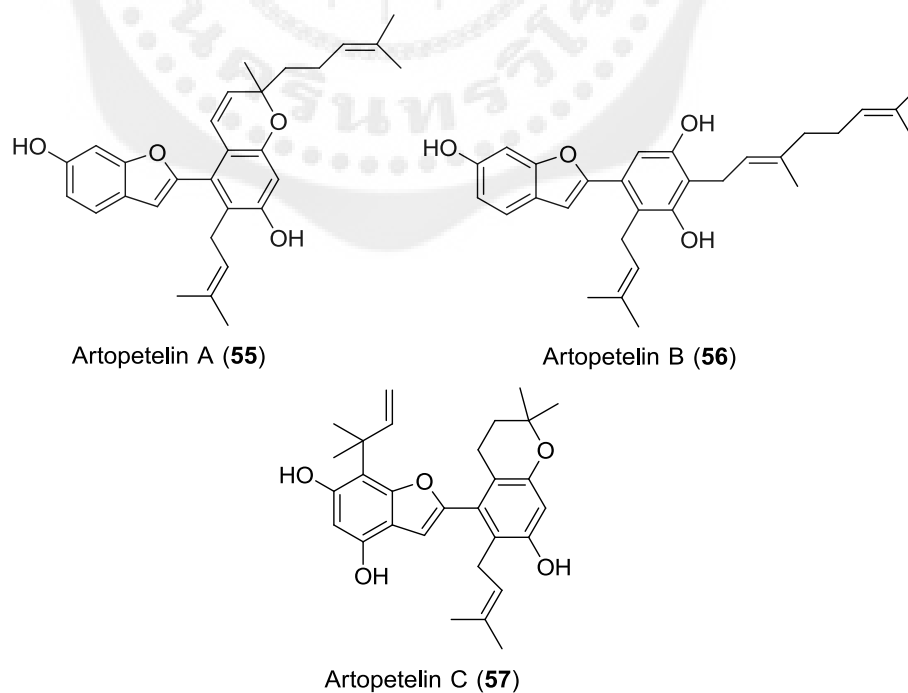


Figure 11 Structures of artopetelins A-C (**55-57**)

Chromatographic separation of the CH_2Cl_2 extract of *A. elasticus* root barks furnished five new prenylated flavonoids, artelastoheterol (**58**), artelasticinol (**59**), cycloartelastoxanthone (**60**), artelastoxanthone (**61**) and cycloartelastoxanthendiol (**62**), together with five known compounds, cyclomorusin (**18**), cycloartobiloxanthone (**25**), artonin F (**63**), artonols A and B (**64** and **65**) by Ko in 2005 (Figure 12). All compounds, except for **18** and **65**, were examined for cytotoxicities against four cell lines, including A549, hepatomacellular carcinoma (Hep3B), human colorectal adenocarcinoma (HT-29), and MCF-7 cells. Compound **64** showed the most active compound for cytotoxicity against the MCF-7, A549, and HT-29 with ED_{50} value in the range of 1.1 to 21.9 $\mu\text{g/mL}$, comparing to 5-flurouracil with ED_{50} values ranging of 0.2-0.4 $\mu\text{g/mL}$ (Ko; et al. 2005: 1692-1695).

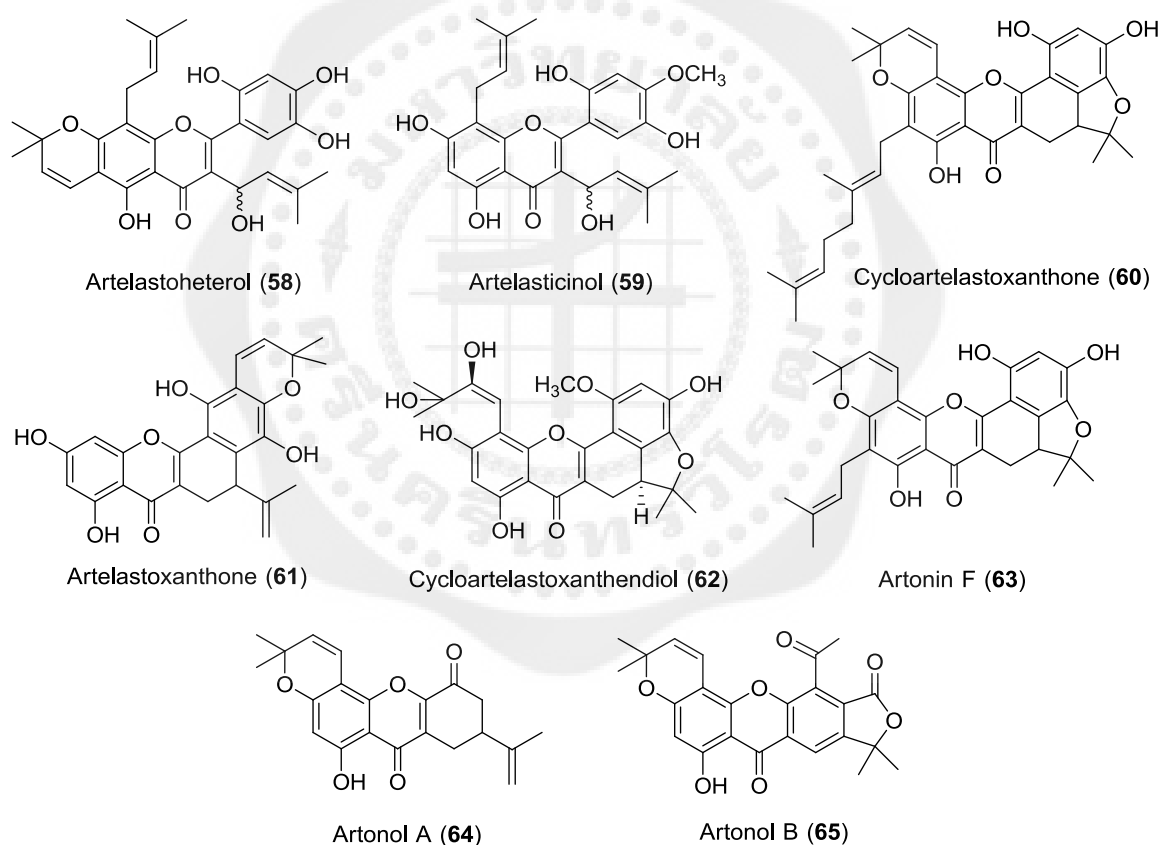


Figure 12 Structures of flavonoids **58-65**

In 2005, Likhitwitayawuid; et al. collected five known flavonoids, including norartocarpetin (**29**), artocarpin (**50**), isocyclomorusin (**66**), cycloartocarpin (**67**) and cudraflavone C (**68**), and two known dimeric stilbenes, artogomezianol (**69**) and andalasin A

(**70**) (Figure 13), from *A. gomezianus* reported on previous researches in order to evaluate on inhibitory effects against herpes simplex virus types 1 and 2 (HSV-1 and -2) and human immunodeficiency virus (HIV-1). They have been possessed antiviral and cytotoxicities as shown in Table 2 (Likhitwitayawuid; 2005: 651-657). One year later, the same group obtained the MeOH extract from the heartwoods of *A. gomezianus* on anti-herpetic activities against HSV-1 and -2 with 90% and 80% inhibition at a concentration of 100 mg/mL, respectively. The active extract furnished a new flavone artogomezianone (**71**), together with six known compounds, oxyresveratrol (**3**), norartocarpetin (**29**), artocarpin (**50**), isocyclomorusin (**66**), cycloartocarpin (**67**) and norcycloartocarpin (**72**). All of them were carried out for their inhibitory activities against both type of HSV. The results have revealed that flavones **3**, **29**, **66** and **67** exhibited moderate property against both types of HSV with IC_{50} values ranging of 11.9-18.0 $\mu\text{g/mL}$, comparing to acyclovir (IC_{50} 0.33 $\mu\text{g/mL}$). Meanwhile, compounds **50**, **71** and **72** were inactive (Likhitwitayawuid; 2006: 1138-1143).

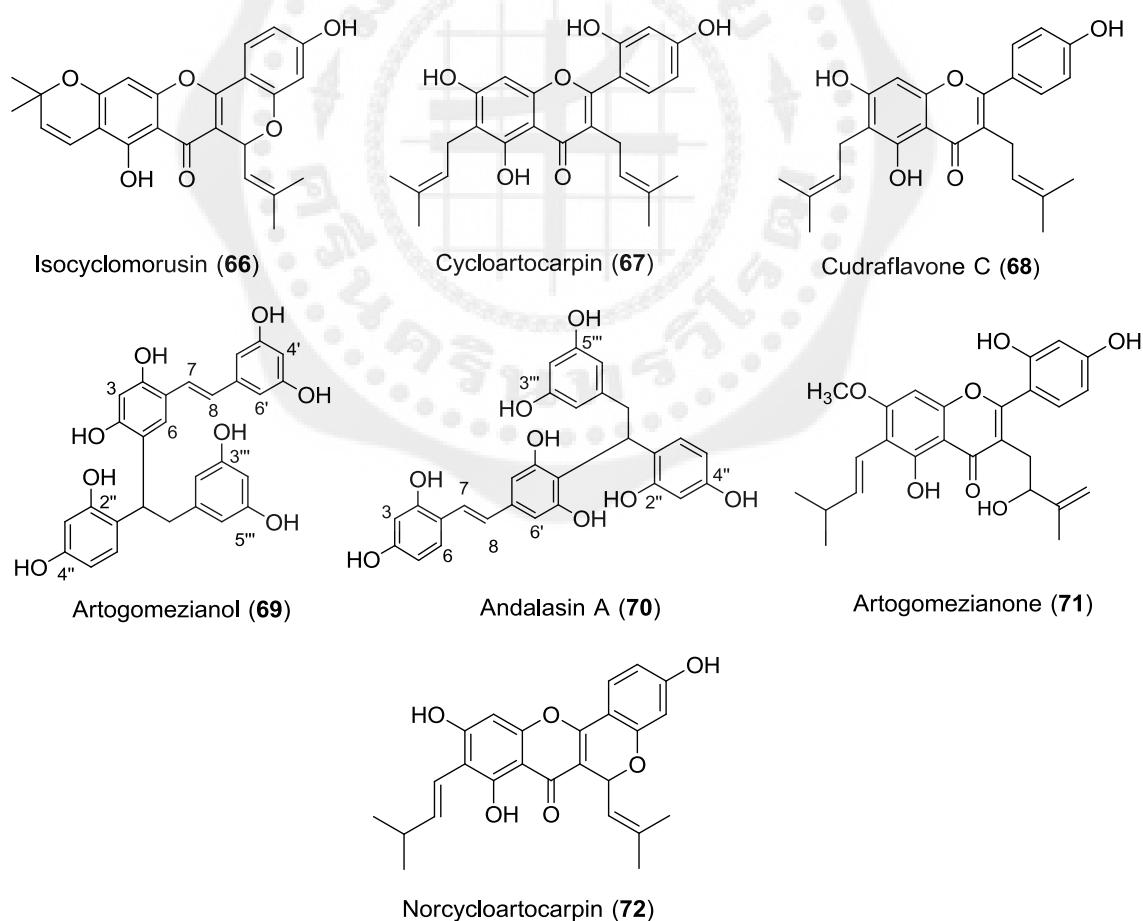


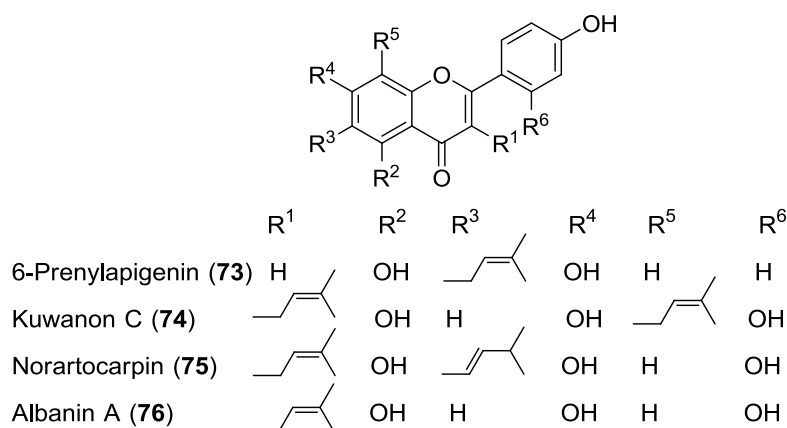
Figure 13 Structures of flavonoids and dimeric stilbenes (**66-72**)

Table 2 Antiviral and cytotoxic activities of compounds **29**, **50** and **66-70**

Compounds	Antiviral activities, (EC ₅₀ , µg/mL)			Cytotoxicities, (IC ₅₀ , µg/mL)	
	HSV-1 ^a	HSV-2 ^b	HIV-1 ^c	Vero cells ^d	PBM cells ^e
29	f	f	0.9	f	7.5
50	f	f	1.2	f	18.2
66	9.4	9.4	8.6	17.3	35.3
67	f	f	4.2	f	6.6
68	f	f	1.9	f	7.7
69	g	g	f	f	f
70	17.1	16.1	f	g	f

^a herpes simplex virus type 1; ^b herpes simplex virus type 2; ^c human immunodeficiency virus; ^d African green monkey kidney; ^e peripheral blood mononuclear cells; ^f not determined; ^g no activity

Phytochemical investigation of the diethyl ether-soluble fraction of *A. heterophyllus* woods led to the isolation of artocarpin (**50**), cudraflavone C (**68**), 6-prenylapigenin (**73**), kuwanon C (**74**), norartocarpin (**75**) and albanin A (**76**) by Arung in 2006 (Figure 14). All constituents were tested for inhibition of mushroom tyrosinase and B16 melanoma cells. For anti-tyrosinase effect, none of them showed higher effect than positive control, kojic acid. In addition, compounds **50**, **68**, and **73-75** exhibited the highest cytotoxic activity on B16 melanoma cells with IC₅₀ values ranging of 1.2-2.9 µg/mL, followed by compound **76** with IC₅₀ value of 14.2 µg/mL, than positive controls, kojic acid and arbutin (Arung; Shimizu; & Kondo. 2006: 847-850).

Figure 14 Structures of prenylated flavonoids **73-76**

In 2006, Chen et al. reported the isolation and structural determination of four new prenylated 2-arylbenzofurans, artopetelins D-G (**77-80**) (Figure 15), from the EtOH extract of the root barks of *A. petelotii* (Chen; Jiang; & Hou. 2006: 1000-1007).

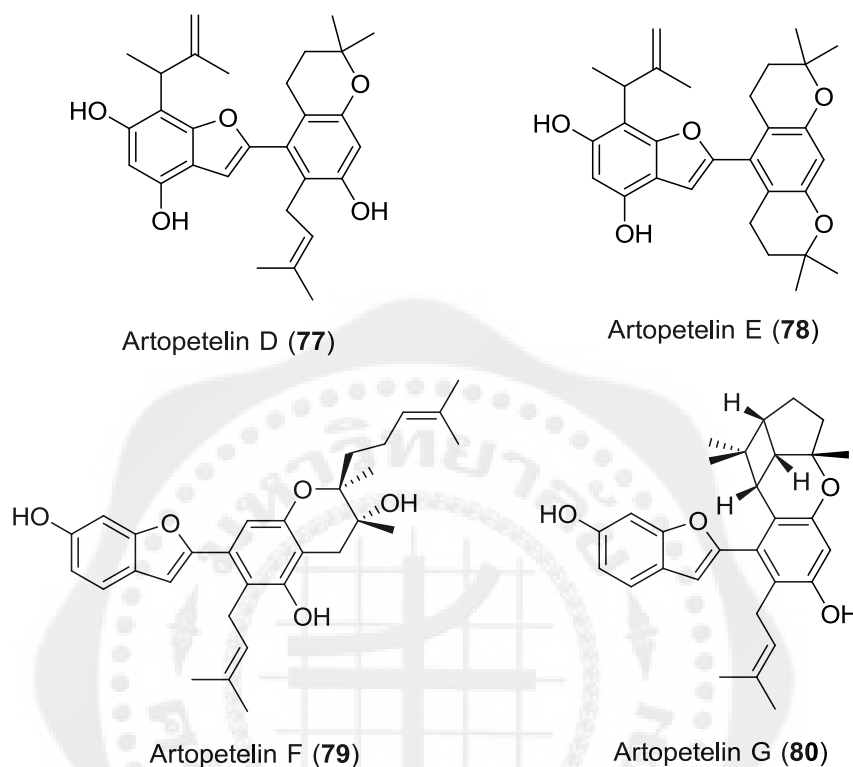
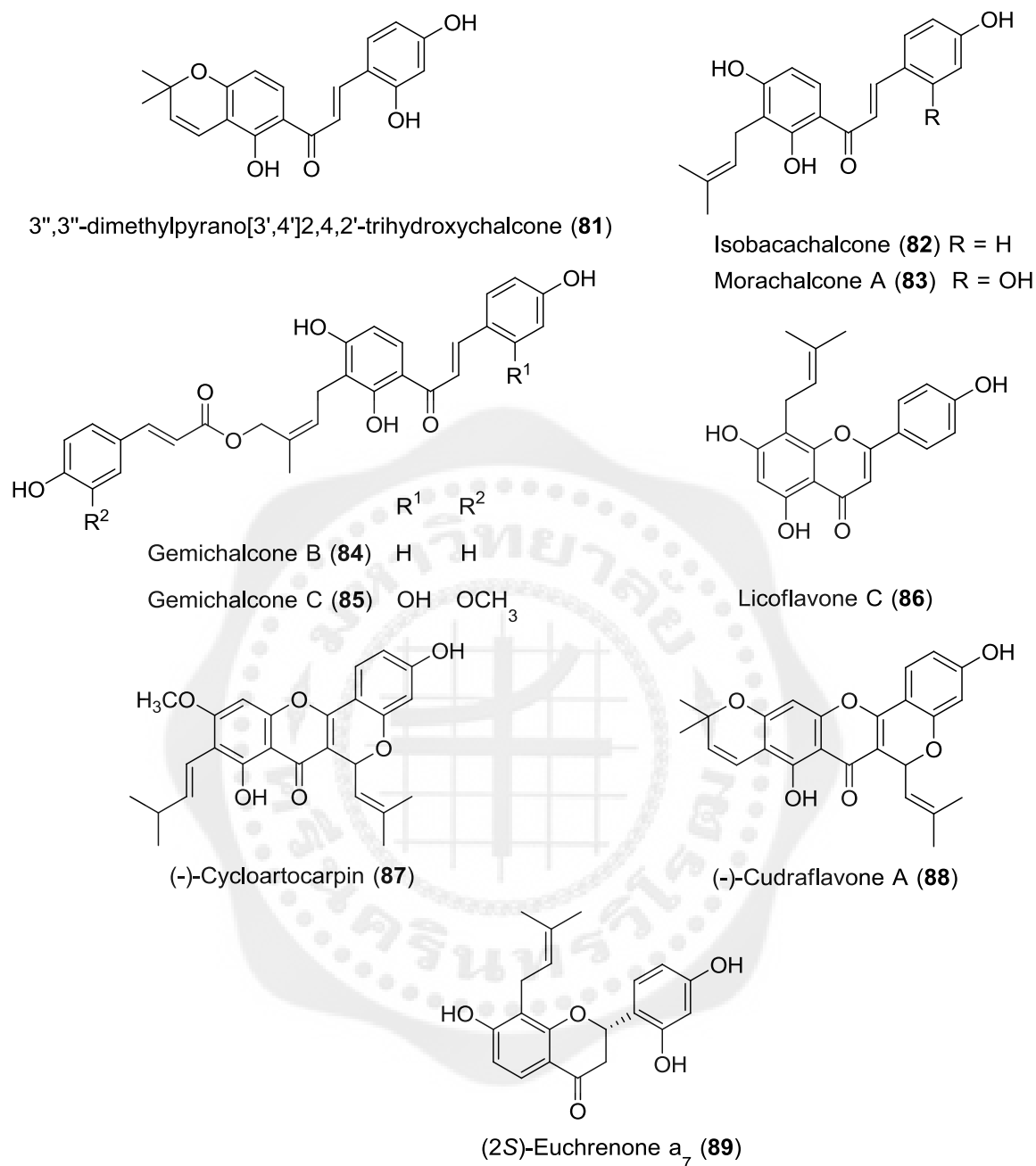


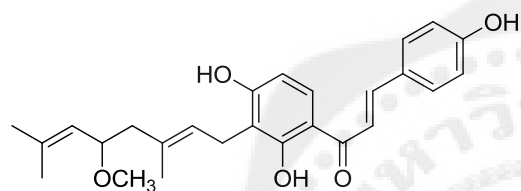
Figure 15 Structures of artopetelins D-G (**77-80**)

In 2006, et al. Han studied on the purification of a new prenylated chalcone, 3'',3''-dimethylpyrano[3',4']2,4,2'-trihydroxychalcone (**81**), together with 10 known flavonoids, artocarpin (**50**), cudraflavone C (**68**), isobacachalcone (**82**), morachalcone A (**83**), gemichalcones B and C (**84** and **85**), licoflavone C (**86**), (-)-cycloartocarpin (**87**) and (-)-cudraflavone A (**88**) and (2*S*)-euchrenone a₇ (**89**) (Figure 16), from the heartwoods of *A. communis*. All compounds were evaluated for inhibitory activity on NO production in LPS-activated mouse macrophage RAW264.7 cells. From the results, compounds **50**, **81-84**, and **89** showed effective inhibitory activity with IC₅₀ values ranging of 2.0-6.3 µg/mL. Compounds **85** and **86** showed inhibitory activity with IC₅₀ values of 12.7 and 6.9 µg/mL, respectively, while the rest **68**, **87**, and **88** exerted weak activity or inactive. *N*^ω Monomethyl-L-arginine (L-NMMA) was used as positive control (IC₅₀ 3.6 µg/mL) (Han; et al. 2006: 719-721).

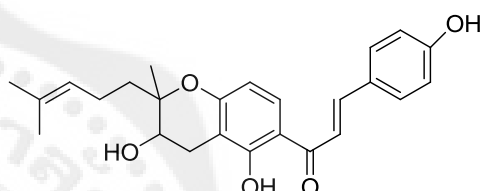
Figure 16 Structures of compounds **81-89**

Four new geranylated phenolic constituents, 2,4,4'-trihydroxy-3-[(2*E*)-5-methoxy-3,7-dimethylocta-2,6-dienyl]chalcone (**90**), 1-(3,4-dihydro-3,5-dihydroxy-2-methyl-2-(3-methyl-2-butenyl)-2*H*-1-benzopyran-6-yl)-3-(4-hydroxyphenyl)-2(*E*)-propen-1-one (**91**), 8-geranyl-3',4',7-trihydroxyflavone (**92**) and 3'-geranyl-4',5,7-trihydroxyflavanone (**93**), together with six known compounds, xanthoangelol (**94**), xanthoangelol B (**95**), 3-geranyl-2,3',4,4'-

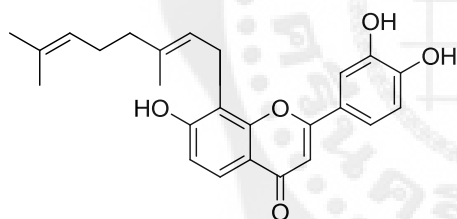
tetrahydroxychalcone (**96**), lespeol (**97**), 8-geranyl-4',7-dihydroxyflavanone (**98**) and isonymphaeol-B (**99**) were obtained from the combined CH₂Cl₂ and EtOAc extracts of the fruits of *A. nobilis* by Jayasinghe in 2006 (Figure 17). All compounds were tested for antioxidant property against DPPH radical by TLC bio-autography method. Compounds **92**, **96** and **99** showed strong off-white spots on TLC, at the concentration 0.1 µg. All of them were then evaluated for antioxidant activity, comparing with α -tocopherol by spectrophotometric method. It was found that compounds **92**, **96** and **99** exhibited strong activity with IC₅₀ values of 6.3, 5.0 and 4.4 µg/mL, respectively, comparing to α -tocopherol with IC₅₀ value of 13.8 µg/mL (Jayasinghe; et al. 2006: 1353-1358).



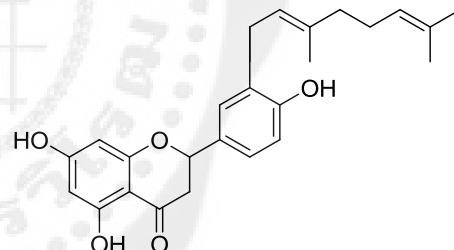
2,4,4'-Trihydroxy-3-[(2E)-5-methoxy-3,7-dimethylocta-2,6-dienyl]chalcone (**90**)



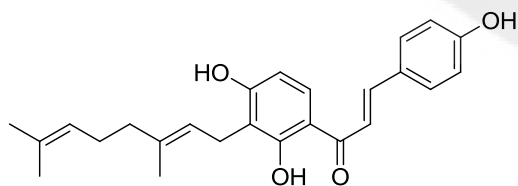
1-(3,4-Dihydro-3,5-dihydroxy-2-methyl-2-(3-methyl-2-butenyl)-2H-1-benzopyran-6-yl-3-(4-hydroxyphenyl)-2(E)-propen-1-one (**91**)



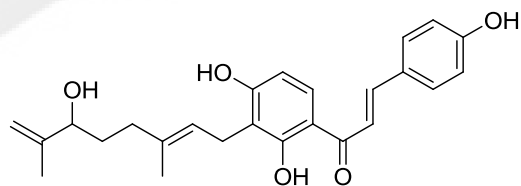
8-Geranyl-3',4',7-trihydroxyflavone (**92**)



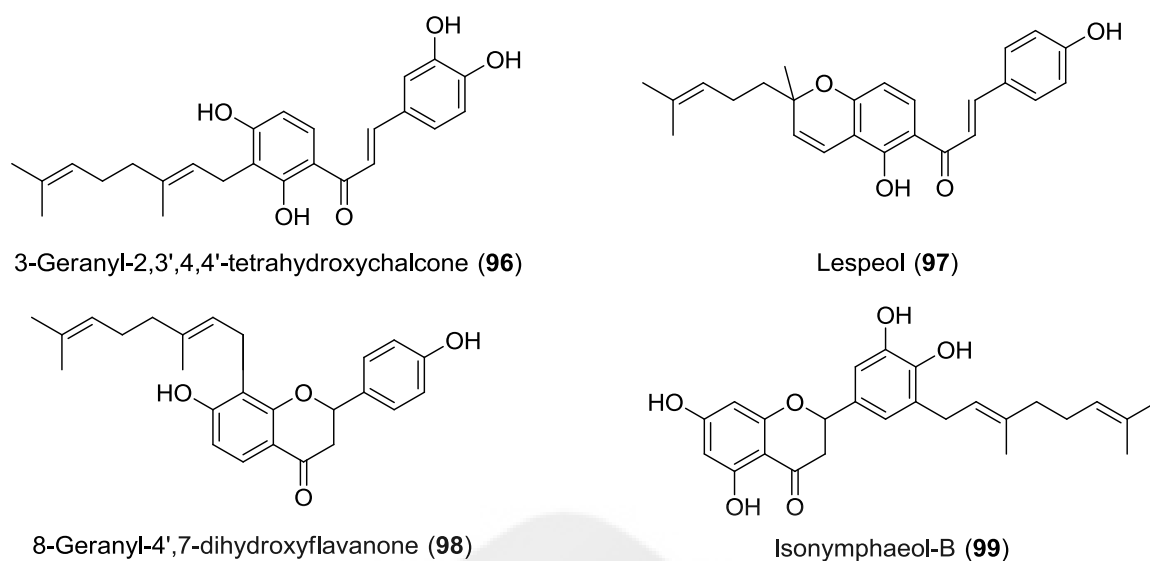
3'-Geranyl-4',5,7-trihydroxyflavanone (**93**)



Xanthoangelol (**94**)



Xanthoangelol B (**95**)

Figure 17 Structures of compounds **90-99**

In 2006, Namdaung et al. purified a new flavonoid, 7-demethylartonol E (**100**) and a new chromone, artorigidusin (**101**), along with four known phenolic compounds, including two xanthones, artanol B (**65**) and artoindonesianin C (**102**), and two flavonoids, cycloartobiloxanthone (**25**) and artonin F (**63**) (Figure 18). All of them were examined for antiplasmodial, antimycobacterial and cytotoxic activities as shown in Table 3 (Namdaung; et al. 2006: 1433-1436).

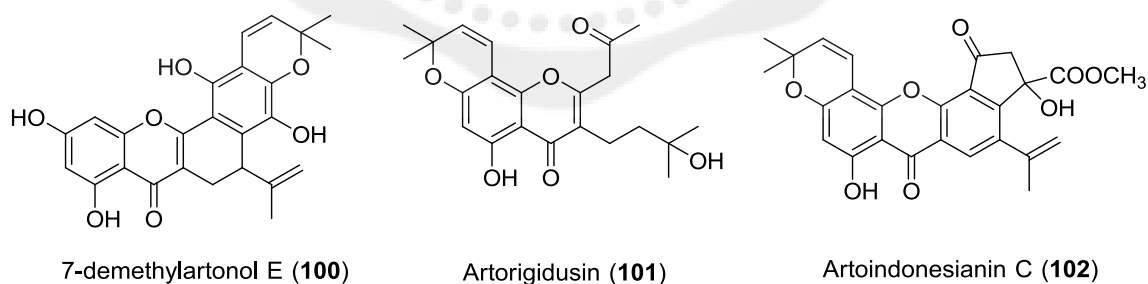
Figure 18 Structures of flavonoids **100-102**

Table 3 Antiplasmodial, antimycobacterial and cytotoxic activities of compounds **25**, **63**, **65**, and **100-102**

Compounds	Antiplasmodial (IC ₅₀ , µg/mL)	Antimycobacterial (IC ₅₀ , µg/mL)	Cytotoxicities (IC ₅₀ , µg/mL)		
			KB	BC	NCI-H187 ^a
25	3.7	25	8.56	4.23	11.83
63	2.4	6.25	inactive	inactive	inactive
65	inactive	100	inactive	inactive	1.26
100	7.9	50	inactive	inactive	5.7
101	inactive	12.5	inactive	12.10	15.63
102	inactive	12.5	8.4	7.7	7.1

^a human small cell lung cancer

The CHCl₃ extract of *A. champeden* heartwoods yielded two new prenylated flavones, artoindonesianins A-2 and A-3 (**103** and **104**), along with three known prenylated flavones, artonin B (**44**), cudraflavone C (**68**) and heterophyllin (**105**) (Figure 19). Cytotoxic activities against murine leukemia P-388 cells of all compound and three prenylated flavones, artoindonesianins Q, R and T (**9-12**) from previous report, were tested. Compounds **12**, **68** and **103-105** displayed strong cytotoxic activity with IC₅₀ values ranging of 1.3-2.8 µg/mL whereas the remaining compounds **9**, **10** and **44** were inactive (Syah; et al. 2006: 308-312).

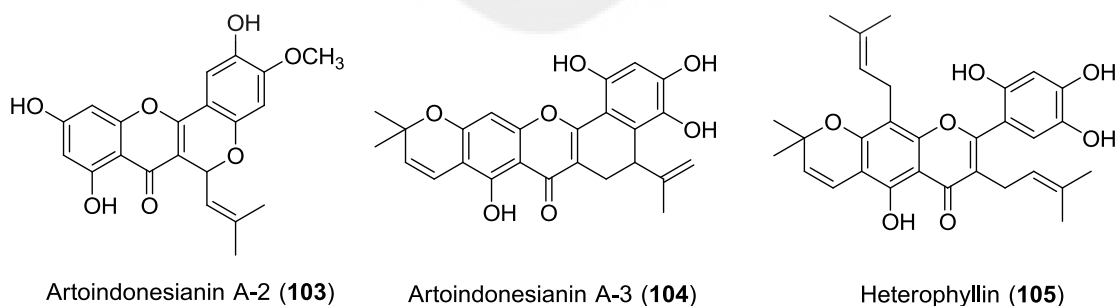


Figure 19 Structures of flavones **103-105**

In 2006, Wang et al. obtained three extracts (PE, EtOAc and *n*-BuOH extracts), from the leaves of *A. altilis*. Among these extracts, the EtOAc extract displayed

cytoprotective activities on human U937 cells, leading to the bioassay-guided fractionation of β -sitosterol (**106**) and six flavonoids, 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone (**107**), 8-geranyl-4',5,7-trihydroxy flavone (**108**), 2-geranyl-2',3,4,4'-tetrahydroxydihydrochalcone (**109**), cyclochampedol (**110**), 2',4',5-trihydroxy-7-methoxy-8-prenylflavone (**111**) and 2'-geranyl-3',4',7-trihydroxyflavone (**112**) (Figure 20) (Wang; et al. 2006: 1052-1055).

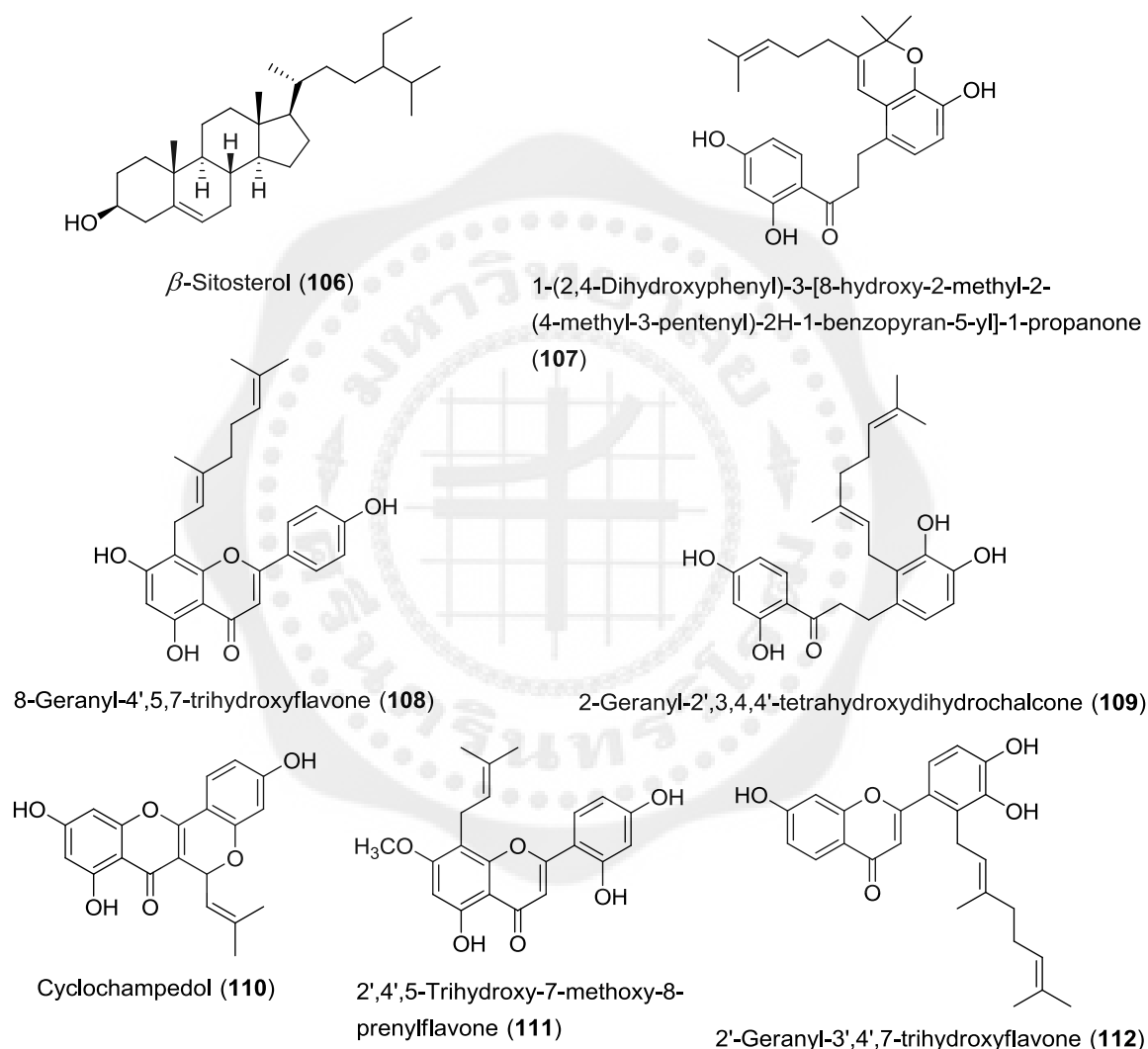


Figure 20 Structures of flavones **106-112**

In 2006, Weng et al. purified four flavonoids, dihydroartomunoxanthone (**113**), artomunoisoxanthone (**114**), cyclocomunomethanol (**115**) and artomunoflavanone (**116**), along with three known compounds, artocommunol CC (**15**), artochamins B and D (**46** and **48**) from the CHCl_3 extract of the heartroots of *A. communis* (Figure 21). All of these

constituents, except for compounds **48** and **116**, were tested for antiplatelet property on human platelet-rich plasma (PRP). It was found that compounds **46**, **48** and **113** had an effective on %inhibition of aggregation induced by adrenaline 51.0%, 65.6% and 46.8%, respectively, comparing to standard drug, aspirin (29.5%). This suggested that antiplatelet effect of the compound may mediated through the suppression of COX-1 which led to decrease thromboxane formation and develop on atherosclerosis in human (Weng; et al. 2006: 824-829).

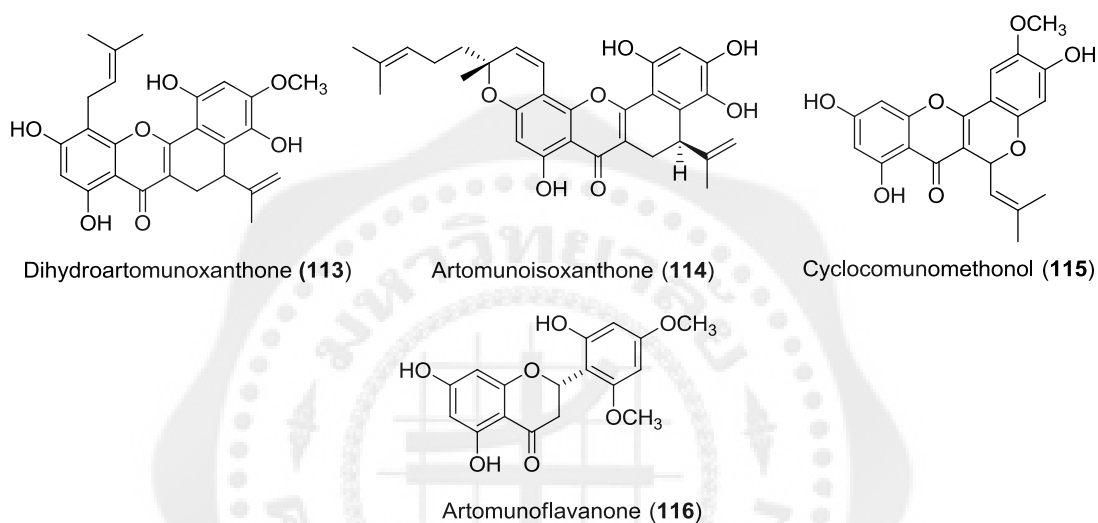


Figure 21 Structures of flavonoids **113-116**

In 2007, Boonphong et al. obtained nine prenylated flavones, including artocarpin (**50**), cycloartocarpin (**67**) and chaplashin (**117**) from the CH_2Cl_2 extract of the root stems, and artonin E (**22**), artobiloxanthone (**24**), cycloartobiloxanthone (**25**), cudraflavone C (**68**), morusin (**118**) and cudraflavone B (**119**) from the root barks of *A. altalis* (Boonphong; et al. 2007: 339-344) (Figure 22). All constituents were tested for antitubercular, antiplasmodial and cytotoxic activities as shown in Table 4.

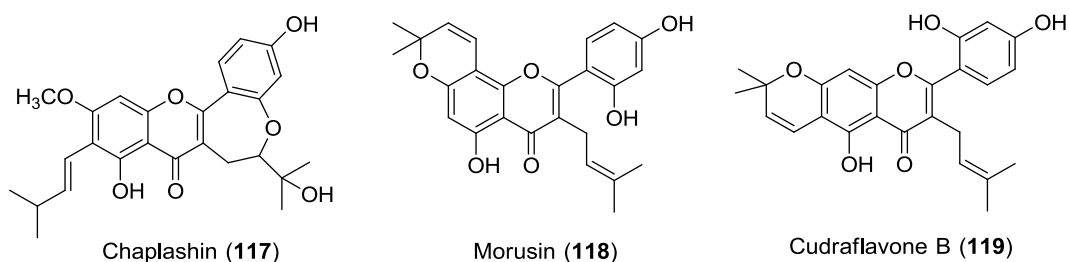


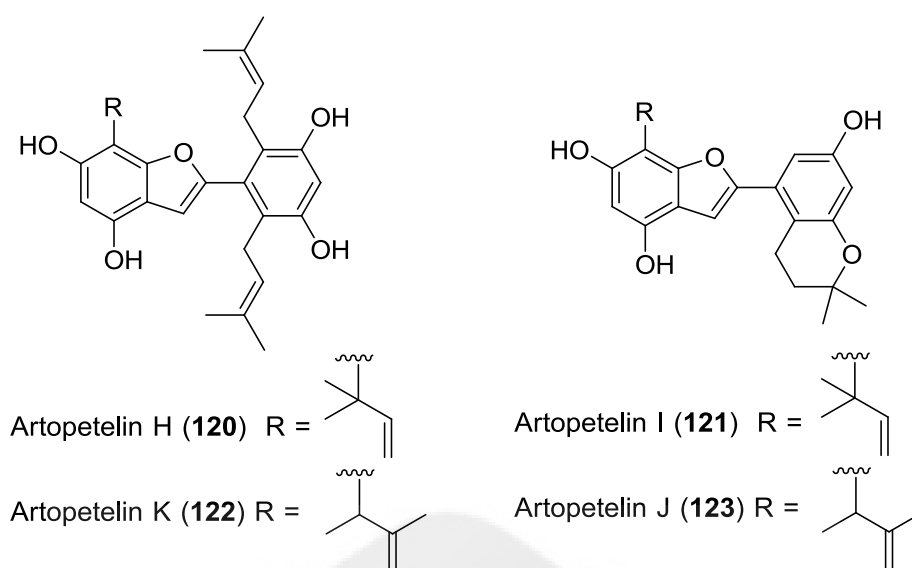
Figure 22 Structures of prenylated flavones **117-119**

Table 4 Antiplasmodial, antimycobacterial and cytotoxic activities of all compounds

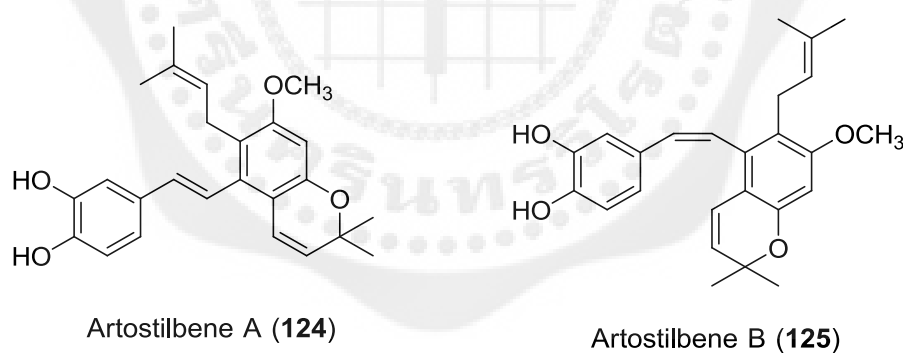
Compounds	Antiplasmodial (IC ₅₀ , µg/mL)	Antimycobacterial (IC ₅₀ , µg/mL)	Cytotoxicity (IC ₅₀ , µg/mL)		
			KB	BC	Vero ^a cells
22	100	2.8 ± 0.3	9.8 ± 0.6	3.5 ± 0.2	6.1 ± 2.8
24	50	3.0 ± 0.4	9.3 ± 1.6	4.9 ± 0.2	7.4 ± 1.7
25	50	2.3 ± 0.6	8.7 ± 0.8	4.1 ± 0.04	7.2 ± 1.5
50	3.12	3.0 ± 0.2	5.1 ± 1.0	3.3 ± 0.6	5.6 ± 2.0
67	12.5	4.3 ± 0.7	5.5 ± 1.1	3.1 ± 0.1	5.5 ± 1.6
68	12.5	^b	8.5 ± 2.5	2.9 ± 0.5	7.4 ± 2.5
117	3.12	3.5 ± 0.4	13.4 ± 0.4	5.5 ± 0.05	8.0 ± 1.9
118	25	1.9 ± 0.2	6.9 ± 2.1	3.3 ± 1.1	10.1 ± 3.2
119	25	2.2 ± 0.3	7.9 ± 1.8	5.2 ± 0.4	14.7 ± 6.2
Rifampicin ^c	0.0023	-	-	-	-
kanamycin ^c	2.5	-	-	-	-
isoniazide ^c	0.1	-	-	-	-
dihydroartemisinin ^c	-	0.0012	-	-	-
ellipticine ^c	-	-	0.2 ± 0.1	0.27 ± 0.1	0.4 ± 0.1

^a African green monkey kidney fibro-blasts; ^b not determined; ^c standard drug

In 2007, Shen et al. obtained four new prenylated 2-arylbenzofurans, artopetelins H-K (**120-123**) (Figure 23), from the root barks of *A. petelotii* (Shen; Hou; & Li. 2007: 1147-1154).

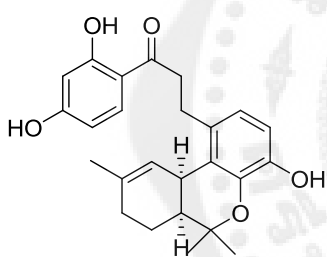
Figure 23 Structures of artopetelins H-K (**120-123**)

In 2007, Wang et al. studied on the isolation of two new isoprenylated stilbenes, artostilbenes A and B (**123** and **124**) (Figure 24), from the CHCl_3 extract of the stems of *A. chama* (Wang; Hou; & Chen. 2007: 605-608).

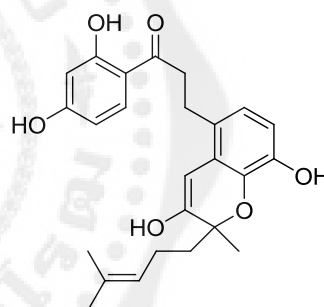
Figure 24 Structures of artostilbenes A and B (**124** and **125**)

In 2007, Wang et al. found five new geranylated 1-(2,4-dihydroxyphenyl)-3-{4-hydroxy-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6H-dibenzo[*b,d*]pyran-5-yl}-1-propanone (**126**), 1-(2,4-dihydroxyphenyl)-3-[3,4-dihydro-3,8-dihydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone (**127**), 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(3,4-epoxy-4-methyl-1-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone (**128**), 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-hydroxy-4-methyl-2-pentenyl)-2H-1-benzopyran-

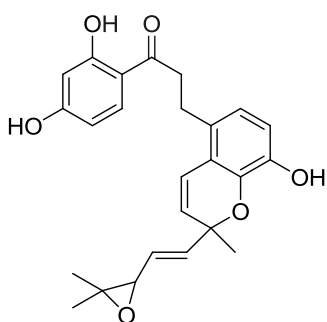
5-yl]-1-propanone (**129**), and 2-[6-hydroxy-3,7-dimethylocta-2(*E*),7-dienyl]-2',3,4,4'-tetrahydroxydihydrochalcone (**130**), together with four known geranyl flavonoids, cycloaltisin 6 (**4**), 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-1-benzopyran-5-yl]-1-propanone (**107**), 2-geranyl-2',3,4,4'-tetrahydroxydihydrochalcone (**109**), 2'-geranyl-3',4',7-trihydroxyflavanone (**112**) (Figure 25), from the EtOAc extract of the leaves of *A. altilis*. Only five new constituents were examined for human lung adenocarcinoma cells (SPC-A-1), human colon carcinoma cells (SW-480), and human hepatocellular carcinoma cells (SMMC-7721). Compounds **126**, **127**, and **130** exerted moderate cytotoxicity against all cancer cell lines with IC₅₀ values ranging of 11.4-28.5 µg/mL. In the contrast, compounds **128** and **129** were inactive for all tests. In addition, compound **126** was the most active effects for SPC-A-1 and SW-480 cell lines and also had a greater activity than standard drug, 9-fluorouracil (IC₅₀ 3.4-6.8 µg/mL) (Wang; et al. 2007: 1300-1306).



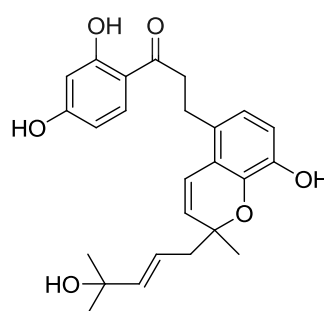
1-(2,4-Dihydroxyphenyl)-3-{4-hydroxy-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6H-dibenzo[b,d]pyran-5-yl}-1-propanone (**126**)



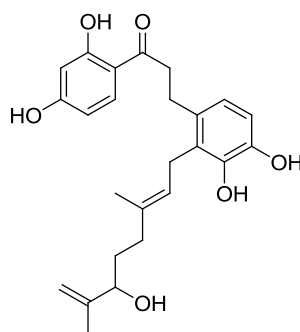
1-(2,4-Dihydroxyphenyl)-3-[3,4-dihydro-3,8-dihydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-1-benzopyran-5-yl]-1-propanone (**127**)



1-(2,4-Dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(3,4-epoxy-4-methyl-1-pentenyl)-2*H*-1-benzopyran-5-yl]-1-propanone (**128**)



1-(2,4-Dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-hydroxy-4-methyl-2-pentenyl)-2*H*-1-benzopyran-5-yl]-1-propanone (**129**)



2-[6-Hydroxy-3,7-dimethylocta-2(E),7-dienyl]-2',3,4,4'-tetrahydroxydihydrochalcone (**130**)

Figure 25 Structures of dihydrochalcones and flavanone **126-130**

The CH₂Cl₂ extract of *A. champeden* stem barks exhibited *in vitro* antimalarial activity against *P. falciparum* 3D7 (IC₅₀ 0.99 µg/mL) and *in vivo* activity against *P. berghei* ANKA strain (an ED₅₀ value of 0.03 mg/kg mouse body weight). The CH₂Cl₂ extract was then purified to yield two new prenylated flavones, artocarpones A and B (**131** and **132**), along with seven known prenylated flavonoids, artonin A (**34**), cycloheterophyllin (**133**), artoindonesianin E (**134**), artoindonesianin R (**10**), heterophyllin (**105**), heteroflavanone C (**135**) and artoindonesianin A-2 (**136**) (Figure 26). All compounds were evaluated for antimalarial activity against *P. falciparum*. Compound **135** displayed the strongest effect with IC₅₀ value of 0.001 µmol/L, followed by compounds **10**, **34**, **105**, **131-133** and **136** had IC₅₀ value in the range of 0.02-1.31 µmol/L and compound **134** exerted the lowest inhibitory activity with IC₅₀ value of 75.3 µmol/L (Widyawaruyanti; et al. 2007: 410-413).

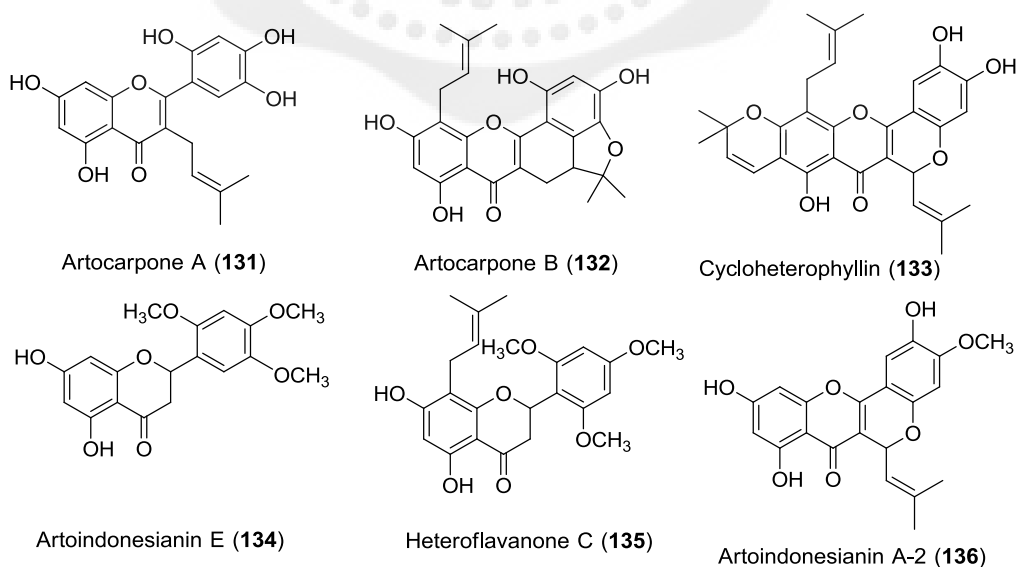


Figure 26 Structures of flavonoids **131-136**

In 2008, Amarasinghe et al. purified three stilbenes, oxyresveratrol (**3**), artoindonesianin F (**137**) and (3-methyl-2-butenyl)-(*E*)-2,3',4,5'-stilbenetetrol (**138**), a known 2-arylbenzofuran, moracin M (**139**), a known flavanone, norartocarpanone (**140**), three known flavones, norartocarpesin (**29**), isoartocarpesin (**141**) and 2',4',5,7-tetrahydroxy-6-(3-methyl-2-butenyl)flavone (**142**), two triterpenes, 3 β -acetoxyolean-12-en-11-one (**143**) and cycloartenyl acetate (**144**) and two sterols, sitosterols (**106**), sitosterol β -D-glucopyranoside (**145**) from the fruits of *A. altilis* (Figure 27) (Amarasinghe; et al. 2008: 323-325).

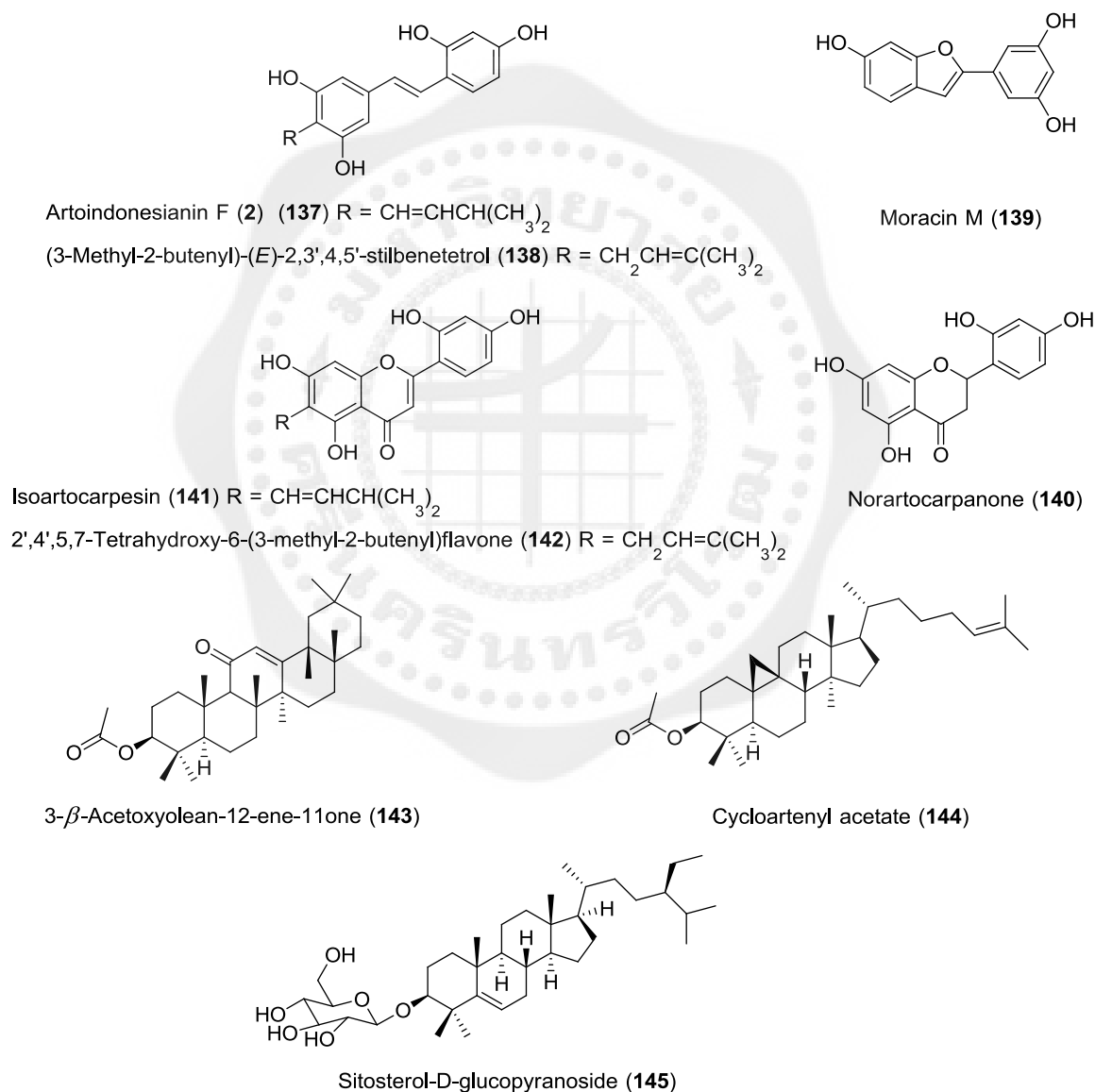


Figure 27 Structures of compounds **137-145**

In 2008, Fang et al. isolated three new geranylated chalcones, isolespeol (**146**), 5'-geranyl-2',4',4-trihydroxychalcone (**147**) and 3,4,2',4'-tetrahydroxy-3'-geranyldihydrochalcone (**148**), together with two known compounds, xanthoangelol (**94**) and lespeol (**95**) (Figure 28), from the leaves of *A. communis*. All compounds, except for compound **95**, were tested for the *in vitro* anticancer activities on seven human cancer cells, including human liposarcoma cells (SW 872), human hepatoblastoma cells (HepG2 and Hep3B), human colorectal carcinoma cells (COLO 205 and HT-29), human hepatocellular carcinoma cells (PLC5 and Huh7). From the results, compound **146** displayed the strongest activities for all cell line tests (Fang; et al. 2008: 8859-8868). Anticancer activities of the compounds were shown in Table 5.

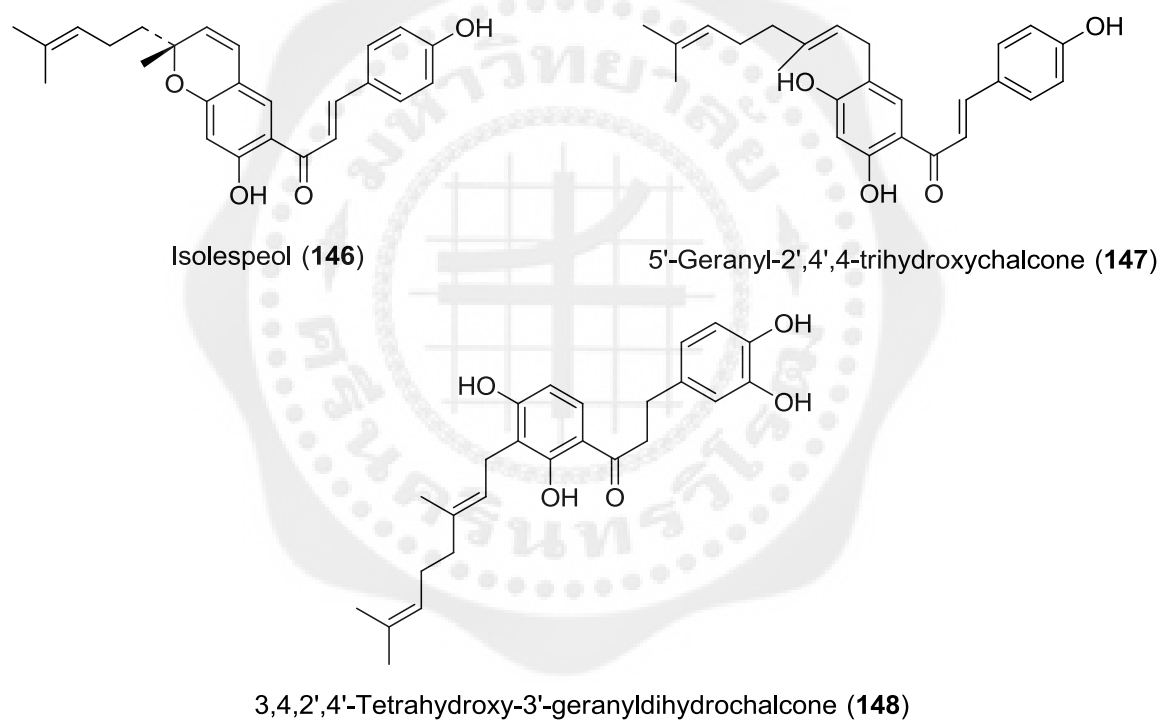


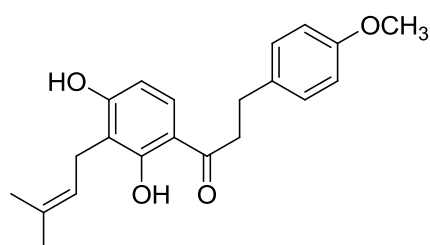
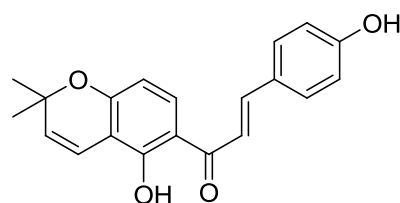
Figure 28 Structures of chalcones **146** and **147**, and dihydrochalcone **148**

Table 5 Anticancer activities of compounds **90** and **146-148** on various human cancer cells

Cell lines	Compounds (IC ₅₀ , µg/mL)			
	94	146	147	148
SW 872	1.7 ± 0.4	1.4 ± 0.2	1.6 ± 0.7	1.7 ± 0.8
HT-29	6.3 ± 0.5	4.2 ± 1.1	4.5 ± 0.9	8.0 ± 3.5
COLO 205	4.7 ± 1.1	6.3 ± 0.8	^a	5.1 ± 0.3
Hep3B	2.3 ± 0.4	6.6 ± 0.2	1.5 ± 0.1	5.6 ± 1.2
PLC5	4.3 ± 0.7	3.3 ± 0.0	3.4 ± 0.2	
Huh7	3.7 ± 0.0	6.8 ± 0.0	3.5 ± 0.3	
HepG2	7.1 ± 0.4		8.6 ± 0.3	

^a IC₅₀ value > 9.8 µg/mL

Chemical investigation of the CH₂Cl₂ extract of the leaves of *A. lowii* furnished a new prenylated dihydrochalcone, 2',4'-dihydroxy-4-methoxy-3'-prenyldihydrochalcone (**149**), together with two known compounds, 2',4',4'-trihydroxy-3'-prenylchalcone (**82**) and 2',4'-dihydroxy-3',4'-(2,2-dimethylchromene)chalcone (**150**) by Jamil in 2008 (Figure 29). All compounds were evaluated for antioxidant effect. From the results, compound **82** was more active with possessed strong DPPH-scavenging activity with 60.2% than compounds **149** and **150** with 41.6% and 48.6%, respectively. Vitamins C and E were used as positive control with 61.9% and 64.4%, respectively. Moreover, compounds **82**, **149** and **150** had IC₅₀ values of 9.7, 81.6 and 48.3 µg/mL, respectively (Jamil; et al. 2008: 321-324).

2',4'-Dihydroxy-4-methoxy-3'-prenyldihydrochalcone (**149**)2',4'-Dihydroxy-3',4'-(2,2-dimethylchromene)chalcone (**150**)Figure 29 Structures of dihydrochalcone **149** and chalcone **150**

In 2008, Jayasinghe et al. found four new prenylated flavones, artonin E 2'-methylether (**151**), isoartonin E 2'-methylether (**152**), dihydroisoartonin E 2'-methylether (**153**), artonin V 2'-methylether (**154**), together with three known flavones artonin E (**22**), artobiloxanthone (**24**) and cycloartobiloxanthone (**25**) (Figure 30) from the root barks of *A. nobilis*. All compounds exhibited strong anti-DPPH radical activity by TLC bio-autography method (Jayasinghe; et al. 2008: 37-41).

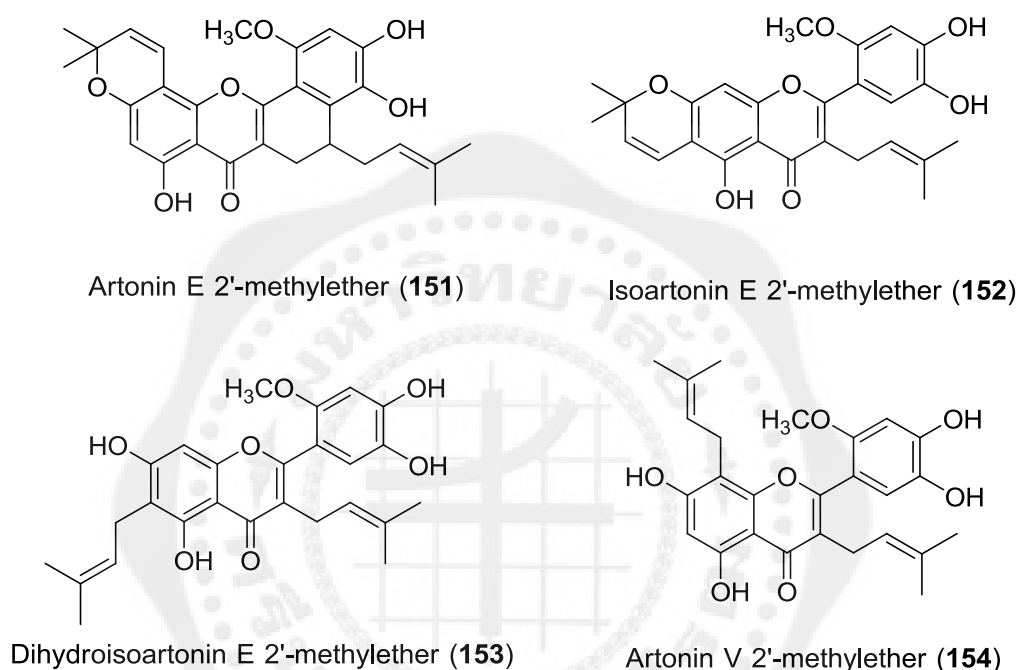
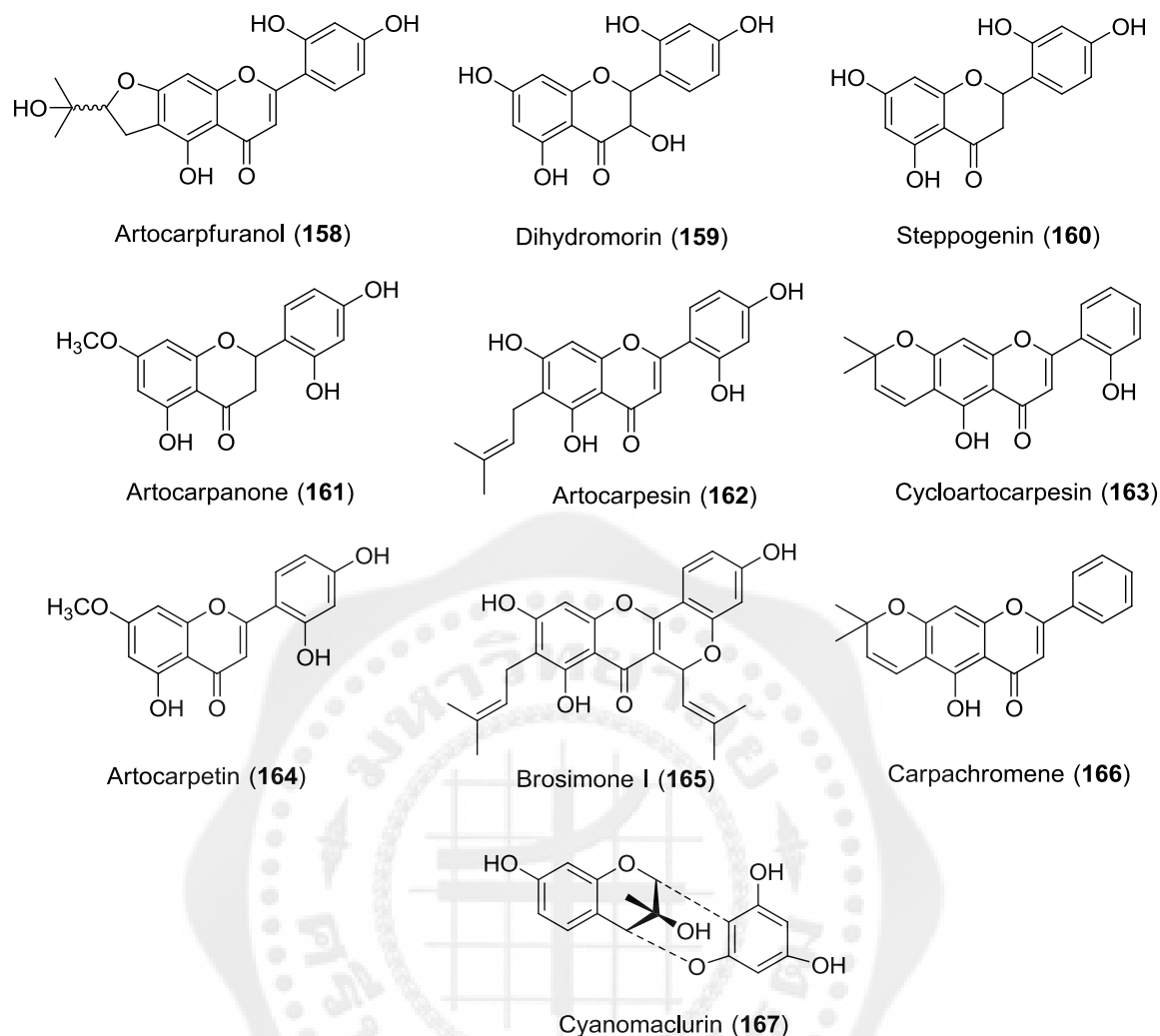


Figure 30 Structures of prenylated flavones **151-154**

In 2008, Lotulung et al. found a prenylated flavonoid, 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]1-propanone (**107**) from the CH_2Cl_2 extract of *A. communis* leaves (Figure 20). This compound was found to be active on cytotoxicity against murine P-388 leukemia cells with IC_{50} value of $6.7 \mu\text{g/mL}$ (Lotulung; et al. 2008: 2517-2520).

In 2008, Shen et al. isolated two new prenylated 2-arylbenzofurans, artopetelins L and M (**155** and **156**), together with a known, lakoochin B (**157**) from *A. petelotii* (Figure 31). Furthermore, artopetelins A-E (**55-57**, **77** and **78**) and G (**80**) were obtained previously from this plant. All of constituents, except for **155** and **156**, were tested for cytotoxicity against HepG2 and MCF-7 cells. From the results, compounds **56**, **57**, **77**, **78** and **157**

The EtOH extract of *A. heterophyllus* woods gave a new furanoflavone, artocarpfuranol (**158**), together with 14 known compounds, norartocarpetin (**29**), artocarpin (**50**), cycloartocarpin (**67**), cudraflavone B (**119**), isoartocarpesin (**140**), dihydromorin (**159**), steppogenin (**160**), artocarpanone (**161**), artocarpesin (**162**), cycloartocarpesin (**163**), artocarpetin (**164**), brosimone I (**165**), carpachromene (**166**) and cyanomaclurin (**167**) (Figure 28). All compounds were evaluated for tyrosinase inhibitory effect. Kojic acid was used as positive control with IC₅₀ value of 10.1 µg/mL in this study. Several constituents (**29**, **158-162**, **165** and **167**) exhibited stronger activity with IC₅₀ value in the range of 0.1-19.7 µg/mL than kojic acid (IC₅₀ 10.1 µg/mL) (Zheng; et al. 2008: 1530-1538).

Figure 32 Structures of compounds **158-167**

In 2009, Lin et al. purified two new prenylflavonoids, cyclogeracommunin (**168**) and artoflavone A (**169**), together with five known flavonoids, artocommunol CC (**15**), artochamin B (**46**), artochamin D (**48**), dihydroartomunoxanthone (**113**), and artomunoisoxanthone (**170**), from the CHCl_3 extract of the heartroots of *A. communis*. Meanwhile, four known flavonoids, cycloartelastoxanthone (**60**), artelastoheterol (**171**), cycloartobiloxanthone (**25**), artonol A (**64**) (Figure 33), were isolated from *A. elasticus*. All compounds were examined for protective effect on DNA damage caused by $\text{O}_2^{\cdot-}$. It was found that compounds **25**, **60**, **64**, **168**, **169** and **171** exhibited potent the effect. Compounds **25**, **60**, **169** and **171** were further tested for anti-DPPH scavenging activity. From the results, antioxidant property of compounds **25**, **60**, **169** and **171** were IC_{50} values of 11.6, 10.6, 10.8 and 21.9 $\mu\text{g/mL}$, respectively (Lin; 2009: 558-562).

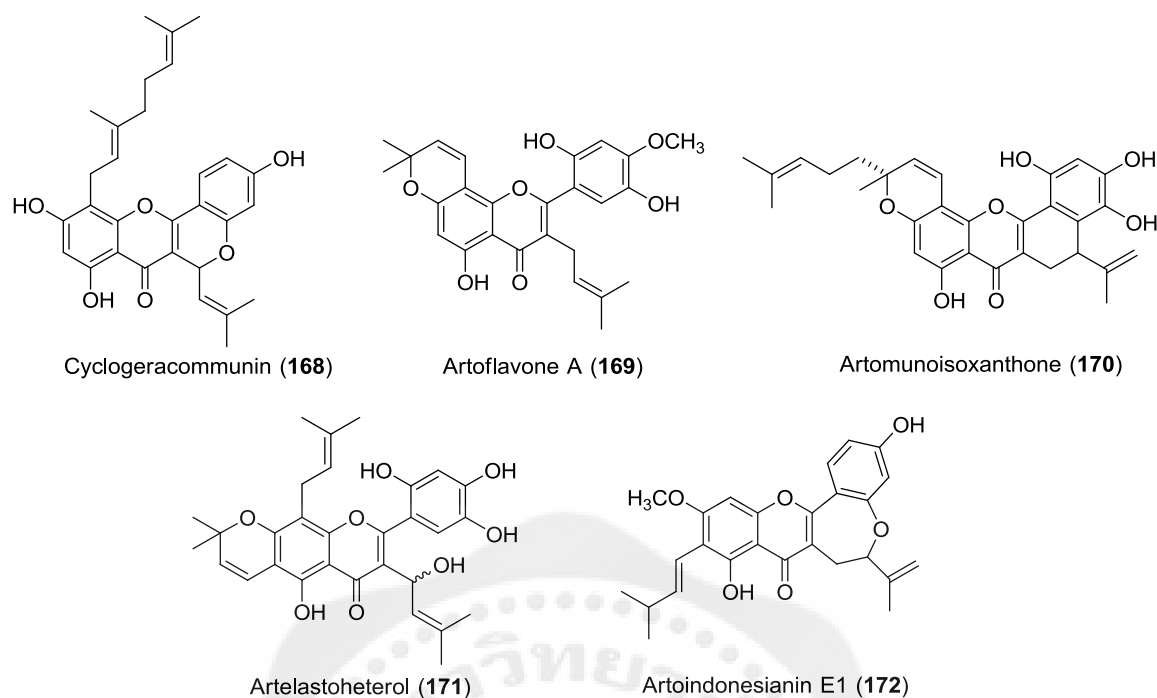


Figure 33 Structures of flavonoids **168-172**

The MeOH extract of *A. elasticus* woods yielded a new oxepinoflavone, artoindonesianin E1 (**172**), along with four known prenylated flavones, artocarpin (**50**), cudraflavone A (**52**), cycloartocarpin (**67**) and cudraflavone C (**68**) (Figure 30). Only compound **172** was tested for cytotoxic activity against murine leukemia P-388 cells because compound **50**, **52**, **67** and **68** were reported in previous investigation. The compound exhibited moderate activity with IC_{50} value of 5.0 $\mu\text{g/mL}$ (Musthapa; et al. 2009: 191-194).

In 2009, Zhao et al. obtained an active CHCl_3 extract of *A. nitidus* stems which showed pancreatic lipase (PL) inhibitory activity *in vitro*. The extract gave two new prenylated 2-arylbenzofurans, artonitidins A and B (**173** and **174**), along with 14 known compounds, artoindonesianin X (**26**), artocarpin (**50**), cycloartocarpin A (**51**), cudraflavones A and C (**52** and **68**), norartocarpin (**75**), albanin A (**76**), chaplashin (**117**), morusin (**118**), cycloartocarpesin (**163**), brosimone I (**165**), artotonkin (**175**), artopetelin M (**156**) and 2,4,2',4'-tetrahydroxy-3'-(3-methylbut-2-en-1-yl)chalcone (**176**) (Figure 34). All compounds, except for compounds **117**, **163**, **173** and **174** were examined for PL inhibitory activity. Compound **75** showed the strongest activity against PL with an IC_{50} value of 0.76 $\mu\text{g/mL}$, followed by **165** (IC_{50} 1.4 $\mu\text{g/mL}$) and **175** (IC_{50} 1.1 $\mu\text{g/mL}$). Compound **68** also exerted

moderate activity (IC_{50} 6.9 $\mu\text{g/mL}$) whereas compounds **76** and **156** showed weak inhibitory activity with IC_{50} values of 22.5 and 18.9 $\mu\text{g/mL}$, respectively. Compounds **26**, **50-52** and **118** did not show the activity. In the tests, orlistat was used as positive control with IC_{50} value of 0.35 $\mu\text{g/mL}$ (Zhao; et al. 2009: 2209-2216).

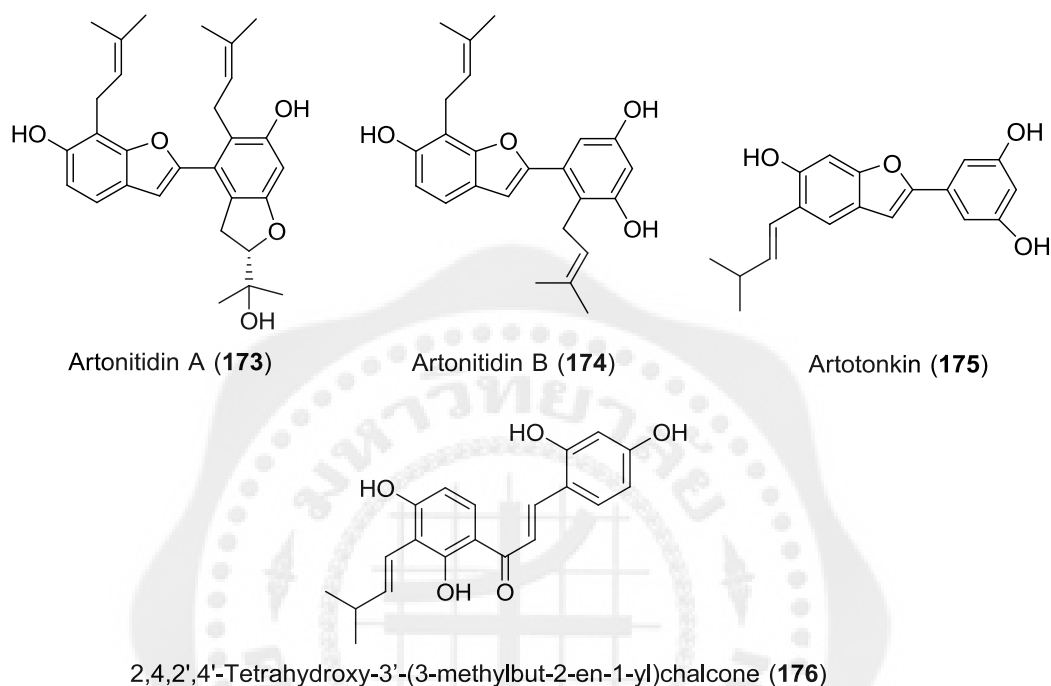


Figure 34 Structures of 2-arylbenzofurans **173-175** and chalcone **176**

In 2009, Zheng et al. isolated a new prenylated 2-arylbenzofuran, artoheterophyllin A (**177**) and three new prenylated flavones, artoheterophyllin B (**178**), artoheterophyllin C (**179**) and artoheterophyllin D (**180**), together with 16 known compounds, norartocarpetin (**29**), artonin A (**34**), artocarpin (**50**), licoflavone C (**86**), cudraflavone B (**119**), cycloheterophyllin (**133**), moracin M (**139**), artocarpesin (**162**), dihydrophaseic acid 4'-O- β -D-glucopyranoside (**181**), *p*-counmaric acid (**182**), vanillic acid (**183**), 4-hydroxybenzoic acid (**184**), (*E*)-5-(6-hydroxybenzofuran-2-yl)-4-(3-methylbut-1-en-1-yl)benzene-1,3-diol (**185**), 6-prenyl-4',5,7-trihydroxyflavone (**186**), artonin J (**187**), and 2-(2,4-dihydroxy-6-methoxyphenyl)-5-hydroxy-7-methoxy-6-(3-methyl-1-buten-1-yl)-3-(3-methyl-2-buten-1-yl)-4*H*-1-benzopyran-4-one (**188**) (Figure 35), from the EtOH extract of the twigs of *A. heterophyllus*. All of them were examined for tyrosinase inhibitory activity. Compounds **29** and **162** exerted strong inhibition with IC_{50} values of 0.2 and 0.6 $\mu\text{g/mL}$, respectively,

followed by compounds **139** and **185** displayed moderate activity with IC_{50} values of 14.0 and 23.9 $\mu\text{g/mL}$, respectively. Kojic acid was used as positive control with IC_{50} value of 6.7 $\mu\text{g/mL}$ (Zheng; et al. 2009: 6649-6655).

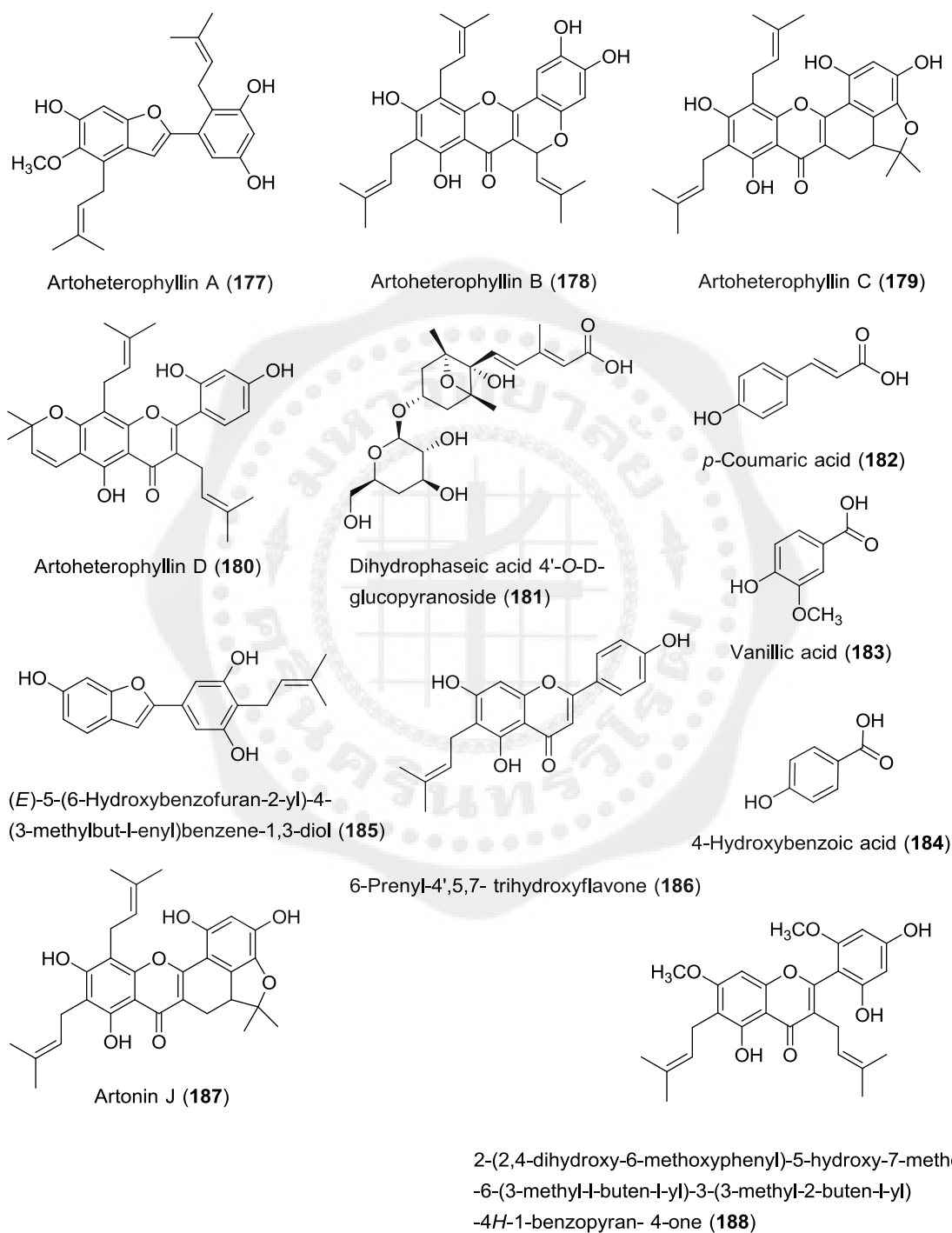
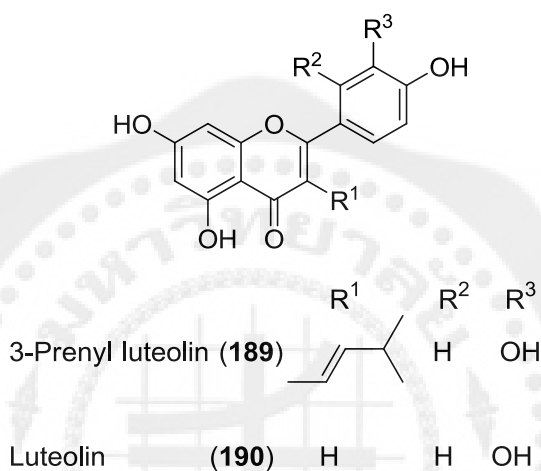


Figure 35 Structures of compounds **177-188**



In 2010, Bourjot et al. investigated on the purification of two new prenylated flavonoids, styracifolins A and B (**191** and **192**), together with six known constituents, artonins A (**34**), B (**44**) and F (**63**), heterophyllin (**105**), artoheterophyllins A and B (**177** and **178**) (Figure 37), from the active EtOAc extract which possessed antiplasmodial activity. All compounds were tested for cytotoxic assays against KB and human diploid embryonic lung cells (MRC-5), antiplasmodial assay against the chloroquine FcB1 resistant strain, and antitrypanosomal assay against the procyclic form (insect stage) of *Trypanosoma brucei brucei*. Compounds **105** and **192** showed the highest cytotoxic activities against both KB and MRC-5 cell lines. Moreover, all compounds exhibited antiplasmodial activity with IC₅₀ values ranging of 0.5-6.9 µg/mL. For antitrypanosomal activity, compounds **44**, **105**, **191** and **192** exhibited higher activity with IC₅₀ values ranging of 3.3-6.7 µg/mL than the standard drug, chloroquine with IC₅₀ value of 47.9 µg/mL (Bourjot; et al. 2010: 1600-1604).

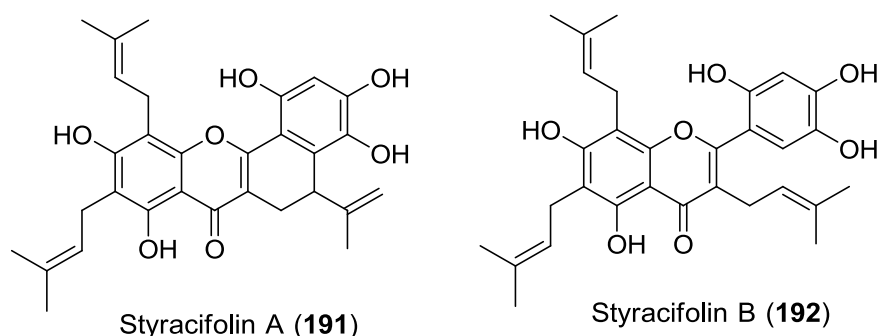


Figure 37 Structures of prenylated flavones **191** and **192**

In 2010, Hashim et al. purified two new flavones, pyranocycloartobiloxanthone A (**193**) and dihydroartoindonesianin C (**194**) (Figure 38), from the CHCl_3 extract of the stem barks of *A. obtusus*. Compound **193** showed strong on antioxidant activity with IC_{50} value of 2.0 $\mu\text{g/mL}$. Furthermore, it exhibited highly active cytotoxicities against three cell lines, K562, HL-60, and MCF7 with IC_{50} values of 0.5, 2.0 and 5.0 $\mu\text{g/mL}$, respectively (Hashim; et al. 2010: 106-112).

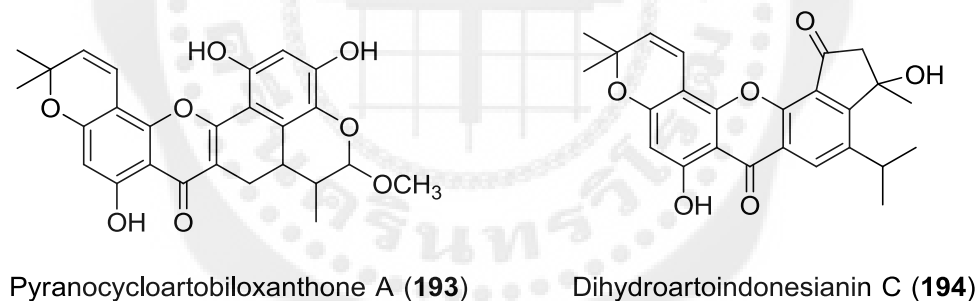


Figure 38 Structures of flavonoids **193** and **194**

In 2010, Iverson et al. investigated on the purification of four known triterpenoids, cyclolaudenyl acetate (**195**), lupeol acetate (**196**), β -amyrine acetate (**197**), and zizphursolic acid (**198**), along with five known flavonoids, artonin E (**22**), artobiloxanthone (**24**), artoindonesianin U (**40**), cyclocommunol (**199**) and multiflorins A (**200**) from the EtOH extract of *A. nobilis* (Figure 39). All compounds were tested for anti-GST (glutathione S-transferase) and anti-leishmanial activities. Compounds **22**, **24**, **40**, **199** and **200** showed significant anti-GST effect with IC_{50} values in the range of 0.4-3.4 $\mu\text{g/mL}$, comparing to ethacrynic acid, a standard GST inhibitor, with IC_{50} value of 4.5 $\mu\text{g/mL}$. For anti-leishmanial

activity, all compounds exhibited the lower activity with IC_{50} value in the range of 25.0-50.2 $\mu\text{g/mL}$ than positive control, miltefosine (IC_{50} 0.4 $\mu\text{g/mL}$). Furthermore, only compounds **22**, **24**, **40**, **199** and **200** were selected for antioxidant effect. It was found that all compounds showed greater activity with IC_{50} value in the range of 5.3-14.0 $\mu\text{g/mL}$ than quercetin (IC_{50} 17.3 $\mu\text{g/mL}$) (Iverson; et al. 2010: 207-211).

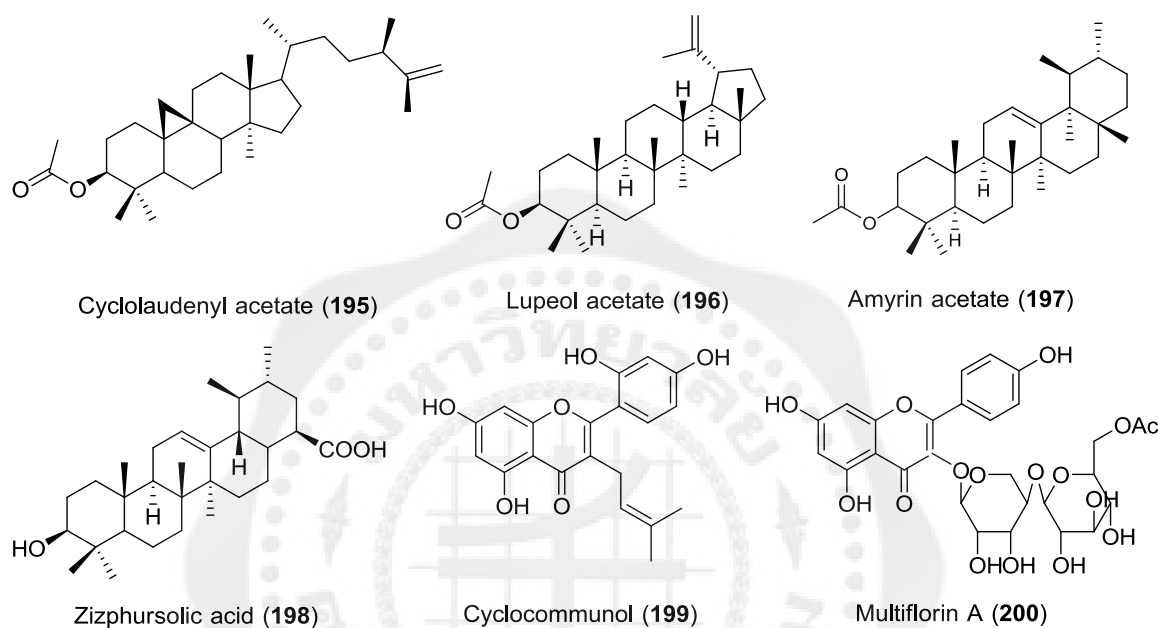


Figure 39 Structures of compounds **195-200**

In 2010, Jantan isolated seven known constituents, including five prenylated flavonoids and two prenylated chalcones. Cycloartobiloxanthone (**25**) and artonol B (**65**) from *A. teysmanii*, isobavachalcone (**82**), cycloheterophyllin (**133**) and 2',4'-dihydroxy-4-methoxy-3'-prenyldihydrochalcone (**149**) from *A. lowii*, and artonin E (**22**) and artobiloxanthone (**24**) from *A. scortechinii* were obtained. Selected compounds **22** and **133** were further acetylated to provided cycloheterophyllin diacetate (**201**) and artonin E tiracetate (**202**) (Figure 40), respectively. All nine compounds were tested on the platelet aggregation induced by arachidonic acid (AA), adenosinediphosphate (ADP) and collagen. Compound **133** displayed strong inhibition against AA and ADP-induced aggregations with %inhibitory properties of 66.7% and 85.7% at 100 $\mu\text{g/mL}$. Compounds **22**, **25**, **82**, **149** and **201** had selective inhibition against ADP-induced aggregation with inhibitory activity of > 55% at 100 $\mu\text{g/mL}$. However, none of compound tested appeared against collagen-induced

aggregation. Furthermore, the IC_{50} values of compound **133** against AA and ADP-induced aggregations were 50.6 and 28.6 $\mu\text{g/mL}$, respectively. Meanwhile, IC_{50} values of compounds **25**, **82**, **133**, **201** and **202** were ranging from 31.0 to 83.7 $\mu\text{g/mL}$ against ADP-induced aggregation. The IC_{50} value of aspirin, a positive control, was 4.2 $\mu\text{g/mL}$ against AA-induced aggregation (Jantan; 2010: 365-369).

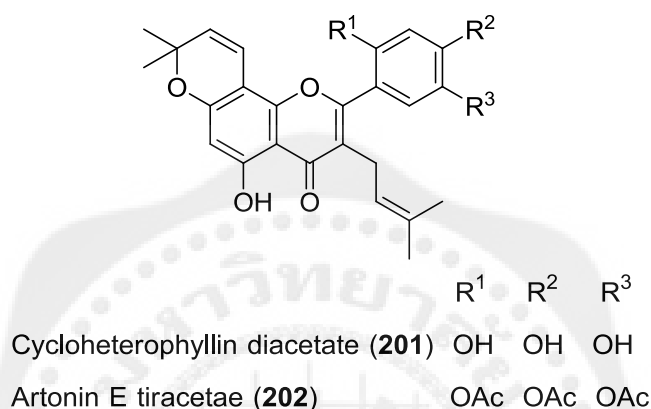


Figure 40 Structures of prenylated flavones **201** and **202**

In 2010, Ren et al. found four new prenylated flavonoids, artorigidins A-C (**203-205**) and cyclorigidol (**206**), and a new stilbenoid, artoristilbene (**207**), together with three known compounds, artobiloxanthone (**24**), cycloartobiloxanthone (**25**) and artonin O (**208**) from the twigs of *A. rigida* (Figure 41). Several of them were tested for cytotoxicity against HT-29 and two assays of $\text{NF-}\kappa\text{B}$ p50 and p6. Compounds **25**, **204** and **208** possessed the most active compounds for cytotoxicity against HT-29 cell line with ED_{50} values of 1.4, 1.6 and 1.8 $\mu\text{g/mL}$, respectively. Meanwhile, compound **203** (IC_{50} 1.0 $\mu\text{g/mL}$) and compound **24** (IC_{50} 0.9 $\mu\text{g/mL}$) showed the strongest effect in the $\text{NF-}\kappa\text{B}$ p50 and p65 assays, respectively (Ren; et al. 2010: 949-955).

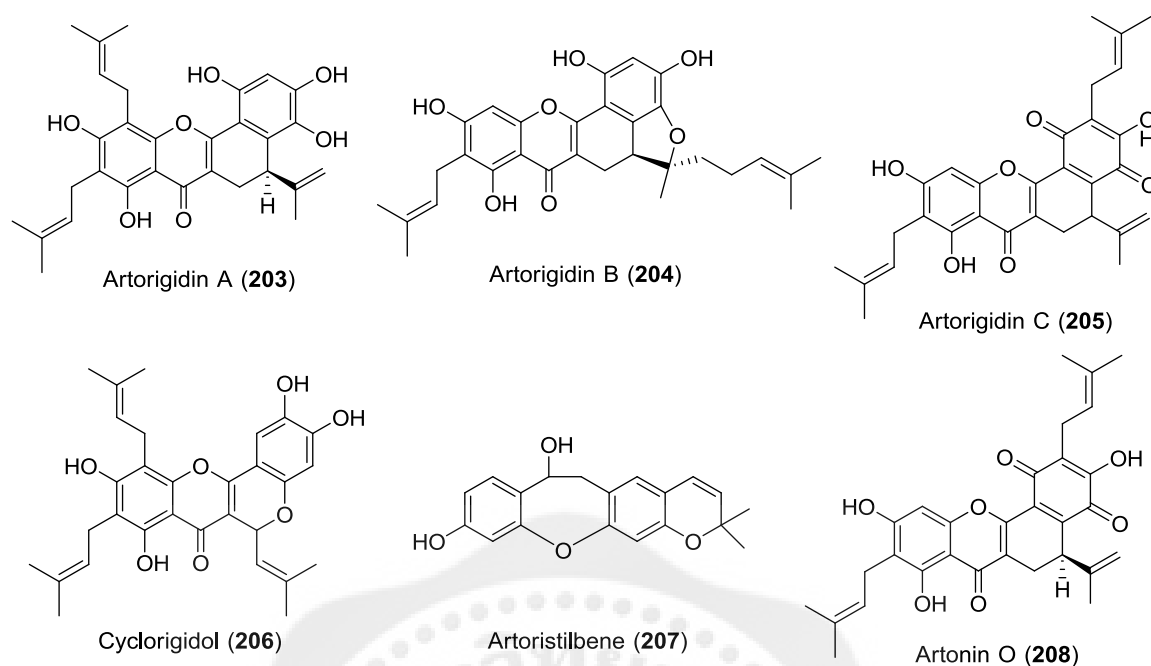


Figure 41 Structures of compounds **203-208**

In 2010, Shamaun et al. reported the isolation and structural determination of a new flavone, hydroxyartocarpin (**209**), and five known flavones, cycloartobiloxanthone (**25**), artoindonesianin V (**41**), artocarpin (**50**), cycloartocarpin A (**51**) and morusin (**118**) from the CHCl_3 extract of the stem barks of *A. altilis* (Figure 42) (Shamaun; et al. 2010: 478-481).

In 2010, Sritularak; et al. studied on the antioxidant effect of the MeOH extract of *A. gomezianus* stem barks using DPPH property, resulting in the isolation of four known flavonoids, including artonin E (**22**), artobiloxanthone (**24**), cycloartobiloxanthone (**25**) and (+)-catechin (**210**) (Figure 42). Evaluation of all isolates for DPPH radical scavenging activity and inhibition against NO production was carried out. All compounds, except for **25**, exhibited anti-DPPH radical activity with IC_{50} ranging of 2.7-3.9 $\mu\text{g/mL}$, comparing to the positive controls, quercetin and trolox (IC_{50} 1.4 and 2.5 $\mu\text{g/mL}$, respectively). Furthermore, compounds **22**, **24** and **25** showed inhibition effect on NO production with IC_{50} values of 7.1, 5.8 and 13.4 $\mu\text{g/mL}$, respectively, whereas **210** was inactive with comparable to those of the positive control, costunolide (IC_{50} 1.9 $\mu\text{g/mL}$) (Sritularak; 2010: 387-392).

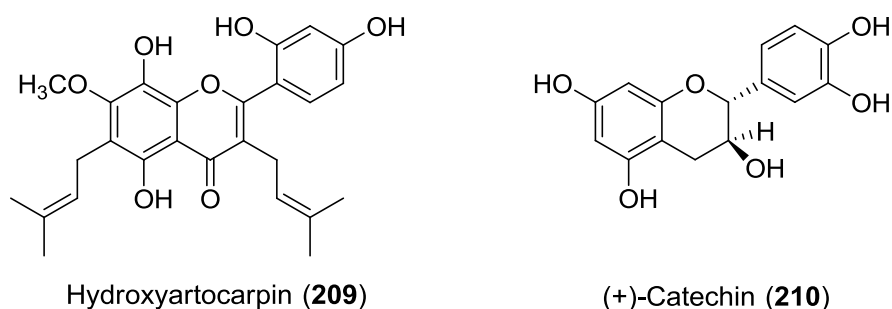


Figure 42 Structures of compounds **209** and **210**

The EtOAc extract from the leaves and twigs of *A. thailandicus* provided two constituents, *p*-hydroxyphenyl-6-acetoxy- β -D-glucopyranoside (**211**) and *p*-hydroxyphenyl- β -D-glucopyranoside or arbutin (**212**) (Figure 43). The compounds were evaluated for antibacterial activity against 11 bacteria strains. Compound **211** was active against three strains of bacteria including *Escherichia coli* (ETEC) DMST 30543, *Salmonella typhi* DMST 22842 and *Proteus mirabilis* DMST 8212. Meanwhile, compound **212** displayed antibacterial activity against five bacteria strains (*E. coli* (ETEC), *P. mirabilis*, *Enterobacter aerogenes* ATCC 13048, *Ent. Cloacae* ATCC 23355 and *Staphylococcus aureus* ATCC 25923) (Boonkla; et al. 2011: 1093-1097).

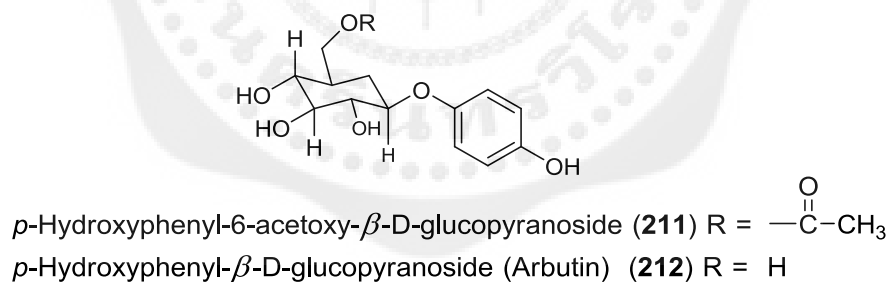
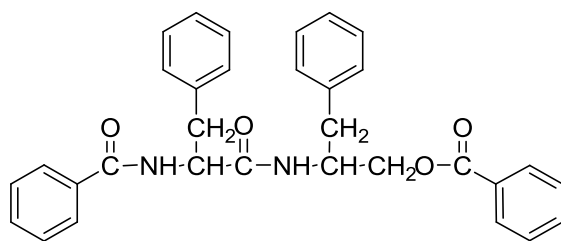
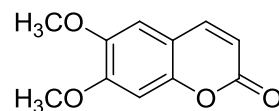
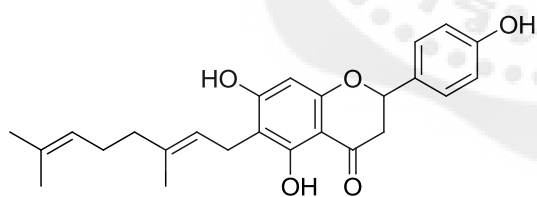
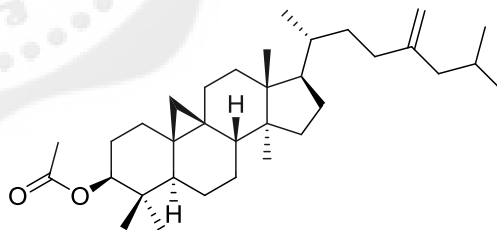


Figure 43 Structures of glucopyranosides **211** and **212**

In 2011, Hashim et al. reported on the purification of a dipeptide, aurantiamide benzoate (**213**), along with three known constituents, cycloartobiloxanthone (**25**), dihydroartoindonesianin C (**194**) and 6,7-dimethoxycoumarin (**214**) (Figure 44), from the CHCl_3 extract of *A. kemando* stem barks (Hashim; et al. 2011: 4224-4230).

Aurantiamide benzoate (**213**)6,7-dimethoxycoumarin (**214**)Figure 44 Structures of aurantiamide benzoate (**213**) and 6,7-dimethoxycoumarin (**214**)

A known flavanone, 5,7,4'-trihydroxy-6-geranylflavanone (**215**), was isolated from the EtOAc soluble fraction of *A. communis* fruits by Lin in 2011 (Figure 45). It was studied on anti-inflammatory effects. In activated monocytes, it displayed great inhibitory effects on the key pro-inflammatory mediators, $\text{TNF-}\alpha$ and $\text{IL-1}\beta$ and it also diminished ROS production in response to S100B, a ligand of the receptor for advanced glycation endproducts (RAGE) receptor, could induce the expression of oxidative stress-sensitive. In this study, RAGE ligation with S100B could trigger a strong inflammatory response in human monocytes, thus, the treatment with the compound could attenuate S100B-induced inflammatory response by decreasing ROS and pro-inflammatory cytokines. Additionally, the compound inhibited on RAGE-dependent and signaling pathways which may associated with the prevention and treatment of inflammation (Lin; et al. 2011: 105-111).

5,7,4'-Trihydroxy-6-geranylflavanone (**215**)24-Methylenecycloartenyl acetate (**216**)Figure 45 Structures of flavanone **215** and triterpenoid **216**

From preliminary screening, three extracts (EtOH, acetone and MeOH extracts) of the stem barks of *A. kamando* exerted cytotoxicities against human promyelocytic leukemia cell (HL-60) with IC_{50} in the range of 15.0-31.5 $\mu\text{g/mL}$ and human neuroblastoma cell (IMR-32) cell lines with IC_{50} in the range of 12.0-13.0 $\mu\text{g/mL}$. Moreover, two known constituents,

cycloartobiloxanthone (**25**) and 24-methylenecycloartenyl acetate (**216**) (Figure 45), were isolated from the EtOH extract from this plant species (Teo; et al. 2011: 74-76).

In 2011, Ti et al. purified two new compounds, artocarpene (**217**) and 2-hydroxynaringenin 4'-O- β -D-glucopyranoside (**218**), together with four known compounds, oxyresveratrol (**3**), 2-hydroxynaringenin (**219**), 3,4',5-trihydroxy-3'-prenylstilbene (**220**), and norartocarpetin (**29**) (Figure 46), from the stems of *A. nitidus* (Ti; et al. 2011: 662-665). All compounds, except for compound **219**, were examined for A549 cells. Compounds **218** and **220** exerted lower activity with IC₅₀ values of 47.3 and 39.9 μ g/mL, respectively, than doxorubicin (IC₅₀ 5.92 μ g/mL). Meanwhile, compounds **3** and **217** were inactive (IC₅₀ > 100 μ g/mL). Moreover, compounds **3**, **29** and **217-219** were evaluated for anti-DPPH activity. Only compounds **3** and **217** exhibited anti-DPPH activity with SC₅₀ values of 31.77 and 116.15 μ g/mL, respectively, and also showed weak potent than L-ascorbic acid (SC₅₀ 18.53 μ g/mL) (Ti; et al. 2011: 662-665).

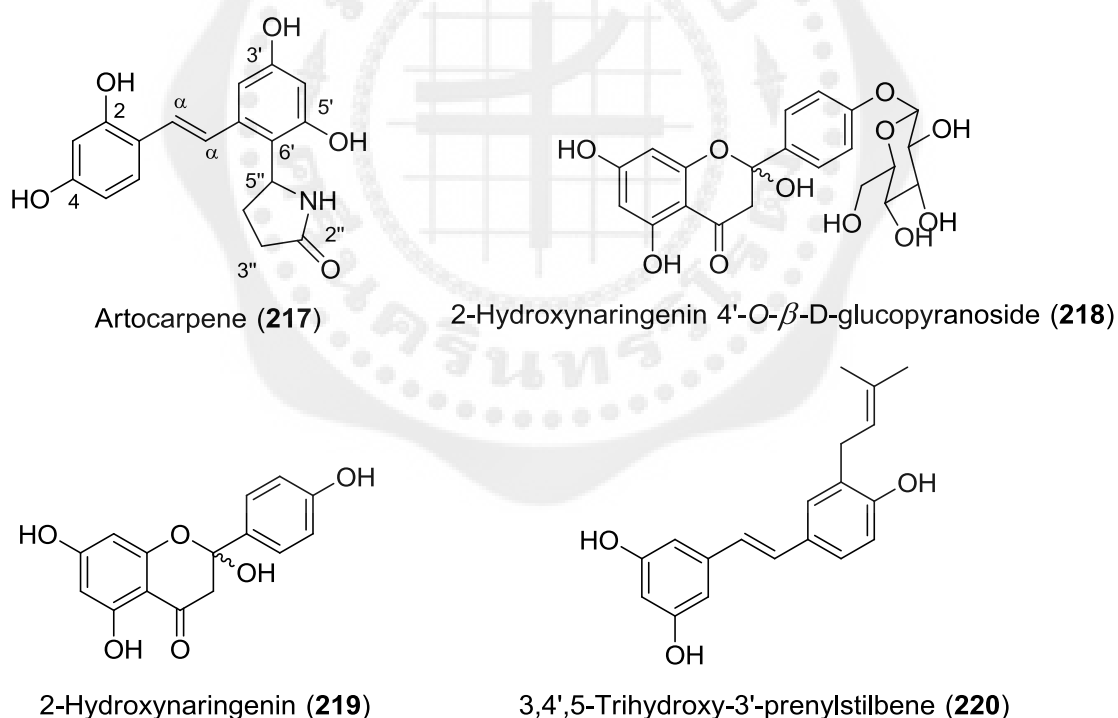


Figure 46 Structures of compounds **217-220**

The EtOH extract of the stem barks of *A. kemando* afforded two known compounds, cycloartobiloxanthone (**25**) and artoindonesianin C (**102**) and the acetone extract gave two known compounds, artonol B (**65**) and artomandin (**221**). While artosimmin

(**222**) were purified from the EtOAc extract of *A. odoratissimus* by Ee in 2012 (Figure 47). In preliminary screening, four extracts (CHCl_3 , acetone, EtOH and MeOH extracts) of *A. kemando* exhibited stronger anti-DPPH scavenging activity than three extracts (CHCl_3 , EtOAc and EtOH extracts) of *A. odoratissimus*. All constituents were then examined for anti-DPPH property. Compounds **221** and **222** displayed the highest effect with IC_{50} values of 38.0 and 32.1 $\mu\text{g/mL}$, respectively, while compound **25** showed weak activity with IC_{50} value of 87.2 $\mu\text{g/mL}$. Vitamin C was used as positive control (IC_{50} 12.2 $\mu\text{g/mL}$) (Ee; et al. 2012: 231-234).

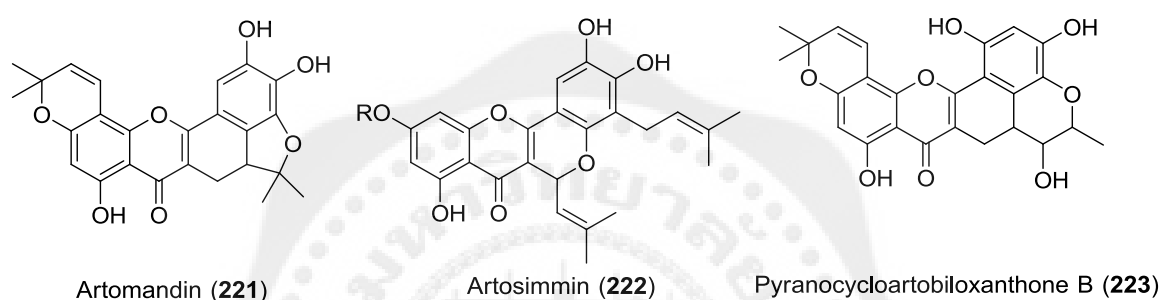
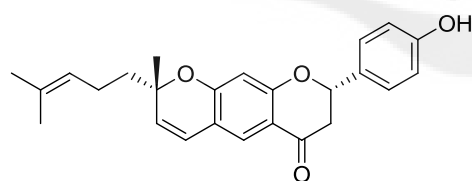
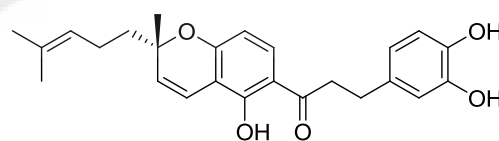
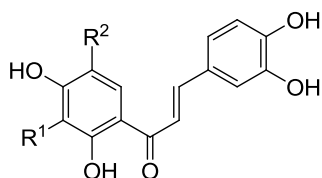


Figure 47 Structures of flavones **221-223**

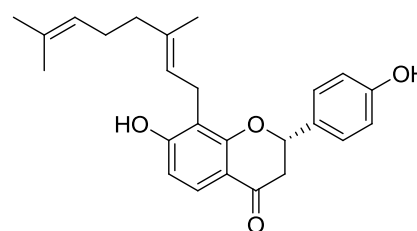
Two previously reported xanthones, pyranocycloartobiloxanthone A (**193**) and dihydroartoindonesianin C (**194**) by Hashim in 2010 and a new xanthone, pyranocycloartobiloxanthone B (**223**) which obtained from the MeOH extract of the stem barks of *A. obtusus* (Figure 47), were tested for antiproliferative assay against HL60 cells, human chronicmyeloid leukemia (K562), and human estrogen receptor (ER+) positive breast cancer (MCF7) cell lines. From the results, compound **193** displayed the stronger effects with IC_{50} value in the range of 0.5-2 $\mu\text{g/mL}$ than the rest, comparing to standard drug, doxorubicin with IC_{50} value of 0.2 $\mu\text{g/mL}$. Only compound **193** was further tested for the cancerous cell lines, including HeLa (human cervical cancer), MDA-MB 231 (human estrogen receptor (ER-) negative), HT29, HepG2, and for the noncancerous cell lines, including 3T3 (Mouse embryo fibroblast), MCF10A (human nontumorigenic breast cell line) and PBMC (human peripheral blood mononuclear cells). The compound had no toxicity on MCF10A and PBMC. In the same year, the same group investigated the antioxidant and antimicrobial activities of all compounds. Compound **193** displayed the highest antioxidant activity with IC_{50} value of 2.0 $\mu\text{g/mL}$, comparing to ascorbic acid, quercetin and α -tocopherol (IC_{50} 20, 40 and 60 $\mu\text{g/mL}$, respectively). For antibacterial activity, compound

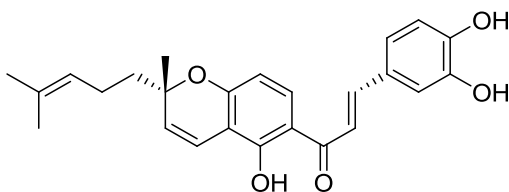
193 showed antibacterial activity against methicillin resistant *Staphylococcus aureus* (ATCC3359) and *Bacillus subtilis* (clinically isolated) with inhibition zone of 20 and 12 mm, respectively, whereas the remaining compounds **194** and **223** were inactive. Furthermore, only compound **193** was examined for tyrosinase inhibitory activity. It was found that the compound showed strong effect with 80% of inhibition, comparing to kojic acid (96% of inhibition) (Hashim; et al. 2012: 6071-6082).

The isolation of three new geranyl flavonoids, arcommunol C (**224**), arcommunol D (**225**), and 5'-geranyl-3,4,2',4'-tetrahydroxychalcone (**226**), along with four known compounds, prostratol (**227**), arcommunol E (**228**), 3-geranyl-3,4,2',4'-tetrahydroxydihydrochalcone (**147**), and 3'-geranyl-3,4,2',4'-tetrahydroxychalcone (**229**) (Figure 48), were obtained from the CHCl₃ extract of the leaves of *A. communis* by Hsu in 2012. All constituents were tested for anti-inflammatory effect on the nitric oxide (NO) production in (lipopolysaccharide) LPS-stimulated RAW 264.7 cells. From the results, compounds **225**, **227** and **228** exhibited the greatest inhibition of LPS-stimulated NO production. Due to the most active inhibitor of compound **227**, it was further determined on various mechanisms on LPS-induced protein expressions of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), p-JNK, JNK, p-ERK, ERK, p-p38, and p38 in RAW 264.7 macrophages. It was found that compound **227** reduced LPS-mediated induction of protein expressions of iNOS and COX-2 and was also suppressed LPS-induced phosphorylation of JNK and p38 mitogen-activated protein kinase (MAPK) signaling (Hsu; et al. 2012: 995-1001).

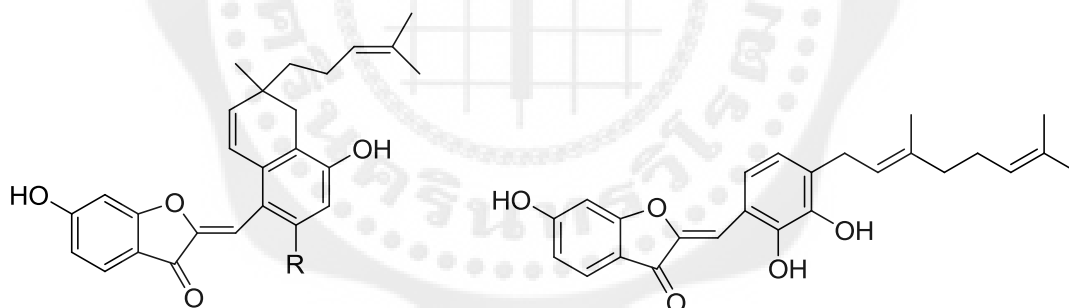
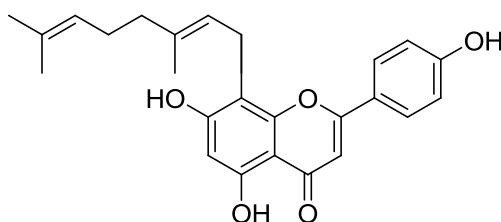
Arcommunol C (**224**)Arcommunol D (**225**)5'-Geranyl-3,4,2',4'-tetrahydroxychalcone (**226**)R¹R²3'-Geranyl-3,4,2',4'-tetrahydroxychalcone (**229**)

geranyl H

Prostratol (**227**)

Arcommunol E (**228**)Figure 48 Structures of compounds **224-229**

Three new aurones, altilisins H-J (**230-232**), along with two known flavonoids, 8-geranyl-4',5,7-trihydroxyflavone (**233**) and cycloaltilisin 7 (**5**) were purified from the CHCl_3 fraction the leaves of *A. atilis* by Mai in 2012 (Figure 49). All of them were evaluated for tyrosinase and α -glucosidase inhibitory activities. Compounds **5** and **233** exhibited tyrosinase inhibitory properties with IC_{50} values of 43.9 and 42.5 $\mu\text{g/mL}$, respectively, comparing to kojic acid (IC_{50} 46.6 $\mu\text{g/mL}$). For α -glucosidase inhibitory activities, compounds **230-232** showed effects with IC_{50} values ranging of 4.9-5.4 $\mu\text{g/mL}$, comparing to acarbose with IC_{50} value of 241.8 $\mu\text{g/mL}$ (Mai; et al. 2012: 647-650).

Altilisin H (**230**) R = HAltilisin J (**232**)Altilisin I (**231**) R = OCH_3 8-Geranyl-4',5,7-trihydroxyflavone (**233**)Figure 49 Structures of compounds **230-233**

Isolation and structure elucidation of four new flavones, artocarmins A-D (**234-237**), and three new chalcones, artocarmittins A-C (**238-240**), together with 13 known compounds, cudraflavone C (**68**), norartocarpin (**75**), albanin A (**76**), gemichalcone B (**84**), *p*-coumaric acid (**182**), vanillic acid (**183**), *p*-hydroxybenzoic acid (**184**), 3'-[γ -hydroxymethyl-(*Z*)- γ -methylallyl]-4,2',4'-trihydroxychalcone (**241**), gemichalcone A (**242**), isogemichalcone B (**243**), morachalcone A (**244**), β -resorcylic acid (**245**), goldfussinol (**246**) were reported by Nguyen in 2012 (Figure 50). These compounds, except for compounds **183**, **238**, **241**, **245** and **246** were purified from the MeOH extract of the woods of *A. heterophyllum*. They were tested for tyrosinase inhibitory activity. Compounds **68**, **75**, **76**, **182**, **184**, **234-236**, **240** and **244** showed more potent inhibitory effect (IC_{50} values 0.004 to 21.2 $\mu\text{g/mL}$) than a positive control, kojic acid (IC_{50} 6.3 $\mu\text{g/mL}$). In addition, compound **244** was found to be the most active tyrosinase inhibitor and it was 3000 times higher active than kojic acid in this assay (Nguyen; et al. 2012: 1951-1955).

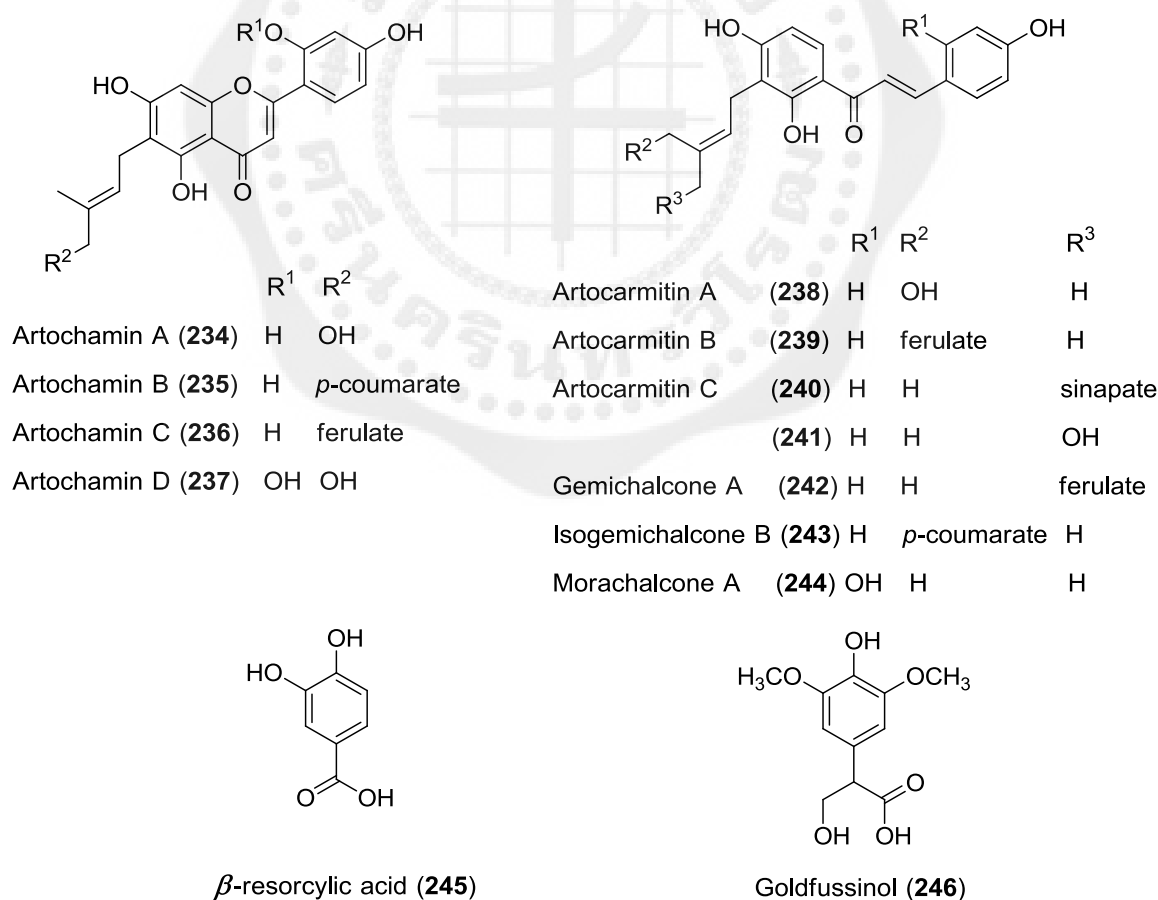


Figure 50 Structures of compounds **234-246**

In 2012, Shahin et al. found six glycosides, n-octadec-9-enoyl α -L-rhamnopyranoside (**247**), n-octadec-9,12-dienoyl α -L-rhamnopyranoside (**248**), n-octadec-9,12-dienoyl- β -D-glucopyranoside (**249**), n-octadec-9-enoyl- β -D-glucopyranoside (**250**), n-octadec-9-enoyl- β -D-arabinopyranoside (**251**) and n-octadec-9-enoyl- α -D-xylopyranoside (**252**) (Figure 51), from the MeOH extract of the leaves of *A. heterophyllus* (Shahin; Kazmi; & Ali. 2012: 449-453).

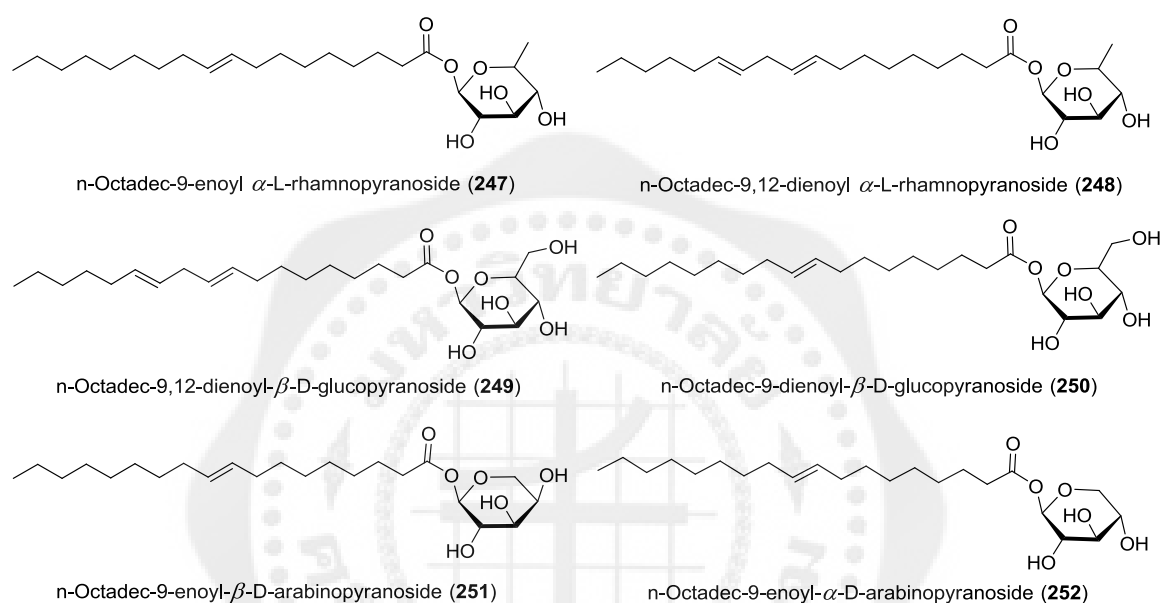
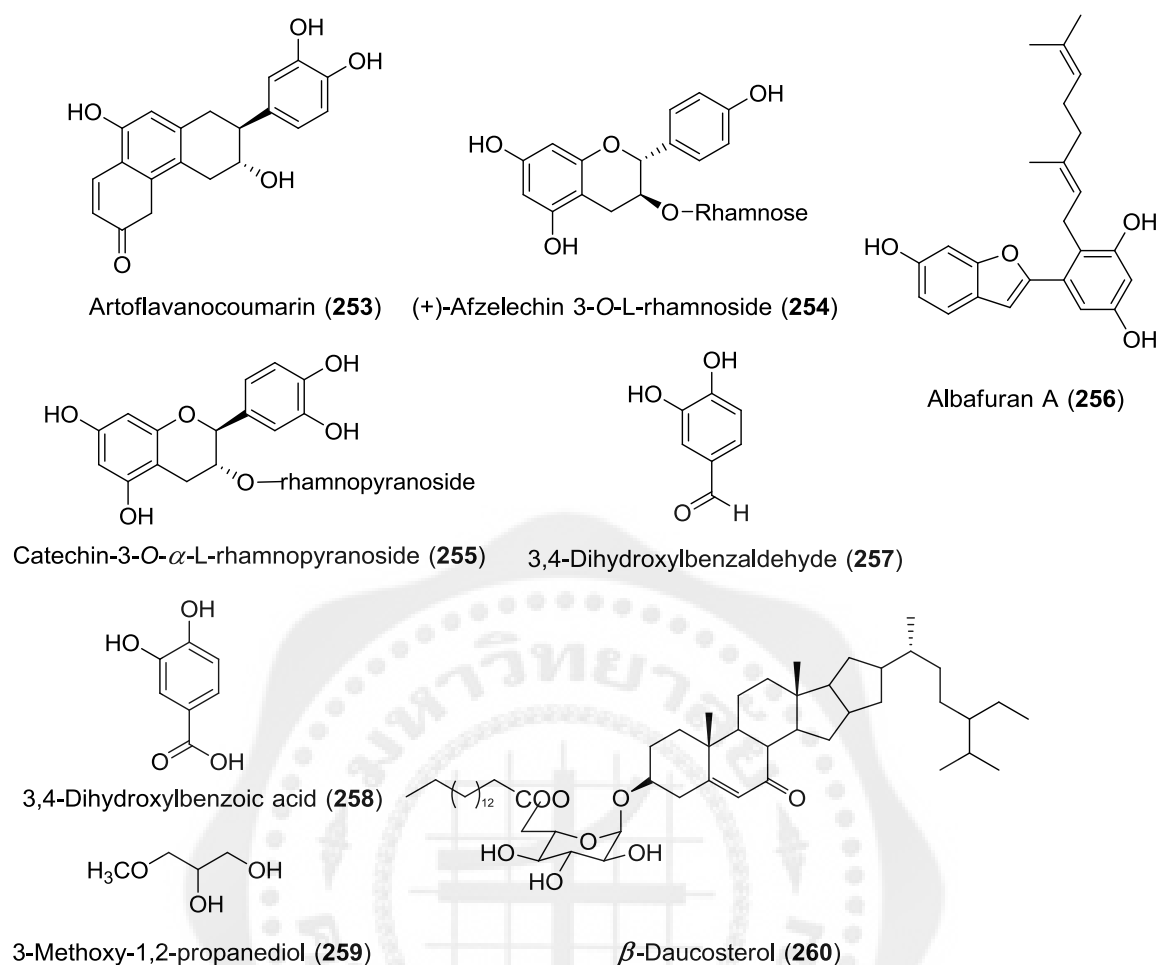
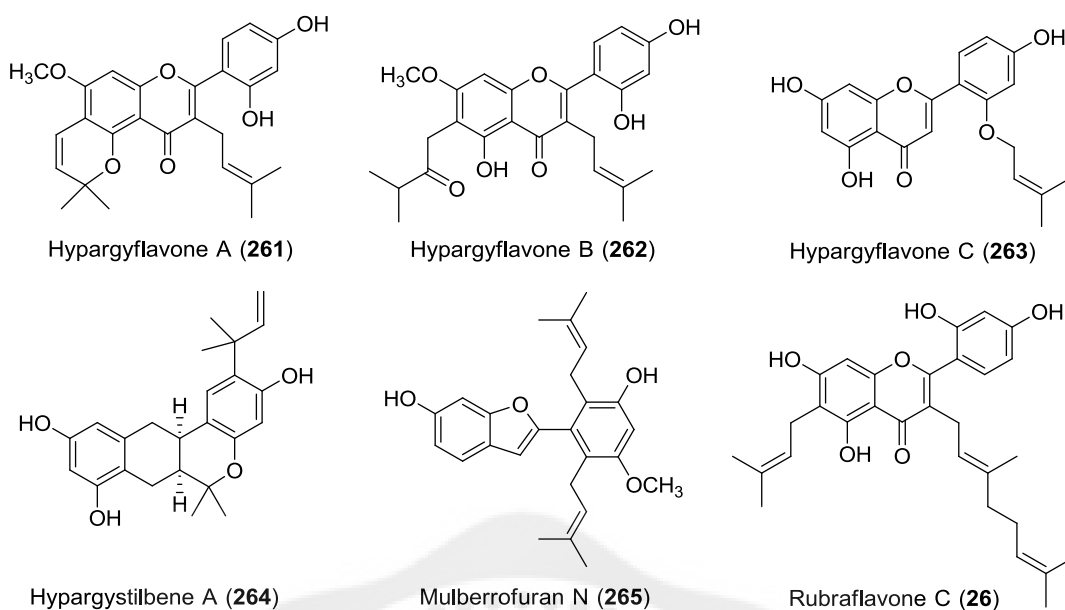


Figure 51 Structures of glycosides **247-252**

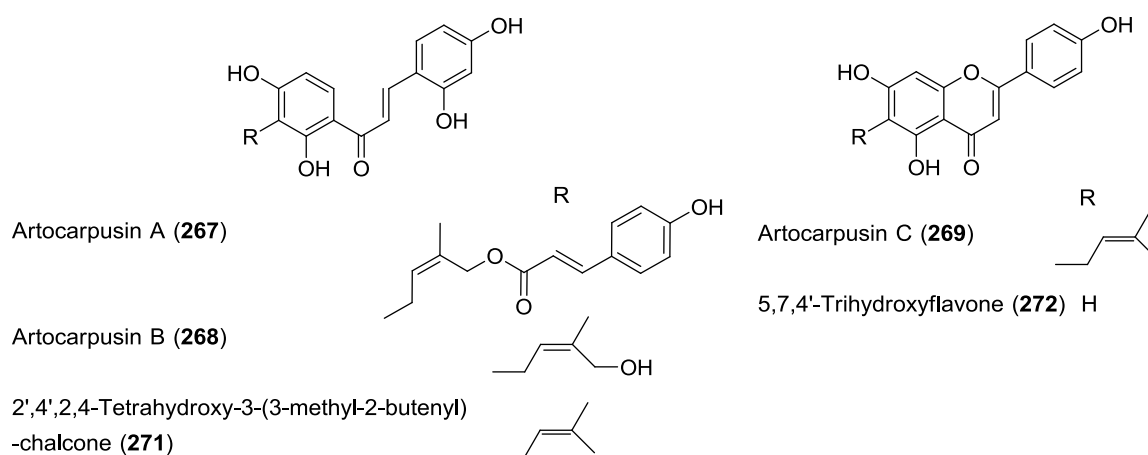
In 2012, Ti et al. purified a new flavan-3-ol, artoflavanocoumarin (**253**), together with three known compounds (+)-catechin (**206**), (+)-afzelechin 3-O- α -L-rhamnoside (**254**), and (+)-catechin-3-O- α -L-rhamnoside (**255**) from the EtOAc extract of the stems of *A. nitidus* (Ti; et al. 2012: 555-558). In the same year, the same group found 13 constituents, artocarpin (**50**), cycloartocarpin A (**51**), artogomezianone (**71**), albanin A (**76**), β -sitosterol (**106**), brosimone I (**165**), artotonkin (**175**), β -amyrin acetate (**186**), albafuran A (**256**), 3,4-dihydroxybenzaldehyde (**257**), 3,4-dihydroxybenzoic acid (**258**), 3-methoxy-1,2-propanediol (**259**), and β -daucosterol (**260**) (Figure 52), from the same plant species (Ti; et al. 2012: 529-532).

Figure 52 Structures of compounds **253-260**

In 2012, Yu et al. studied the isolation of three new isoprenylated flavones, hypargyflavones A-C (**261-263**), and a novel stilbene derivative, hypargystilbene A (**264**), together with seven known compounds, cycloartocarpin A (**51**), cudraflavones A and C (**52** and **68**), norartocarpin (**75**), brosimone I (**165**), mulberrofuran N (**265**) and rubraflavone C (**266**) from the EtOH extract of *A. hypargyreus* stems (Figure 53). Selected compounds **261-266** were examined for PL-inhibitory activity. Only compound **261** displayed significant inhibitory effect on pancreatic lipase (PL) with IC_{50} value of 0.9 $\mu\text{g/mL}$, comparing to orlistat (IC_{50} 0.54 $\mu\text{g/mL}$) whereas the rest **262-266** did not show the activity (Yu; et al. 2012: 394-402).

Figure 53 Structures of compounds **261-266**

In 2013, Di et al. found two new chalcones, artocarpusins A and B (**267** and **268**), a flavone, artocarpusin C (**269**) and a new 2-arylbenzofuran derivative, artocarstilbene A (**270**), along with 15 known flavonoids, artocarpin (**50**), cudraflavone C (**68**), 6-(3-methylbut-2-enyl)-apigenin (**73**), norartocarpin (**75**), isobavachalcone (**82**), artocarpesin (**162**), artocarmitins A and B (**238-239**), 3'-[γ -hydroxymethyl-(Z)- γ -methylallyl]-2',4',4'-trihydroxychalcone (**241**), gemichalcones A (**242**) and B (**84**), isogemichalcone B (**243**), 2',4',2,4-tetrahydroxy-3-(3-methyl-2-butenyl)-chalcone (**271**), 5,7,4'-trihydroxyflavone (**272**) and norartocarpetin (**29**) (Figure 54), from the combined PE and EtOAc soluble fractions twigs of *A. heterophyllus*. Cytotoxic activities against human prostate adenocarcinoma (PC-3) and NCI-H460 cells of all compounds were shown as in Table 6. (Di; et al. 2013: 24-28).



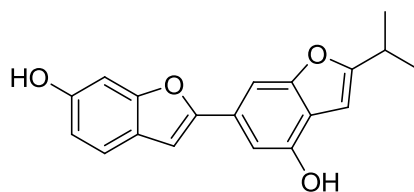
Artocarstilbene A (**272**)Figure 54 Structures of compounds **267-272**

Table 6 Cytotoxic activities of all compounds against PC-3 and NCI-H460 cells

Compounds	Cytotoxicities (μg/mL)	
	PC-3	NCI-H460
29	11.8 ± 0.5	5.3 ± 3.0
50	3.4 ± 0.6	3.6 ± 0.4
68	6.4 ± 0.1	8.0 ± 1.9
73	14.8 ± 1.9	13.7 ± 1.7
75	9.4 ± 0.9	8.7 ± 0.7
82	5.0 ± 0.0	5.2 ± 1.6
84	4.7 ± 0.2	6.3 ± 1.6
162	5.7 ± 0.1	4.1 ± 0.3
242	4.2 ± 0.3	4.9 ± 0.4
238	14.7 ± 0.5	13.2 ± 4.4
239	5.7 ± 0.7	8.3 ± 0.5
241	9.2 ± 2.8	8.1 ± 1.0
243	7.2 ± 0.5	8.5 ± 1.1
267	7.0 ± 0.3	8.1 ± 0.5
268	>100	>100
269	>100	22.2 ± 7.1
270	>100	>100
271	7.5 ± 0.5	10.1 ± 0.5
272	20.3 ± 1.5	12.2 ± 1.1
Doxorubicin	0.11 ± 0.1	0.17 ± 0.1

In 2013, Hoi et al. studied the isolation of six known compounds, artochamin B (**46**), β -sitosterol (**106**), α -amyrin 3-acetate (**274**), betulinic acid (**275**), friedelan-3-one (**276**), and 2-C-methyl-D-erythritol 4-O- α -D-glucopyranoside (**277**) from the CH_2Cl_2 extract stem barks and leaves of *A. nigrifolius* (Figure 55). All of them, except for **106** and **275**, were examined for cytotoxicities against MCF-7, Lu, HepG2 and KB cell lines. Compound **46** displayed the greatest cytotoxicities of all human cancer cell lines with IC_{50} values ranging of 1.18-20.0 $\mu\text{g/mL}$, comparing to ellipticin with IC_{50} values ranging of 0.35-0.53 $\mu\text{g/mL}$ whereas compound **276** displayed moderate activities on MCF7, HepG2, and KB cells with IC_{50} values of 20.0, 14.0, and 8.0 $\mu\text{g/mL}$, respectively. In contrast, the remaining compounds were inactive. Furthermore **46**, **106** and **276** were selected for antibacterial activity against *Lactobacillus fermentum*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and antifungal activity against *Candida albican*. Only compound **46** displayed inhibitory effect on only two growth of *Bacillus subtilis* and *Staphylococcus aureus*, with IC_{50} values of 21.7 and 25.1 $\mu\text{g/mL}$, respectively, whereas the rest had no effect on all bacteria (Hoi; et al. 2013: 667-672).

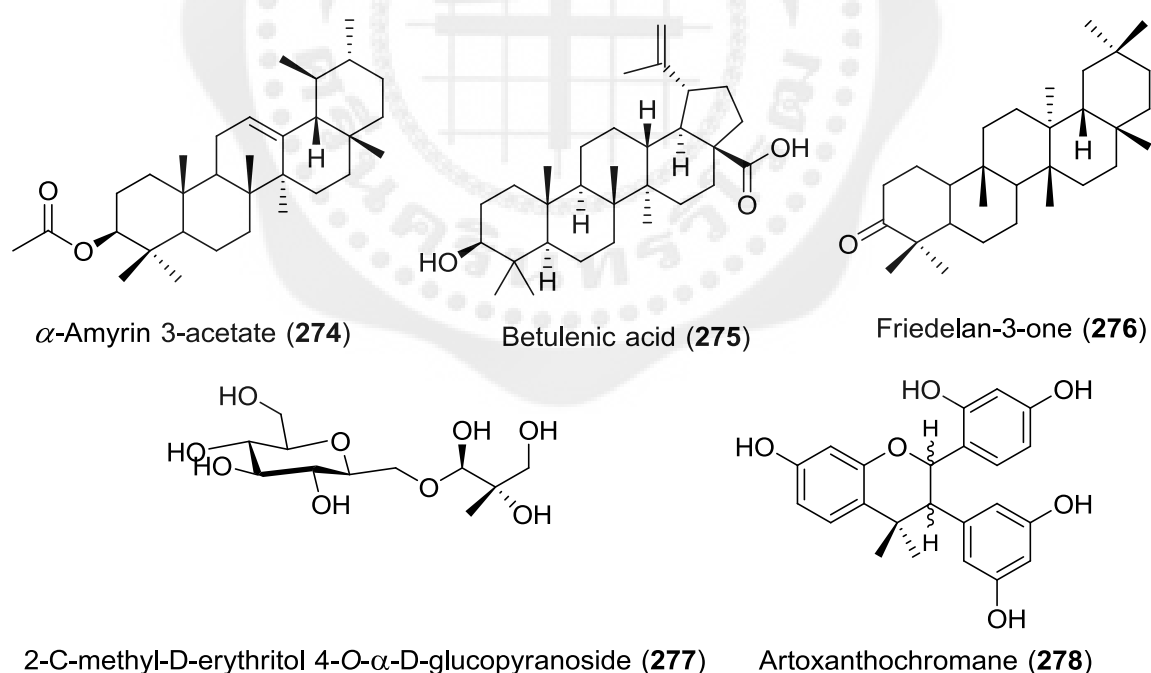


Figure 55 Structures of compounds **274-278**

In 2013, Ko et al. further investigated on cytotoxicity skin irritation of norartocarpetin (**29**) which obtained from the heartwoods of *A. communis*. It was found that the compound had no effect on cell viability B16F10 melanoma and human dermal fibroblasts. No skin irritation effect of the compound on BALB/c nude mice. In addition, the compound can inhibit α -MSH-induced melanogenesis which led to tyrosinase synthesis and enhancement of melanoma (Ko; et al. 2013: 348).

The MeOH extract of *A. xanthocarpus* heartwoods afforded a new compound, artoxanthochromane (**278**). This compound was examined for tyrosinase inhibition and radical-scavenging assays on ABTS⁺ and superoxide anion ($O_2^{\cdot-}$). However, the compound showed weak for all activity tests, comparing to positive control, arbutin and vitamin C (Ko; et al. 2013: 1269-1275).

In 2013, Lan et al found two new prenylated flavones, 10-oxoartogomezianone (**279**), hydroxyartoflavone A (**280**), together with 11 known compounds, cyclomorusin (**18**), norartocarpetin (**29**), artocarpin (**50**), artonol B (**65**), cycloartocarpin (**67**), artogomezianone (**71**), 8-geranylapigenin (**281**), cudraflavone A (**52**), cyclogeracommunin (**168**), artoflavone A (**169**), artonin M (**282**), from the CH_2Cl_2 extract *A. atilis*. In addition, the $CHCl_3$ extract of the cortex of this plant gave three new prenylated flavones, 8-geranyl-3-(hydroxyphenyl)isoetin (**283**), isocycloartobiloxanthone (**284**) and furanocyclocommunin (**285**), along with a known compound, cyclocommunol (**199**) (Figure 56). All compounds, except for **65** and **285**, were selected for anti-tyrosinase and antioxidative activities. Furthermore, inhibition effect of compounds **29**, **50**, **52**, **71** and **282** on B16F10 melanoma cells were performed. From the results, compounds **29** was the most potent inhibitor on tyrosinase with IC_{50} values of 0.12 $\mu g/mL$, followed by compounds **52**, **71** and **282** with IC_{50} values in the range of 30.9 to 39.9 $\mu g/mL$, respectively. Arbutin (IC_{50} 84.1 $\mu g/mL$), kojic acid (IC_{50} 25.1 $\mu g/mL$), apigenin (IC_{50} 82.2 $\mu g/mL$) and quercetin (IC_{50} 0.75 $\mu g/mL$) were used as positive control. For antioxidant properties, compounds **169**, **280** and **284** showed moderate activity on DPPH radical (IC_{50} 9.0-24.0 $\mu g/mL$). While compounds **29**, **71** and **284** exhibited moderate property on ABTS⁺ radical (IC_{50} 3.1-15.2 $\mu g/mL$) and compounds **29**, **50** and **71** also showed moderate activity on $O_2^{\cdot-}$ radical (IC_{50} 17.9-41.0 $\mu g/mL$) (Lan; et al. 2013: 78-88).

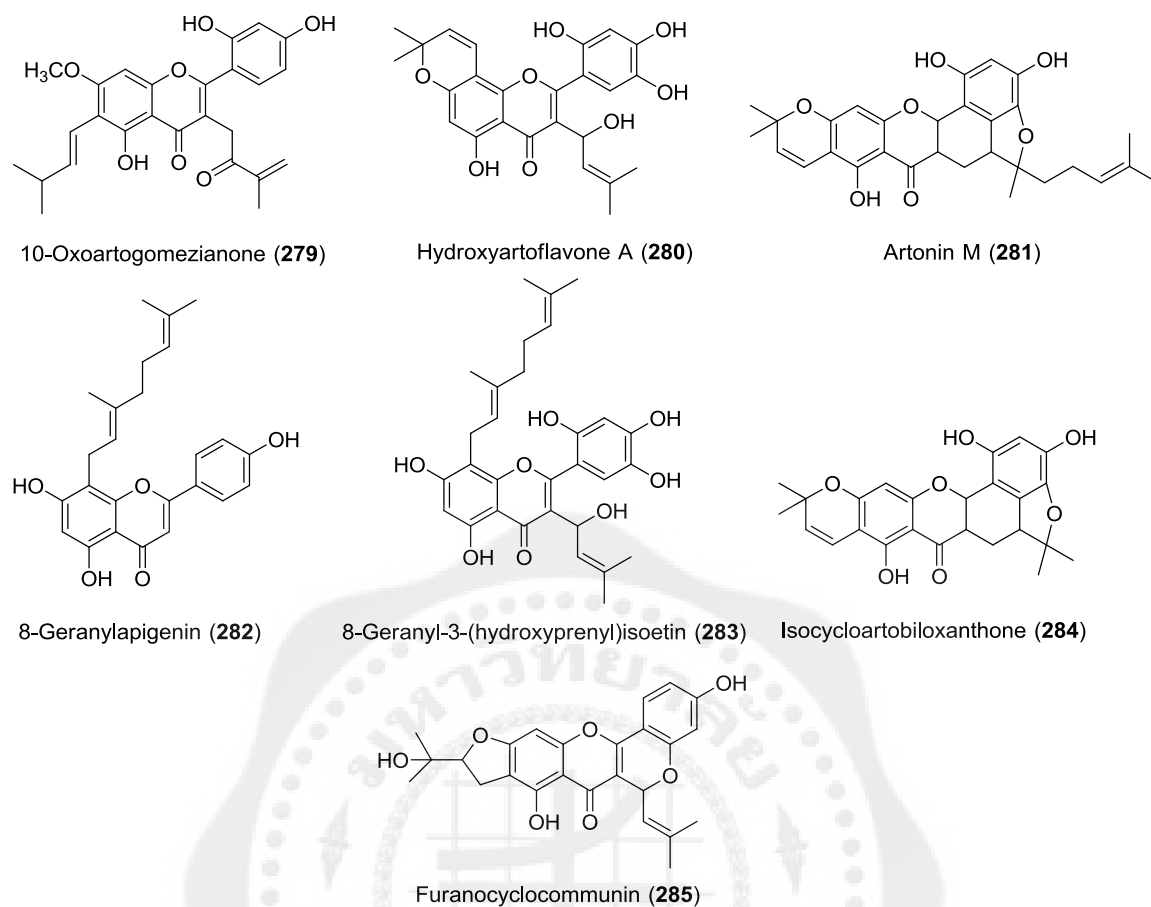
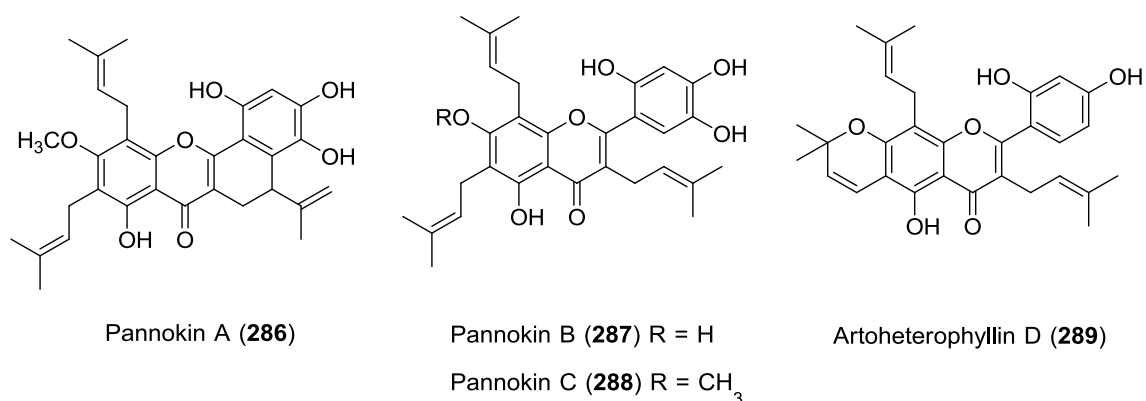
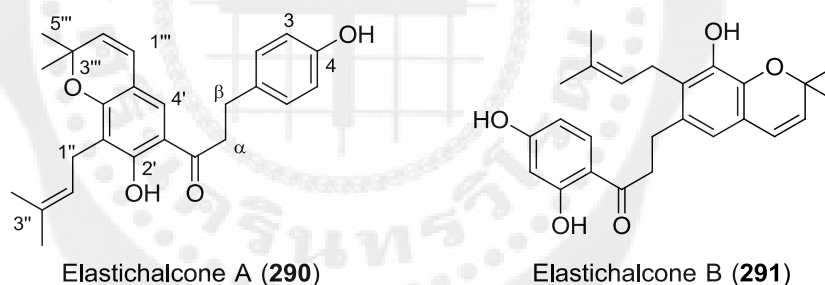


Figure 56 Structures of flavones **279-285**

In 2013, Minakawa et al. obtained three new flavonoids, pannokins A-C (**286-288**), together with three known compounds, artonin B (**44**), heterophyllin (**105**) and artoheterophyllin D (**289**) (Figure 57), from the most active EtOAc-soluble fraction of *A. champeden* roots. All these compounds were evaluated for their activity in overcoming TRAIL-resistance in human gastric adenocarcinoma (AGS) cells. From the results, compound **105** exhibited the highest activity and it was further studied using the AGS cells which related to apoptosis. It was found that the compound enhanced capases 3/7 activity and the expression of DR₄ and DR₅ mRNA, leading to cause a CHOP-dependent up-regulation of DR5 expression in apoptosis in AGS cells (Minakawa; et al. 2013: 299-304).

Figure 57 Structures of prenylated flavones **286-289**

Two dihydrochalcones, elastichalcones A and B (**290** and **291**), together with a known flavone, cycloartocarpesin (**163**) (Figure 58), were isolated from the hexane extract of the leaves of *A. elasticus* by Ramli in 2013. Compounds **163** and **291** possess good free radical scavenging activity with IC₅₀ values of 11.89 and 11.30 µg/mL, respectively (Ramli; et al. 2013: 582-585).

Figure 58 Structures of dihydrochalcones **290** and **291**

In 2013, Ren et al. purified a new flavone, styracifolin C (**292**), together with nine known compounds, artonin A (**34**), artopetelin B (**56**), albanin A (**76**), artocarpone B (**132**), lakoochin A (**293**), (-)-catechin (**294**), (-)-catechin-3-O- α -L-rhamnopyranoside (**295**), lup-12-ene-3 β -ol acetate (**296**) and lupeol acetate (**196**), from the PE-soluble fraction of *A. styracifolius* root barks (Ren; et al. 2013: 97-100). In same year, the same group isolated 10 known constituents, diosgenin (**297**), prosapogenin of dioscin (**298**), progenin II (**299**), diosgenyl-3-O- α -L-rhamnopyranosyl (1 $\rightarrow$ 3)-[α -L-rhamnopyranosyl (1 $\rightarrow$ 4)]- β -D-glucoside (**300**), 7-oxositosterol acilglicosilado (**301**), ethyl caffeate (**302**), dibutyl phthalate (**303**), pinioresinol (**304**), (7S, 8S, 8'R)-5,5'-dimethoxylariciresinol (**305**) and palmitic acid (**306**)

(Figure 59), from the EtOH extract of the fruiting branches of *A. nanchuanensis* (Ren; et al. 2013: 97-100).

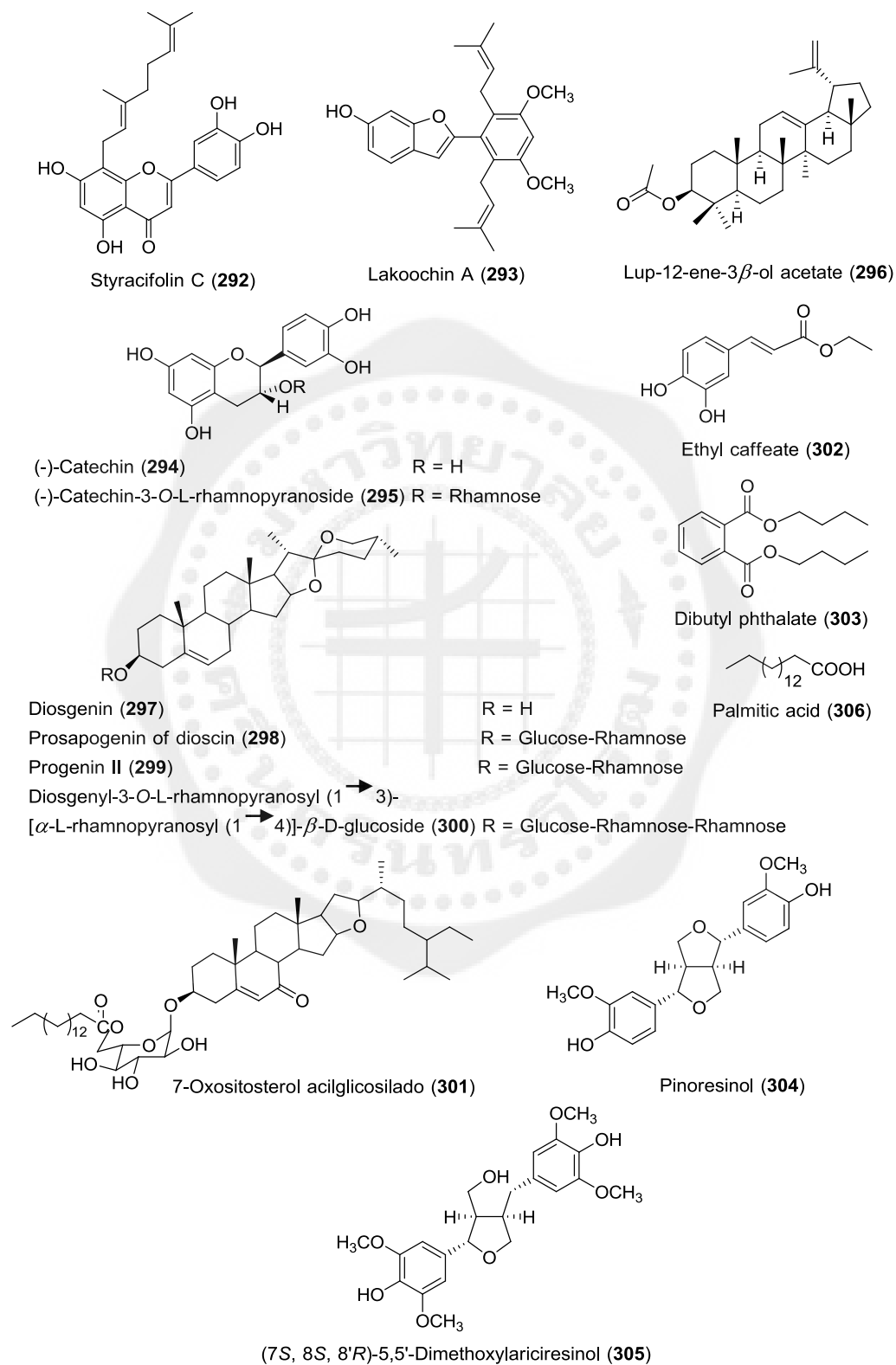


Figure 59 Structures of compounds **292-306**

In 2013, Suhartati et al. reported the isolation and structural determination of two flavans, afzelecin-3-O- α -L-rhamnoside (**254**) and catechin (**30**) from the root barks of *A. dadah*. In addition, both compounds were examined for cytotoxicity against murine leukemia P388 cancer cell. It was found that compounds **254** and **30** were inactive with $IC_{50} > 100$ μ g/mL (Suhartati; et al. 2013: 1050-1056).

Antidiabetic activity of the most active hexane extract of *A. camansi* leaves was studied by Nasution in 2014. It resulted in the isolation of a known compound, β -sitosterol propionate (**307**) (Figure 60). In the antidiabetic activity assay, this compound was had a higher activity than the hexane extract. After loading the compound at 30, 60 and 90 minutes, blood glucose of mice was diminished as much as 87.67, 89.0 and 22.0 mg/dL, respectively (Nasution; et al. 2014: 1279-1285).

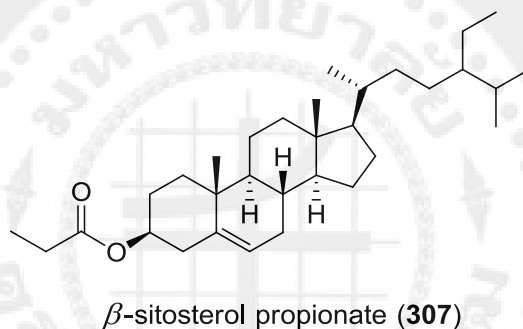


Figure 60 Structures of β -sitosterol propionate **307**

In 2014, Nguyen et al. studied on the isolation of eight new dihydrochalcones, sakenins (**308-315**), together with four known constituents, 1-(2,4-dihydroxyphenyl)-3-[3,4-dihydro-3,8-dihydroxy-2-methyl-2-(4-methylpent-3-enyl)-2H-1-benzopyran-5-yl]propan-1-one (**127**), 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-methylpent-3-enyl)-2H-1-benzopyran-5-yl] propan-1-one (**107**), 2-geranyl-2',3,4,4'-tetrahydroxydihydrochalcone (**109**) and cycloaltilisins (Figure 61). All these compounds were tested for cytotoxicity against PANC-1 (human pancreatic cancer cells). Compounds **313** and **315** were cytotoxic candidates with PC_{50} values of 3.4 and 3.6 μ g/mL, respectively, comparing to arctigenin with PC_{50} value of 2.9 μ g/mL. (Nguyen; et al. 2014: 193-200).

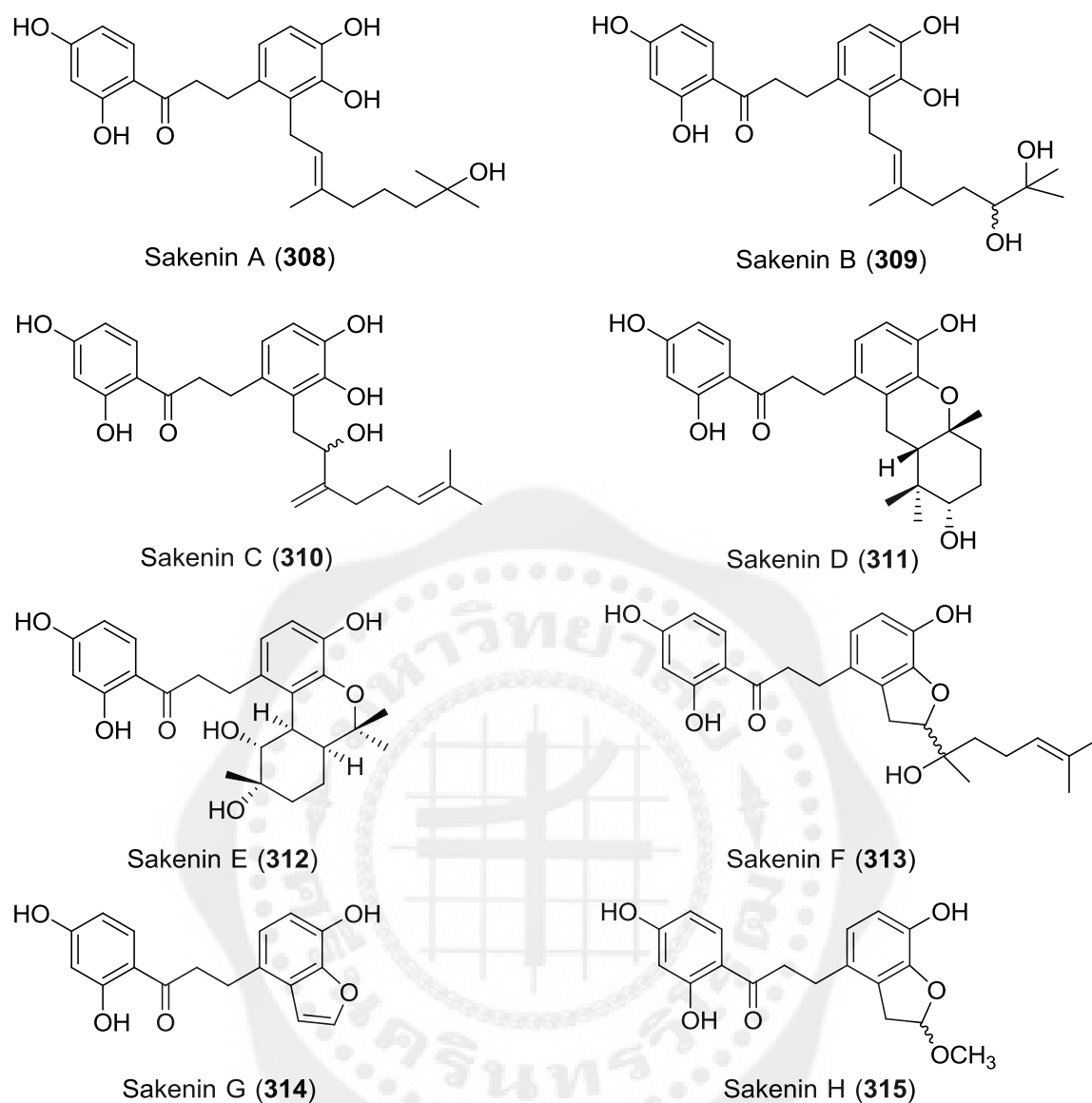
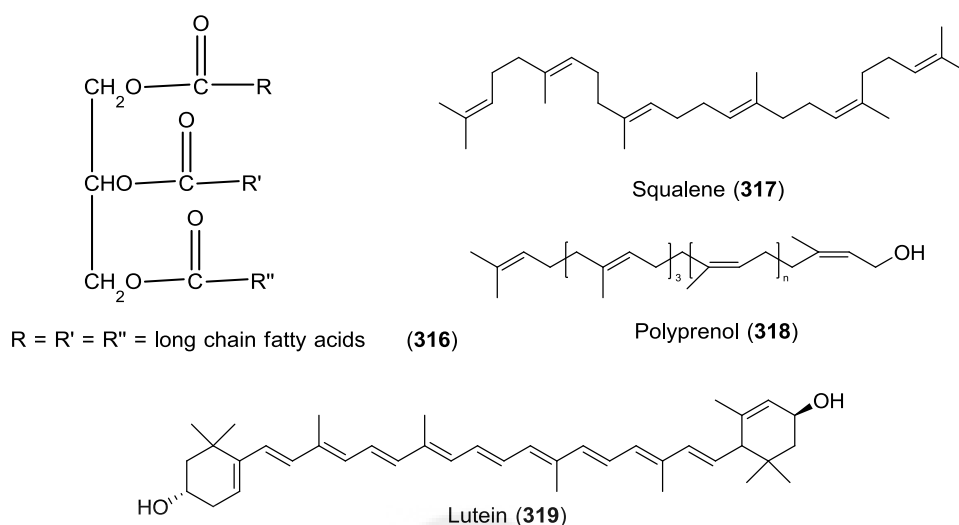
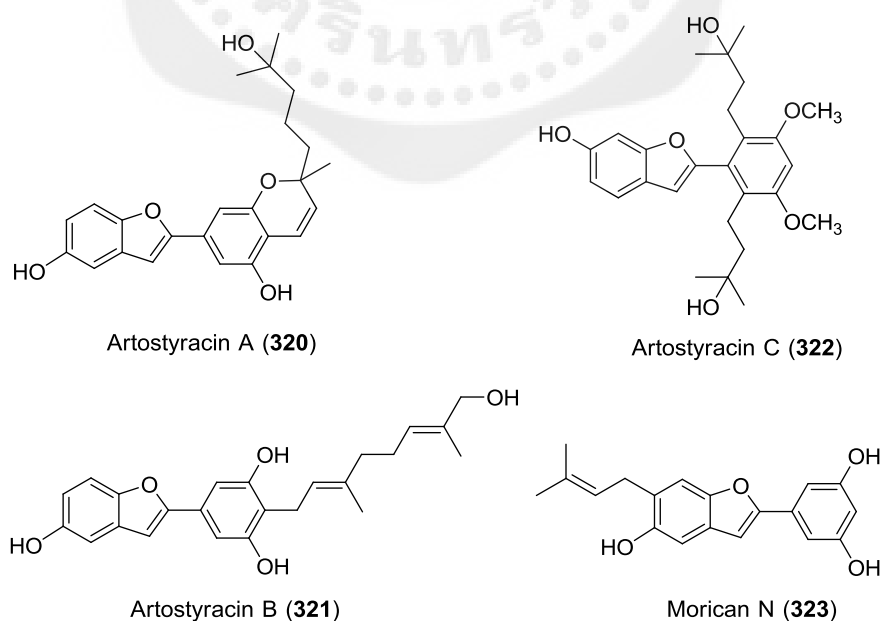


Figure 61 Structures of sakenins A-H (**308-315**)

In 2014, Ragasa reported on the isolation of β -sitosterol (**106**), unsaturated triglycerides (**309**), squalene (**310**), polyprenol (**311**), lutein (**312**) and unsaturated fatty acids from the CH_2Cl_2 extract of the leaves of *A. altilis*. For compounds **106**, **309** and unsaturated fatty acids were also derived from the CH_2Cl_2 extract of the fruits and seeds of *A. odorarissimus* (Ragasa; et al. 2014: 1081-1087) (Figure 62).

Figure 62 Structures of compounds **316-319**

Chemical investigation of the CHCl_3 -soluble potion of *A. styracifolius* dried roots afforded three new 2-arylbenzofurans, artostyracins A-C (**320-322**), along with a known 2-arylbenzofuran, moracin N (**323**) by Ren in 2014 (Figure 63). All compounds were evaluated for inhibitory effect on respiratory burst of rat neutrophils PMNs. From the results, compounds **320** and **322** exhibited strong anti-respiratory burst activity with IC_{50} values of 0.55 and 0.84 $\mu\text{g/mL}$, respectively, whereas compounds **321** and **323** showed moderate activity with IC_{50} values of 4.5 and 14.5 $\mu\text{g/mL}$, respectively (Ren; et al. 2014: 235-239).

Figure 63 Structures of artostyracins A-C (**320-322**) and Moracin N (**323**)

It is noted that the genus *Artocarpus* has a close relationship with the genus *Morus*, which supports from interesting studies, DNA sequence data (Datwyler; et al. 2004: 767-777 & Matsuda; et al. 2005: 231-233). Consequently, the phytochemicals found in both genera are possibly similar.

2.2 Botanical description of *Artocarpus lakoocha* Roxb.

A. lakoocha is commonly known in Thai as “Ma-Haad”. For Thai folk medicine, its heartwoods is well-known for the treatment of tapeworm infection and its edible fruit pulps used as a tonic for the liver. Tree to 40 m tall, deciduous. Leaf twigs 3-5 mm thick, densely brown (to whitish) puberulous with straight or also some uncinata hairs, smooth, drying brown (to blackish). Leaves distichous; lamina subcoriaceous to chartaceous, elliptic to oblong or ovate, 8-30 by 4-17 cm, apex short- acuminate to acute (or rounded), base subcordate to rounded to broadly cuneate, often slightly inequilateral, margin denticulate towards the apex to entire (or, when juvenile, 3-lobed to pinnately incised), $\pm$ revolute to flat; upper surface brownish puberulous with straight and some uncinata hairs (mainly) on the main veins, smooth, lower surface brownish puberulous to subtomtose on the veins, smooth; lateral veins (7-)10-15 pairs, none (or some of them in the upper part of lamina) forked away from the margin, tertiary venation scalariform, prominent; petiole 0.5-2(-4.5) cm long, 1.5-2.5 mm thick, densely brown puberulous, the epidermis persistent; stipules lateral, 0.3-0.5(-1.2) cm long, brown (to whitish) appressed-puberulous, caduceus (or subpersistent) or persistent at the shoot-apices forming terminal buds (or also in the leaf axils forming lateral buds). Staminate inflorescences axillary (or just below the leaves), solitary; peduncle 0.6-1.5(-2) cm long, densely brown puberulous with straight and uncinata hairs; head globose to semi-globose or discoid and 0.8-1.5 cm diam. or ellipsoid to clavate-spicate and to 4.5 cm long; perianth 2-3 partite, ca. 0.3 mm long; stamen ca. 0.5 mm long, anther ca. 0.2 mm long; ; interfloral bracts (sub)peltate, caduceus or persistent, the apical part 0.3-0.4 mm diam., puberulous. Infructescences subglobose, to ca. 7 cm diam., often lobed, densely, minutely, brown puberulous (to velutinous); fruits ellipsoid. ca. 1-1.2 cm long (Berg; et al. 2011, 480-497).



Figure 64 Pictures of *A. lakoocha* leaves and fruits

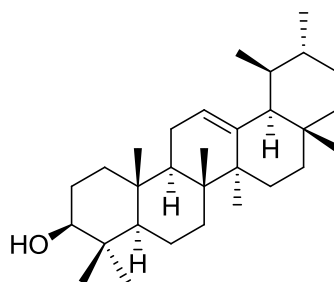
2.3 Chemical constituents and their bioactivities of *A. lakoocha*

Various flavonoids, stilbenes, 2-arylbenzofurans and triterpenes have been found in *A. lakoocha*. Many of which have shown significant pharmacological properties. However, oxyresveratrol or 2, 4, 3', 5'-tetrahydroxystilbene has reported in many researches as the major compound in the heartwoods of this plant species.

In 1957, Mongolsuk; et al. isolated oxyresveratrol (**3**) from the hot aqueous extract of the powdered woods of *A. lakoocha* (Mongolsuk; Robertson; & Towers. 1957: 2231-2233).

In 1960, Kapil; et al. studied on the hot petroleum ether extract of the air-dried stem barks of *A. lakoocha*. The extract yielded two triterpenoids, including lupeol acetate (**196**) and amyrin acetate (**324**) (Figure 65) (Kapil; & Joshi. 1960: 498).

Two known triterpenoids, lupeol acetate (**196**) and α -amyrin (**317**) were isolated from the petroleum ether extract of the air-dried stem barks of *A. lakoocha* by Mahato in 1996 (Figure 64) (Mahato; Banerjee; & Chakravarti. 1996: 16).



α -Amyrin (**324**)

Figure 65 Structure of compound **324**

In 1979, Chauhan; et al. isolated and characterized a new flavonol glycoside, galangin-3-O- β -galactopyranosyl(1 $\rightarrow$ 4)- α -L-rhamnopyranoside (**325**) from the MeOH extract of the root barks of *A. lakoocha* (Chauhan; Kumari; & Saraswat. 1979: 473-475). In the same year, the same group found a new glycoflavonol, galangin-3-O- α -L-(-)-rhamnopyranoside (**326**), together with two known compounds, lupeol (**327**) and β -sitosterol (**106**) (Chauhan; & Kumari. 1979: 86-88). Three years later, they also purified quercetin 3-O- α -L-rhamnopyranoside (**328**) and kaempferol 3-O- β -D-xylanopyranoside (**329**) from the hot EtOH ext. of the air-dried root barks of this plant (Figure 66) (Chauhan; et al. 1982: 217-218).

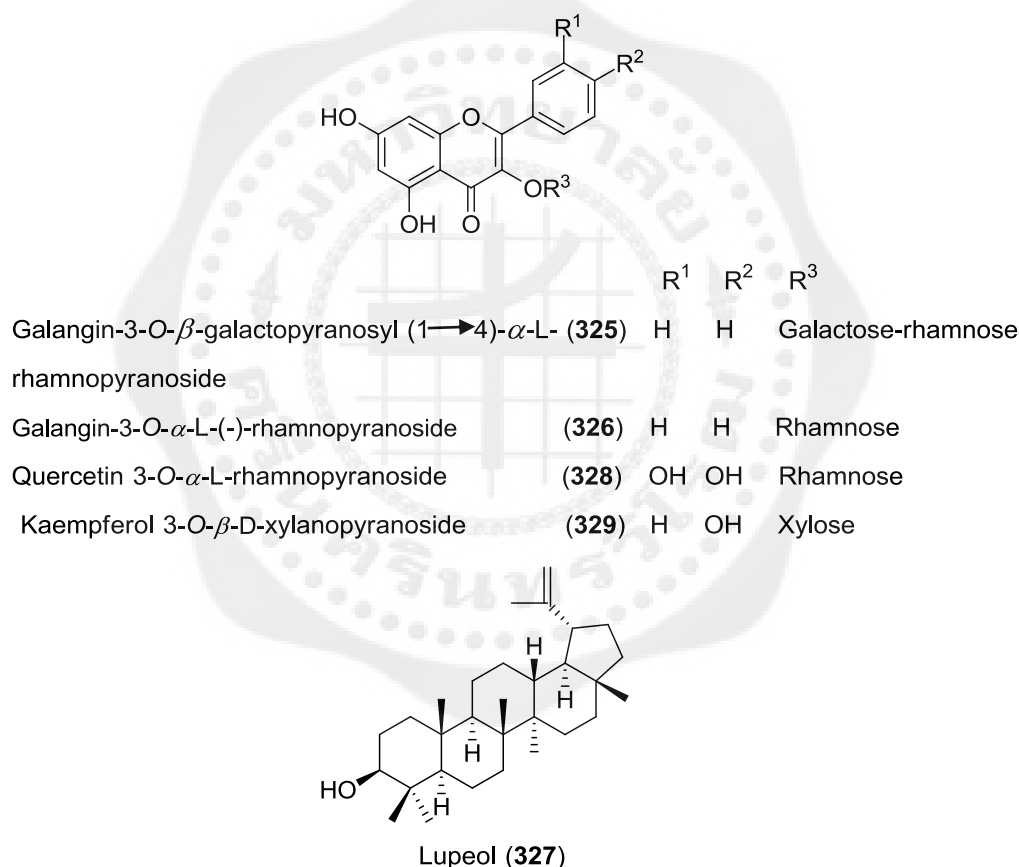


Figure 66 Structures of compounds **325-329**

In 1991, Chowdhury; et al. investigated on the effect of chemical modification on an anti T-like lectin or artocarpin, isolated from *A. lakoocha* seeds, was investigated in order to identify the type of amino acids involved in its agglutinating activity (Chowdhury; Ahmed; & Chatterjee. 1991: 563-571).

In 1995, Wongkham; et al. isolated two isolectins, ALA-I and ALA-II, from the seed extracts of *A. lakoocha*, which was collected in Thailand by anion exchange chromatography in order to study on the properties of these isolectins (Wongkham; et al. 1995: 1331-1334).

In 1999, Subhasis; et al. separated artocarpin, a α -D-galactosyl specific lectin from *A. lakoocha* seeds was purified by ammonium sulfate fractionation followed by affinity chromatography. From the result, localization of artocarpin using monospecific anti-artocarpin and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG by immunofluorescent staining technique revealed that the lectin was present in the entire cytosolic region and the cell wall (Subhasis; Majumder; & Chatterjee. 1999: 285-290).

Two new 2-arylbenzofurans, lakoochins A and B (**293** and **157**) were purified and determined their chemical structures from the CH_2Cl_2 extract of the air-dried ground roots of *A. lakoocha* which were collected in Thailand by Puntumchai in 2004. Both compounds **293** and **157** were tested for antimycobacterial, and cytotoxic activities against BC and KB cell lines. For antimycobacterial property, compound **293** with MIC value of 12.5 $\mu\text{g/mL}$ was more active than compound **157** with MIC value of 50 $\mu\text{g/mL}$. Furthermore, compound **157** showed a greater effect on both anti-BC and KB with IC_{50} values of 3.1 and 6.1 $\mu\text{g/mL}$, respectively, than compound **293** exhibited lower anti-BC activity with IC_{50} value 6.1 $\mu\text{g/mL}$ and it was also inactive for anti-KB activity (Puntumchai; et al. 2004: 485-486).

In 2005, Likhitwitayawuid; et al. found oxyresveratrol (**3**) from the heartwoods of *A. lakoocha*. The compound was evaluated for anti-HSV types-1 and -2, and anti-HIV (a wild-type human immunodeficiency virus type 1 (HIV-1/LAI)) activities. From the results, it possessed moderate activity against both types of HSV-1 and -2 with EC_{50} values of 15.4 and 13.4 $\mu\text{g/mL}$, respectively. Additionally, it displayed moderate anti-HIV-1 activity with EC_{50} value of 7.0 $\mu\text{g/mL}$, comparing with the positive control, azidothymidine (AZT) (EC_{50} 0.003 $\mu\text{g/mL}$) and it was also inactive for toxicities in peripheral blood mononuclear (PBM), CEM and vero cells (Likhitwitayawuid; et al. 2005: 177-182).

In 2006, Tengamnuay; et al. prepared the heartwood aqueous extract and oxyresveratrol (**3**) isolated in order to investigate on the *in vitro* anti-tyrosinase and *in vivo* skin whitening activities. For anti-tyrosinase property, the extract and compound **3** exhibited higher potency with IC_{50} values of 0.76 and 0.83 $\mu\text{g/mL}$, respectively, than those of kojic acid (IC_{50} 5.8 $\mu\text{g/mL}$) and the licorice extract (IC_{50} 12.9 $\mu\text{g/mL}$). Moreover, evaluation of skin whitening activity revealed that the *A. lakoocha* extract at 0.25% in propylene glycol

appeared had more effective than 3% kojic acid and 0.25% licorice extract (Tengamnuy; et al. 2006: 269-276).

In 2008, Chatterjee; et al. isolated agglutinin (ALA) from the seeds of *A. lakoocha* in order to examine on mitogenic and antiproliferative activities, including Jurkat, U937 and K562 cells. In mitogenic assay, ALA was found to be as a very potent mitogen for human PBMC. Furthermore, ALA showed antiproliferative activities on Jurkat and U937 cells with IC_{50} values of 13.15 ng/ml and 25.14 ng/ml, respectively (Chatterjee; et al. 2008: 741-752).

In 2008, Chuanasa; et al. obtained an active fraction from the MeOH extract of the heartwoods of *A. lakoocha* and it was then purified using vacuum liquid chromatography to furnish oxyresveratrol (**3**). This compound was determined for its antiviral activity by the plaque reduction assays against HSV-1 strains including wild type (7401H), clinical isolates 1-3, TK-deficient and PAA-resistant with IC_{50} value in the range of 19.8-25.5 μ g/mL. Furthermore, various antiviral activities of the compound against HSV-1 (KOS and 7401H), HSV-2 (Baylor186), poliovirus type 1 (Sabin strain) and measles virus (Tanabe strain) were carried out, and had IC_{50} value in the range of 18.7-90.0 μ g/mL (Chuanasa; et al. 2008: 62-70).

In 2009, Sasivimolphan; et al. studied on anti-varicella-zoster virus (VZV) activity of oxyresveratrol (**3**) obtained from *A. lakoocha* heartwoods. The compound was tested with various strains, including wild type (kawaguchi strain), TK-deficient and two types of DNA polymerase mutant (ACV-resistances A3 and A6). The IC_{50} values of the compound were in the range of 12.80-12.99 μ g/mL, indicating that there were no significant differences among the IC_{50} values of it. Meanwhile, the reference drug, acyclovir, had the IC_{50} values of 0.17 ± 0.06 μ g/mL (Sasivimolphan; et al. 2009: 95-97).

In 2009, Pandey; et al. examined on the antioxidant effect of the MeOH extracts of the barks of *A. lakoocha* using DPPH and ferric ion reducing properties. They found that the MeOH extracts showed a strong antioxidant activity with 53.2 mg/g of flavonoids content and 0.360 μ g/mL of phenolic content (Pandey; & Bhatnagar. 2009: 23-28).

In 2010, Kumar; et al. reported on some biological activities of the MeOH extract of the fruit pericarps of *A. lakoocha*. In this study, antibacterial effect of the extract against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* by agar well diffusion method. The extract was more effective against on *S. aureus* than *K. pneumoniae* and *P. aeruginosa*. In addition, the extract exhibited antioxidant activity with IC_{50} value of 49.42 μ g/mL whereas the ascorbic had an IC_{50} value of 6.09 μ g/mL. For anthelmintic

activity, the extract at concentration of 100 mg/mL had greater effect than standard drug, 1% piperazine citrate, by paralysis and death time. The insecticidal activity of the extract was determined against against 2nd and 3rd instar larvae of *Aedes aegypti*. It was found that 2nd instar larvae were shown to be more susceptible than 3rd instar larvae. The preliminary phytochemical analysis of the extract was also studied. The extract had revealed the presence of tannins and alkaloids. The biological activities of the extract in this study could thus be due to the presence of tannins and alkaloids (Kumar; et al. 2010: 239-245).

In 2010, Nguyen; et al. isolated four known triterpenoids, lupeol (lup-20(29)-ene-3b-ol) (**327**), lupeol acetate (lup-20(29)-ene-3b-acetate) (**196**), lup-20(29)-ene-3-one (**330**) and betulinic acid (**275**) from the *n*-hexane and EtOAc extracts of *A. lakoocha* (Nguyen; Nguyen; & Tran. 2010: 507-510). In the same year, the same group further obtained three compounds, friedelan-3-one (**276**), stigmast-4-en-3-one (**331**) and 1-tricosanoyl-glycerol (**332**) from the same plant species (Figure 67) (Nguyen; et al. 2010: 754-757).

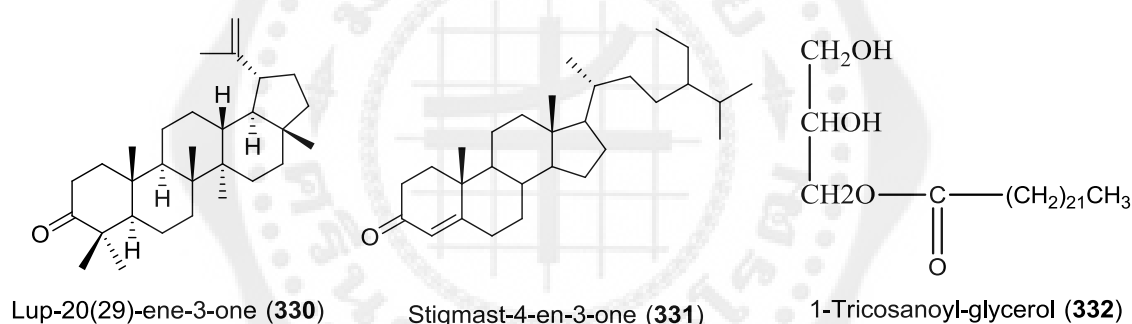


Figure 67 Structures of compounds **330-332**

In 2010, Povichit; et al. investigate on 15 medicinal plant extracts for the studies of total phenolic content and free radical scavenging activities by DPPH and ABTS methods following anti-lipid peroxidation by TBARS, and antiglycation activities. In this study, *A. lakoocha* is one of selected plants which extracted with 95% EtOH to give EtOH extract. From the results, the EtOH extract contained high phenolic content with GAE value 0.48 ± 0.01 . It also showed high antioxidant activity on both DPPH and ABTS assays. Furthermore, high anti-lipid peroxidation activity of this plant extract had IC₅₀ of 1,220 µg/mL. The extract also exerted promising antiglycation activity (Povichit; et al. 2010: 403-408). Consequently, the same group further purified oxyresvertarol (**3**) from the EtOH extract of *A. lakoocha* heartwoods in the same year. The compound was evaluated for

antiglycation and antioxidant activities (DPPH method) and anti-lipid peroxidation activity (TBARS method). The results showed antiglycation activity of the compound with IC_{50} value of $2.0 \pm 0.03 \mu\text{g/mL}$ which was five times greater than positive control, aminoguanidine. Moreover, the compound possessed antioxidant activity by DPPH and TBARS methods, with IC_{50} values of 100 ± 0.01 and $430 \pm 0.03 \mu\text{g/mL}$, respectively (Povichit; et al. 2010: 454-461).

In 2010, Singhatong; et al. studied on the antioxidant activity of the EtOH extract of *A. lakoocha* heartwoods. In chemical profiling using HPLC, the EtOH extract was found to contain rutin (**333**), quercetin (**334**), pyrogallol (**335**), gallic acid (**336**), resorcinol (**337**), caffeic acid (**338**) and catechin (**30**) (Figure 68). The extract also displayed antioxidant activities with 128.30 ± 0.13 , 55.86 ± 0.01 , and $463.49 \pm 0.01 \mu\text{mol trolox/g}$ extracted by ABTS, DPPH, and H_2O_2 scavenging methods, respectively. In addition, the extract was further investigated for totals of phenolic compounds. A 1g of dry weight extract contained a total amount of phenols, flavonoids and tannins as follows: 325.63 mg GE, 521.98 mg QE and 124.03 g TE, respectively (Singhatong; & Leelarungrayub. 2010: 947-953).

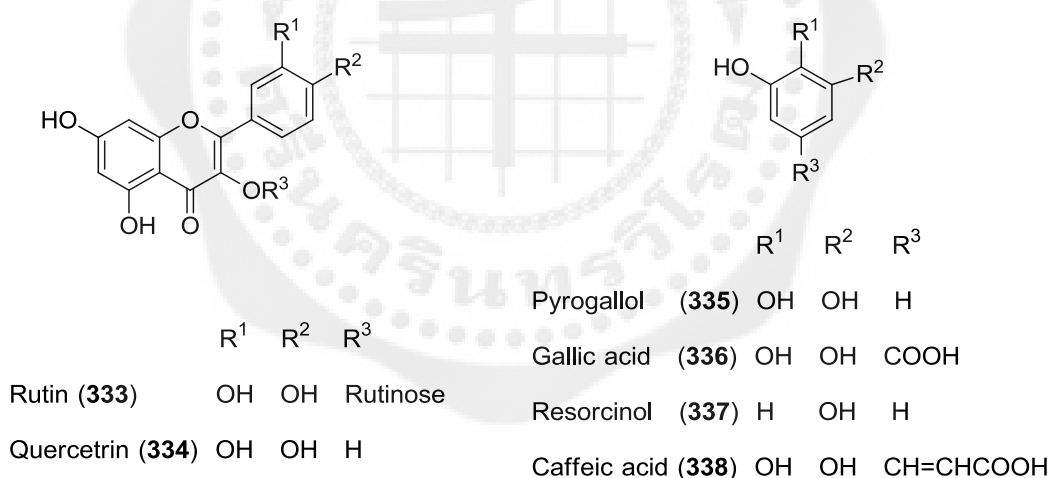


Figure 68 Structures of compounds **333-338**

In 2010, Sritularak; et al. reported on the isolation and structure determination of three new 2-arylbenzofurans, artolakoochol (**339**), 4-hydroxyartolakoochol (**340**) and cycloartolakoochol (**341**) from the EtOAc extract of the root barks of *A. lakoocha* (Figure 69). All of them were evaluated for their inhibitory activity against both HSV types-1 and -2 using the plaque reduction assay. They were inactive at the concentration of $100 \mu\text{g/mL}$ (Sritularak; et al. 2010: 6548-6558).

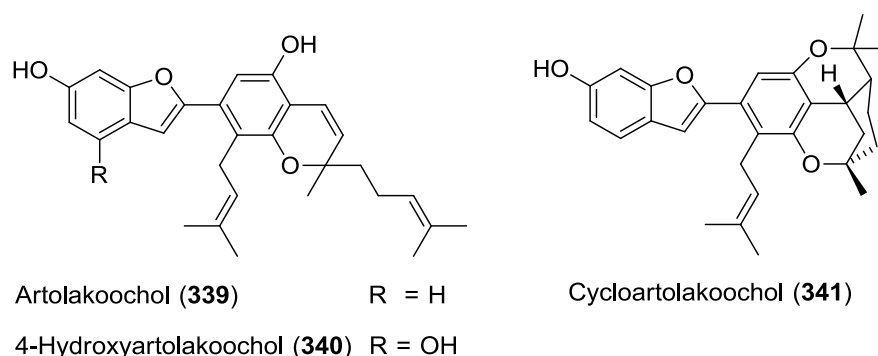


Figure 69 Structures of 2-arylbenzofurans **339-341**

In 2011, Lipipun; et al. investigated on inhibitory property on HSV-1 clinical isolates and HSV-2 clinical isolates as well as wild-type HSV-1 and HSV-2 *in vitro* in the plaque reduction assay of oxyresveratrol (**3**) from the heartwoods of *A. lakoocha*. From the results, the compound displayed anti-HSV-1 and -2 with IC_{50} values ranging of 20.9-29.5 and 22.2-27.5 $\mu\text{g/mL}$, respectively. Meanwhile, the IC_{50} values of positive drug, acyclovir, for HSV-1 and -2 were 0.07-0.31 and 0.11-0.43 $\mu\text{g/mL}$, respectively. Additionally, the compound at 2.5%, 5%, 10% and 20% w/w was also incorporated in cream base and was used in animal experiments. From the results, the IC_{50} values of the compound against HSV-1 (KOS) and HSV-2 (Baylor186) were 22.5 ± 3.4 and 25.0 ± 2.5 $\mu\text{g/mL}$ in post-treatment assay and the IC_{50} values of 22.5 ± 2.5 and 25.0 ± 3.0 $\mu\text{g/mL}$ in the inactivation-treatment assay, respectively (Lipipun; et al. 2011: 154-160).

In 2011, Raote; et al. developed to quantify rutin (**333**) and quercetin (**334**) from the leaves of *A. lakoocha* using liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (Raote; et al. 2011: 848-853). In the same year, the same group analyzed on the quantitation of two triterpenoids, lupeol (**327**) and β -sitosterol (**106**) (Figure 66), from the leaves of *A. lakoocha* by high-performance thin layer chromatographic technique (HPTLC). This investigation will help in qualitative analysis of plant material using fingerprint pattern and quantitative analysis of compounds **327** and **106** (Raote; Jangid; & Tale. 2011: 860-866).

Callus cultures of *A. lakoocha* seedling were carried out by Maneechai in 2012. They were further isolated for the structure elucidation, and for inhibitory activity against the DPPH free radical and the enzyme tyrosinase. Four new compounds, 2'-O-methylalbanin A (**342**), cycloisoartocarpesin (**343**), artolacuchin (**344**) and isochlorophorin (**345**), were obtained and characterized, along with nine polyphenolics including cudraflavone C (**68**),

norartocarpin (**75**), albanin A (**76**), cudraflavone B (**119**), isoartocarpesin (**140**), artotonkin (**174**), (-)-catechin (**287**), chlorophorin (**349**) and aesculin (**350**) (Figure 70). From the results, compounds **344**, **345**, **119** and **349** exhibited antioxidant activity with IC_{50} values in the range of $12.5 \pm 38.06 \mu\text{g/mL}$, comparing to quercetin with IC_{50} value of $10.3 \mu\text{g/mL}$. For tyrosinase inhibitory effect, compounds **344**, **345**, **119** and **346** also had IC_{50} values in the range of $3.0\text{--}25.0 \mu\text{g/mL}$, comparing to oxyresveratrol with IC_{50} value of $0.41 \mu\text{g/mL}$ (Maneechai; et al. 2012: 42-49).

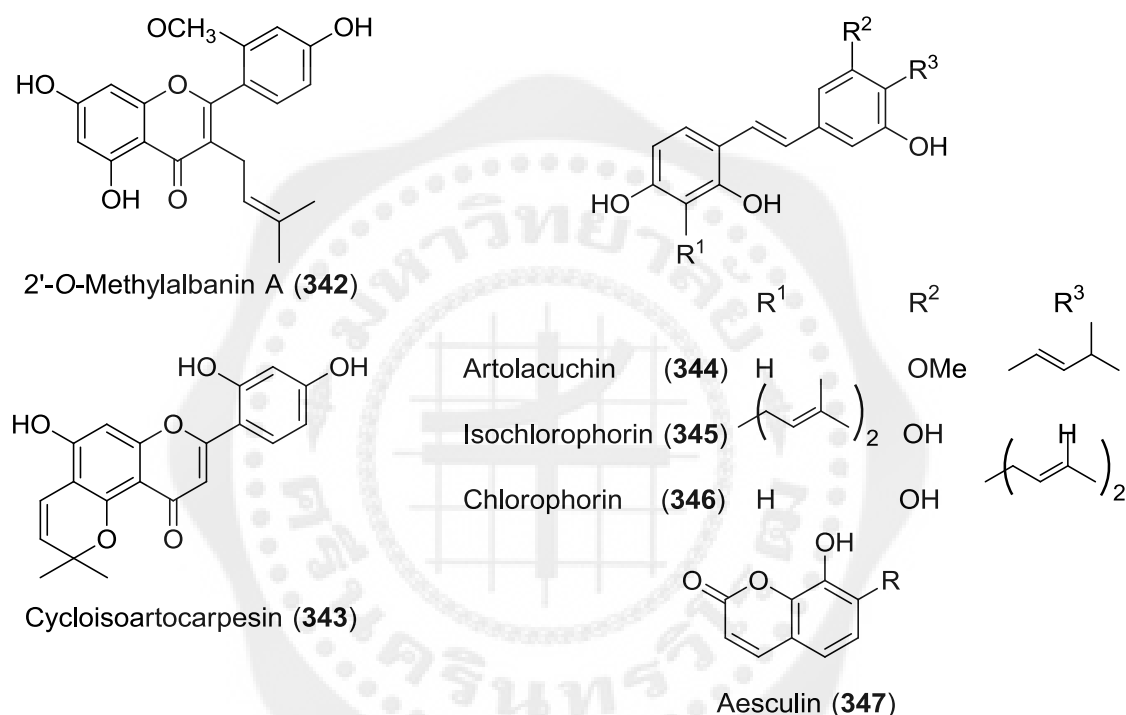
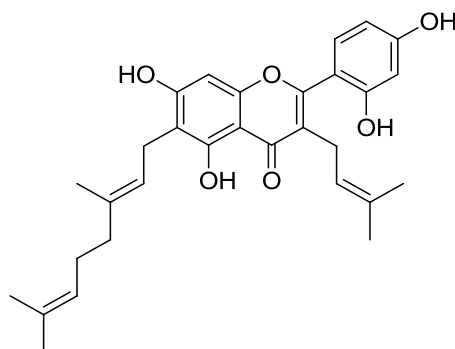


Figure 70 Structures of compounds **342-347**

In 2013, Sritularak; et al. purified a new compound 5,7,2',4'-tetrahydroxy-6-geranyl-3-prenyl-flavone (**348**), along with three known flavonoids, cudraflavone C (**68**), (+)-catechin (**210**) and (+)-afzelechin-3-O- α -L-rhamnopyranoside (**254**) from the MeOH extract of the root barks of *A. lakoocha* (Figure 71). The inhibitory effects against HSV types-1 and -2 of all compounds were carried out. Compounds **68** and **348** exerted moderate and weak activities against both types of HSV, respectively, whereas compounds **210** and **254** were inactive (Sritularak; et al. 2013: 1079-1080).



5,7,2',4'-Tetrahydroxy-6-geranyl-3-prenyl-flavone (**348**)

Figure 71 Structure of 5,7,2',4'-tetrahydroxy-6-geranyl-3-prenyl-flavone (**348**)

The MeOH extract of the shedded leaves of *A. lakoocha* were partitioned to furnish four fractions, petroleum benzene, hexane, ethyl acetate, acetone and methanol fractions by Biswas in 2013. All fractions were tested for antioxidant activity. Among these, the hexane fraction displayed the most activity with IC_{50} value of 15 $\mu\text{g/mL}$. The hexane fraction was thus purified to afford squalene (**317**). Compound **317** was then examined for antifungal and antibacterial effects. For antifungal activity, it exerted maximum inhibitory effects on *A. fumigates* at 1500 and 2000 ppm, *A. niger* at 500 ppm, and *A. tamari* at 1000 and 2000 ppm. Moreover, it had much effect on *E. coli* and *S. lutea* but no effect on *S. aureus* (Biswas; & Chakraborty. 2013: 21-27).

2.4 2-Arylbenzofurans

2-arylbenzofurans or 2-phenylbenzofurans, are important family of naturally occurring oxygen-containing heterocycles, are classified in polyphenol/phenolic compounds and have been recognized as antifungal phytoalexins (Ghosh; Banerjee; & Baul. 1999: 11537-11546). These compounds with various substituents, hydroxyl, methoxy or prenyl groups, have been extensively reported to possess significantly pharmacological activities including antifungal, antibacterial, insect feeding deterrent, free radical scavenging, antioxidant, cyclooxygenase inhibiting and estrogenic activities. Most of them, especially prenylated 2-arylbenzofurans contain in the family Moraceae and Fabaceae, originated from the phenylpropanoid-polymalonate pathway. Previous investigation on comparison of substitution patterns and labeling experiments has revealed that there are possibly two main 2-arylbenzofuran biogenesis. Depending upon the botanical family, Moraceae produces 3',5',6-trioxygenated 2-arylbenzofurans deriving from stilbene whereas Fabaceae

produces 2',4',6-trioxygenated 2-arylbenzofurans deriving from isoflavonoids (Reuß; & König. 2004: 3113-3118).

2.5 2-Arylbenzofurans from *Artocarpus* plants and their bioactivities except

A. lakoocha

As mentioned above, 2-arylbenzofurans is one of the components found in *Artocarpus* plants. They possessed several biological activities. It should be noted that 2-arylbenzofurans from Moraceae having 3',5',6-trioxygenated 2-arylbenzofurans skeleton (Table 7).

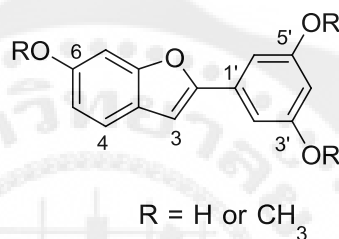


Figure 72 3',5',6-Trioxygenated 2-arylbenzofuran skeleton

Table 7 Bioactivities of 2-arylbenzofurans from *Artocarpus* plants

<i>Artocarpus</i> sp./ Compounds	Plant source	Bioactivities	References
<i>A. altilis</i>			
Moracin M (139)	fruits	-	Amarasinghe et al. 2008: 323-325
<i>A. gomezianus</i>			
Artoindonesianin O (2)	tree barks	-	Hakim et al. 2002: 597-603

Table 7 (continued)

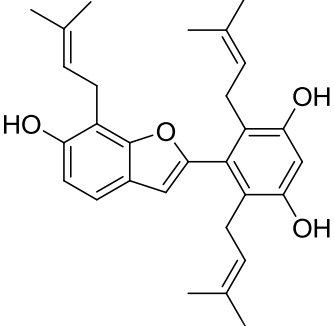
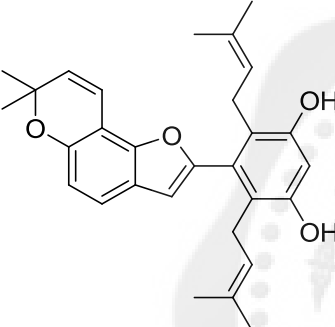
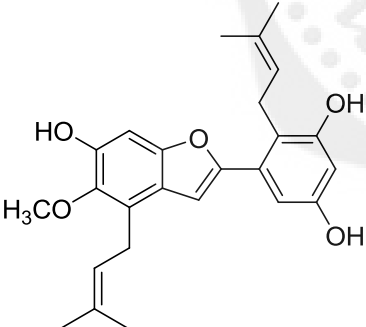
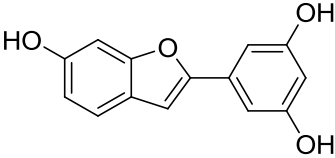
<i>Artocarpus</i> sp./ Compounds	Plant source	Bioactivities	References
 Artoindonesianins X (26)	root barks	moderate activity against the brine shrimp (<i>Artemia</i> <i>salina</i>)	Soekamto; et al. 2003: 831-834
 Artoindonesianins Y (27)	root barks	moderate activity against the brine shrimp (<i>Artemia</i> <i>salina</i>)	Soekamto; et al. 2003: 831-834
<i>A. heterophyllus</i>  Artoheterophyllin A (177)	twigs	-	Zheng; et al. 2009: 6649- 6655
 Moracin M (139)	twigs	moderate inhibitory activities against mushroom tyrosianse	Zheng; et al. 2009: 6649- 6655

Table 7 (continued)

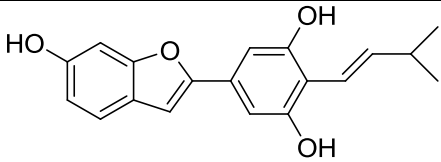
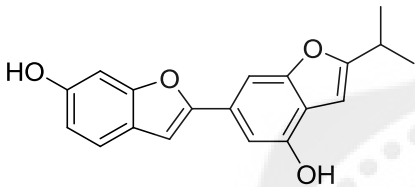
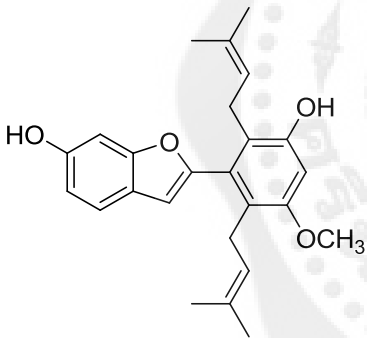
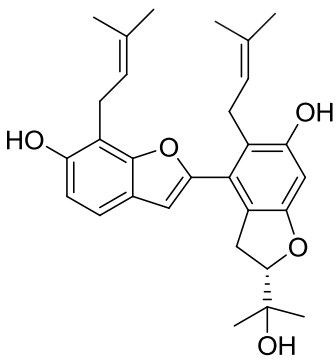
<i>Artocarpus</i> sp./ Compounds	Plant source	Bioactivities	References
 (E)-5-(6-Hydroxybenzofuran-2-yl)-4-(3-methylbut-1-enyl)benzene-1,3-diol (185)	twigs	moderate inhibitory activities against mushroom tyrosianse	Zheng; et al. 2009: 6649- 6655
 Artocarstilbene A (270)	twigs	-	Di; et al. 2013: 24-28
<i>A. hypargyreus</i>  Mulberrofuran N (265)	stems	-	Yu; et al. 2012: 394-402
<i>A. nitidus</i>  Artonitidin A (173)	roots	-	Zhao; et al. 2009: 2209- 2216

Table 7 (continued)

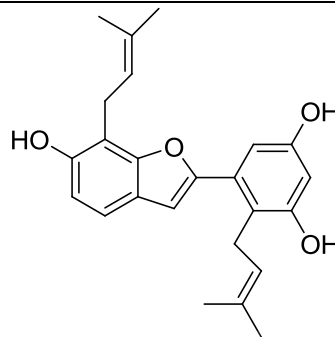
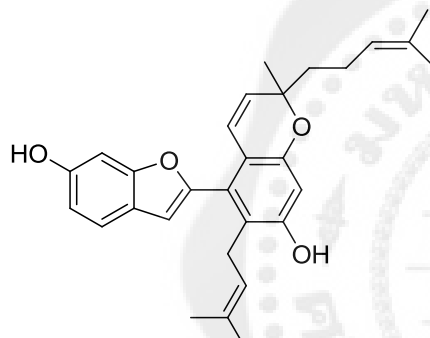
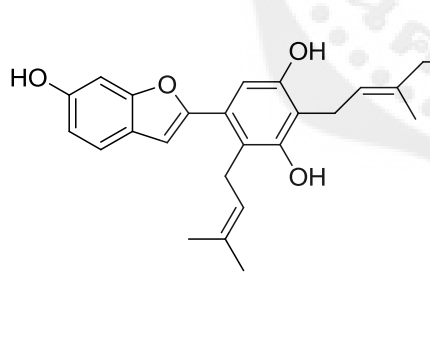
<i>Artocarpus</i> sp./ Compounds	Plant source	Bioactivities	References
 Artocarpin B (174)	roots	-	Zhao; et al. 2009: 2209- 2216
<i>Artocarpus petelotii</i>  Artopetelin A (55)	root barks	inactive cytotoxicities against HepG2 and MCF	Chen; et al. 2005: 2554- 2560 Shen; et al. 2008: 1451- 1456
 Artopetelin B (56)	root barks	potent cytotoxicities against HepG2 and MCF	Chen; et al. 2005: 2554- 2560 Shen; et al. 2008: 1451- 1456 Ren; et al. 2013: 97-100

Table 7 (continued)

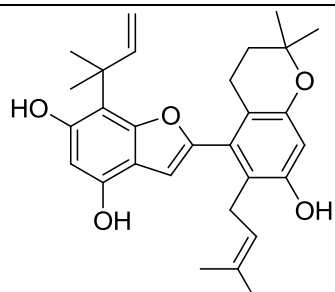
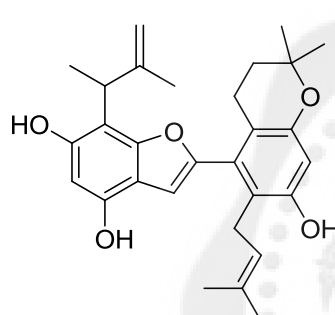
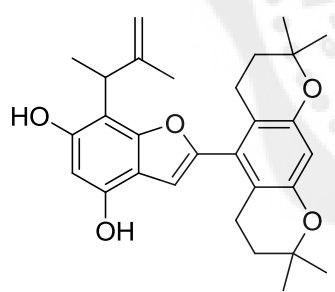
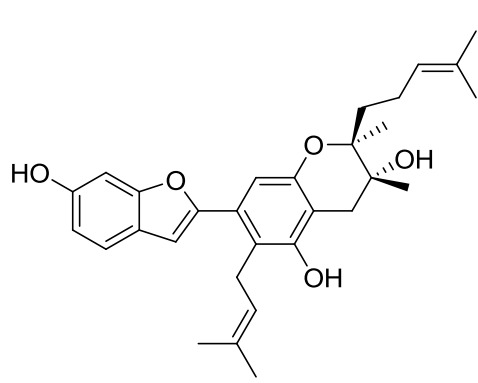
<i>Artocarpus</i> sp./ Compounds	Plant source	Bioactivities	References
 Artopetelin C (57)	root barks	potent cytotoxicities against HepG2 and MCF	Chen; et al. 2005: 2554-2560 Shen; et al. 2008: 1451-1456
 Artopetelin D (77)	root barks	potent cytotoxicities against HepG2 and MCF	Chen; et al. 2006, 89: 1000-1007 Shen; et al. 2008: 1451-1456
 Artopetelin E (78)	root barks	potent cytotoxicities against HepG2 and MCF	Chen; et al. 2006: 1000-1007 Shen; et al. 2008: 1451-1456
 Artopetelin F (79)	root barks	-	Chen; et al. 2006: 1000-1007 Shen; et al. 2008: 1451-1456

Table 7 (continued)

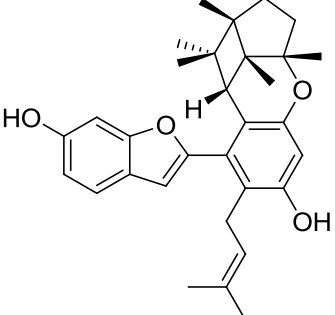
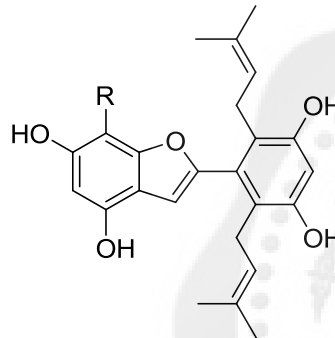
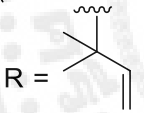
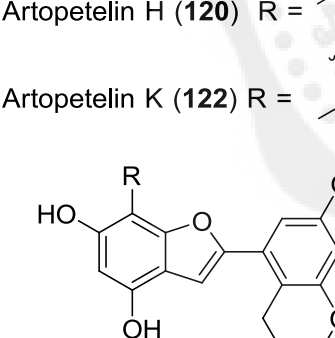
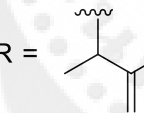
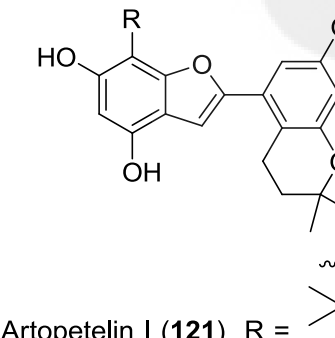
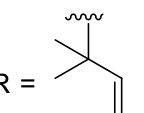
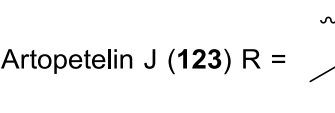
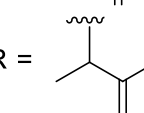
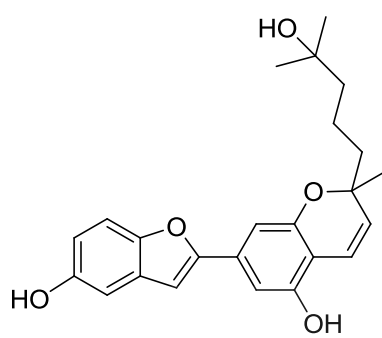
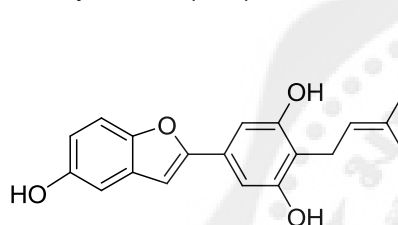
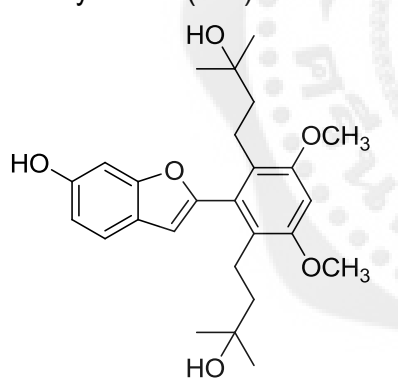
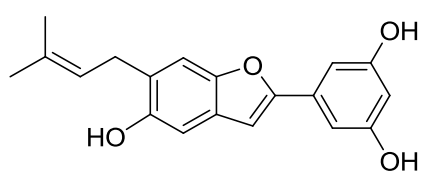
<i>Artocarpus</i> sp./ Compounds	Plant source	Bioactivities	References
 Artopetelin G (80)	root barks	inactive cytotoxicities against HepG2 and MCF	Chen; et al. 2006: 1000- 1007 Shen; et al. 2008: 1451- 1456
 Artopetelin H (120) R = 	root barks	-	Shen; et al. 2007: 1147- 1155
 Artopetelin K (122) R = 			
 Artopetelin I (121) R = 			
 Artopetelin J (123) R = 			

Table 7 (continued)

<i>Artocarpus</i> sp./ Compounds	Plant source	Bioactivities	References
<i>A. styracifolius</i>			
 Artostyracin A (320)	roots	strong anti-respiratory burst activity	Ren; et al. 2014: 235-239
 Artostyracin B (321)	roots	moderate anti-respiratory burst activity	Ren; et al. 2014: 235-239
 Artostyracin C (322)	roots	strong anti-respiratory burst activity	Ren; et al. 2014: 235-239
 Morican N (323)	roots	moderate anti-respiratory burst activity	Ren; et al. 2014: 235-239

2.6 2-Arylbenzofurans from other plant species and their significant bioactivities (e.g. Fabaceous plants)

However, 2-arylbenzofurans can be also found in other plant species, some of them showed interesting biological activities below (Table 8).

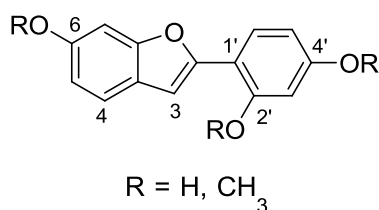


Figure 73 2',4',6-Trioxygenated 2-arylbenzofuran skeleton

Table 8 Significant bioactivities of 2-arylbenzofurans from other plant species

Plant sp./ Compounds	Plant source	Bioactivities	References
<i>Andira inermis</i>			
 Andinermal A (349)	leaves	strong antiplasmodial activity	Kraft; et al. 2001: 769-774
 Andinermal C (350)	leaves	strong antiplasmodial activity	Kraft; et al. 2001: 769-774
<i>Bolusanthus speciosus</i>			
 Bolusanthin IV (351)	root woods	moderate antimicrobial and antioxidant activities	Erasto; et al. 2004: 875-880
<i>Cicer bijugum</i>			
 Cicerfuran (352)	roots	antibacterial against <i>B. subtilis</i> and <i>P. syringae</i> , and antifungal activities against <i>B. cinerea</i> and <i>A. niger</i>	Aslam; et al. 2009: 191-195

Table 8 (continued)

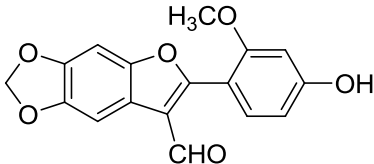
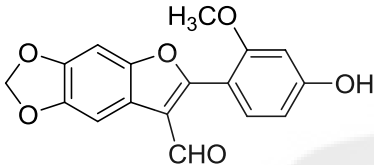
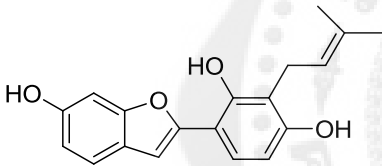
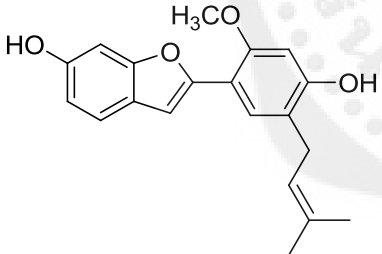
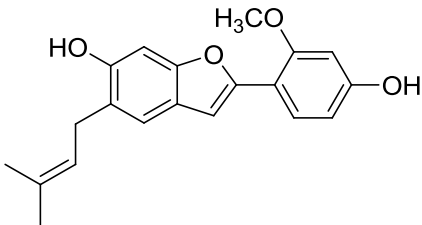
Plant sp./ Compounds	Plant source	Bioactivities	References
<i>Dalea spinosa</i>			
 Spinosan A (353)	barks	-	Belofsky; et al. 2006: 261-264
 Spinosan A (354)	barks	-	Belofsky; et al. 2006: 261-264 303-310
<i>Erythrina addisoniae</i>			
 2'-O-demethylbidwillol B (355)	stem barks	protein tyrosine phosphatase 1B (PTP1B) inhibitory activity	Na; et al. 2007: 3868-3871
 Addisofuran A (356)	stem barks	PTP1B inhibitory activity	Na; et al. 2007: 3868-3871
 Addisofuran B (357)	stem barks	PTP1B inhibitory activity	Na; et al. 2007: 3868-3871

Table 8 (continued)

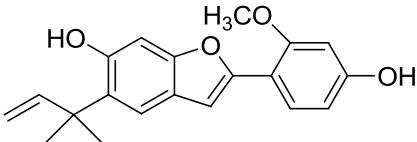
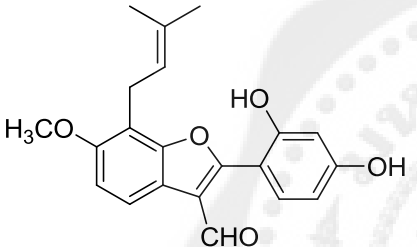
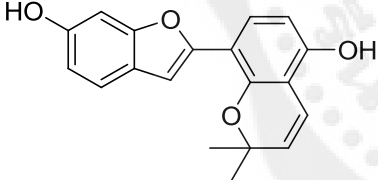
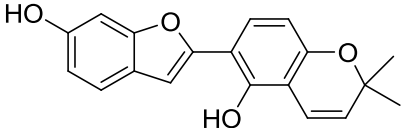
Plant sp./ Compounds	Plant source	Bioactivities	References
<i>Erythrina burtii</i>			
 Burtinol-D (358)	root barks and stem barks		Yenesew; et al. 2002: 337-341
		strong antiplasmodial and antioxidant activities	Yenesew; et al. 2012: 123-127
<i>Erythrina poeppigiana</i>			
 Erypoeigin F (359)	roots	anti-MRSA (methicillin-resistant <i>Staphylococcus aureus</i>) activity	Tanaka; et al. 2003: 597-602
<i>Glycyrrhiza glabra</i>			
 4',6-dihydroxy-[6'',6''-dimethylpyrano (2'',3'' : 2',3')-2-arylbenzofuran (360)	roots	-	Kinoshita; et al. 1996: 1218-1221
 2',6-dihydroxy-[6'',6''-dimethylpyrano (2'',3'' : 4',3')-2-arylbenzofuran (361)	roots	-	Kinoshita; et al. 1996: 1218-1221

Table 8 (continued)

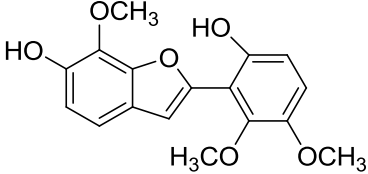
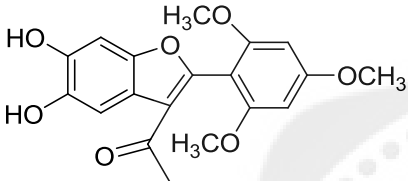
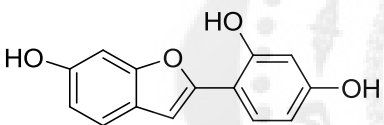
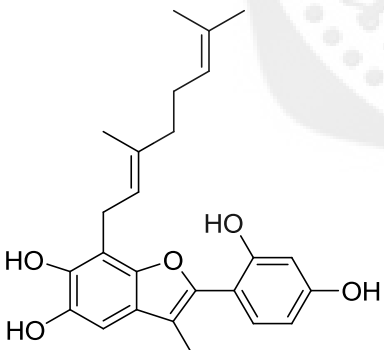
Plant sp./ Compounds	Plant source	Bioactivities	References
<i>Lespedeza virgata</i>			
 Lespedezavirgatalol (362)	aerial	strong inhibitory activity against lipid peroxidation in rat kidney homogenate and plasma	Chen; et al. 2008: 929-932
 Lespedezavirgatal (363)	aerial	strong inhibitory activity against lipid peroxidation in rat kidney homogenate and plasma	Chen; et al. 2008: 929-932
<i>Lespedeza homoloba</i>			
 2-(2,4-dihydroxyphenyl)-6-hydroxybenzofuran (364)	stems	antioxidant activity	Miyase; et al. 1999: 303-310
 Lespedezol B ₁ (365)	stems	antioxidant activity	Miyase; et al. 1999: 303-310

Table 8 (continued)

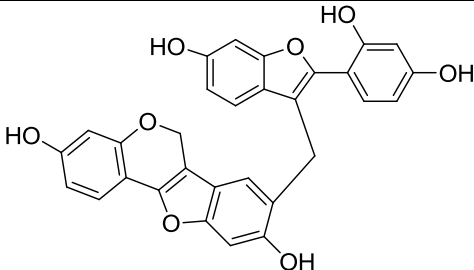
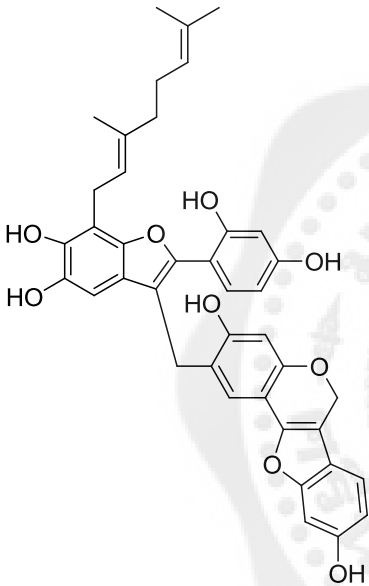
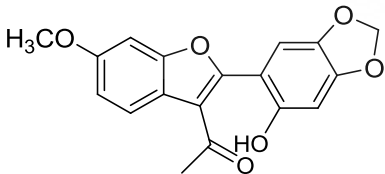
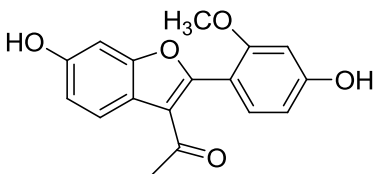
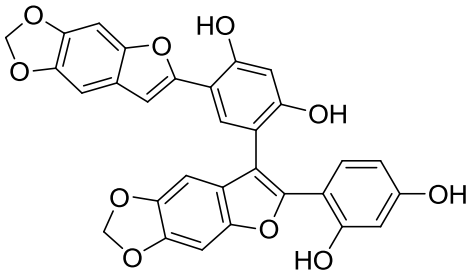
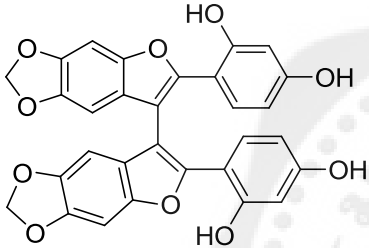
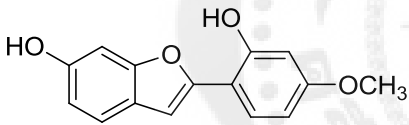
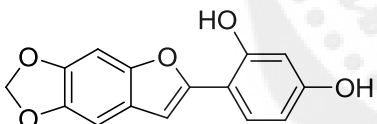
Plant sp./ Compounds	Plant source	Bioactivities	References
 Lespedezol B₂ (366)	stems	antioxidant activity	Miyase; et al. 1999: 303-310
 Lespedezol B₃ (367)	stems	antioxidant activity	Miyase; et al. 1999: 303-310
<i>Psorothamnus arborescens</i>  2-(2'-hydroxy-4',5'-methylenedioxyphenyl)-6-methoxybenzofuran-3-carbaldehyde (368)	root	antiprotozoal activity	Salem; & Werbovetz. 2006: 43-49
<i>Pueraria lobata</i>  Puerariafuran (369)	roots	strong inhibitory activity on AGEs formation	Jang; et al. 2006: 1315-1317

Table 8 (continued)

Plant sp./ Compounds	Plant source	Bioactivities	References
<i>Sophora tonkinensis</i>			
 Shandougenine A (370)	roots	antioxidant activity	Luo; et al. 2014: 21-27
 Shandougenine B (371)	roots	antioxidant activity	Luo; et al. 2014: 21-27
 Bolusanthin IV (372)	roots	antioxidant activity	Luo; et al. 2014: 21-27
 2-(2',4'-Dihydroxyphenyl)-5,6-methylenedioxybenzofuran (373)	roots	antioxidant activity	Luo; et al. 2014: 21-27

2.7 Alzheimer's disease (AD)

AD, the leading cause of dementia in elderly, is a serious neurodegenerative disorder which expresses many symptoms, including memory loss, emotional behavior and cognitive impairment (Singh; et al. 2013: 165-188). The estimated number of AD patients worldwide is forecasted to elevate to 106.8 million in 2050 (Lee; et al. 2014: 913-918). Although, the certain cause of this disorder still unknown. But, there have been three main hypotheses to explain the possible cause of AD as follows:

Tau protein hypothesis

Tau, a highly soluble microtubule-binding protein, abundantly present in neurons which stabilizes the microtubules. The function of microtubule is to provide structural support to neurons. In the process, tau can be hyperphosphorylated to destabilize microtubule, then hyperphosphorylated tau can self-assemble to form neurofibrillary tangles NFT (Figure 74). The formation of NFTs present as one of the hallmarks of AD (Duan; et al. 2012: 1-7).

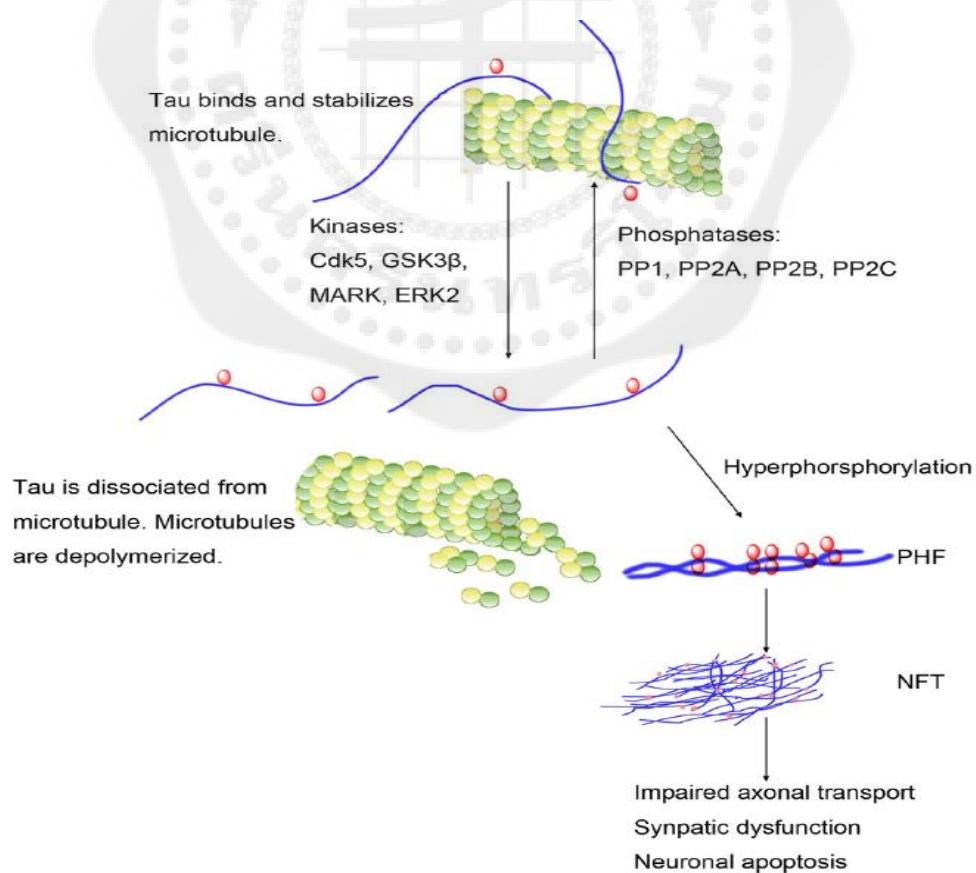


Figure 74 Effects of tau hyperphosphorylation on axonal function

Amyloid β peptide ($A\beta$) hypothesis

$A\beta$ is generated by the continuous cleavage of amyloid precursor protein (APP) via two main proteases, β - and γ -secretases. APP plays a key role in amyloid plaque. It is a single pass transmembrane which commonly expressed in integral membrane protein. It is first cleaved by β -secretase at the N-terminus of the $A\beta$ domain, and this is followed by γ -secretase cleavage at the C-terminus to generate the $A\beta$ amylogenic peptides (Figure 75). $A\beta$ is secreted into the extracellular and can then aggregate into oligomers and form neuritic plaques which are toxic to neuron system (Rohn; et al. 2013: 14908-14922).

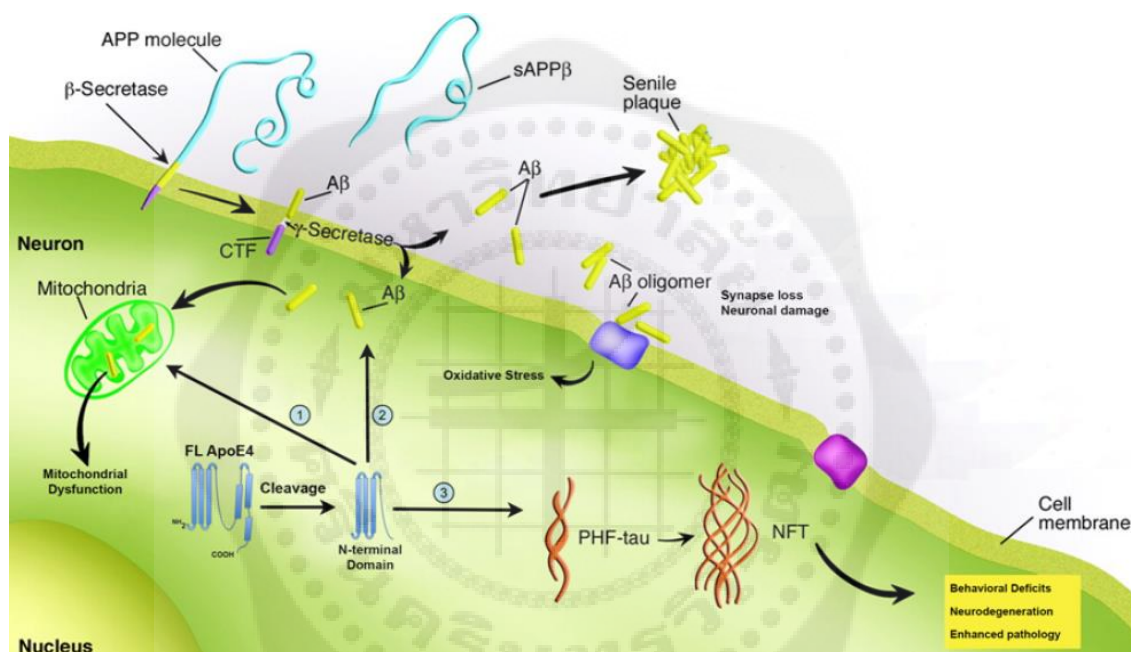


Figure 75 APP processing and AD

Cholinergic system hypothesis

This system in AD patients involves in two major cholinesterase enzymes (ChE), acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BuChE, EC 3.1.1.8) (Jeger. 2013: 424-435). AChE is selectively responsible for hydrolyzing neurotransmitter acetylcholine (ACh) in the early stages of AD and expresses mainly in the neurons, axons and blood. During neurotransmission, ACh is synthesized by choline acetyltransferase in pre-synaptic membrane (Figure 76). It then released into the synaptic cleft and binds to ACh receptors on the postsynaptic membrane. The free molecule of choline (Ch) is taken up again to presynaptic and it combines to acetyl-CoA through the action of choline

acetyltransferase. However, in AD brain AChE presents in synaptic area can also hydrolyze ACh and led to the depletion of ACh.

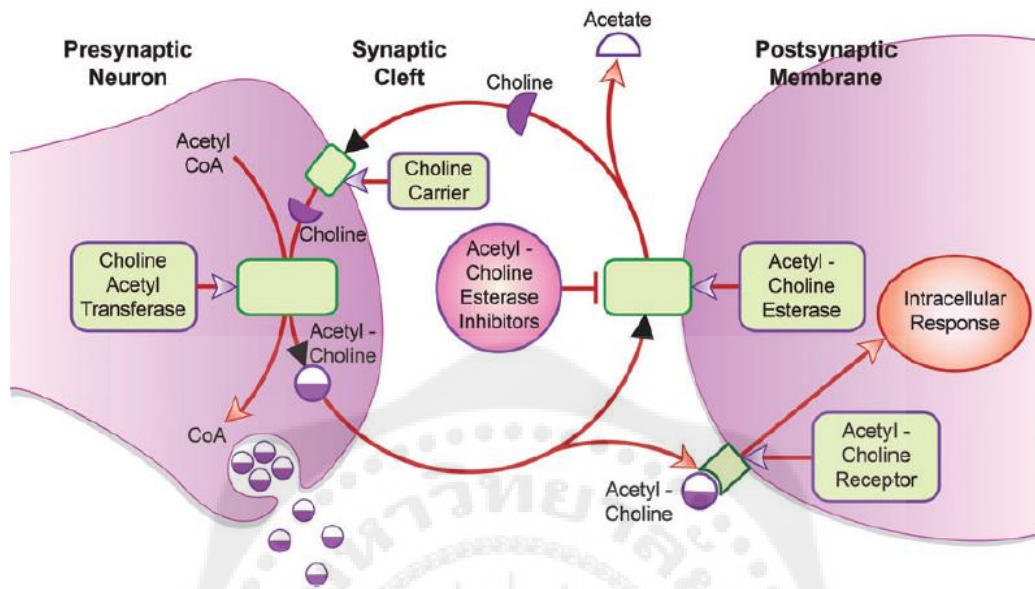


Figure 76 Cholinergic system in neurotransmission

Structure of AChE

The structure of AChE is a deep and narrow gorge, $\sim 20 \text{ \AA}$ long (Figure 77). This enzyme has two main active sites, anionic and esteratic subsites. When ACh binds to the enzyme, it is then hydrolyzed. Quaternary ammonium group of ACh interacts with anionic subsite. Then, the acetyl group is transferred to the serine group at the esteratic site and a free molecule of choline is released. Furthermore, AChE contains a peripheral anionic site (PAS) located at the mouth of the active site gorge, which is regarded as the recognition binding site (Houghton; Ren; & Howes. 2006: 181-199).

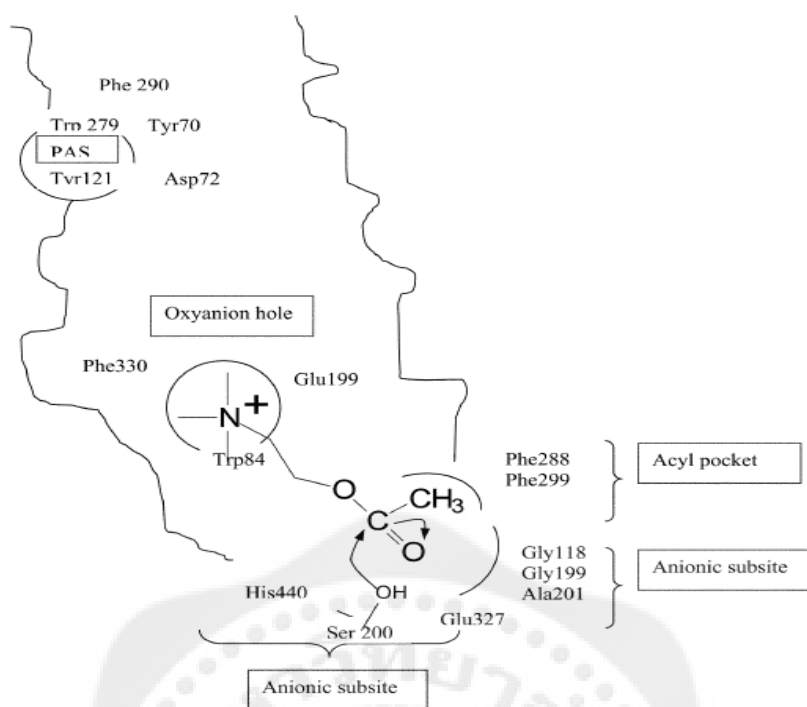


Figure 77 The binding regions of the AChE gorge

CholinEsterase Inhibitors (ChEIs)

Currently, four cholinesterase inhibitors (ChEIs) approved by the U.S. Food and Drug Administration (FDA) are tacrine (**374**), donepezil (**375**), rivastigmine (**376**), and galantamine (**377**) (Figure 78), some of which possesses hepatotoxicity, vomiting and diarrhea. They also treat only mild to moderate levels of disease. Among these medications, donepezil and galantamine found to be a selective AChEI whereas rivastigmine is a dual AChE/BuChE inhibitor (Ballard 2002; Furukawa-Hibi., 2011). So far, many efforts to seek lead compounds from natural sources for the treatment of AD have been carried out (Mukherjee; et al. 2007: 289-300). Thai higher plant is thus taken into account to be one of the target sources for achieving new anti-AChE drugs discovery.

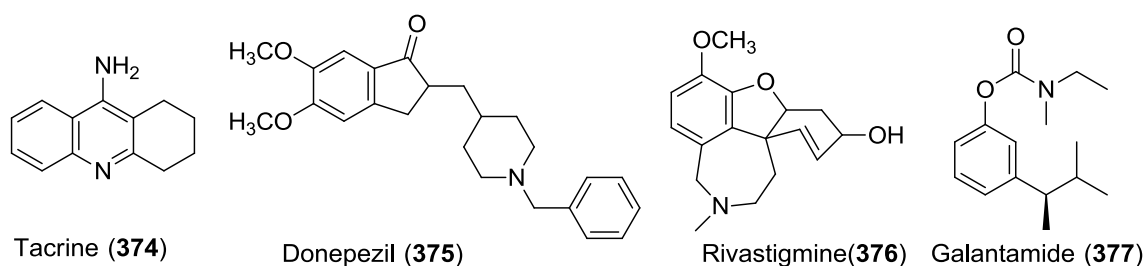


Figure 78 Structures of ChEIs **374-377**

2.8 Anticholinesterase activity in *Artocarpus* species or 2-arylbenzofurans

To date, four reports on anti-AChE property of *Artocarpus* species or 2-arylbenzofurans have been published, as follows:

In 2007, Zahid et al isolated two new cycloartane-type triterpenoids from the EtOH extract of *A. nobilis* barks yielding artocarpuates A and B (**378** and **379**) (Figure 79). The compounds possessed weak acetylcholinesterase inhibitory activity with IC_{50} values of 0.195 and 0.146 mM, respectively (Zahid; Ata; & Samarasekera. 2007: 280-284).

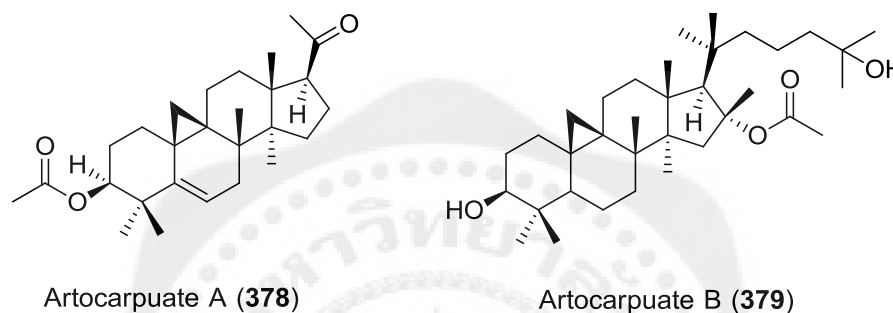


Figure 79 Structures of artocarpuates A and B (**378** and **379**)

In 2008, Rizzo et al synthesized benzofuran-based hybrid molecules which are a series of potential multifunctional drugs for AD. The molecules were prepared by linking a benzofuran ring deriving from $A\beta$ antiaggregant (SKF64346) to *N*-methyl-*N*-benzylamine through a heptyloxy chain. All compounds were tested for AChE inhibitory activity. Only compounds **380** and **381** exhibited the highest anti-AChE activity with IC_{50} values of 17.4 and 10.5 μ M (Figure 80), respectively, comparing to standard drugs, rivastigmine with IC_{50} value of 3.0 μ M (Rizzo; et al. 2008: 2883-2886). Four years later, benzofuran-based hybrid molecules were prepared by the same group. The lead compound **382** was modified by varying the length of spacer and substituent in position 3 to afford various compounds. All modified compounds were evaluated for anti-AChE activity. Only compounds **383** and **384** displayed the strongest inhibitory activity with IC_{50} values of 0.34 and 0.24 μ M respectively, comparing to standard drugs, including rivastigmine, tacrine and galantamine with IC_{50} value in the range of 0.42-3.01 μ M (Rizzo; et al. 2012: 519-532).

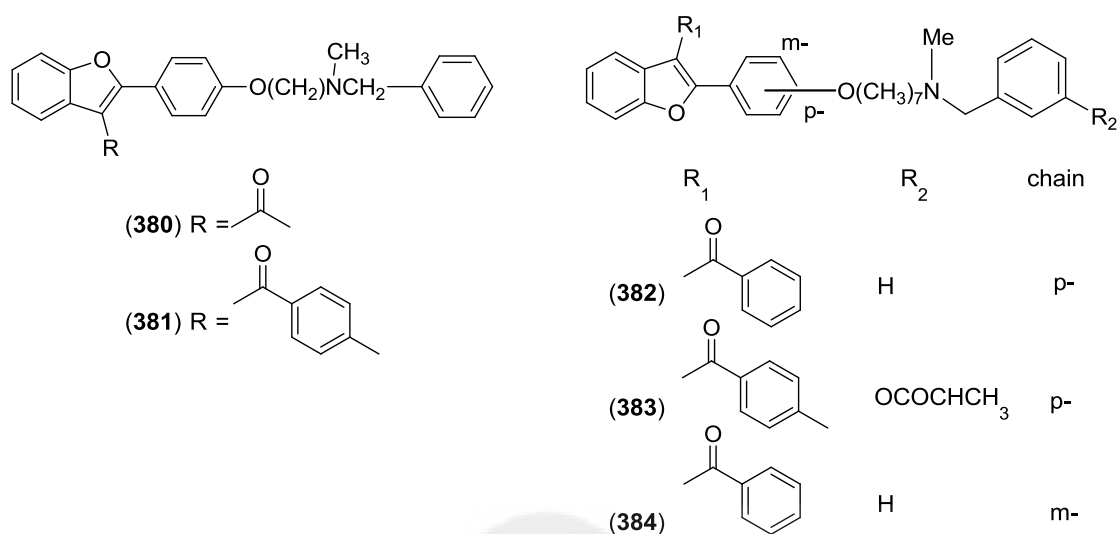


Figure 80 Structures of compounds **380-384**

In 2011, Chromatographic separation of the CH₂Cl₂ extract of egonol-type benzofurans from *Styrax agrestis* fruits furnished three new benzofurans, egonol-9(Z),12(Z) linoleate (**385**), 7-demethoxyegonol-9(Z),12(Z) linoleate (**386**) and 7-demethoxyegonol oleate (**388**), along with 10 known benzofurans, egonol oleate (**387**), egonol-2-methylbutanoate (**389**), 7-demethoxyegonol-2-methylbutanoate (**390**), 7-demethoxyegonol acetate (**391**), egonol propanoate (**392**), egonol-2-methylpropanoate (**393**), egonol butanoate (**394**), egonol acetate (**395**), 7-demethoxyegonol (**396**), and egonol (**397**) (Figure 81). Furthermore, egonol-type analogues, 3-[2-(1,3-benzodioxol-5-yl)-7-methoxy-1-benzofuran-5-yl]propanal (**400**), egonoic acid (**401**), *N*-tertbutyl egonolcarbamate (**402**) *N*-phenyl egonolcarbamate (**403**) and were prepared from compound **395** in order to figure out SAR. All natural or modified egonol-type benzofurans, were examined for inhibition of EeAChE and hAChE. From the results, compounds **385-390** and **403** showed greater inhibitions of EeAChE and hAChE with IC₅₀ value in the range of 1.4-5.5 and 1.7-6.1 μM, respectively, than standard drug, tacrine with IC₅₀ value of 50 and 1026 μM, respectively (Liu; et al. 2011: 2081-2088).

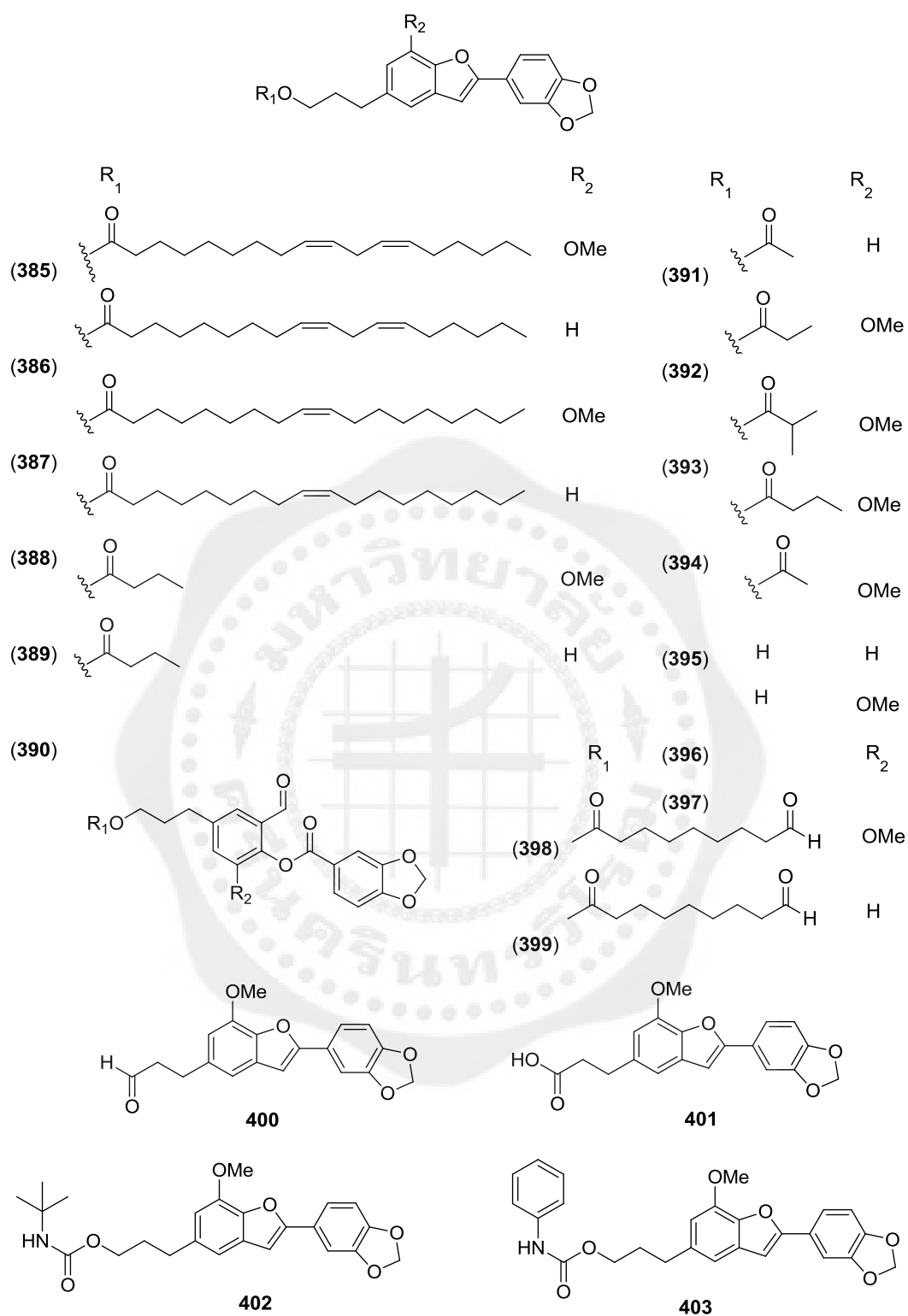


Figure 81 Structures of natural and synthesized egonol-type benzofurans **385-403**

CHAPTER 3

EXPERIMENTAL

General techniques

Thin-Layer Chromatography (TLC)

Technique: One dimension, ascending

Adsorbent: Silica gel 60 GF₂₅₄ 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-H₂SO₄ reagent (2.5% v/v in absolute MeOH containing 3.4% v/v H₂SO₄ acid and 1.0% v/v glacial acetic acid). After heating of TLC plate at 100-110 °C for 10-30 seconds, the spots of organic compounds will give specific colors with this reagent.

Column chromatography (CC)

Liquid column chromatography

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

2) Silica gel 60 particle size 0.063-0.0200 mm (Merck 1.07734)

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

Packing method: Slurry packing method

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 0.063-0.0200 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.

Quick column chromatography (QCC)

Adsorbent: Silica gel particle size 40-63 µm (SiliCycle, SILIAFLASH®F60 230-400 mesh)

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 Merck silica gel 60 GF₂₅₄. 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.

Physical Properties

Optical rotation: Optical rotation was recorded in MeOH, CHCl₃ and acetone on a JASCO-1020 digital polarimeter

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

Spectroscopy

Infrared (IR) Spectra

IR spectra were measured on Perkin Elmer FT-IR spectrum BX spectrometer using a solution in CHCl₃.

Ultraviolet (UV) Absorption Spectra

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

Mass Spectra

Electrospray ionization mass spectra (ESIMS) were measured on Finnigan LC-Q mass spectrometer (Department of Chemistry, Faculty of Science, Ramkhamhaeng University)

High resolution electrospray ionization mass spectra (HRESIMS) were recorded on a Bruker Daltonics micro TOF mass spectrometer (Department of Chemistry, Faculty of Science, Ramkhamhaeng University)

Nuclear Magnetic Resonance (NMR) Spectra

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

Plant materials

Air-dried roots, twigs and leaves of *A. lakoocha* were collected from Pa Phayom District, Patthalung Province, Thailand, June 2011 (voucher specimen: Umalee Namdaung 001). Air-dried root barks of *A. lakoocha* were collected from Buachad District, Surin Province, Thailand, January 2013 (voucher specimen: Natthakaln Lomchoey 005). Both voucher specimens were indentified by Professor Nopporn Damrongsiri and have been deposited at the herbarium of Department of Chemistry, Faculty of Science, Srinakharinwirot University, Thailand.

Phytochemicals content screening by TLC technique

A small quantity (15-20 g) of each part (root barks, heartroots, twig barks, twig heartwoods and leaves) of *A. lakoocha* from Patthalung province was macerated with EtOAc (40 mL), followed by with MeOH (40 mL) at room temperature for each 3 days. All obtained extracts were monitored by TLC technique. It was found that the EtOAc and MeOH extracts from the root barks provided obviously most interesting spots with anisaldehyde- H_2SO_4 reagent. The TLC patterns of the EtOAc and MeOH extracts from the heartroots and twig heartwoods were similar and gave few interesting spots. In contrast, these patterns differ from the EtOAc and MeOH extracts from the twig barks. In addition, the EtOAc and MeOH extracts from the leaves afforded only one green spot. Therefore, the root barks, heartroots and twig barks were selected for further extraction in order to submit for anti-AChE and BuChE activities.

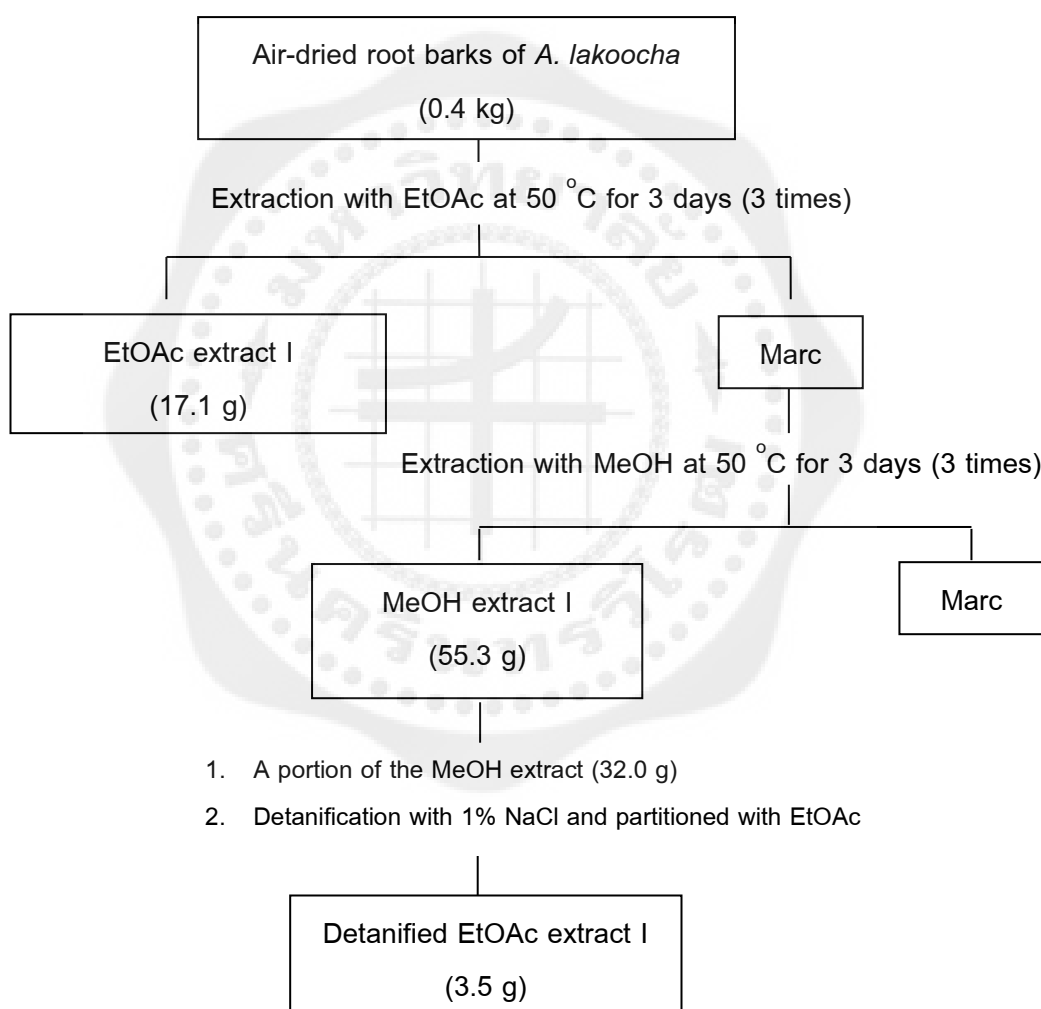
In the likewise manner, the EtOAc and MeOH extracts from the root barks collected from Surin province were also prepared. In comparison of the EtOAc and MeOH extracts from Patthalung and Surin, the EtOAc extracts gave different major spots whereas the MeOH extracts exhibited the same pattern on TLC. Thus, the root barks obtained from Surin were only extracted with EtOAc in order to obtain various chemical constituents.

Extraction and isolation procedures

Extraction of the air-dried root barks of *A. lakoocha* collected from Patthalung Province

The air-dried root barks of *A. lakoocha* (0.4 kg) from Patthalung province were pulverized and extracted successively with EtOAc (3 x 2 L, 3 days for each), followed by

MeOH (3 x 2 L, 3 days for each) at 50 °C in a water bath. The extracts were evaporated to dryness under reduced pressure at about 45 °C. The EtOAc extract I (black gum, 17.1 g) and MeOH extract (dark brown gum, 55.3 g) were obtained, respectively. The EtOAc extract I gave some typical intense orangish brown coloration with anisaldehyde-H₂SO₄ reagent. In addition, a portion of MeOH extract (32 g) was further tannin removed by 1%NaCl and then partitioned with EtOAc by tannin separation method (Wall; et al. 1996: 281-285) to furnish detanified extract I (dark brown viscous, 3.5 g). The extraction procedure is shown in Scheme 1.



Scheme 1 Extraction procedure for the root barks of *A. lakoocha*
collected from Patthalung Province

Isolation of compounds from the EtOAc extract I

A portion of the EtOAc extract (13.5 g) was fractionated by silica gel column chromatography (finer than 0.040-0.063 mm, $\Phi = 5$, $L = 10$ cm), eluting with a gradient conditions of *n*-hexane-EtOAc, EtOAc and EtOAc-MeOH (3-10% increment of more polar solvent, each 70 mL). The eluates were examined by TLC analysis to afford 20 fractions (1-20) (Scheme 2).

Isolation of compounds A (lakoochin D, sss5311), B (lakoochin A, sss5617) and C (cycloartolakoochol, sss5420)

Fraction 3 (0.51 g) was chromatographed over silica gel column chromatography (finer than 0.063 mm, 18 g), eluted with an isocratic system of *n*-hexane-CH₂Cl₂ (40:60) to furnish 10 subfractions (3A-3J). Subfraction 3D (111 mg) was further purified by silica gel CC with an isocratic system of *n*-hexane-CH₂Cl₂ (50:50) to give four subfractions (3D1-3D4). Fractions 3D2-3D3 (90 mg) were combined and applied twice to silica gel column using isocratic system of *n*-hexane-CH₂Cl₂ (10:90) yielded compound **A** (sss5311, 17 mg). Subfraction 3G (29 mg) was applied to silica gel column (finer than 0.063 mm, 7 g) using *n*-hexane-CH₂Cl₂ (10:90) as isocratic system, followed by silica gel CC isocratic condition of *n*-hexane-CH₂Cl₂ (15:85) afforded compound **B** (sss5617, 5 mg). Compound **C** (sss5420, 8 mg), pale yellow sticky oil, was obtained from silica gel CC (finer than 0.063 mm, 7g) with isocratic system of *n*-hexane-CH₂Cl₂ (5:95) of fraction 3J (17 mg).

Isolation of compound D (lakoochin C, sss5286)

Fraction 4 (0.33 g) was rechromatographed over silica gel column (finer than 0.063 mm, 30 g), eluted with a gradient system of *n*-hexane-EtOAc (85:15 to 0:100) to give nine subfractions (4A-4I). Subfraction 4F (35 mg) was subjected to silica gel CC (finer than 0.063 mm, 7 g) using *n*-hexane-CH₂Cl₂ (20:80) as isocratic system to afford compound **D** (sss5286, 19 mg).

Isolation of compound E (artolakoochol, sss5190 and sss5975)

Fraction 6 (1.08 g) was separated on silica gel CC twice with isocratic elution of CH₂Cl₂-MeOH (50:1) furnished compound **E** (sss5190, 178 mg)

Fraction 5 (0.66 g) was subjected to silica gel CC (finer than 0.063 mm, 22 g), eluted with a gradient system of CH_2Cl_2 -MeOH (50:1 to 50:3) to furnish 13 subfractions (5A-5M). As for, fraction 7 (0.17 g) was rechromatographed on silica gel CC (finer than mm, 7 g) and eluted with isocratic elution of CH_2Cl_2 -MeOH (70:1) to give nine subfractions (7A-7I). From the observation of TLC profile, subfractions 5I and 7E showed a similar pattern and gave a big purple black spot as the major compound with R_f value of 0.32 (CH_2Cl_2 -MeOH 50:1), corresponded to R_f value of artolakoochol. Subfractions 5I and 7E were thus combined and purified by silica gel CC with an isocratic system of CH_2Cl_2 -MeOH (70:1) to afford compound **E** as yellow sticky oil (244 mg).

Isolation of compound F (4-hydroxyartolakoochol, sss5303)

Fraction 11 (0.54 g) was separated using silica gel CC (finer than 0.063 mm, 30 g) with CH_2Cl_2 -MeOH (20:1) to give 13 subfractions (11A-11M). Purification of fraction 11J (130 mg) by silica gel CC (finer than 0.063 mm, 9 g) with isocratic system of CH_2Cl_2 -MeOH (30:1) yielded compound **F** (sss5303, 14 mg).

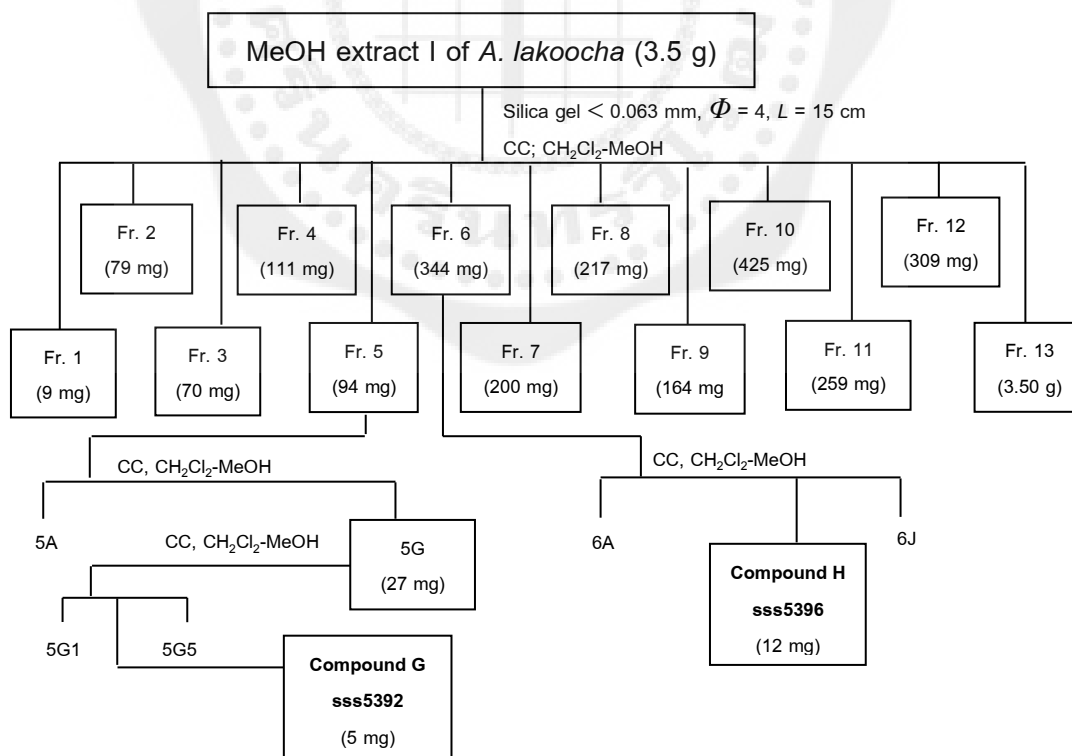
Scheme 2 Isolation scheme of the EtOAc extract I of the root barks of *A. lakoocha*

Isolation of compounds from the MeOH extract I

A portion of MeOH extract I (32 g) was further tannin removed by washing with a 1% aqueous solution of NaCl (Wall; et al. 1996: 281-285) and then partitioned with EtOAc to provide detannified extract I (dark brown viscous, 3.5 g). The detannified EtOAc extract I was fractionated by column chromatography silica gel (finer than 0.063 mm, $\Phi = 4$, $L = 15$ cm), eluting with a gradient condition of CH_2Cl_2 -MeOH (85:15 to 60:40). The eluates were examined by TLC and 13 groups of eluting fractions (fractions 1-13) were obtained (Scheme 3).

Isolation of compounds **G** (oxyresveratrol, sss5392) and **H** ((+)-catechin, sss5396)

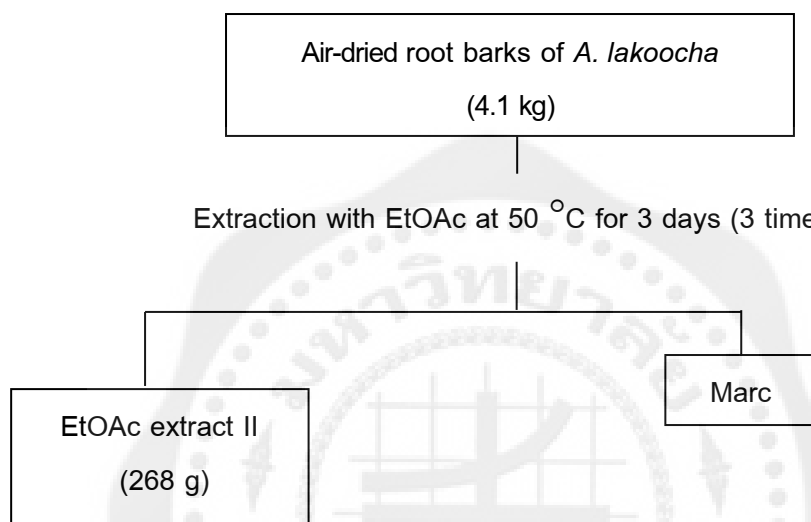
Fraction 5 (94 mg) was separated twice by silica gel CC (finer than 0.063 mm, 17 g) with an isocratic system CH_2Cl_2 -MeOH (92:8) to give compound **G** (5 mg). Fraction 6 (344 mg) was rechromatographed over silica gel column (finer than 0.063 mm, 22 g) using system CH_2Cl_2 -MeOH (90:10) as isocratic system to furnish compound **H** (sss5396, 12 mg).



Scheme 3 Isolation scheme of the MeOH extract I from the root barks of *A. lakoocha*

Extraction of the air-dried root barks of *A. lakoocha* collected from Surin Province

The air-dried root barks of *A. lakoocha* (4.1 kg) from Surin province were milled and extracted successively with EtOAc (27 L, 3 days for each) by maceration at 50 °C. The EtOAc solution was filtered and concentrated to dryness under reduced pressure at temperature 45 °C to yield an EtOAc extract II (black gum, 268 g).



Scheme 4 Extraction procedure for the root barks of *A. lakoocha* collected from Surin Province

Isolation of compounds from the EtOAc extract II

A portion of the EtOAc extract II (90 g) was fractionated by quick column chromatography (finer than 40-63 μm , $\Phi = 10$, $L = 3.5$ cm), eluting with a gradient conditions of *n*-hexane-EtOAc, EtOAc and EtOAc-MeOH (3-20% increment of more polar solvent, each 70 mL). The eluates were examined by TLC analysis to afford 11 fractions (1-11) (Scheme 5).

Isolation of compound I (albafuran B, sss5827)

Fraction 3 (1.90 g) was chromatographed over silica gel CC (finer than 0.063 mm, 30 g) and eluted with a gradient condition of 100% CH_2Cl_2 and CH_2Cl_2 -MeOH (99:1 to 90:10) to give eight subfractions (3A-3H). Subfraction 3E (127 mg) was further purified by silica gel CC (finer than 0.063 mm, 7 g) with CH_2Cl_2 -MeOH (98:2) as eluent to

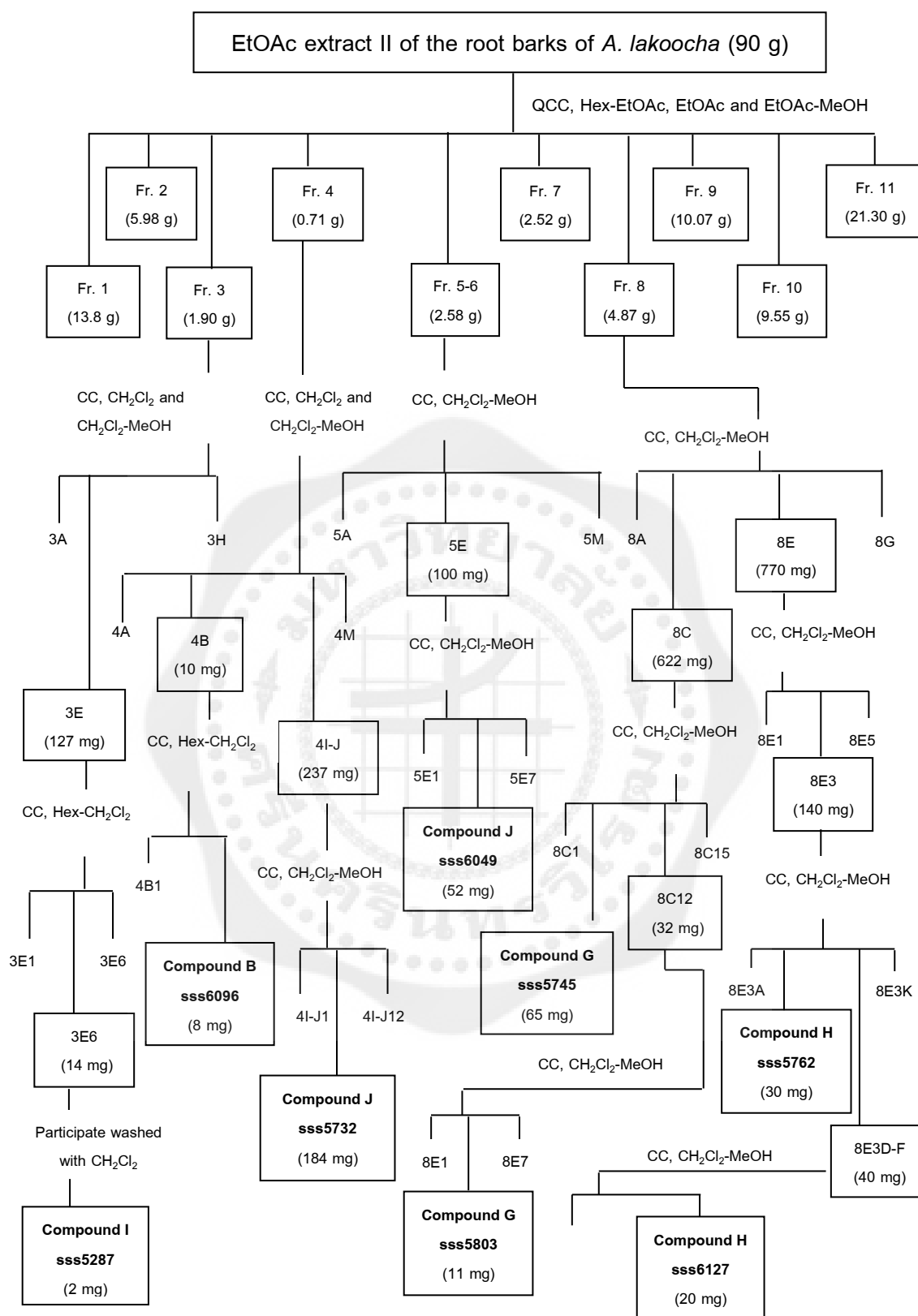
yield as brown amorphous which was then washed with 100% CH_2Cl_2 to afford compound **I** as white amorphous (sss5827, 2 mg).

Isolation of compounds B (lakoochin A, sss6096) and J (lakoochin B, sss5732 and sss6049)

Fraction 4 (0.71 g) was applied to silica gel CC (35 g), eluting with a gradient system of 100% CH_2Cl_2 and CH_2Cl_2 -MeOH (99:1 to 70:30) to afford 13 subfractions (4A-4M). Purification of subfraction 4B (10 mg) by silica gel CC with n-hexane- CH_2Cl_2 (15:85) yielded compound **B** (sss6096, 8 mg). Isolation of combined subfractions 4I and 4J (237 mg) were separated on silica gel CC with CH_2Cl_2 -MeOH (99:1 to 98:2) yielding compound **J** (sss5732, 184 mg). Two successive silica gel columns of combined fractions 5 and 6 (2.58 g) by of CH_2Cl_2 -MeOH (70:1), as an isocratic eluent to provide compound **J** (sss6049, 52 mg).

Isolation of compounds G (oxyresveratrol, sss5745 and sss5803) and H ((+)-catechin, sss5762 and sss6127)

Fraction 8 (4.87 g) was separated using silica gel CC (finer than 0.063 mm, 30 g) and eluted with a gradient condition of CH_2Cl_2 -MeOH (96:4 to 80:20) yielding seven subfractions (8A-8G). Subfraction 8C (622 mg) was applied to silica gel CC using system CH_2Cl_2 -MeOH (96:4 to 94:6) as gradient system furnished 15 subfractions (8C1-8C15) and subfraction 8C9 yielded compound **G** (sss5745, 65 mg) as white crystal. Isolation of subfraction 8C12 by silica gel CC (finer than 0.063 mm, 2.5 g) with gradient system of CH_2Cl_2 -MeOH (96:4 to 94:6) gave compound **G** (sss5803, 11 mg). Whlist, the more polar subfraction 8E (770 mg) was further purified by silica gel CC (finer than 0.063 mm, 20 g), eluting with a gradient system of CH_2Cl_2 -MeOH (95:5 to 92:8) gave five subfractions (8E1-8E5). Subfraction 8E3 was further isolated on silica gel CC with CH_2Cl_2 -MeOH (99:5 to 91:9) as eluent to furnish 11 subfractions (8E3A-8E3K). Compound **H** (30 mg) was obtained as white needles from subfraction 8E3B. Additionally, combined subfraction 8E3D-8E3F (40 mg) was purified by silica gel CC (finer than 0.063 mm, 2.5 g) with CH_2Cl_2 -MeOH (93:7) as eluent to yield compound **H** as white crystal (sss6127, 20 mg).

Scheme 5 Isolation scheme of the EtOAc extract II from the root barks of *A. lakoocha*

In the likewise manner, another portion of the EtOAc extract III (150 g) was subjected to isolation and purification to yield lakoochins A (3 mg) and B (138 mg).

Physical and spectral data of compounds A-J

Compound A (lakoochin D, sss5311 AL(RB)-Et-73/8-18)

Colorless sticky oil 17.0 mg (4.2×10^{-3} %), soluble in CH_2Cl_2

R_f : 0.36 (CH_2Cl_2), a purple coloration with anisaldehyde- H_2SO_4 reagent

$[\alpha]_D^{29.1}$: -2.2° ($c = 0.21$, CHCl_3)

UV (MeOH) λ_{max} (log ϵ) nm : 241 (sh 4.32), 254 (sh 4.15), 295 (4.28)

IR (film) ν_{max} cm^{-1} : 3403, 2925, 2854, 1625, 1585, 1487, 1444, 1376, 1148, 1115, 823

ESMS (-ve) (%rel. intensity) : m/z 473 $[\text{M}-\text{H}]^-$ (100)

HRESIMS (+ve) : m/z 497.2642 $[\text{M}+\text{Na}]^+$ (calcd 497.2662 for $\text{C}_{31}\text{H}_{38}\text{O}_4+\text{Na}$)

^1H NMR : δ ppm, 300 Hz, in CDCl_3 ; Table 15 and Figure 87

Compound B (lakoochin A)

Pale yellow sticky oil 5.0 mg (1.2×10^{-3} %) (sss5617 AL(RB)-Et-130/17-20 from Pattalung), soluble in CH_2Cl_2

Pale yellow sticky oil 8.0 mg (2.0×10^{-3} %) (sss6096 AL(RB)-2/2-Et-134/14-28 from Surin), soluble in CH_2Cl_2

R_f : 0.29 (n -hexane- $\text{CH}_2\text{Cl}_2 = 1:4$), a purple coloration with anisaldehyde- H_2SO_4 reagent

UV (MeOH) λ_{max} (log ϵ) nm : 239 (3.90), 296 (3.82) [lit. λ_{max} (log ϵ) nm : 209 (4.76), 239 (4.35), 295 (4.26) (Puntumchai; et al. 2004: 485-486)]

IR (film) ν_{max} cm^{-1} : 3376, 2959, 2925, 2856, 1623, 1585, 1443, 1318, 1114, 1031, 823 [lit.

ν_{max} cm^{-1} : 3329, 3007, 2965, 2929, 2856, 1625, 1586, 1489, 1461, 1438, 1321, 1115, 1033, 823 (Puntumchai; et al. 2004: 485-486)]

ESMS (+ve) (%rel. intensity) : m/z 407 $[\text{M}+\text{H}]^+$ (100)

^1H NMR : δ ppm, 300 Hz, in CDCl_3 ; Table 16 and Figure 88

Compound C (cycloartolakoochol, sss5256 AL(RB)-Et-46/1-4 and sss5420 AL(RB)-Et-101/17-28)

Pale yellow sticky oil 14.0 mg (3.5×10^{-3} %), soluble in CH_2Cl_2

R_f : 0.36 (CH_2Cl_2), a dark purple coloration with anisaldehyde- H_2SO_4 reagent

UV (MeOH) $\lambda_{\max}$ (log ϵ) nm : 220 (4.40), 313 (4.49) [lit. $\lambda_{\max}$ (log ϵ) 226 (2.50), 316 (2.35) (Sritularak; et al. 2010: 6548-6558)]

IR (film) $\nu_{\max}$ cm^{-1} : 3370, 2966, 2928, 2870, 1619, 1576, 1483, 1446, 1369, 1127, 969 [lit (film) $\nu_{\max}$ cm^{-1} ; 3365, 2925, 2873, 2854, 1622, 1489, 1455 (Sritularak; et al. 2010: 6548-6558)]

ESMS (+ve) (%rel. intensity) : m/z 445 $[\text{M}+\text{H}]^+$ (18)

^1H NMR : δ ppm, 300 Hz, in CDCl_3 ; Table 13 and Figure 85

Compound D (Iakoochin C, sss5286 AL(RB)-Et-53/1-10)

Off-white amorphous solid 19.0 mg (4.7×10^{-3} %), soluble in CH_2Cl_2

R_f : 0.35 (n -hexane- CH_2Cl_2 = 1:4), a purple coloration with anisaldehyde- H_2SO_4 reagent

$[\alpha]_{\text{D}}^{28.9}$: -3.4° (c = 0.21, CHCl_3)

UV (MeOH) $\lambda_{\max}$ (log ϵ) nm : 230 (sh 4.61), 277 (sh 4.26), 310 (4.50)

IR (Neat) $\nu_{\max}$ cm^{-1} : 3398, 2966, 2921, 2856, 1657, 1613, 1487, 1369, 1155, 1045, 822

ESMS (-ve) (%rel. intensity) : m/z 459 $[\text{M}-\text{H}]^-$ (100)

HRESIMS (-ve) : m/z 459.2547 $[\text{M}-\text{H}]^-$ (calcd 459.2540 for $\text{C}_{30}\text{H}_{36}\text{O}_4-\text{H}$)

^1H NMR : δ ppm, 300 Hz, in CDCl_3 ; Table 18 and Figure 90

Compound E (artolakoochol, sss5190 AL(RB)-Et-28/32-37 and sss5975 AL(RB)-Et-177/30-54)

Yellow sticky oil 422 mg (0.1 %), soluble in CH_2Cl_2

R_f : 0.58 (CH_2Cl_2 -MeOH = 20:1), a dark purple coloration with anisaldehyde- H_2SO_4 reagent

$[\alpha]_{\text{D}}^{30}$: -76.6° (c = 0.25, MeOH) [lit. $[\alpha]_{\text{D}}^{20}$: -86.1° (c = 0.03, MeOH)]

UV (MeOH) $\lambda_{\max}$ (log ϵ) nm : 232 (4.47), 332 (4.57) [lit. (log ϵ): 234 (3.16), 329 (3.23) (Sritularak; et al. 2010: 6548-6558)]

IR (film) $\nu_{\max}$ cm^{-1} : 3369, 2969, 2922, 2863, 1621, 1605, 1490, 1421, 1146, 968, 822 [lit. (film) $\nu_{\max}$ cm^{-1} ; 3377, 2966, 2924, 2854, 1623, 1607, 1489, 1445 (Sritularak; et al. 2010: 6548-6558)]

ESMS (-ve) (%rel. intensity) : 443 $[\text{M}-\text{H}]^-$ (29)

^1H NMR : δ ppm, 300 Hz, in CDCl_3 ; Table 11 and Figure 83

Compound F (4-hydroxyartolakochole, sss5303 AL(RB)-Et-62/27-43 (P))

Off-white powder 14 mg (3.5×10^{-3} %), soluble in MeOH

R_f : 0.23 (CH_2Cl_2 -MeOH = 20:1), a dark brown coloration with anisaldehyde- H_2SO_4 reagent

$[\alpha]_D^{30}$: -103.6° ($c = 0.25$, MeOH) [lit. $[\alpha]_D^{20}$ -117.6° ($c = 0.03$, MeOH)]

UV (MeOH) $\lambda_{\max}$ (log ϵ) nm : - [lit. $\lambda_{\max}$ (log ϵ) nm : 240 (3.04), 341 (3.02) (Sritularak; et al. 2010: 6548-6558)]

IR (film) $\nu_{\max}$ cm^{-1} : - [lit (film) $\nu_{\max}$ cm^{-1} ; 3417, 2965, 2920, 2855, 1633, 1609, 1447, 1418 (Sritularak; et al. 2010: 6548-6558)]

ESMS (-ve) (%rel. intensity) : m/z 459 $[\text{M}-\text{H}]^-$ (100)

^1H NMR : δ ppm, 300 Hz, in CDCl_3 + 2 drops CD_3OD ; Table 12 and Figure 84

Compound G (oxyresveratrol)

Yellow amorphous solid 5 mg (1.2×10^{-3} %) (sss5392 AL(RB)-Et-94/14-20 from Pattalung), soluble in MeOH

Pale yellow crystal 76 mg (1.9×10^{-3} %) (sss5745 AL(RB)-2/2-Et-24/5C and sss5803 AL(RB)-2/2-Et-64/7-14(C) from Surin), soluble in MeOH

R_f : 0.23 (CH_2Cl_2 -MeOH = 5:1), a reddish brown coloration with anisaldehyde- H_2SO_4 reagent

UV (MeOH) $\lambda_{\max}$ (log ϵ) nm : 215 (4.29), 235 (4.21), 289 (4.19), 301 (4.22), 327 (4.33)

IR (ATR) $\nu_{\max}$ cm^{-1} : 3360, 3178, 1590, 1517, 1479, 1348, 1304, 1249, 1172, 1155, 1116, 978, 820 cm^{-1}

ESMS (-ve) (%rel. intensity) : m/z 283 $[\text{M}+\text{K}]^+$ (100)

^1H NMR : δ ppm, 300 Hz, in CDCl_3 + 7 drops $\text{DMSO}-d_6$; Table 19 and Figure 92

Compound H ((+)-catechin)

White amorphous solid 12 mg (3.0×10^{-3} %) (sss5396 AL(RB)-Et-94/12-14 from Pattalung), soluble in MeOH

White needles 50 mg (1.2×10^{-3} %) (sss5762 AL(RB)-2/2-Et-38/27-44(P) and sss6127 AL(RB)-2/2-Et-143/7-26 from Surin), soluble in MeOH

R_f : 0.20 (CH_2Cl_2 -MeOH = 5:1), an orange coloration with anisaldehyde- H_2SO_4 reagent

$[\alpha]_D^{28.8}$: $+9.4^\circ$ ($c = 0.33$, acetone) from sss5396 sample [lit $[\alpha]_D^{25}$ $+14^\circ$ ($c = 0.4$, acetone) (EL-Razek. 2007: 4867-4872)]

$[\alpha]_D^{28.8}$: +18.3° (c = 0.33, acetone) from sss5826 sample [lit $[\alpha]_D^{25}$ +14° (c= 0.4, acetone) (EL-Razek. 2007: 4867-4872)]

UV (MeOH) $\lambda_{\max}$ (log ϵ) nm : 232 (4.18), 280 (3.62)

IR (ATR) $\nu_{\max}$ cm⁻¹ : 3304, 1607, 1518, 1461, 1141, 1028 [Lit. (KBr) $\nu_{\max}$ cm⁻¹; 3404, 1621, 1514, 1285, 1243, 1180, 1145, 1112, 1073 (EL-Razek. 2007: 4867-4872)]

ESMS (-ve) (%rel. intensity) : m/z 138 [M+H]⁻ (100)

¹H NMR : δ ppm, 300 Hz, in CDCl₃+ 8 drops DMSO-*d*₆; Table 20 and Figure 93

Compound I (albafuran B, sss5287 AL(RB)-2/2-Et-71/53-55)

White amorphous 2.0 mg (5.0 x 10⁻⁵ %), soluble in CH₂Cl₂

R_f : 0.38 (CH₂Cl₂-MeOH = 20:1), a reddish purple coloration with anisaldehyde-H₂SO₄ reagent

UV (MeOH) $\lambda_{\max}$ (log ϵ) nm : - [lit. $\lambda_{\max}$ (log ϵ) nm : 320 (34000), 311 (sh 24000), 298 (sh 15400), 286 (sh 13100), 219 (sh 27000) (Takasugi; et al. 1982: 1221-1222)]

ESMS (-ve) (%rel. intensity) : m/z 377 [M-H]⁻ (38)

¹H NMR : δ ppm, 300 Hz, in CDCl₃+ 1 drop CD₃OD; Table 17 and Figure 89

Compound J (lakoochin B, sss5732 AL(RB)-2/2-Et-146/12-14 and sss6049 AL(RB)-2/3-Et-131/16-20)

Dark yellow sticky oil 236 mg (5.9 x 10⁻³ %), soluble in CH₂Cl₂

R_f : 0.64 (CH₂Cl₂-MeOH = 20:1), a dark purple coloration with anisaldehyde-H₂SO₄ reagent
 $[\alpha]_D^{29.2}$: +0.9° (c = 0.46, CHCl₃)

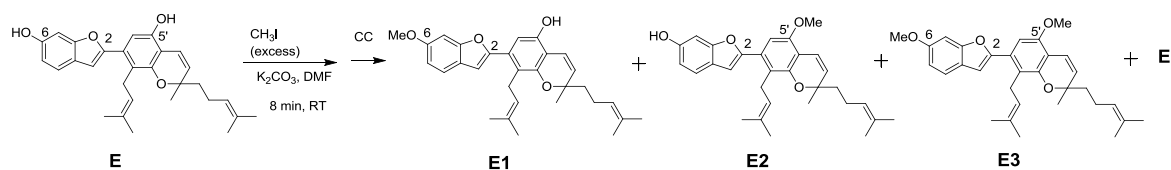
UV (MeOH) $\lambda_{\max}$ (log ϵ) nm : 253 (4.14), 296 (4.26) [lit. $\lambda_{\max}$ (log ϵ) nm : 205 (4.49), 250 (3.77), 296 (4.88) (Puntumchai; et al. 2004: 485-486)]

IR (film) $\nu_{\max}$ cm⁻¹ : 3400, 2976, 2922, 2853, 1621, 1597, 1488, 1440, 1144, 1112, 966, 824 [lit. (film) $\nu_{\max}$ cm⁻¹ 3427, 3009, 2972, 2917, 2857, 1625, 1602, 1488, 1440, 1304, 1145, 1112, 966, 824 (Puntumchai; et al. 2004: 485-486)]

ESMS (-ve) (%rel. intensity) : m/z 446 [M]⁻ (34)

¹H NMR : δ ppm, 300 Hz, in CDCl₃; Table 14 and Figure 85

Preparation of artolakoochol methyl ether derivatives



A mixture of compound **E** (106.2 mg, 0.24 mmol) and K_2CO_3 (33.5 mg, 0.24 mmol) in anhydrous DMF (1.0 mL) was added with excess methyl iodide (3 drops). The reaction mixture was stirred for 8 min at room temperature and the reaction progress was monitored by TLC (CH_2Cl_2). Water was then added and the mixture was extracted with ethyl acetate (3 x 30 mL). The combined organic phase was washed with H_2O (3 x 30 mL), dried over anhydrous Na_2SO_4 and the solvent was removed under vacuum. The crude product was purified by column chromatography using hexane- CH_2Cl_2 to yield the mono methyl ethers **E1** (5.9 mg, 5.5%), **E2** (6.6 mg, 6.2%), and the fully methylated **E3** (5.6 mg, 5.2%), together with the unreacted starting compound **E** (64 mg, 60.2%).

Artolakoochol-6-O-methyl ether **E1**, sss6068

pale yellow sticky oil 5.9 mg, soluble in CH_2Cl_2

R_f : 0.36 (CH_2Cl_2), dark purple coloration with anisaldehyde- H_2SO_4 reagent

UV (MeOH) λ_{max} (log ϵ) nm : 227 (4.30), 329 (4.20)

IR (ATR) ν_{max} cm^{-1} : 3394, 2965, 2923, 2856, 1623, 1607, 1491, 1375, 1108, 820

HRESIMS (-ve): m/z 457.2396 $[\text{M}-\text{H}]^-$ (calcd 457.2384 for $\text{C}_{30}\text{H}_{34}\text{O}_4-\text{H}$)

^1H NMR : δ ppm, 300 Hz, in CDCl_3 ; Table 21 and Figure 93

Artolakoochol-5'-O-methyl ether **E2**, sss6021

colorless sticky oil 6.6 mg, soluble in CH_2Cl_2

R_f : 0.31 (CH_2Cl_2), purple coloration with anisaldehyde- H_2SO_4 reagent

UV (MeOH) λ_{max} (log ϵ) nm : 227 (4.65), 333 (4.66)

IR (ATR) ν_{max} cm^{-1} : 3352, 2923, 2856, 1665, 1597, 1489, 1375, 1111, 820

HRESIMS (-ve): m/z 457.2373 $[\text{M}-\text{H}]^-$ (calcd 457.2384 for $\text{C}_{30}\text{H}_{34}\text{O}_4-\text{H}$)

^1H NMR : δ ppm, 300 Hz, in CDCl_3 ; Table 21 and Figure 93

Artolakoochol-6,5'-di-O-methyl ether **E3**, sss6020

yellow sticky oil, soluble in CH_2Cl_2

R_f : 0.73 (CH_2Cl_2), dark purple coloration with anisaldehyde- H_2SO_4 reagent

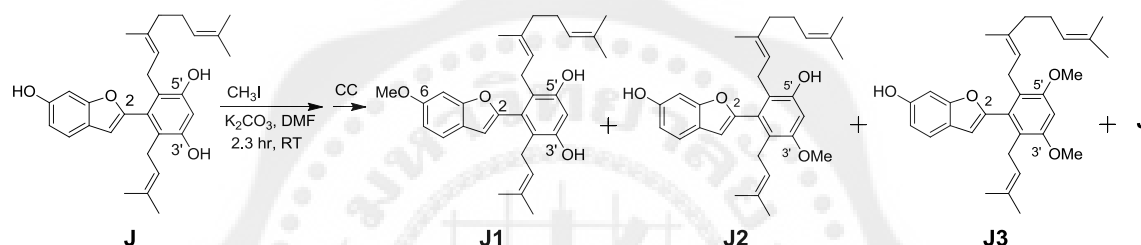
UV (MeOH) λ_{max} (log ϵ) nm: 217 (4.17), 227 (4.13), 328 (4.12)

IR (ATR) ν_{max} cm^{-1} : 2958, 2924, 2850, 1622, 1595, 1491, 1108, 820

HRESIMS (-ve): m/z 471.2540 $[\text{M}-\text{H}]^-$ (calcd 471.2529 for $\text{C}_{31}\text{H}_{36}\text{O}_4-\text{H}$)

^1H NMR: δ ppm, 300 Hz, in CDCl_3 ; Table 21 and Figure 93

Preparation of lakoochin B methyl ether derivatives



In the same manner that as for compound **J** (96.9 mg, 0.22 mmol), after stirring for 2.5 h at room temperature, the reaction mixture was then added with water and extracted with EtOAc (3×30 mL). The combined organic phase was washed with water, dried over anhydrous Na_2SO_4 , filtered and concentrated in *vacuo*. The crude product was purified by column chromatography eluting with hexane- CH_2Cl_2 to give the mono methyl ethers **J1** (4.0 mg, 4.1%), **J2** (2.0 mg, 2.0%) and lakoochin B-3',5'-di-O-methyl ether **J3** (1.2 mg, 1.2%), together with the unreacted starting compound **J** (44.2 mg, 45.6%).

Lakoochin B-6-O-methyl ether **J1**, sss6139

yellow sticky oil 4.0 mg, soluble in CH_2Cl_2

R_f : 0.24 (*n*-hexane-EtOAc = 5:1 x3), purple coloration with anisaldehyde- H_2SO_4 reagent

UV (MeOH) λ_{max} (log ϵ) nm: 255 (3.81), 295 (3.89)

IR (ATR) ν_{max} cm^{-1} : 3352, 2923, 2854, 1656, 1620, 1591, 1491, 1438, 1147, 962, 822

HRESIMS (+ve): m/z 483.2492 $[\text{M}+\text{Na}]^+$ (calcd 483.2505 for $\text{C}_{30}\text{H}_{36}\text{O}_4-\text{H}$)

^1H NMR: δ ppm, 300 Hz, in CDCl_3 ; Table 22 and Figure 94

Lakoochin B-3'-O-methyl ether **J2**, sss6069

pale yellow sticky oil 2.0 mg, soluble in CH_2Cl_2

R_f : 0.36 (*n*-hexane-EtOAc = 5:1 x3), purple coloration with anisaldehyde- H_2SO_4 reagent

UV (MeOH) λ_{max} (log ϵ) nm : 254 (3.86), 295 (3.97)

IR (ATR) ν_{max} cm^{-1} : 3358, 2958, 2923, 2823, 1657, 1624, 1589, 1444, 1143, 1112, 963, 820

HRESIMS (-ve) : m/z 459.2558 $[\text{M}-\text{H}]^-$ (calcd 459.2540 for $\text{C}_{30}\text{H}_{36}\text{O}_4\text{-H}$)

^1H NMR : δ ppm, 300 Hz, in CDCl_3 ; Table 22 and Figure 94

Lakoochin B-3',5'-di-*O*-methyl ether **J3**, sss6076

pale yellow sticky oil 1.2 mg, soluble in CH_2Cl_2

R_f : 0.41 (*n*-hexane-EtOAc = 5:1 x3), purple coloration with anisaldehyde- H_2SO_4 reagent

^1H NMR : δ ppm, 300 Hz, in CDCl_3 ; Table 22 and Figure 94

AChE and BuChE inhibitory assay

Enzymes *Electrophorus electricus* EeAChE and EeBuChE were purchased from Sigma Aldrich and anti-AChE and BuChE activities were determined by Ellman's method (Ellman et al., 1961). In brief, 140 μL of 10 mM sodium phosphate buffer (pH 8.0), 20 μL of a solution of EeAChE (EeAChE, 0.2 unit/mL in 10 mM sodium phosphate buffer, pH 8.0) and 20 μL of test compound solution dissolved in 80% MeOH (a final concentration of 0.1 mg/mL) were mixed in 96-well plates, and then incubated at RT for 15 min. The reaction was initiated by adding 20 μL of mixture solution of 5 mM DTNB in 10 mM sodium phosphate buffer (pH 8.0), containing 0.1% bovine serum albumin (BSA) and 5 mM acetylthiocholine (ASCh) in 10 mM sodium phosphate buffer, pH 8.0 (5:1). The hydrolysis of ASCh was monitored the formation of the yellow 5-thio-2-nitrobenzoate anion resulting from reaction with DTNB with thiocholines (SCh), then catalyzed by enzymes at a wavelength of 405 nm and the absorbance was measured after 2 min of incubation at RT. Percentage inhibition was calculated by comparison of rate of enzymatic hydrolysis of ASCh for the sample to that of the blank (80% MeOH in buffer). Galantamine was used as a standard. Inhibition of BuChE was measured same as described above.

CHAPTER 4

RESULTS AND DISCUSSION

Phytochemical contents screening by TLC technique on plant part of *A. lakoocha* collected from Pattalung province revealed that the root bark extracts showed the most interesting spots observed compared to that of other parts (Table 9). In addition, comparative phytochemical screening study of the root barks collected from different areas, Patthalung and Surin, gave different major spots on TLC. Consequently, extraction and purification on only the root barks were carried out.

Table 9 Phytochemical contents screening by TLC of *A. lakoocha* plant parts

Part	EtOAc extract			MeOH extract		
	2-arylbenzofuran	oxyresveratrol	catechin	oxyresveratrol	catechin	Other compounds
Root barks						
-Patthalung	√√√	√	√	√	√	-
-Surin	√√√	√	√	√	√	-
Heartroots	√	√	nd	√	√	-
Twig barks	nd	nd	nd	nd	nd	√√
Twig-heartwoods	√	√	-	√√√	√√√	-
Leaves ^a	nd	nd	nd	nd	nd	√

√√√ in higher quantity

√ in lower quantity

nd not detected

Chromatographic separation of the EtOAc and MeOH extracts furnished arylbenzofuran-type compounds (**A-J**), two of which were new compounds (**A** and **D**) and other phenolic compounds, a stilbene (**G**) and a flavonoid (**H**) (Table 10). The structures of the compounds were achieved base on spectroscopic data analysis (NMR, MS UV and MS) and comparison of their spectral data with those published in the literature.

Table 10 List of isolated compounds obtained from the dried root barks collected from Patthalung and Surin provinces

Compounds		EtOAc extract	MeOH extract
		Weight (mg)	Weight (mg)
Patthalung			
A (Lakoochin D)	sss5311	17	
B (Lakoochin A)	sss5617	5	
C (Cycloartolakoochol)	sss5256	6	
	sss5420	8	
D (Lakoochin C)	sss5286	19	
E (Artolakoochol)	sss5190	178	
	sss5975	244	
F (4-Hydroxyartolakoochol)	sss5303	14	
G (Oxyresveratrol)	sss5392		5
H ((+)-Catechin)	sss5396		12
Surin			
B (Lakoochin A)	sss6096	8	
I (Albafuran B)	sss5827	2	
J (Lakoochin B)	sss5732	184	
	sss6049	52	
G (Oxyresveratrol)	sss5745	65	
	sss5803	11	
H ((+)-Catechin)	sss5762	30	
	sss6127	20	

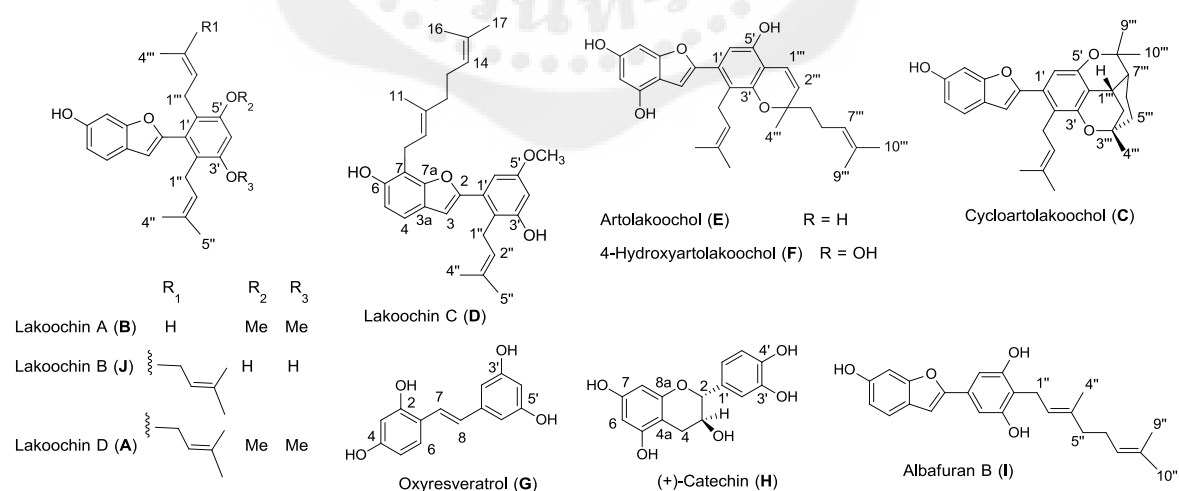


Figure 82 Isolated compounds obtained from the root barks of *A. lakoocha*

4.1 Structural determination of the isolated 2-arylbenzofurans

In general, the obtained 3',5',6-trioxygenated 2-arylbenzofurans showed some characteristic physical and spectral data as follows:

1. Most compounds gave purple or purple-like color with anisaldehyde reagent on TLC.
2. The UV spectra exhibited two major maximal absorption at $\lambda_{\max}$ 207-239 and 290-320 nm, indicating of an 2-arylbenzofuran-type skeleton (Kapche; et al. 2009: 216-221).
3. The IR absorption bands for hydroxyl ($\sim 3400\text{ cm}^{-1}$), aliphatic (2966, 2929 and 2854 cm^{-1}) and aromatic (1625 and 1444 cm^{-1}) functionalities were observed.
4. The ^1H -NMR spectra displayed aromatic proton signals on ring A, an ABX system at δ_{H} 7.32-7.39 (d, J ca 8.3 Hz, H-4), 6.93-6.97 (d, J ca 2.2 Hz, H-7) and 6.75-6.79 (d, J ca 8.3 and 2.2 Hz, H-5), and a singlet signal for H-3 at δ_{H} 6.50-6.73, together with a singlet resonance of either H-4' or H-6' on ring B at δ_{H} 6.50-6.70, which corresponding for the presence of 3',5',6-trioxygenated 2-arylbenzofuran moiety.

4.1.1 Compound E (Artolakoochol, **sss5190** and **sss5975**)

Compound **E** was isolated as the major 2-arylbenzofuran from Phattalung source gave a dark purple coloration with anisaldehyde- H_2SO_4 reagent indication of arylbenzofuran skeleton. Its UV spectrum in MeOH showed the typical bands at $\lambda_{\max}$ 211, 232 and 332 nm and its IR spectrum exhibited absorption bands for hydroxyl (3369 cm^{-1}), aliphatic (2969, 2922 and 2863 cm^{-1}) and aromatic (1621 and 1490 cm^{-1}), which are characteristic absorptions for 2-arylbenzofuran skeleton. The molecular formula of this compound was established as $\text{C}_{29}\text{H}_{32}\text{O}_4$ by ESMS at m/z 443 $[\text{M}+\text{H}]^+$.

The ^1H NMR spectrum (Figure 98, Table 11) displayed the following signals for 2-arylbenzofuran skeleton: one set of ABX aromatic protons at δ_{H} 7.38 (d, J = 8.3 Hz, H-4), 6.95 (d, J = 1.9 Hz, H-7) and 6.75 (dd, J = 8.3, 1.9 Hz, H-5), along with two singlet signals at δ_{H} 6.73 (H-3) and 6.70 (H-6'). Additionally, a set of the signals in upfield region at δ_{H} 5.16 (br t, J = 6.4 Hz, H-2''), 3.44 (d, J = 6.4 Hz, H-1''), 1.71 (s, H-4'') and 1.67 (s, H-5'') was characteristic for an isoprene unit in ^1H NMR data. The remaining signals appeared as the typical signals of modified geranyl substituent. These signals were two doublet signals at δ_{H} 6.66 (H-1''') and 5.57 (H-2''') with a coupling constant value of 10.0 Hz which were assignable to two *cis*-coupled olefinic protons of a pyrano ring moiety, a broad triplet of

olefinic proton at δ_{H} 5.09 ($J = 7.0$ Hz, H-7'''), two multiplets of methylene protons at δ_{H} 2.10 (H-6''') and 1.75 (H-5'''), and three singlets of methyl protons at δ_{H} 1.64 (H-10'''), 1.56 (H-9''') and 1.36 (H-4'''). The ^{13}C NMR spectrum provided 29 signals due to five methyls, three methylenes, nine methines and twelve quaternary carbons (Figure 99). In the HMBC experiment, the correlations of H-1''/C-2' and H-1''/C-3' revealed the position of the isoprene unit to be at C-2' (Figure 83). The HMBC correlations of the olefinic protons at δ_{H} 6.66 (H-1''') and 5.57 (H-2''') to C-3' (δ_{C} 152.3) and to quaternary carbon C-4' (δ_{C} 109.5), and also δ_{H} 6.66 (H-1''') to quaternary carbon C-5' (δ_{C} 149.1) confirmed that the pyrano system was located at C-3' and C-4'. HMBC cross-peaks of the two olefinic protons (H-1''' and H-2''') to quaternary carbon (C-3'''), methyl proton (H-4''') to the carbons (C-2''', C-3''' and C-5'''), and two methyl protons (H-9''' and H-10''') to olefinic carbon (C-7''') and quaternary carbon (C-8''') were also revealed. The correlation between aromatic proton (H-6') to quaternary carbon (C-2) was further observed in the HMBC experiment, suggesting there is no substituent at C-6'. The structure of this compound was confirmed by comparison of spectroscopic data with those of the literature values (Sritularak; et al. 2010: 6548-6558), compound **E** was identified as 7-(6-hydroxybenzofuran-2-yl)-2-methyl-8-(3-methylbut-2-enyl)-2-(4-methylpent-3-enyl)-2H-chromen-5-ol or artolakoochol (**339**).

Compound **E** displayed levorotary optical rotation value of $[\alpha]_{\text{D}}^{30} -76.6^{\circ}$ ($c = 0.25$, MeOH) similar to that of reported artolakoochol $[\alpha]_{\text{D}}^{20} -86.1^{\circ}$ ($c = 0.03$, MeOH) (Sritularak; et al. 2010: 6548-6558), the structure of compound **E** should have the same stereostructure as that of artolakoochol (**339**).

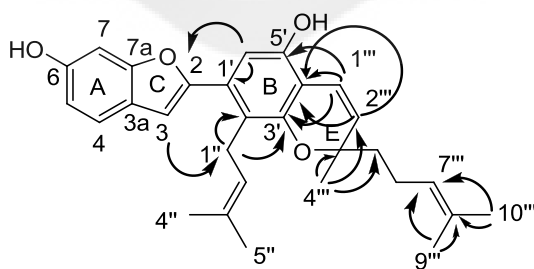


Figure 83 Selected HMBC correlations of compound **E**

Table 11 Comparison of ^1H - and ^{13}C -NMR data of compound **E** with artolakoochol (**339**) in CDCl_3

Position	δ_{H}		δ_{C}	
	Compound E	Artolakoochol ^a (339)	Compound E	Artolakoochol ^a (339)
2	-	-	154.4	154.4
3	6.73 (1H, s)	6.72 (1H, d, J=0.5)	105.0	105.0
3a	-	-	122.9	122.9
4	7.38 (1H, d, J=8.3)	7.36 (1H, d, J=8.5)	121.1	121.2
5	6.75 (1H, dd, J=8.3, 1.9)	6.75 (1H, dd, J=8.5, 2.0)	111.8	111.9
6	-	-	153.5	153.4
7	6.95 (1H, d, J=1.9)	6.95 (1H, d, J=2.0)	98.1	98.2
7a	-	-	155.3	155.2
1'	-	-	130.3	130.2
2'	-	-	120.1	120.1
3'	-	-	152.3	152.3
4'	-	-	109.5	109.6
5'	-	-	149.1	149.1
6'	6.70 (1H, s)	6.70 (1H, s)	106.9	106.9
1''	3.44 (2H, d, J=6.4)	3.45 (2H, d, J=6.5)	25.6	25.6
2''	5.16 (1H, br t, J=6.4)	5.17 (1H, br t, J=6.5)	123.6	123.6
3''	-	-	131.1	131.2
4''	1.71 (3H, s)	1.72 (3H, s)	18.1	18.1
5''	1.67 (3H, s)	1.67 (3H, s)	25.7	25.7
1'''	6.66 (1H, d, J=10.0)	6.68 (1H, d, J=10.0)	116.9	117.0
2'''	5.57 (1H, d, J=10.0)	5.57 (1H, d, J=10.0)	128.6	128.6
3'''	-	-	78.4	78.5
4'''	1.36 (3H, s)	1.36 (3H, s)	26.2	26.2
5'''	1.75 (2H, m)	1.71 (2H, m)	41.3	41.3
6'''	2.10 (2H, m)	2.10 (2H, m)	22.9	22.9
7'''	5.09 (1H, br t, J=7.0)	5.10 (1H, br t, J=7.0)	124.1	124.2
8'''	-	-	131.7	131.7
9'''	1.56 (3H, s)	1.57 (3H, s)	17.6	17.6
10'''	1.64 (3H, s)	1.65 (3H, s)	25.6	25.6
-OH-4	-	-	-	-
-OH-6	-	5.29 (1H, br s)	-	-
-OH-5'	-	5.19 (1H, br s)	-	-

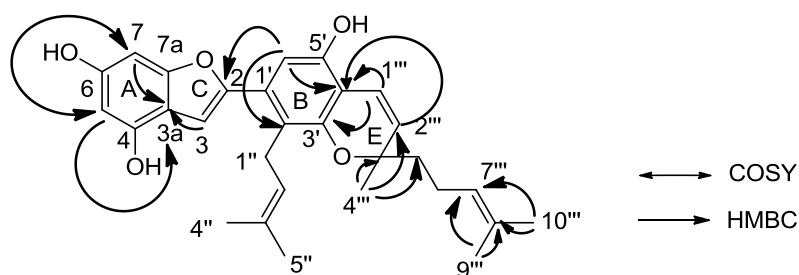
^a Sritularak; et al. 2010: 6548-6558

4.1.2 Compound F (4-hydroxyartolakoochol, sss5303)

Compound **F** was obtained as off-white powder and gave a dark brown coloration with anisaldehyde-H₂SO₄ reagent. It has higher polarity, with *R_f* value of 0.23 (CH₂Cl₂-MeOH = 20:1), than compound **E**. Due to a small amount and unstable compound, compound **F** have no UV and IR recorded.

In the ¹H NMR spectrum (Figure 100, Table 12), compound **F** appeared similar to compound **E** in upfield shift, except for the absence of an ABX system of ring A in compound **F** which was placed by two doublet signals of *meta*-coupled protons on ring A at δ_{H} 6.50 (*J* = 1.9 Hz, H-7) and 6.19 (*J* = 1.9 Hz, H-5). This was further supported by COSY cross-peak of H-7 to H-5. The ¹³C NMR spectrum of compound **F** (Figure 101) was also resembled to those of compound **E**, except for the appearance of C-4 as oxygenated quaternary carbon at δ_{C} 150.1 in compound **F** instead of C-4 as aromatic carbon at δ_{C} 121.1 in compound **E**. These findings suggested that there is a substituent at C-4. In addition, compound **F** had a molecular formula of C₃₀H₃₆O₄ as determined by ESMS ion at *m/z* 459 [M+H]⁺, which was 16 mass units differing to compound **E**. Thus, the structure of compound **F** contained extra hydroxyl group comparing to compound **E**. The correlations between the olefinic proton at δ_{H} 6.77 (H-3) to quaternary carbon at δ_{C} 112.0 (C-3a) and δ_{C} 156.6 (C-7a), and two aromatic protons at δ_{H} 6.19 (H-5) and 6.50 (H-7) to quaternary carbon δ_{C} 90.5 (C-3a), were observed in HMBC data. The other correlations were shown in Figure 84. This compound was identified by comparison of spectroscopic data with the literature data (Table 12) (Sritularak; et al. 2010: 6548-6558), the structure of compound **F** was established to be 4-hydroxyartolakoochol (**340**).

Comparison of the optical rotation of both compound **F** [α_{D}^{30}]-103.6° (*c* = 0.25, MeOH) to that of the reported 4-hydroxyartolakoochol [α_{D}^{20}]-117.6° (*c* = 0.03, MeOH) (Sritularak; et al. 2010: 6548-6558), they should have the same stereostructure. The structure of compound **F** was therefore identified as hydroxyartolakoochol (**340**) or 2-(5-hydroxy-2-methyl-8-(3-methylbut-2-enyl)-2-(4-methylpent-3-enyl)-2H-chromen-7-yl) benzofuran-4,6-diol.

Figure 84 Selected COSY and HMBC correlations of compound **F**Table 12 Comparison of ^1H - and ^{13}C -NMR data of compound **F** in CDCl_3 + 2 drops CD_3OD with 4-hydroxyartolakochole (**340**) in acetone- d_6

Position	δ_{H}		δ_{C}	
	Compound F	4-Hydroxyartolakochole ^a (340)	Compound F	4-Hydroxyartolakochole ^a (340)
2	-	-	152.9	153.2
3	6.77 (1H, s)	6.86 (1H, d, J=1.0)	101.9	103.1
3a	-	-	112.0	112.3
4	-	-	150.1	152.0
5	6.19 (1H, d, J=1.9)	6.29 (1H, d, J=2.0)	97.6	98.6
6	-	-	155.1	157.6
7	6.50 (1H, d, J=1.9)	6.49 (1H, d, J=2.0)	90.5	90.4
7a	-	-	156.6	157.7
1'	-	-	130.3	131.9
2'	-	-	119.3	119.0
3'	-	-	152.2	153.0
4'	-	-	109.5	110.2
5'	-	-	149.8	151.8
6'	6.64 (1H, s)	6.80 (1H, s)	106.8	107.6
1''	3.42 (2H, d, J=6.5)	3.47 (2H, d, J=6.5)	25.7	26.2
2''	5.14 (1H, br t, J=6.5)	5.17 (1H, br t, J=6.5)	123.7	124.8
3''	-	-	130.8	131.1
4''	1.69 (3H, s)	1.74 (3H, s)	18.0	18.3
5''	1.62 (3H, s)	1.65 (3H, s)	25.7	25.9
1'''	6.67 (1H, d, J=10.0)	6.74 (1H, d, J=10.5)	117.3	118.4
2'''	5.52 (1H, d, J=10.0)	5.56 (1H, d, J=10.5)	128.0	128.5
3'''	-	-	78.3	79.0

Table 12 (continued)

Position	δ_{H}		δ_{C}	
	Compound F	4-Hydroxyartolakoochol ^a (340)	Compound F	4-Hydroxyartolakoochol ^a (340)
4'''	1.32 (3H, s)	1.37 (3H, s)	26.2	26.6
5'''	1.75 (2H, m)	1.75 (2H, m)	41.3	42.0
6'''	2.07 (2H, m)	2.07 (2H, m)	22.8	23.6
7'''	5.07 (1H, br t, J=7.0)	5.13 (1H, br t, J=7.0)	124.2	125.1
8'''	-	-	131.6	131.4
9'''	1.54 (3H, s)	1.57 (3H, s)	17.5	17.7
10'''	1.62 (3H, s)	1.64 (3H, s)	25.6	25.8
-OH-4	-	8.83 (1H, br s)	-	-
-OH-6	-	8.38 (1H, br s)	-	-
-OH-5'	-	8.48 (1H, br s)	-	-

^a Sritularak; et al. 2010: 6548-6558

4.1.3 Compound **C** (cycloartolakoochol, sss5256 and sss5420)

Compound **C** was obtained as pale yellow sticky oil with R_f value of 0.36 (CH_2Cl_2) and gave a dark purple coloration with anisaldehyde- H_2SO_4 reagent. Its UV spectrum also showed the typical bands at λ_{max} 220 and 313 nm and its IR spectrum displayed absorption bands for hydroxyl (3370 cm^{-1}), aliphatic (2966 , 2928 and 2870 cm^{-1}) and aromatic (1619 and 1446 cm^{-1}), indicating that the presence of 2-arylbenzofuran moiety though. The ESMS showed a molecular ion at m/z 445 $[\text{M}+\text{H}]^+$, which corresponds to the formula $\text{C}_{29}\text{H}_{32}\text{O}_4$.

Comparison of the ^1H -NMR spectrum between compounds **C** and **E** exhibited the same patterns of ABX system of ring A and isoprene unit in downfield region. The ^1H -NMR data of compound **C** (Figure 102, Table 13) showed the absence of two typical doublet signals of pyrano system and methylene protons (H-1'') were clearly splitted into two doublet of doublets when compared to that of compound **E**, suggesting that the substituent patterns on ring B were changed. Furthermore, the additional signals at high field region displayed complicated patterns as follows: δ_{H} 2.90 (br t, J=2.0 Hz, H-1'''), 2.21 (m, H-2'''_{eq}), 2.04 (m, H-7'''), 1.82 (dd, J = 13.1 and 1.1 Hz, H-2'''_{ax}), 1.73 (m, H-5'''_{ax}), 1.52 (s, H-9'''), 1.45 (m, H-5'''_{eq}), 1.38 (s, H-4'''), 1.26 (m, H-6'''_{eq}), 1.03 (s, H-10''') and 0.69 (m,

6'''_{ax}) were appeared. These patterns were observed as a tricyclic structure at C-3' and C-5' which are from arrangement of monoterpene moiety attaching at C-4'. The ^{13}C NMR (Figure 103) and DEPT spectra showed 29 carbons due to five methyls, four methylenes, eight methane and 12 quaternary carbons. In COSY experiment, the cross peak of H-1''' at ring F to axial proton (H-2'''_{ax}), equatorial protons (H-2'''_{eq}) and proton H-7''' were appeared, as well as, the correlations of axial proton (H-6'''_{ax}) to axial proton (H-5'''_{ax}), two equatorial protons (H-5'''_{eq} and 6'''_{eq}) and methine proton (H-7'''). In ring F, the HMBC interactions from an axial proton (H-2'''_{ax}) to carbons C-4' (δ_{C} 117.2), C-1''' (δ_{C} 28.5) and C-7''' (δ_{C} 46.9) indicated that ring F was at C-4'. Moreover, the HMBC correlations of methyl proton (H-4''') at δ_{H} 1.38 to two methylene carbons at δ_{C} 35.1 (C-2''') and 37.5 (C-5''') were revealed (Figure 85). Obvious cross-peak between methyl proton H-9''' and methine proton H-1''' were further shown in the NOESY spectrum. This compound was identified as cycloartolakoochol (**341**) by comparison of its spectral data (^1H - and ^{13}C -NMR) with these reported in literature (Sritularak; et al. 2010: 6548-6558).

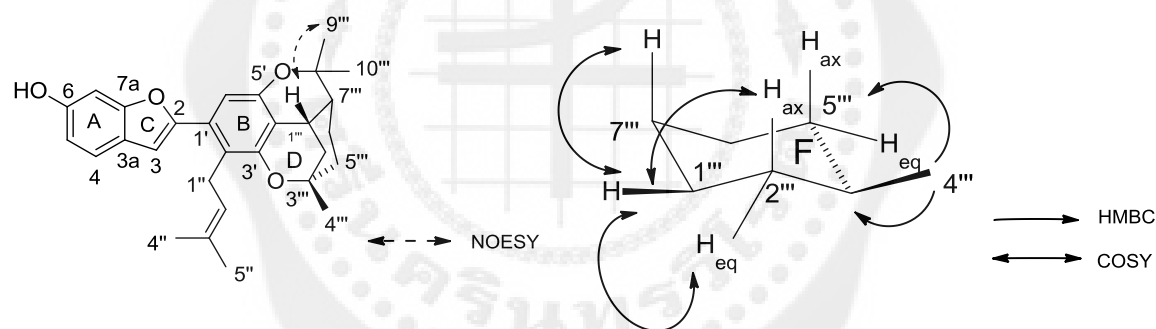


Figure 85 Selected COSY, HMBC and NOESY correlations of compound **C**

Table 13 Comparison of ^1H - and ^{13}C -NMR data of compound **C** with cycloartyolakoochol (**341**) in CDCl_3

Position	δ_{H}		δ_{C}	
	Compound C	Cycloartolakoochol ^a (341)	Compound C	Cycloartolakoochol ^a (341)
2	-	-	155.3	155.4
3	6.68 (1H, s)	6.68 (1H, d, J=1.0)	104.3	104.3
3a	-	-	122.9	123.0
4	7.36 (1H, d, J=8.3)	7.36 (1H, d, J=8.0)	120.8	120.9
5	6.74 (1H, dd, J=8.3, 2.2)	6.73 (1H, dd, J=8.0, 2.0)	111.6	111.6

Table 13 (continued)

Position	δ_{H}		δ_{C}	
	Compound C	Cycloartolakoochol ^a (341)	Compound C	Cycloartolakoochol ^a (341)
6	-	-	153.3	153.3
7	6.97 (1H, br d, J=2.2)	6.96 (1H, d, J=2.0)	98.1	98.2
7a	-	-	155.4	155.5
1'	-	-	128.4	128.4
2'	-	-	120.7	120.8
3'	-	-	154.9	154.9
4'	-	-	117.2	117.2
5'	-	-	154.5	154.5
6'	6.82 (1H, s)	6.81 (1H, s)	109.2	109.2
1'' _{ax}	3.40 (1H, dd, J=14.6,6.5)	3.40 (1H, dd, J=14.5,7.0)	25.5	25.5
1'' _{eq}	3.55 (1H, dd, J=14.6,6.5)	3.54 (1H, dd, J=14.5,7.0)		
2''	5.18 (1H, br t, J=6.5)	5.19 (t, J=7.0)	123.9	123.9
3''	-	-	130.8	130.9
4''	1.69 (3H, s)	1.69 (3H, s)	18.0	18.1
5''	1.66 (3H, s)	1.66 (3H, s)	25.8	25.8
1'''	2.90 (1H, br t, J = 2.0)	2.89 (1H, br t, J = 2.0)	28.5	28.6
2''' _{ax}	1.82 (1H, dd, J=13.2 and 1.1)	1.82 (dd, J=13.0 and 1.5)	35.1	35.1
2''' _{eq}	2.21 (1H, m)	2.21 (m)		
3'''	-	-	74.5	74.6
4'''	1.38 (3H, s)	1.39 (3H, s)	29.1	29.2
5''' _{ax}	1.73 (2H, m)	1.71 (2H, m)	37.5	37.5
5''' _{eq}	1.45 (2H, m)	1.42 (2H, m)		
6''' _{ax}	0.69 (1H, m)	0.70 (1H, m)	22.2	22.3
6''' _{eq}	1.26 (1H, m)	1.25 (1H, m)		
7'''	2.04 (1H, m)	2.05 (1H, m)	46.9	46.9
8'''	-	-	83.5	83.5
9'''	1.52 (3H, s)	1.52 (3H, s)	29.7	29.8
10'''	1.03 (3H, s)	1.04 (3H, s)	23.7	23.8

^a Sritularak; et al. 2010: 6548-6558

4.1.4 Compound J (Iakoochin B, **sss5732** and **sss6049**)

Compound **J** was the major 2-arylbenzofuran isolated from Surin source and obtained as dark yellow sticky oil. In the ESMS analysis, the compound showed a molecular ion at m/z 446 [M] corresponding to the formula $C_{29}H_{34}O_4$. Its UV spectrum in MeOH showed the typical bands at λ_{max} 208 and 296 nm and its IR spectrum exhibited absorption bands for hydroxyl (3400 cm^{-1}), aliphatic (2976 , 2922 and 2853 cm^{-1}) and aromatic (1621 and 1440 cm^{-1}), which are characteristic absorptions for 2-arylbenzofuran skeleton.

The ^1H -NMR data of this compound was similar to compound **E**, except for the disappearance of two doublet signals at δ_{H} 6.66 ($J = 9.9\text{ Hz}$, H-1'') and 5.57 ($J = 9.9\text{ Hz}$, H-2'') belonging to two *cis*-coupled olefinic protons of pyrano ring in compound **E**. The ^1H -NMR spectrum of compound **J** (Figure 104, Table 14), exhibited the following signals for 2-arylbenzofuran skeleton: one set of ABX aromatic protons at δ_{H} 7.39 (d, $J = 8.3\text{ Hz}$, H-4), 6.96 (d, $J = 2.1\text{ Hz}$, H-7) and 6.78 (dd, $J = 8.3$ and 2.1 Hz , H-5), along with two singlet signals at δ_{H} 6.53 (H-3) and 6.49 (H-4'). Furthermore, a set of the signals in upfield shift at δ_{H} 5.17 (br t, $J = 6.7\text{ Hz}$, H-2''), 3.13 (d, $J = 6.7\text{ Hz}$, H-1''), 1.67 (s, H-5'') and 1.61 (s, H-4'') which corresponding for the presence of an isoprene unit in ^1H NMR data. The rest of the signals presented as the typical signals of geranyl group. These signals were two broad triplets of olefinic protons at δ_{H} 5.17 ($J = 6.7\text{ Hz}$, H-2'') and 5.00 ($J = 6.5\text{ Hz}$, H-7''), one doublet of methylene proton at δ_{H} 3.13 ($J = 6.7\text{ Hz}$, H-1''), two multiplets of methylene protons δ_{H} 2.03 (H-6'') and 2.00 (H-5''), four siglets of methyl protons at δ_{H} 1.67 (H-5''), 1.65 (H-10''), 1.61 (H-4'',4'') and 1.56 (H-9''). Analysis of ^{13}C NMR (Figure 105) and DEPT spectra displayed 29 carbons, belonging to five methyls, four methylenes, eight methines and 12 quaternary carbons. The HMBC correlations of methylene proton at δ_{H} 3.13 (H-1'') to quaternary carbons C-1' (δ_{C} 131.3), C-5' (δ_{C} 154.0) and C-6' (δ_{C} 120.0) confirmed that the geranyl group was located at C-6', as well as, correlations of methylene proton at δ_{H} 3.13 (H-1'') to quaternary carbons C-1', C-2' (δ_{C} 120.1) and C-3' (δ_{C} 154.2), confirmed that the attachment of the prenyl group at C-2' (Figure 86). In addition, the correlations of the methylene proton (H-1'') to C-2'' (δ_{C} 122.4) and C-3'' (δ_{C} 138.1), the methyl proton (H-4'') to C-2'', C-3'' and C-5'' (δ_{C} 39.6), the methylene proton (H-5'') to C-6'' (δ_{C} 26.3) and two methyl protons (H-9'' and H-10'') to C-7'' (δ_{C} 123.7) and C-8'' (δ_{C} 132.0) were observed.

The correlations of the methylene proton (H-1'') to C-2'' (δ_{C} 122.4) and C-3'' (δ_{C} 134.3), and two methyl protons (H-4'' and H-5'') to C-2'' and C-3'' were also revealed. HMBC cross-peaks of the singlet aromatic proton at δ_{H} 6.50 (H-4') to two oxygenated quaternary carbons (C-3' and C-5'), and two quaternary carbons (C-2' and C-6') suggested that there is no substituent at C-4'. Comparison of ^1H and ^{13}C -NMR data between compound **J** and reported lakoochin B, it was shown that they were also very similar (Puntumchai; et al. 2004: 485-456). Compound **J** was therefore identified as (*E*)-4-(3,7-dimethylocta-2,6-dienyl)-5-(6-hydroxybenzofuran-2-yl)-6-(3-methylbut-2-enyl)benzene-1,3-diol or lakoochin B (**157**).

Lakoochin B (**157**) was firstly isolated from *A. lakoocha* (Puntumchai; et al. 2004: 485-456) and also found in *A. Petelotii* (Shen; et al. 2008: 1451-1456). It possessed antimycobacterial, and cytotoxic activities against BC, KB, HepG2 and MCF-7 cell lines (Puntumchai; et al. 2004: 485-486; Shen; & Hou. 2008: 1451-1456).

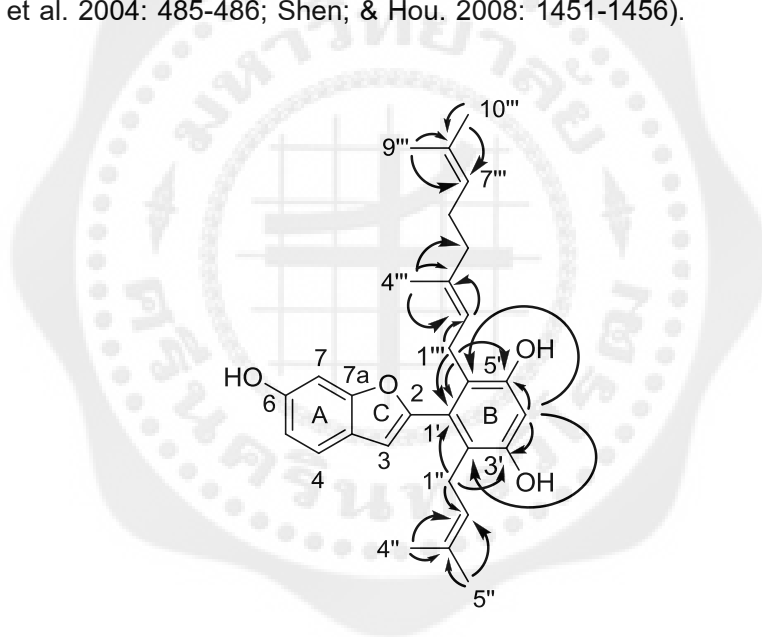


Figure 86 Selected HMBC correlations of compound **J**

Table 14 Comparison of ^1H - and ^{13}C -NMR data of compound **J** with lakoochin B (**157**) in CDCl_3

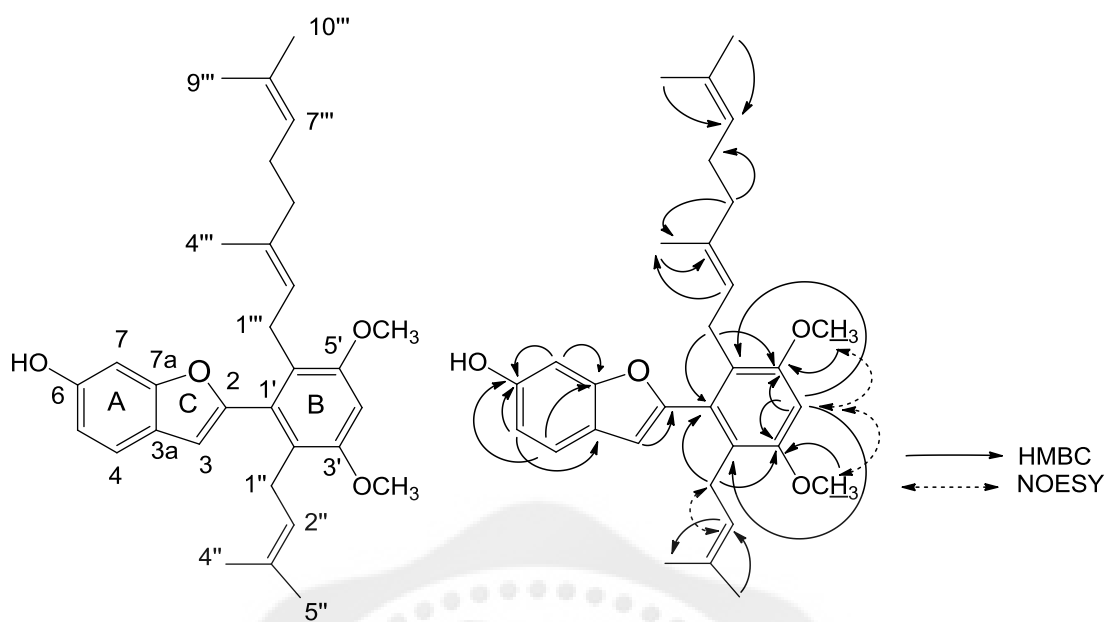
Position	δ_{H}		δ_{C}	
	Compound J	Lakoochin B ^a (157)	Compound J	Lakoochin B ^a (157)
2	-	-	153.2	153.1
3	6.53 (1H, s)	6.58 (1H, s)	106.4	106.4
3a	-	-	122.2	122.1
4	7.39 (1H, d, J=8.3)	7.44 (1H, d, J=8.4)	121.0	120.9
5	6.78 (1H, dd, J=8.3, 2.1)	6.83 (1H, dd, J=8.4, 2.1)	111.8	111.8
6	-	-	153.3	153.3
7	6.96 (1H, d, J=2.1)	7.01 (1H, d, J=2.1)	98.3	98.3
7a	-	-	155.6	155.6
1'	-	-	131.3	131.4
2'	-	-	120.1	120.2
3'	-	-	154.2	154.0
4'	6.49 (1H, s)	6.55 (1H, s)	105.6	105.6
5'	-	-	154.0	153.8
6'	-	-	120.0	120.1
1''	3.13 (2H, d, J=6.7)	3.19 (2H, d, J=6.8)	27.4	27.4
2''	5.17 (1H, br t, J=6.7)	5.21 (1H, br t, J=6.5)	122.4	122.4
3''	-	-	134.3	134.2
4''	1.61 (3H, s)	1.64 (3H, s)	17.7	17.7
5''	1.67 (3H, s)	1.71 (3H, s)	25.7	25.7
1'''	3.13 (2H, d, J=6.7)	3.19 (2H, d, J=6.8)	27.4	27.4
2'''	5.17 (1H, br t, J=6.7)	5.21 (1H, br t, J=6.5)	122.4	122.3
3'''	-	-	138.1	138.0
4'''	1.61 (3H, s)	1.64 (3H, s)	16.0	16.0
5'''	2.00 (2H, m)	2.03 (2H, m)	39.6	39.6
6'''	2.03 (2H, m)	2.08 (2H, m)	26.3	26.3
7'''	5.00 (1H, br t, J=6.5)	5.01 (1H, br t, J=6.6)	123.7	123.7
8'''	-	-	132.0	132.0
9'''	1.56 (3H, s)	1.60 (3H, s)	17.6	17.6
10'''	1.65 (3H, s)	1.69 (3H, s)	25.6	25.6
-OH-6	-	5.37 (br s)	-	-
-OH-3'	-	5.43 (br s)	-	-
-OH-5'	-	5.43 (br s)	-	-

^aPuntumchai; et al. 2004: 485-456

4.1.5 Compound A (lakoochin D, sss5311)

Compound **A** obtained as colorless sticky oil and the molecular formula was determined to be $C_{31}H_{38}O_4$ by HRESIMS at m/z 497.2642 $[M+Na]^+$ (calcd for $C_{31}H_{38}O_4Na$, 497.2662). Its UV and IR data were closely resembled those of compound **J** or lakoochin **B**, indicating that this compound has similar 2-arylbenzofuran skeleton.

The ^1H and ^{13}C -NMR spectrum of compound **A** or lakoochin **D** (Figure 106, Table 15) was very similar to those of compound **J** or lakoochin **B**, except for an additional singlet signal at δ_H 3.84 (6H) and δ_C 55.9 was observed as two methoxy groups. The ^{13}C -NMR and DEPT spectra showed 31 carbons, including two methoxys, five methyls, four methylenes, eight methines and 12 quaternary carbons. An obvious downfield shift in ring **B** at δ_C 133.9 of C-3''' and δ_C 97.3 of C-4' in compound **A**, compared to that of the respective δ_C 138.1 and 105.6 in **J**, indicated for the methylation at C-3',5' was observed. The HMBC experiment were revealed the correlations between the methoxy protons, and two oxygenated quaternary carbons at δ_C 156.4 (C-3') and 156.3 (C-5') (Figure 87). The correlations of the singlet aromatic proton at δ_H 6.58 (H-4') to two oxygenated quaternary carbons (C-3' and C-5'), and two quaternary carbons (C-2' and C-6') at δ_C 123.3 and 123.2, respectively, suggesting that no substituent attached to C-4'. The NOESY spectrum also showed intense cross-peak between the singlet aromatic proton of ring **B** at H-4' and the methoxy signal. These findings suggested that the location of the methoxy groups was at C-3' and -5' of ring **B**. Additionally, the positions of prenyl and geranyl groups were confirmed by HMBC experiment and revealed correlations of a methylene proton at δ_H 3.12 (H-1'') to C-1', C-2', C-3' and from a methylene proton H-1''' (δ_H 3.12) to C-1', C-5', C-6'. The other correlations were shown in Figure 87. Accordingly, the structure of compound **A** was established as (*E*)-2-(2-(3,7-dimethylocta-2,6-dienyl)-3,5-dimethoxy-6-(3-methylbut-2-enyl)phenyl)benzofuran-6-ol or lakoochin **B**-3',5'-di-O-methyl ether and named lakoochin **D** (406).

Figure 87 Selected HMBC and NOESY correlations of compound **A**Table 15 Comparison of ^1H - and ^{13}C -NMR data of compound **A** with compound **J** in CDCl_3

Position	δ_{H}		δ_{C}	
	Compound A	Compound J	Compound A	Compound J
2	-	-	153.3	153.2
3	6.50 (1H, s)	6.53 (1H, s)	106.2	106.4
3a	-	-	122.5	122.2
4	7.36 (1H, d, J=8.3)	7.39 (1H, d, J=8.3)	120.8	121.0
5	6.75 (1H, dd, J=8.3, 2.1)	6.78 (1H, dd, J=8.3, 2.1)	111.5	111.8
6	-	-	153.0	153.3
7	6.93 (1H, d, J=2.1)	6.96 (1H, d, J=2.1)	98.2	98.3
7a	-	-	155.6	155.6
1'	-	-	131.9	131.3
2'	-	-	123.3	120.1
3'	-	-	156.4	154.2
4'	6.58 (1H, s)	6.49 (1H, s)	97.3	105.6
5'	-	-	156.3	154.0
6'	-	-	123.2	120.0

Table 15 (continued)

Position	δ_{H}		δ_{C}	
	Compound A	Compound J	Compound A	Compound J
1''	3.12 (2H, d, J=6.6)	3.13 (2H, d, J=6.7)	26.4	27.4
2''	5.06 (1H, br d, J=6.6)	5.17 (1H, br t, J=6.7)	123.6	122.4
3''	-	-	130.3	134.3
4''	1.36 (3H, s)	1.61 (3H, s)	17.6	17.7
5''	1.55 (3H, s)	1.67 (3H, s)	25.7	25.7
1'''	3.12 (2H, d, J=6.6)	3.13 (2H, d, J=6.7)	26.6	27.4
2'''	5.06 (1H, br d, J=6.6)	5.17 (1H, br t, J=6.7)	123.6	122.4
3'''	-	-	133.9	138.1
4'''	1.35 (3H, s)	1.61 (3H, s)	15.8	16.0
5'''	1.87 (2H, m)	2.00 (2H, m)	39.7	39.6
6'''	1.97 (2H, m)	2.03 (2H, m)	26.6	26.3
7'''	5.04 (1H, br d, J=6.6)	5.00 (1H, br t, J=6.5)	124.5	123.7
8'''	-	-	131.0	132.0
9'''	1.55 (3H, s)	1.56 (3H, s)	17.6	17.7
10'''	1.63 (3H, s)	1.65 (3H, s)	25.6	25.6
-OMe-	3.84 (6H, s)	-	55.9	-
3',5'				

4.1.6 Compound **B** (lakoochin **A**, **sss5617** and **sss6096**)

Compound **B** was obtained as pale yellow sticky oil with R_f value of 0.29 (n-hexane-CH₂Cl₂ 1:4) and gave a purple coloration with anisaldehyde-H₂SO₄ reagent. Its UV and IR spectrum were similar to compound **J** or lakoochin **B**, indicating that this compound has similar 2-arylbenzofuran skeleton. The ESMS showed the molecular ion at m/z 407 $[M+H]^+$, corresponding to the formula of C₂₆H₃₀O₄.

The ¹H NMR data of compound **B** (Figure 108, Table 16) closely resembled those of compound **J** except for the absence of two multiplet signals in compound **B** at δ_{H} 2.03 (C-6''') and 2.00 (C-5'''), which is typical signals of geranyl unit indicating that no geranyl group contained in the structure. From the observation, two sets of signals at δ_{H} 5.04 (2x2H, d, J = 6.8 Hz, H-2'' and H-2'''), 3.11 (2x2H, d, J = 6.8 Hz, H-1'' and H-1'''), 1.55 (2x3H, s, H-4'' and H-4''') and 1.36 (2x3H, s, H-5'' and H-5''') were characterised for two

prenyl units. These are in agreement with correlations between methylene protons H-1'' with aromatic carbon at δ_C 131.8 (C-1') and oxygenated carbon at δ_C 156.3 (C-3'), as well as, correlations between methylene protons H-1''' with aromatic carbon at δ_C 131.8 (C-1') and oxygenated carbon at δ_C 156.3 (C-5') in the HMBC spectrum (Figure 88). Analysis of ^{13}C NMR (Figure 109) and DEPT spectra showed 26 carbons, belonging to four methyls, two methylenes, seven methines and 13 quaternary carbons. In NOESY spectrum, the location of two methoxy groups were also confirmed by intense cross-peak of OCH_3 group to aromatic proton H-4'. Furthermore, the correlations between aromatic protons (H-4') to two oxygenated quaternary carbon (C-3' and C-5') at δ_C 156.3 and to two quaternary carbon (C-2' and C-6') at δ_C 122.5 were revealed in the HMBC technique. This compound was also confirmed by comparison of spectroscopic data with those of the literature values (Puntumchai; et al. 2004: 485-456). The structure of the compound was thus concluded as 2-(3,5-dimethoxy-2,6-bis(3-methylbut-2-enyl)phenyl)benzofuran-6-ol or lakoochin A (**293**).

Lakoochin A (**293**) was found in *Artocarpus* plants such as *A. lakoocha* (Puntumchai; et al. 2004: 485-456), *A. styrcifolius* (Ren; et al. 2013: 97-100) and *A. xanthocarpus* (Jin; et al. 2015: 424-435). It exhibited antimycobacterial, antityrosinase, antioxidant and cytotoxicities against BC, KB and B16F10 cells (Puntumchai; et al. 2004: 485-486; Jin; et al. 2015: 424-435).

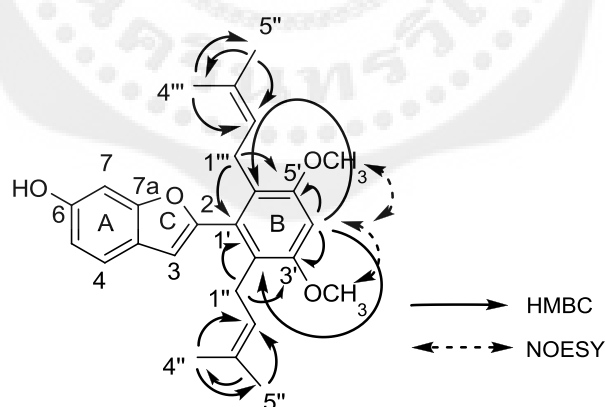


Figure 88 Selected HMBC and NOESY correlations of compound **B**

Table 16 Comparison of ^1H - and ^{13}C NMR data of compound **B** with lakoochin A (**293**) in CDCl_3

Position	δ_{H}		δ_{C}	
	Compound B	lakoochin A ^a (293)	Compound B	lakoochin A ^a (293)
2	-	-	153.1	153.0
3	6.50 (1H, s)	6.54 (1H, s)	106.2	106.2
3a	-	-	123.3	123.2
4	7.37 (1H, d, J=8.3)	7.40 (1H, d, J=8.1)	120.8	120.8
5	6.75 (1H, dd, J=8.3, 2.2)	6.78 (1H, dd, J=8.1, 2.1)	111.5	111.5
6	-	-	153.3	153.3
7	6.94 (1H, d, J=2.2)	6.97 (1H, d, J=2.1)	98.2	98.2
7a	-	-	155.6	155.6
1'	-	-	131.8	131.8
2'	-	-	122.5	122.5
3'	-	-	156.3	156.3
4'	6.57 (1H, s)	6.61 (1H, s)	97.4	97.3
5'	-	-	156.3	156.3
6'	-	-	122.5	122.5
1''	3.11 (2H, d, J=6.8)	3.15 (2H, d, J=6.8)	26.6	26.5
2''	5.04 (1H, br t, J=6.8)	5.05 (1H, br t, J=7.1)	123.6	123.6
3''	-	-	130.3	130.3
4''	1.55 (3H, s)	1.58 (3H, s)	25.7	25.7
5''	1.36 (3H, s)	1.39 (3H, s)	17.6	17.6
1'''	3.11 (2H, d, J=6.8)	3.15 (2H, d, J=6.8)	26.6	26.5
2'''	5.04 (1H, br t, J=6.8)	5.05 (1H, br t, J=7.1)	123.6	123.6
3'''	-	-	130.3	130.3
4'''	1.36 (3H, s)	1.39 (3H, s)	17.6	17.6
5'''	1.55 (3H, s)	1.58 (3H, s)	25.7	25.7
-OMe-3'	3.83 (3H, s)	3.87 (3H, s)	55.9	55.9
-OMe-5'	3.83 (3H, s)	3.87 (3H, s)	55.9	55.9

^aPuntumchai; et al. 2004: 485-486

4.1.7 Compound I (albafuran B, sss5827)

Compound I was obtained as a minor compound from Surin source. It was obtained as white amorphous with R_f value of 0.29 (n-hexane-CH₂Cl₂ 1:4) and gave a purple coloration with anisaldehyde-H₂SO₄ reagent indication of arylbenzofuran. This compound has no UV and IR data recorded because of its small amount.

Comparison of the ¹H-NMR spectra between compounds I and J (lakoochin B) showed the similar pattern except for the presence of symmetrically substituted on ring B at two aromatic protons δ_H 6.82 (2H) were assignable to H-2' and H-6' in compound I. In addition, a set of the signals in upfield shift at δ_H 5.27 (br t, J = 6.9 Hz, H-2''), 5.03 (br t, J = 6.2 Hz, H-7''), 3.42 (d, J = 6.9 Hz, H-1''), 2.05 (2H, m, H-5''), 2.07 (2H, m, H-6''), 1.79 (s, H-10'') 1.65 (s, H-9'') and 1.56 (s, H-4'') was characteristic for a geranyl group in ¹H NMR data (Figure 110, Table 17). These findings suggested that there is a geranyl group in the structure. In HMBC spectrum, the correlations from methylene protons at δ_H 3.42 (H-1'') to C-4' (δ_C 113.9), and two oxygenated carbons (C-3' and C-5' at δ_C 155.5), confirmed that the geranyl group was attached to C-4' (Figure 89). Analysis of ¹³C NMR spectrum (Figure 111) presented 24 carbons, belonging to the 14 carbons of 2-arylbenzofuran skeleton and 10 carbons of a geranyl group, which indicated that no prenyl group in the structure. This was consistent with a molecular ion at m/z 377 [M-H]⁻ in the ESMS, which provided the formula C₂₄H₂₆O₄. HMBC cross-peaks of the singlet aromatic protons of H-2',6' to two oxygenated quaternary carbons (C-3' and C-5') and a quaternary carbon (C-4') were observed. Furthermore, the correlations of the methylene proton (H-1'') to C-2'' (δ_C 121.3) and C-3'' (δ_C 139.1), the methyl proton (H-4'') to C-2'', C-3'' and C-5'' (δ_C 39.7), the methylene proton (H-5'') to C-4'' (δ_C 16.2) and two methyl protons (H-9'' and H-10'') to C-7'' and C-8'' (δ_C 123.8 and 132.0) were revealed (Figure 89). So, only the structure of compound I revealed the absence of the prenyl unit at C-2' in a series of isolated 2-arylbenzofurans. This compound was also identified by comparison of spectroscopic data with those of the literature values (Takasuki; Ishikawa; & Masamune. 1982: 1221-1222). The compound was therefore concluded to be albafuran B (**407**) or (*E*)-2-(3,7-dimethylocta-2,6-dienyl)-5-(6-hydroxybenzofuran-2-yl)benzene-1,3-diol. This is the first report on ¹³C NMR data for albafuran B.

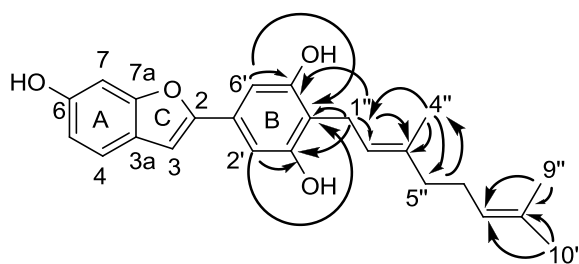


Figure 89 Selected HMBC correlations of compound I

Table 17 Comparison of ^1H - and ^{13}C NMR data of compound I in CDCl_3 +1 drop CD_3OD with albafrican B (**407**) in CD_3OD

Position	δ_{H}		δ_{C}	
	Compound I	Albafrican B ^a (407)	Compound I	Albafrican B ^a
2	-	-	154.0	-
3	6.79 (1H, s)	6.89 (1H, br s)	101.0	-
3a	-	-	122.5	-
4	7.32 (1H, d, J=8.4)	7.35 (1H, d, J=8.0)	120.9	-
5	6.73 (1H, dd, J=8.4, 1.9)	6.79 (1H, dd, J=8.0, 2.0)	112.0	-
6	-	-	154.7	-
7	6.93 (1H, br d, J=1.9)	6.93 (1H, br d, J=2.0)	98.1	-
7a	-	-	155.6	-
1'	-	-	129.7	-
2'	6.82 (1H, s)	6.89 (1H, br s)	104.3	-
3'	-	-	155.5	-
4'	-	-	113.9	-
5'	-	-	155.5	-
6'	6.82 (1H, s)	6.89 (1H, br s)	104.3	-
1''	3.42 (2H, d, J=6.9)	3.39	22.4	-
2''	5.27 (1H, br t, J=6.9)	5.33	121.3	-
3''	-	-	139.1	-
4''	1.56 (3H, s)	1.55	16.2	-
5''	2.05 (2H, m)	2.05	39.7	-
6''	2.07 (2H, m)	2.05	26.3	-
7''	5.03 (1H, br t, J=6.7)	5.09	123.7	-

Table 17 (continued)

Position	δ_{H}		δ_{C}	
	Compound I	Albafuran B^a (407)	Compound I	Albafuran B^a
8''	-	-	132.0	-
9''	1.65 (3H, s)	1.61	17.6	-
10''	1.79 (3H, s)	1.79	25.6	-
-OH-3	-	8.30 (3H, br s)		

^aTakasuki; et al. 1982: 1221-1222

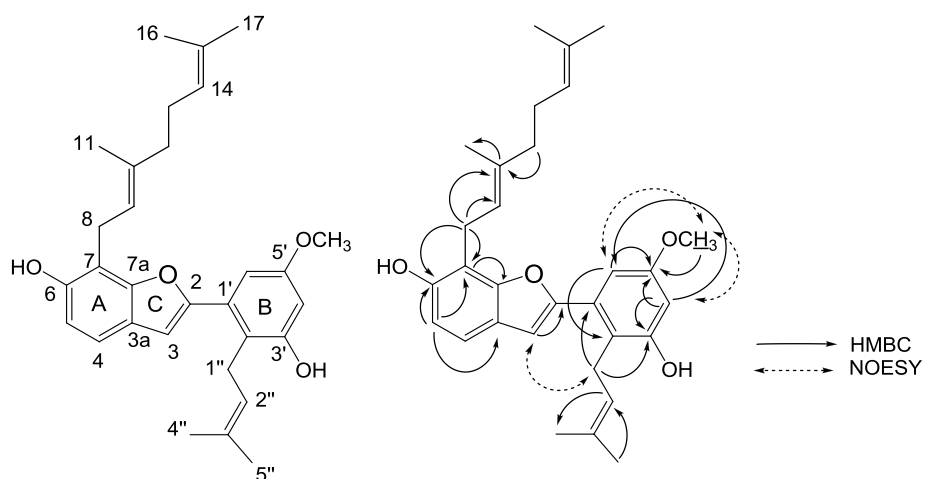
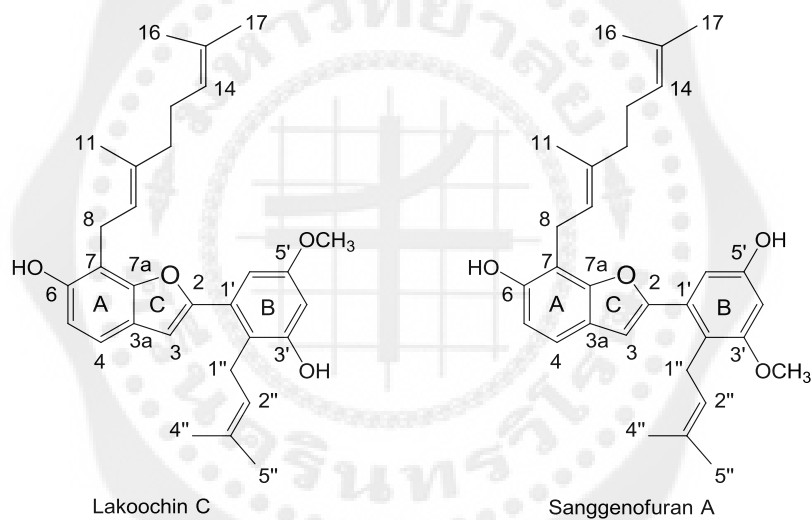
1.8 Compound **D** (Iakoochin **C**, sss5286)

Compound **D** had a molecular formula of $\text{C}_{30}\text{H}_{36}\text{O}_4$ as determined by HRESIMS ion at m/z 459.2547 $[\text{M}-\text{H}]^-$ (calcd for $\text{C}_{30}\text{H}_{36}\text{O}_4$, 459.2540). It gave a purple color with anisaldehyde to follow with its UV absorption at 208 and 310 nm indicating of arylbenzofuran skeleton for compound **D**.

The ^1H -NMR spectrum of compound **D** in CDCl_3 (Figure 112, Table 18) exhibited the similar patterns in upfield region comparing to compound **A** (Iakoochin **D**), except for the absence of an ABX system of ring A in compound **D** which was placed by two sets of aromatic protons: *ortho*-coupled protons on ring A at δ_{H} 7.27 (d, $J = 8.3$ Hz, H-4) and 6.77 (d, $J = 8.3$ Hz, H-5) and *meta*-coupled protons in ring B at δ_{H} 6.81 (d, $J = 2.4$ Hz, H-6') and 6.46 (d, $J = 2.4$ Hz, H-4'). This was further supported by COSY cross-peak of H-4 to H-5 and H-6' to H-4'. In addition, a singlet methoxyl signal at δ_{H} 3.79 (3H) and δ_{C} 55.4 were observed in the ^1H -NMR data. Analysis of ^1H spectrum further showed two sets of signals for geranyl and prenyl moieties. A set of signals of two broad triplets of olefinic protons at δ_{H} 5.38 ($J = 7.1$ Hz, H-9) and 5.03 ($J = 6.9$ Hz, H-14), one doublet of methylene proton at δ_{H} 3.66 ($J = 7.1$ Hz, H-8), two multiplets of methylene protons δ_{H} 2.06 (H-13) and 2.03 (H-12), and three siglets of methyl protons at δ_{H} 1.83 (H-11), 1.64 (H-16) and 1.55 (H-17) were characterised for the geranyl unit. The other set of signals of a broad triplet of olefinic protons at δ_{H} 5.28 ($J = 6.5$ Hz, H-2''), one doublet of methylene proton at δ_{H} 3.50 ($J = 6.5$ Hz, H-1''), and two siglets of methyl protons at δ_{H} 1.76 (H-4'') and 1.75 (H-5'') were

the typical signals of the prenyl group. The ^{13}C -NMR (Figure 113) and DEPT data provided signals for 30 signals due to five methyls, one methoxyl, four methylenes, eight methines and twelve quaternary carbons. The HMBC spectrum revealed correlations between the methylene signal at δ_{H} 3.66 (H-8) and two quaternary carbons at δ_{C} 152.3 (C-6) and 110.3 (C-7), confirming the location of the geranyl group was at C-7 on ring A (Figure 90). Additionally, the methylene proton signal at δ_{H} 3.50 (H-1'') showed correlations with two aromatic carbon signals at δ_{C} 132.0 (C-1') and 117.6 (C-2'), and a quaternary oxygenated carbon 156.2 (C-3'), indicating that the prenyl group was located at C-2' on ring B. From these data, the presence of a set of aromatic protons, one geranyl, one prenyl and one methoxy signal are consistent with those reported for sanggenofuran A (**408**) which previously isolated from *Morus australis* (Shi; et al. 2001: 143-146). Importantly, the NOESY spectrum appeared intense cross peaks of methoxy proton to two aromatic proton signals at H-4' and H-6'. These findings confirmed that compound **D** was a structural isomer of sanggenofuran A. They are isomer differing from each other only by the position of hydroxyl and methoxy groups. The remaining signals in the HMBC spectrum were shown in Figure 90. Furthermore, the ^1H and ^{13}C -NMR data of compound **D** and reported sanggenofuran A measured in acetone- d_6 , were very similar. However, in NOESY spectrum of compound **D** in acetone- d_6 , there was no obvious cross peak indicating the difference of the structures between compound **D** and sanggenofuran A. The structure of compound **D** was therefore elucidated namely lakoochin C or (*E*)-7-(3,7-dimethylocta-2,6-dienyl)-2-(3-hydroxy-5-methoxy-2-(3-methylbut-2-enyl)phenyl)benzofuran-6-ol.

To our knowledge, This is the first time for the isolation of 3',5',6-triyoxygenated 2-arylbenzofurans with a geranyl substituted in ring A at C-7 in *Artocarpus* species, although similar geranylated 2-arylbenzofurans have been found in *Morus* species (Nomura; et al. 1983: 90-94; Shi; et al. 2001: 143-146; Yang; et al. 2010: 257-260; Yang; et al. 2011: 6010-6022; Tan; et al. 2010: 742-746). The present study agrees with an earlier reported observation that 3',5',6-triyoxygenated 2-arylbenzofurans occurs in Moraceous plants (Reuß et al., 2004). Furthermore, previous important evidences have been shown that two genera, *Artocarpus* and *Morus*, are closely related inferring from DNA sequence data (Datwyler; & Weibleno. 2004; Matsuda; et al., 2005).

Figure 90 Selected HMBC and NOESY correlations for compound **D**Figure 91 Structures of compound **D** (Lakoochin C) and Sanggenofuran A (**408**)Table 18 Comparison of ^1H - and ^{13}C NMR data of compound **D** in CDCl_3 with sanggenofuran A (**408**) in acetone- d_6

Position	δ_{H}			δ_{C}		
	Compd D ^a	Compd D ^b	Sanggenofuran A ^{b,c} (408)	Compd D ^a	Compd D ^b	Sanggenofuran A ^{b,c}
2	-	-	-	153.9	155.3	155.3
3	6.70 (1H, s)	6.77 (1H,s)	6.78 (1H,s)	105.5	105.9	106.1
3a	-	-	-	122.1	122.2	122.4

Table 18 (continued)

Position	δ_{H}			δ_{C}		
	Compd D ^a	Compd D ^b	Sanggenofuran A ^{b,c} (408)	Compd D ^a	Compd D ^b	Sanggenofuran A ^{b,c}
4	7.27 (1H, d, J=8.3)	7.21 (1H, d, J=8.3)	7.24 (1H, d, J=8.5)	118.5	118.7	118.9
5	6.77 (1H, d, J=8.3)	6.84 (1H,d, J=8.3)	6.84 (1H,d, J=8.5)	112.7	112.9	113.0
6	-	-	-	152.3	153.5	153.4
7	-	-	-	110.3	111.9	112.0
7a	-	-	-	154.7	155.2	153.4
8	3.66 (2H, br d, J=7.1)	3.61 (2H, br d, J=7.1)	3.62 (2H, br d, J=7.0)	23.1	23.2	23.3
9	5.38 (1H, br t, J=7.1)	5.43 (1H, br t, J=7.1)	5.45 (1H, m (tqq-like), J= 1,6,5)	120.9	123.1	123.1
10	-	-	-	138.9	135.4	135.6
11	1.83 (1H, s)	1.83 (3H,s)	1.84 (3H,s)	16.2	16.3	16.4
12	2.03 (2H, m)	1.96 (2H,m)	1.96 (2H,m)	39.6	40.3	40.5
13	2.06 (2H, m)	2.03 (2H,m)	2.03 (2H,m)	26.4	27.2	27.3
14	5.03 (br t, J=6.9)	5.02 (1H, br t,J=6.7)	5.04 (1H, m (tqq-like), J=1,6,5)	123.7	124.9	125.0
15	-	-	-	132.0	131.6	131.6
16	1.64 (3H, s)	1.48 (3H, s)	1.50 (3H, br s)	17.6	17.5	17.6
17	1.55 (3H, s)	1.53 (3H, s)	1.55 (3H, d , J=1)	25.6	25.6	25.7
1'	-	-	-	132.0	132.7	132.6
2'	-	-	-	117.6	119.8	120.1
3'	-	-	-	156.2	157.6	160.0
4'	6.46 (1H, d, J=2.4)	6.53 (1H, d, J=2.3)	6.53 (1H, d, J=2.5)	102.5	102.5	100.2
5'	-	-	-	158.7	159.2	157.2
6'	6.81 (1H, d, J=2.4)	6.78 (1H,br s)	6.82 (1H,d, J=2.5)	107.1	105.5	108.0
1''	3.50 (1H, br d, J=6.5)	3.51 (2H, br d, J=6.4)	3.48 (2H, br d, J=6.5)	26.5	26.2	26.3
2''	5.28 (1H, br t, J=6.5)	5.17 (1H, br t, J=6.4)	5.17 (1H, m (tqq-like), J=1,6,5)	122.5	125.1	125.3
3''	-	-	-	134.6	131.0	131.0
4''	1.75 (3H, s)	1.63 (3H, s)	1.65 (3H, s)	17.9	17.9	17.8
5''	1.76 (3H, s)	1.61 (3H, s)	1.63 (3H, s)	25.7	25.7	25.9
-OH-6	-	9.01 (1H, s)	8.39 (1H, br s)			
-OH-3'	-	8.86 (1H, s)	8.31 (1H, br s)			
-OCH ₃ -5'	3.79 (3H, s)	3.76 (3H, s)	3.82 (3H, s)	55.4		56.0

^a in CDCl₃, ^b in acetone-*d*₆, ^c Shi; et al. 2001: 143-146

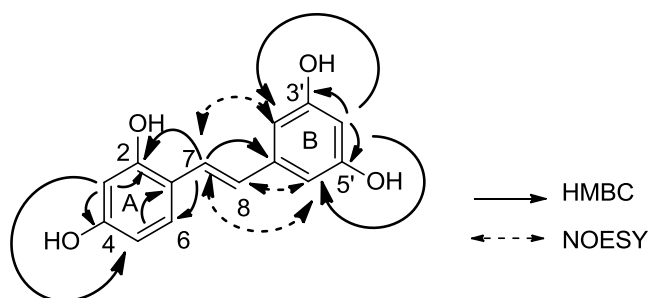
4.2 Structural determination of non 2-arylbenzofurans

4.2.1 Compound **G** (oxyresveratrol, sss5745)

Compound **G** was isolated as pale yellow crystal with the R_f values of 0.23 (CH_2Cl_2 -MeOH = 5:1 (x2)) and gave a reddish brown coloration with anisaldehyde- H_2SO_4 reagent on TLC. Its IR spectrum exhibited only absorption bands for hydroxyl (3360 cm^{-1}) and aromatic (1590 and 1479 cm^{-1}), in addition, the absence of absorption bands for aliphatic were observed. The UV spectrum of the compound displayed various maximal absorptions at λ_{max} 215, 235, 289, 301 and 327 nm. From these UV and IR spectra, compound **G** should have different skeleton from previous isolated compounds. On the basis of its ESMS ($[\text{M}+\text{K}]^+$ at m/z 283), a molecular formula of compound **G** was established as $\text{C}_{14}\text{H}_{12}\text{O}_4$ with the support of ^{13}C NMR data.

The ^1H NMR data of compound **G** (Figure 114, Table 19) (in CDCl_3 + 7 drops $\text{DMSO}-d_6$) showed an ABX aromatic system in ring A [δ_{H} 7.31 (d, $J = 8.4\text{ Hz}$, H-6), 6.42 (d, $J = 2.0\text{ Hz}$, H-3)] and 6.35 (dd, $J = 8.4$ and 2.0 Hz , H-5)], and AB_2 aromatic system in ring B [δ_{H} 6.50 (d, $J = 1.6\text{ Hz}$, H-2',6') and 6.24 (br s, H-4')]. The coupling constant value of 16.3 Hz of two doublet signals at δ_{H} 7.29 (H-7) and 6.78 (H-8) were characteristic of a *trans* conjugated double bond. The ^{13}C NMR (Figure 115) and DEPT spectra displayed 14 carbons due to two methylenes, two methines, six aromatic and four oxygenated quaternary carbons. These data implied that the presence of stilbene skeleton in compound **G**. The HMBC spectrum showed a long-range correlation between the aromatic proton signal at δ_{H} 6.50 (H-2',6') and olefinic methine carbon at δ_{C} 125.0 (C-8) (Figure 92). This was supported by NOESY experiment which presented the cross-peaks between H-8, H-2' and H-6' and also found cross-peaks between H-7, H-2' and H-6' (Figure 91). Moreover, the correlations between the olefinic proton signals at δ_{H} 7.29 (H-7) to quaternary carbon δ_{C} 116.0 (C-1), 155.6 (C-2) and 140.1 (C-1') and were observed in HMBC data. These evidences confirmed that the conjugated double bond connected to two rings A and B. The rest of HMBC correlations were shown in Figure 91. This compound was identified as oxyresveratrol or (*E*)-4-(3,5-dihydroxystyryl)benzene-1,3-diol (**3**) by comparison of its spectroscopic data (^1H - and ^{13}C -NMR) with those reported in literature (Likhitwitayawuid; & Sritularak. 2001: 1457-1459).

Several reports have revealed that oxyresveratrol commonly found as the major compound in *A. lakoocha* heartwoods (Mongolsuk; Robertson; & Towers. 1957: 2231-2233).

Figure 92 Selected HMBC and NOESY correlations of compound **G**Table 19 Comparison of ^1H - and ^{13}C NMR data of compound **G** in CDCl_3 + 7 drops $\text{DMSO}-d_6$ with oxyresveratrol (**3**) in $\text{DMSO}-d_6$

Position	δ_{H}		δ_{C}	
	Compound G	Oxyresveratrol ^a (3)	Compound G	Oxyresveratrol ^a (3)
1	-	-	116.0	115.4
2	-	-	155.6	156.1
3	6.42 (1H, d, J=2.0)	6.33 (1H, d, J=2.4)	102.8	102.7
4	-	-	157.5	158.2
5	6.35 (1H, dd, J=8.4, 2.0)	6.25 (1H, dd, J=8.4, 2.4)	107.2	107.4
6	7.31 (1H, d, J=8.4)	7.34 (1H, d, J=8.4)	126.7	127.3
7	7.29 (1H, d, J=16.3)	7.15 (1H, d, J=16.5)	123.4	123.3
8	6.78 (1H, d, J=16.3)	6.77 (1H, d, J=16.5)	125.0	124.7
1'	-	-	140.1	140.1
2'	6.50 (1H, br d, J=1.6) ^b	6.35 (1H, d, J=1.8)	104.5	104.2
3'	-	-	157.9	158.5
4'	6.24 (1H, br s)	6.08 (1H, br s)	101.5	101.5
5'	-	-	157.9	158.5
6'	6.50 (1H, br d, J=1.6) ^b	6.35 (1H, d, J=1.8)	104.5	104.2
-OH	-	-	-	-

^aLikhitwitayawuid; et. al. 2001: 1457-1459^bSuperscript within the same column may be interchanged.

4.2.2 Compound H ((+)-catechin, sss6127)

Compound **H** was the most polar compound among the isolated with the R_f values of 0.20 (CH_2Cl_2 -MeOH = 5:1). For ESMS, a major peak of m/z 139 was characterized as a flavan-3-ol which based on the retro Diels-Alder cleavage of C-ring bond.

The ^1H NMR experiment of compound **H** (Figure 116, Table 20) (in CDCl_3 + 8 drops $\text{DMSO}-d_6$) revealed the characteristic signals of flavan-3-ol: one set of an AMX aromatic system in ring B [δ_{H} 6.73 (d, $J = 1.2$ Hz, H-2'), 6.63 (d, $J = 8.1$ Hz, H-5')] and 6.56 (dd, $J = 8.1$ and 1.2 Hz, H-6'); two doublets of *meta*-coupled protons in ring A at δ_{H} 5.84 ($J = 1.8$, H-6) and 5.74 ($J = 1.8$ Hz, H-8), and one doublet signal of methine proton [δ_{H} 4.40 ($J = 7.7$ Hz, H-2)], one multiplet signal of methine proton at δ_{H} 4.60 (H-3), two doublet signals of methylene proton [δ_{H} 2.73 ($J = 16.1$ and 5.5 Hz, H-4_{eq}) and 2.35 ($J = 16.1$ and 8.1 Hz, H-4_{ax})]. The coupling constant of H-2 ($J = 7.6$ Hz) suggested that 2,3-*trans* configuration between H-2 and H-3 which is characteristic for catechin. This was further supported by ^{13}C NMR data (Figure 117), which displayed 15 carbons (one methylene, seven methines and seven quaternary carbons), suggesting that compound **H** was a flavan-3-ol skeleton. The downfield chemical shift of H-2 and H-3 suggested that these two protons were closed to oxygen-bearing carbons. The relative stereochemistry at C-2 and C-3, were observed in the NOESY spectrum which was in agreement with axial for H-2 and axial for H-3. In the aromatic ring A, the HMBC interactions from a hydroxyl proton at δ_{H} 8.38 (s, OH-5) to aromatic carbons C-4a (δ_{C} 99.1), C-6 (δ_{C} 95.4) and C-8a (δ_{C} 155.1) indicated that C-5 to be hydroxylated, and from a hydroxyl proton at δ_{H} 8.28 (s, OH-7) to aromatic carbon C-8 (δ_{C} 94.3) and C-8a suggested that C-7 to be hydroxylated. In addition, HMBC correlations from aromatic proton H-6 to C-4a, C-5 (δ_{C} 155.8), C-7 (δ_{C} 156.1) and C-8, as well as, from aromatic proton H-8 to C-4a, C-6 and C-8a were observed (Figure 93). This assignment was supported by cross-peak between OH-7/H-8 and OH-5/H-6 in NOESY spectrum. In the aromatic ring B, the HMBC correlations from a hydroxyl proton at δ_{H} 7.94 (s, OH-3') to aromatic carbons C-2' (δ_{C} 113.8) and C-3' (δ_{C} 144.3) indicated that C-3' to be hydroxylated, and from a hydroxyl proton at δ_{H} 7.62 (s, OH-4') to aromatic carbon C-4' (δ_{C} 144.3) and C-5' (δ_{C} 114.8) suggested that C-4' to be hydroxylated. This observation was in agreement with NOESY experiment where cross-peak -OH-3'/H-5' and -

OH-4'/H-6' were found. HMBC interactions from aromatic proton H-2' to C-2 (δ_C 81.0), C-3' and C-6' (δ_C 118.7), from aromatic proton H-5' to C-1' (δ_C 130.1) and C-4' and from aromatic proton H-6' to C-2' and C-4' were also observed. In addition, the HMBC spectrum revealed cross peaks of ring C between methine proton (H-2) with C-3, C-4 (δ_C 27.6), C-1', C-2' and C-6', as well as, cross peaks between methylene proton (H-4_{eq}) with C-2, C-3, C-4a, C-1'. Based on comparison of NMR data of compound **H** and reported (+)-catechin (**210**) or (2*R*,3*S*)-2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol (Shen; et al. 1993: 843-845) were similar. Moreover, compound **H** gave the levorotary optical rotation value of $[\alpha]_D^{28.8} +18.3^\circ$ ($c = 0.33$, acetone) which comparable to that of reported (+)-catechin (**3**) $[\alpha]_D^{25} +14^\circ$ ($c = 0.4$, acetone) (EL-Razek; et al. 2007: 4867-4872). So, the structure of compound **H** was determined to be (+)-catechin.

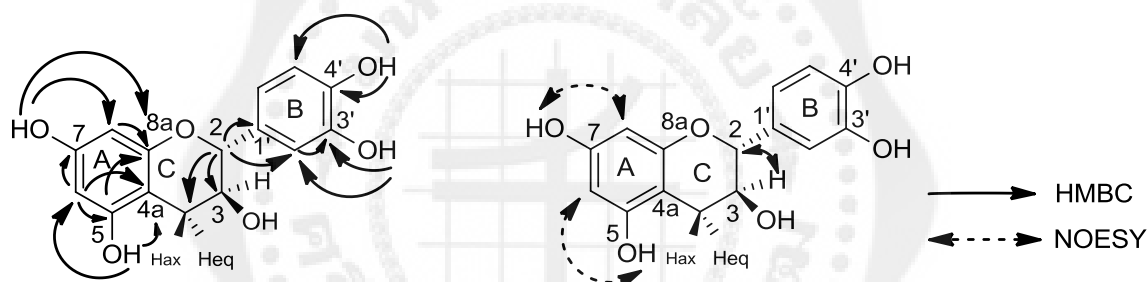


Figure 93 Selected HMBC and NOESY correlations of compound **H**

Table 20 Comparison of ^1H - and ^{13}C NMR data of compound **H** in CDCl_3 + 8 drops $\text{DMSO-}d_6$ with (+)-catechin (**210**) in $\text{DMSO-}d_6$

Position	δ_{H}		δ_{C}	
	Compound H	(+)-Catechin ^a	Compound H	(+)-Catechin ^a
2	4.40 (d, J=7.7)	4.51 (d, J=7.3)	81.0	81.0
3	3.80 (1H, m)	3.84 (1H, m)	67.2	66.4
4ax	2.35 (1H, dd, J=16.1, 8.1)	2.38 (1H, dd, J=16.0, 7.9)	27.1	27.7
4eq	2.73 (1H, dd, J=16.1, 5.5)	2.68 (1H, dd, J=16.0, 5.3)		27.7
4a	-	-	99.1	99.2
5	-	-	155.8	156.1
6	5.84 (1H, d, J=1.8)	5.90 (1H, d, J=2.2)	95.4	95.3
7		-	156.1	155.4
8	5.74 (1H, d, J=1.8)	5.72 (1H, d, J=2.2)	94.3	94.0
8a	-	-	155.1	155.3
1'	-	-	130.1	130.7
2'	6.73 (1H, d, J=1.2)	6.74 (1H, d, J=1.9)	113.8	114.5
3'	-	-	144.3	144.8
4'	-	-	144.4	144.8
5'	6.63 (1H, d, J=8.1)	6.70 (1H, d, J=8.0)	114.8	115.1
6'	6.56 (1H, dd, J=8.1, 1.2)	6.61 (1H, dd, J=8.0, 1.9)	118.7	118.4
-OH-3	-	4.75 (d, 4.7)	-	-
-OH-5	8.38 (1H, s)	-	-	-
-OH-7	8.28 (1H, s)	-	-	-
-OH-3'	7.94 (1H, s)	-	-	-
-OH-4'	7.62 (1H, s)	-	-	-

^aShen; et al. 1993: 843-845

4.3 Synthesis of some arylbenzofuran methyl ethers

4.3.1 Synthesis of artolakoochol analogs (E1-E3)

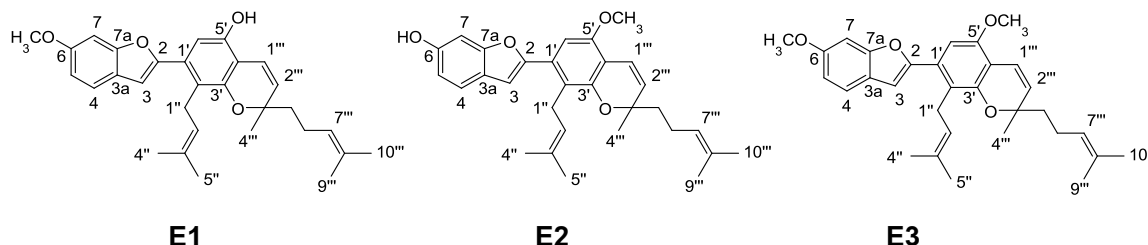


Figure 94 Structures of artolakoochol derivatives (**E1-E3**)

Methylation reaction of artolakoochol (compound **E**) was employed under the standard procedure using excess methyl iodide in anhydrous DMF (1.0 mL) in the presence of K_2CO_3 (33.5 mg, 0.24 mmol) afforded its derivative of **E**, 6-O-methyl (**E1**), 5'-O-methyl ethers (**E2**), and 6,5'-di-O-methyl ether (**E3**) in 5.5, 6.2 and 5.2% yield, respectively. The ^1H and ^{13}C -NMR spectra (Figure 118-121, Table 21) of compounds **E1** and **E2** were similar to that of parent compound **E** except for the presence of an extra methyl ether signal at δ_H 3.85, δ_C 55.7 for **E1** and δ_H 3.84, δ_C 55.6 for **E2**. Moreover, both compounds **E1** and **E2** had the same molecular formula, $C_{30}H_{34}O_4$, by HRESIMS, confirming that they were monomethyl ether derivatives. A clearly downfield shift in ring A at δ_C 158.0 of C-6 and an upfield shift at δ_C 95.8 of C-7 in **E1**, compared to that of the respective δ_C 153.3 and 98.3 in **E**, indicated for the methylation at C-6 was observed. A HMBC correlation between H-7 and C-6 further supported this fact. The HMBC correlations between aromatic protons (H-4 and H-7) and C-6 were also observed. In contrast, the ^1H -NMR data of compound **E2** had no change in the resonance in ring A. The ^{13}C -NMR spectrum obviously exhibited a downfield shift at δ_C 153.1 of C-5' and an upfield shift at δ_C 104.9 ppm of C-6', suggested that a methyl ether was at C-5' in ring B. HMBC cross-peaks of singlet aromatic proton H-6' to oxygenated quaternary (C-5') confirmed for the methylation at C-5'. These observation suggested that compounds **E1** and **E2** were artolakoochol-6-O-methyl ether and artolakoochol-5'-O-methyl ether, respectively.

Compound **E3** revealed two singlet methoxy signals at δ_H 3.85 (6-OCH₃ and 5'-OCH₃) and δ_C 55.7 and 55.6 in the NMR data. Analysis of HMBC data, the correlations of the OCH₃-6 to C-6 and OCH₃-5' to C-5' further confirmed the methylations taking place at C-6 and C-5', respectively. Its HRESIMS also showed molecular formula (C₃₁H₃₆O₄), indicating that both C-6 and C-5' were fully substituted with two methyl ethers. Thus, the structure of compound **E3** was proved to be artolakoochol-6,5'-di-O-methyl ether.

Table 21 Comparison of ¹H and ¹³C-NMR data of artolakoochol derivatives **E1-E3** in CDCl₃

Position	δ_H			δ_C		
	Compd E1	Compd E2	Compd E3	Compd E1	Compd E2	Compd E3
2	-	-	-	154.7	154.1	154.8
3	6.74 (1H, s)	6.74 (1H, s)	6.75 (1H, s)	105.0	104.9	104.9
3a	-	-	-	122.6	122.8	122.6
4	7.41 (1H, d, J=8.5)	7.38 (1H, d, J=8.4)	7.42 (1H, d, J=8.5)	120.9	121.0	120.9
5	6.85 (1H, dd, J=8.5, 1.9)	6.76 (1H, d, J=8.4, 2.0)	6.85 (1H, dd, J=8.5, 2.2)	111.7	111.8	111.7
6	-	-	-	158.0	153.6	157.9
7	7.01 (1H, d, J=1.9)	6.99 (1H, d, J=2.0)	7.04 (1H, d, J=2.2)	95.8	98.1	95.8
7a	-	-	-	155.4	154.9	155.4
1'	-	-	-	130.4	130.6	130.3
2'	-	-	-	120.1	120.4	120.4
3'	-	-	-	152.3	152.0	153.1
4'	-	-	-	109.5	110.6	110.5
5'	-	-	-	149.2	153.1	153.1
6'	6.70 (1H, s)	6.75 (1H, s)	6.76 (1H, s)	106.9	102.8	102.7
1''	3.45 (2H, d, J=6.3)	3.45 (2H, d, J=6.3)	3.46 (2H, d, J=6.3)	25.7	25.7	25.7
2''	5.17 (1H, br t, J=6.3)	5.17 (1H, br t, J=6.3)	5.17 (1H, br t, J=6.3)	123.6	123.7	123.7
3''	-	-	-	131.0	130.9	131.0
4''	1.72 (3H, s)	1.70 (3H, s)	1.71 (3H, s)	18.1	18.1	18.1
5''	1.67 (3H, s)	1.67 (3H, s)	1.67 (3H, s)	25.7	25.7	25.7
1'''	6.66 (1H, d, J=10.0)	6.70 (1H, d, J=10.0)	6.70 (1H, d, J=10.0)	117.0	117.3	117.3
2'''	5.57 (1H, d, J=10.0)	5.54 (1H, d, J=10.0)	5.54 (1H, d, J=10.0)	128.5	128.2	128.2
3'''	-	-	-	78.4	78.3	78.3
4'''	1.36 (3H, s)	1.34 (3H, s)	1.35 (3H, s)	26.2	26.2	26.2

Table 21 (continued)

Position	δ_{H}			δ_{C}		
	Compd E1	Compd E2	Compd E3	Compd E1	Compd E2	Compd E3
5'''	1.75 (2H, m)	1.75 (2H, m)	1.75 (2H, m)	41.3	41.3	41.3
6'''	2.10 (2H, m)	2.09 (2H, m)	2.10 (2H, m)	22.9	22.9	22.9
7'''	5.09 (1H, br t, J=7.1)	5.08 (1H, br t, J=6.8)	5.08 (1H, br t, J=7.0)	124.2	124.2	124.2
8'''	-	-	-	131.6	131.6	131.6
9'''	1.57 (3H, s)	1.57 (3H, s)	1.56 (3H, s)	17.6	17.5	17.5
10'''	1.64 (3H, s)	1.64 (3H, s)	1.64 (3H, s)	25.6	25.6	25.6
-OCH ₃ -6	3.85 (3H, s)	-	3.85 (3H, s)	55.7	-	55.7
-OCH ₃ -5'	-	3.84 (3H, s)	3.85 (3H, s)	-	55.6	55.6

4.3.2 Synthesis of lakoochin B analogs (J1-J3)

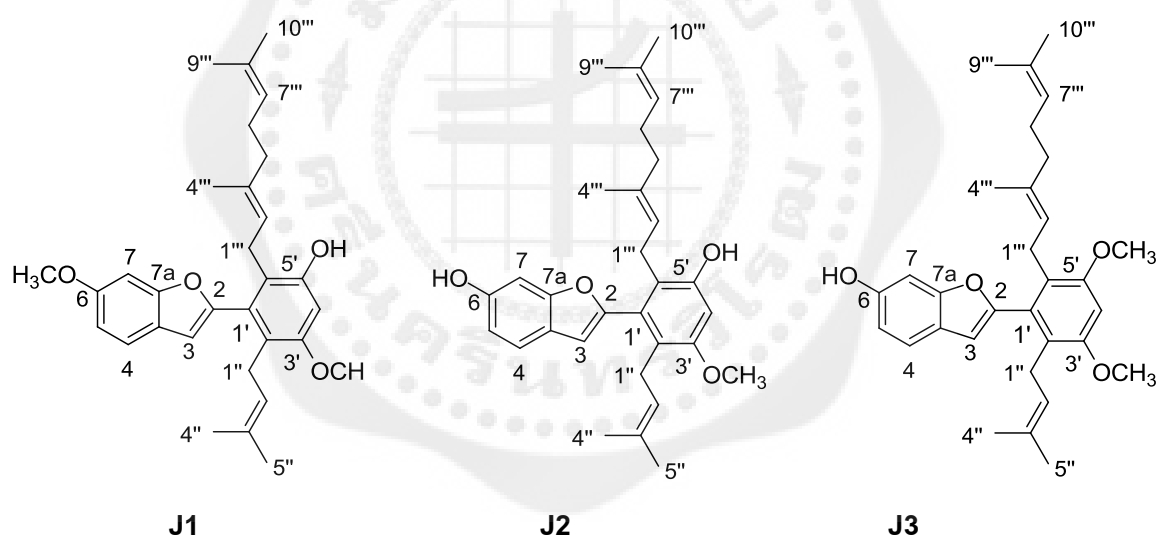


Figure 95 Structures of lakoochin B derivatives (J1-J3)

Compounds **J1**, **J2** and **J3** were obtained from the treatment of lakoochin B (compound **J**) with methyl iodide in the presence of K₂CO₃ (34.0 mg, 0.24 mmol) in anhydrous DMF (4 mL) at room temperature in 4.1%, 2.0% and 1.2% yield, respectively. The ¹H and ¹³C-NMR spectra (Figure 122-125, Table 22) of compounds **J1**, **J2** and **J3** were similar to that of parent **J** except for the presence of an extra methyl ether signal, δ_{H} 3.84,

δ_{C} 55.7 for **J1** and δ_{H} 3.79, δ_{C} 55.7 for **J2**. Furthermore, both compounds **J1** and **J2** had the same molecular formula, $\text{C}_{30}\text{H}_{36}\text{O}_4$, by HRESIMS, confirming that they were monomethyl ether derivatives. An obvious downfield shift in ring A at δ_{C} 157.8 of C-6 and an upfield shift at δ_{C} 96.0 of C-7 in **J1**, compared to that of the respective δ_{C} 153.3 and 98.3 in **J**, indicated for the methylation at C-6 was observed. This was consistent with the presence of HMBC cross peak between C-6 and H-4, H-7 and $-\text{OCH}_3$ -6. For the ^1H spectrum of compound **J2**, it has no change in the resonance in ring A. While the ^{13}C -NMR chemical shift of compound **J2** in ring B at δ_{C} 156.8 (C-3'), 123.6 (C-2'') and 132.0 (C-3'') differ from the parent compound **J** with the respective δ_{C} 154.2, 123.4 and 134.3. Furthermore, a downfield shift at δ_{C} 123.7 was assigned to C-7''' in compound **J2**, comparing to the same chemical shift of C-7''' in compound **J**. This implied that C-3' was methylated and C-5' was hydroxylated in compound **J2**. These observations suggested that compounds **J1** and **J2** were lakoochin B-6-O-methyl ether and lakoochin B-3'-O-methyl ether.

Compound **J3** revealed two singlet signals of two methoxy groups at δ_{H} 3.84 (6 and 5'- OCH_3) and δ_{C} 55.7 and 55.6 (6 and 5'- OCH_3) in the ^1H and ^{13}C -NMR spectral data. HMBC correlations between methoxy group at C-6 and H-7, and methoxy group at C-5' and H-6' were observed. Its HRESIMS also showed molecular formula ($\text{C}_{31}\text{H}_{36}\text{O}_4$), indicating that both C-6 and C-5' were substituted by two methyl ethers. Thus, the structure of compound **J3** was proved to be lakoochin B-3',5'-di-O-methyl ether.

Table 22 Comparison of ^1H - and ^{13}C NMR data of lakoochin B derivatives **J1-J3** in CDCl_3

Position	δ_{H}			δ_{C}		
	Compound J1	Compound J2	Compound J3	Compd J1	Compd J2	Compd J3
2	-	-	-	153.1	153.2	-
3	6.54 (1H, s)	6.51 (1H, s)	6.50 (1H, s)	106.4	106.3	-
3a	-	-	-	121.9	122.2	-
4	7.43 (1H, d, J=8.5)	7.38 (1H, d, J=8.3)	7.36 (1H, d, J=8.3)	120.8	120.9	-
5	6.87 (1H, dd, J=8.5, 2.0)	6.76 (1H, dd, J=8.3, 1.8)	6.75 (1H, d, J=8.3, 2.0)	111.8	111.7	-
6	-	-	-	157.8	153.4	-
7	7.01 (1H, br d, J=2.0)	6.94 (1H, br d, J=1.8)	6.93 (1H, d, J=2.0)	96.0	98.3	-
7a	-	-	-	155.8	155.6	-
1'	-	-	-	131.5	131.5	-
2'	-	-	-	120.1	118.9	-
3'	-	-	-	154.0	156.8	-
4'	6.50 (1H, s)	6.53 (1H, s)	6.57 (1H, s)	105.6	101.2	-
5'	-	-	-	154.2	153.9	-
6'	-	-	-	120.0	118.9	-
1''	3.14 (2H, d, J=6.5)	3.11 (2H, d, J=6.5)	3.12 (2H, d, J=6.5)	27.4	26.7	-
2''	5.17 (1H, br t, J=6.5)	5.04 (1H, br t, J=6.5)	5.06 (1H, br t, J=6.5)	122.5	123.6	-
3''	-	-	-	134.2	132.0	-
4''	1.61 (3H, s)	1.36 (3H, s)	1.36 (3H, s)	17.7	17.6	-
5''	1.67 (3H, s)	1.56 (3H, s)	1.55 (3H, s)	25.7	25.7	-
1'''	3.14 (2H, d, J=6.5)	3.11 (2H, d, J=6.5)	3.12 (2H, d, J=6.5)	27.4	27.3	-
2'''	5.17 (1H, br t, J=6.5)	5.18 (1H, br t, J=6.5)	5.06 (1H, br t, J=6.5)	122.4	122.4	-
3'''	-	-	-	138.1	138.3	-
4'''	1.61 (3H, s)	1.62 (3H, s)	1.35 (3H, s)	16.0	16.0	-
5'''	2.00 (2H, m)	2.00 (2H, m)	1.87 (2H, m)	39.6	39.6	-
6'''	2.03 (2H, m)	2.03 (2H, m)	1.97 (2H, m)	26.3	26.3	-
7'''	5.01 (1H, br t, J=6.4)	5.00 (1H, br t, J=6.8)	5.04 (1H, br t, J=6.3)	123.8	123.7	-
8'''	-	-	-	132.0	130.4	-
9'''	1.56 (3H, s)	1.56 (3H, s)	1.55 (3H, s)	17.6	17.6	-
10'''	1.65 (3H, s)	1.65 (3H, s)	1.62 (3H, s)	25.6	25.6	-

Table 22 (continued)

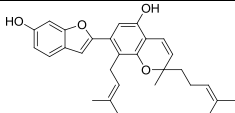
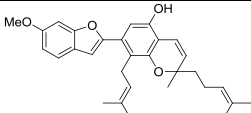
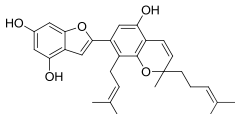
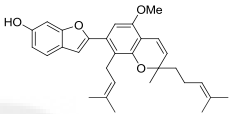
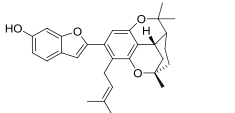
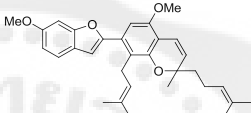
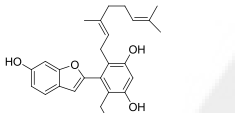
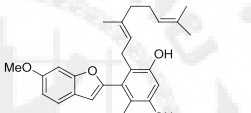
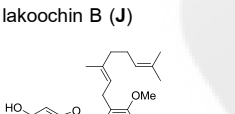
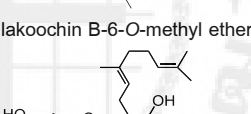
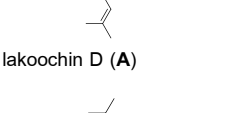
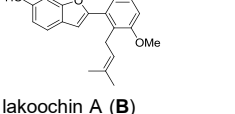
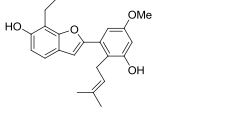
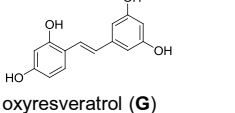
Position	δ_{H}			δ_{C}		
	Compound J1	Compound J2	Compound J3	Compd J1	Compd J2	Compd J3
-OCH ₃ -6	3.84 (3H, s)	-	-	55.7	-	-
-OCH ₃ -3'	-	3.79 (3H, s)	3.84 (3H, s)	-	55.7	-
-OCH ₃ -5'	-	-	3.84 (3H, s)	-	-	-
-OH-6	-	-	-	-	-	-
-OH-3'	5.30 (1H, br s)	-	-	-	-	-
-OH-5'	5.30 (1H, br s)	5.41 (1H, br s)	-	-	-	-

4.4 Anti-AChE and BuChE activities of the isolated compounds and the methylated derivatives

From bioassay-guided screening, the EtOAc extract I had greater inhibitions of AChE and BuChE (IC_{50} 5.3 and 0.83 $\mu\text{g/mL}$, respectively) than the MeOH extract I (IC_{50} 21.8 and 18.5 $\mu\text{g/mL}$, respectively), comparing to galantamine (IC_{50} 0.41 and 1.06 $\mu\text{g/mL}$, respectively). These results led to further isolation using chromatographic separation of the EtOAc I, EtOAc II and the MeOH I extracts afforded arylbenzofuran type compounds (**A-J**), two of which are new compounds (**A** and **D**) and other phenolic compounds a stilbene (**G**) and a flavonoid (**H**) (Table 9).

The inhibitory potency of all isolated compounds (**A-J**), except for albufuran **B** (**I**), and the methylated derivatives (**E1-E3**, and **J1-J2**), comparing to the standard drug galantamine, are summarized in Table 23.

Table 23 Anti-AChE and BuChE activities of the isolated compounds and the methylated derivatives

Natural Compounds	AChE; IC ₅₀ (μ M)	BuChE; IC ₅₀ (μ M)	Derivatives	AChE; IC ₅₀ (μ M)	BuChE; IC ₅₀ (μ M)
 artolakochole (E)	0.87	14.93	 artolakochole-6-O-methyl ether (E1)	inactive	1.11
 4-hydroxyartolakochole (F)	1.10	8.86	 artolakochole-5'-O-methyl ether (E2)	inactive	0.30
 cycloartolakochole (C)	inactive	1.56	 artolakochole-6,5'-di-O-methylether (E3)	inactive	inactive
 lakoochin B (J)	1.08	0.10	 lakoochin B -6-O-methyl ether (J1)	inactive	0.99
 lakoochin D (A)	inactive	inactive	 lakoochin B -3'-O-methyl ether (J2)	inactive	0.50
 lakoochin A (B)	27.42	13.88			
 lakoochin C (D)	61.86	47.21	Galantamine	1.45 μ M 0.41 μ g/mL	3.72 μ M 1.06 μ g/mL
 oxyresveratrol (G)	inactive	2.95			
 (+)-catechin (H)	inactive	inactive			

The AChE and BuChE inhibitions of all isolated compounds **A-J**, except for compound **I** (albafuran **B**), had IC_{50} values in the range of 0.87-61.86 μ M and 0.10-47.21 μ M, respectively. The results indicated that artolakoochol (**E**) possessed the most potent inhibition of AChE (IC_{50} 0.87 μ M), followed by lakoochin B (**J**) (IC_{50} 1.08 μ M) and 4-hydroxyartolakoochol (**F**) (IC_{50} 1.10 μ M), which were also greater than standard drug galantamine (IC_{50} 1.45 μ M). While lakoochins A (**B**) and C (**D**) showed moderate and weak AChE inhibitory, respectively. The remaining cycloartolakoochol (**C**), lakoochin D (**A**), oxyresveratrol (**G**) and (+)-catechin (**H**) were inactive. In case of BuChE inhibition, lakoochin B exhibited the most potent inhibitor (IC_{50} 0.10 μ M), followed by cycloartolakoochol (IC_{50} 1.56 μ M) and oxyresveratrol (IC_{50} 2.95 μ M) which were also greater than galatamine (IC_{50} 3.72 μ M). While artolakoochol, 4-hydroxyartolakoochol, lakoochins A and C showed moderate to weak inhibitory activity, along with the remaining lakoochin D and (+)-catechin were inactive.

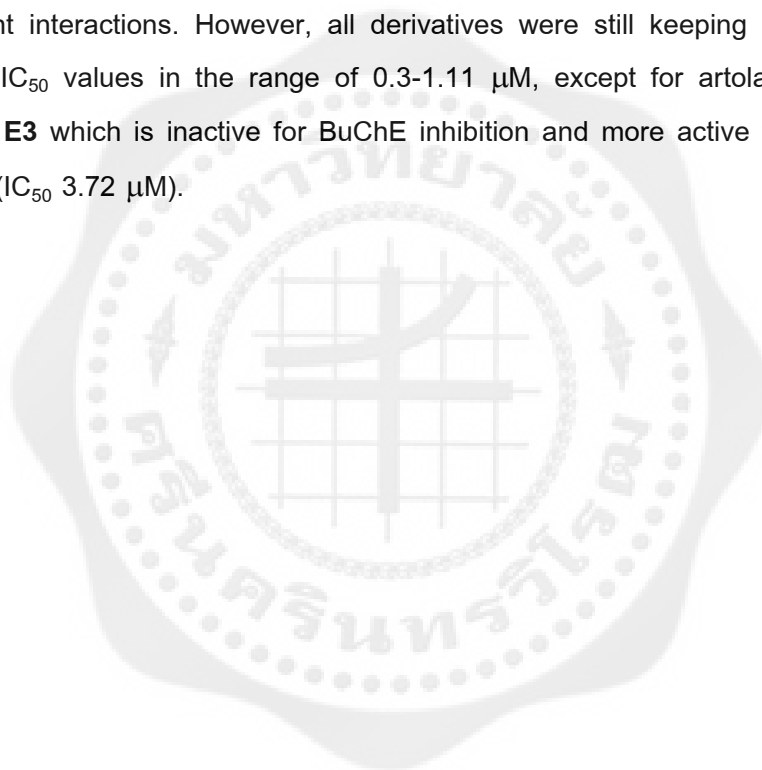
As shown in Table 23, the core structures of isolated 2-arylbenzofurans (**A-F** and **J**) are 3',5',6-trixyoxxygenated 2-arylbenzofurans with prenyl at C-2'. Their structures have been categorized into two types, 2-arylbenzofurans with pyrano system (**C**, **E** and **F**) and 2-arylbenzofurans comprising geranyl group (**A**, **B**, **D** and **J**).

The results revealed that the degree of AChE inhibition is related to pyrano system and the free hydroxyl groups. It was found that artolakoochol (**E**) with pyrano ring exhibited the greatest inhibitory effect against AChE and also showed a better effect than lakoochin B (**J**). Additionally, the presence of an additional hydroxyl group on meta-substituent on ring A in 4-hydroxyartolakoochol (**F**) displayed a little lower inhibitory effect against AChE than artolakoochol (**E**). This may be due to the steric hindrance of meta-substituent (Razavi et al., 2013). Among compounds **A**, **B**, **D** and **J**, lakoochin B (**J**) was the highest potent inhibition than the rest. Interestingly, the replacement of one and two hydroxyl groups by methyl ether (s) observed in lakoochins D (**A**), A (**B**) and C (**D**) led to reduce and loss the inhibitory property. Besides, there is a complexity in structure of cycloartolakoochol (**C**) which causes to be completely inactive, as well as, oxyresveratrol (**G**) and (+)-catechin (**H**) were inactive.

As for BuChE inhibition, lakoochin B (**J**) with geranyl group showed clearly greater effect than artolakoochol (**E**) and 4-hydroxyartolakoochol (**F**). In addition, the additional hydroxyl group on meta-substituent in 4-hydroxyartolakoochol displayed better

inhibitory activity than artolakoochol. Interestingly, the presence of one or two methyl ether (s) in lakoochins A, C and D exhibited moderate to loss anti-BuChE property.

In order to figure out SAR data in a new series of 2-arylbenzofuran, all methylated derivatives (**E1-E3** and **J1-J2**) were evaluated for anti-AChE and BChE effects. Interestingly, all artolakoochol and lakoochin B derivatives were inactive for anti-AChE activity. In contrast, artolakoochol derivatives (**E1-E3**) enhanced the BuChE inhibition compared to their parent artolakoochol (**E**). Unlike lakoochin B derivatives (**J1-J2**), all of them had a little lower BuChE inhibitory effect, comparing to their parent lakoochin B (**J**). It seemed that the substitution of hydroxyl group by methyl ether at C-6 or C-3' established less important interactions. However, all derivatives were still keeping good inhibition of BuChE with IC_{50} values in the range of 0.3-1.11 μ M, except for artolakoochol-6,5'-di-O-methyl ether **E3** which is inactive for BuChE inhibition and more active than the standard galantamine (IC_{50} 3.72 μ M).



CHAPTER 5

CONCLUSION

From phytochemical contents screening using TLC technique, the root bark extracts of *A. lakoocha* provided some major spots of 2-arylbenzofuran and other phenolic constituents. In this investigation, *A. lakoocha* root barks from different areas, Patthalung and Surin provinces were collected in order to search for various chemical constituents. In addition, a preliminary screening for AChE and BuChE inhibitory activities using Ellman's assay, the EtOAc and MeOH extracts were found to be active extracts for AChE and BuChE inhibitions, comparing to standard drug, galantamine. These findings resulted in the purification and structural elucidation of two new 2-arylbenzofurans, lakoochins D (**A**) and C (**D**), along with six known 2-arylbenzofurans, artolakoochol (**E**), 4-hydroxyartolakoochol (**F**), cycloartolakoochol (**C**), lakoochins A (**B**) and B (**J**), albafrican B (**I**), and phenolic compounds a stilbene, oxyresveratrol (**G**) and a flavonoid, (+)-catechin (**H**) from the EtOAc and MeOH extracts. The structures of these constituents were established by spectroscopic techniques (NMR, MS, UV and IR) and comparison of their spectral data with the previously reported data.

AChE and BuChE inhibitory effects of the isolated (**A-J**), except for compound **I**, revealed that artolakoochol (**E**) displayed the strongest AChE inhibitory effect, followed by lakoochin B (**J**) and 4-hydroxyartolakoochol (**F**), comparing to galatamine. It was found that the replacement of hydroxyl groups by methyl ether (s) led to obviously reduce or loss the activity. While lakoochin B (**J**) was the most potent BuChE inhibitor, followed by cycloartolakoochol (**C**) and oxyresveratrol (**G**), comparing to galatamine. Therefore, two highly active arylbenzofurans, artolakoochol (**E**) and lakoochin B (**J**) were selected for further methylation in order to figure out SAR. All methylated derivatives (**E1-E3** and **J1-J2**) were evaluated for their AChE and BuChE inhibitions. All of them were inactive for AChE inhibition due to the replacement of hydroxyl groups by methyl ether (s) led to loss the activity. For BuChE inhibition, artolakoochol methylated (**E1** and **E2**), except for fully methylated (**E3**), showed greater inhibitions than their parent **E** and galantamine due to the replacement of hydroxyl groups by methyl ether (s) led to enhance the activity. However, this was inconsistent with lakoochin B derivatives (**J1** and **J2**) which exhibited lower activity than their parent **J** when hydroxyl groups were substituted by methyl ether (s).

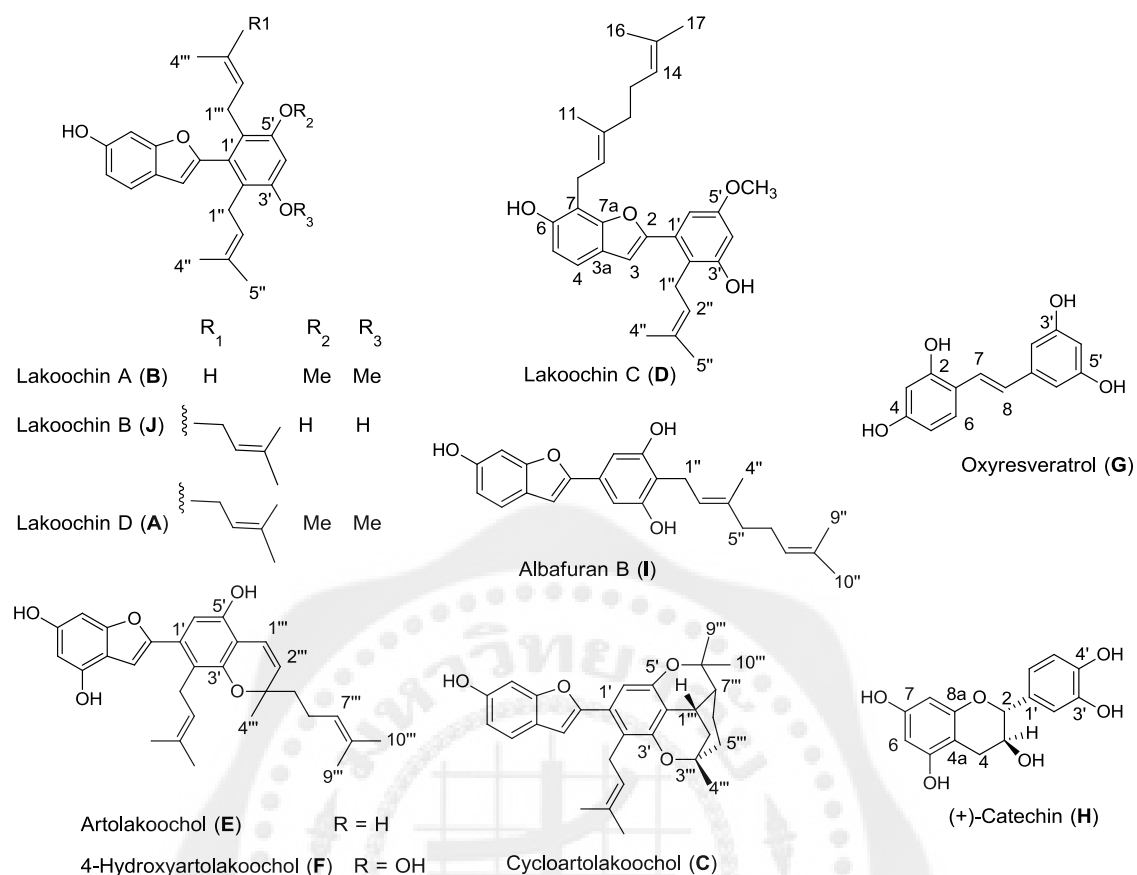
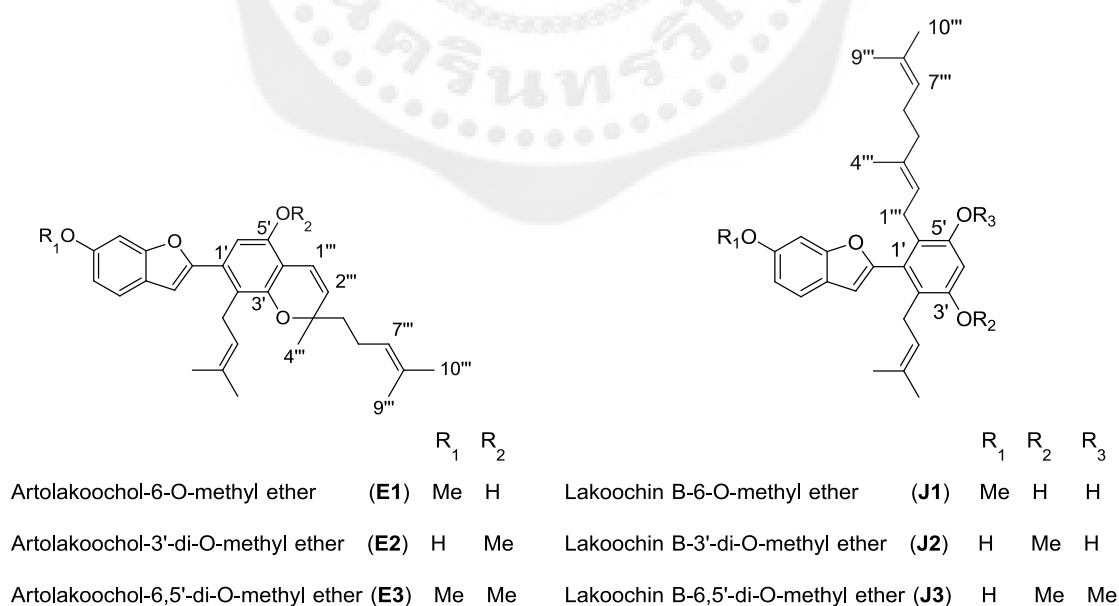
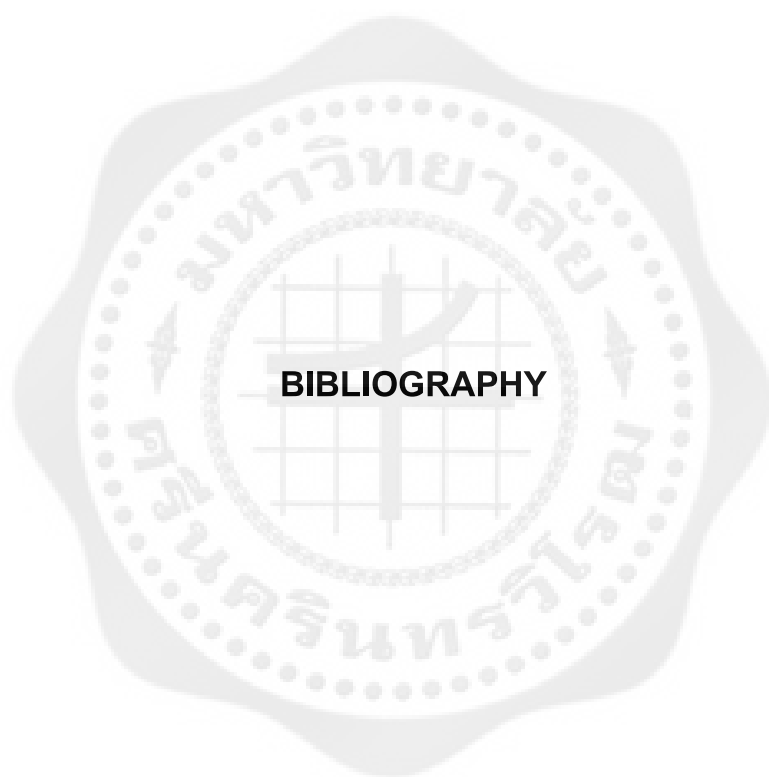
Figure 96 Structures of the isolated compounds from *A. lakoocha* root barks

Figure 97 Structures of artolakochole and lakoochin B derivatives



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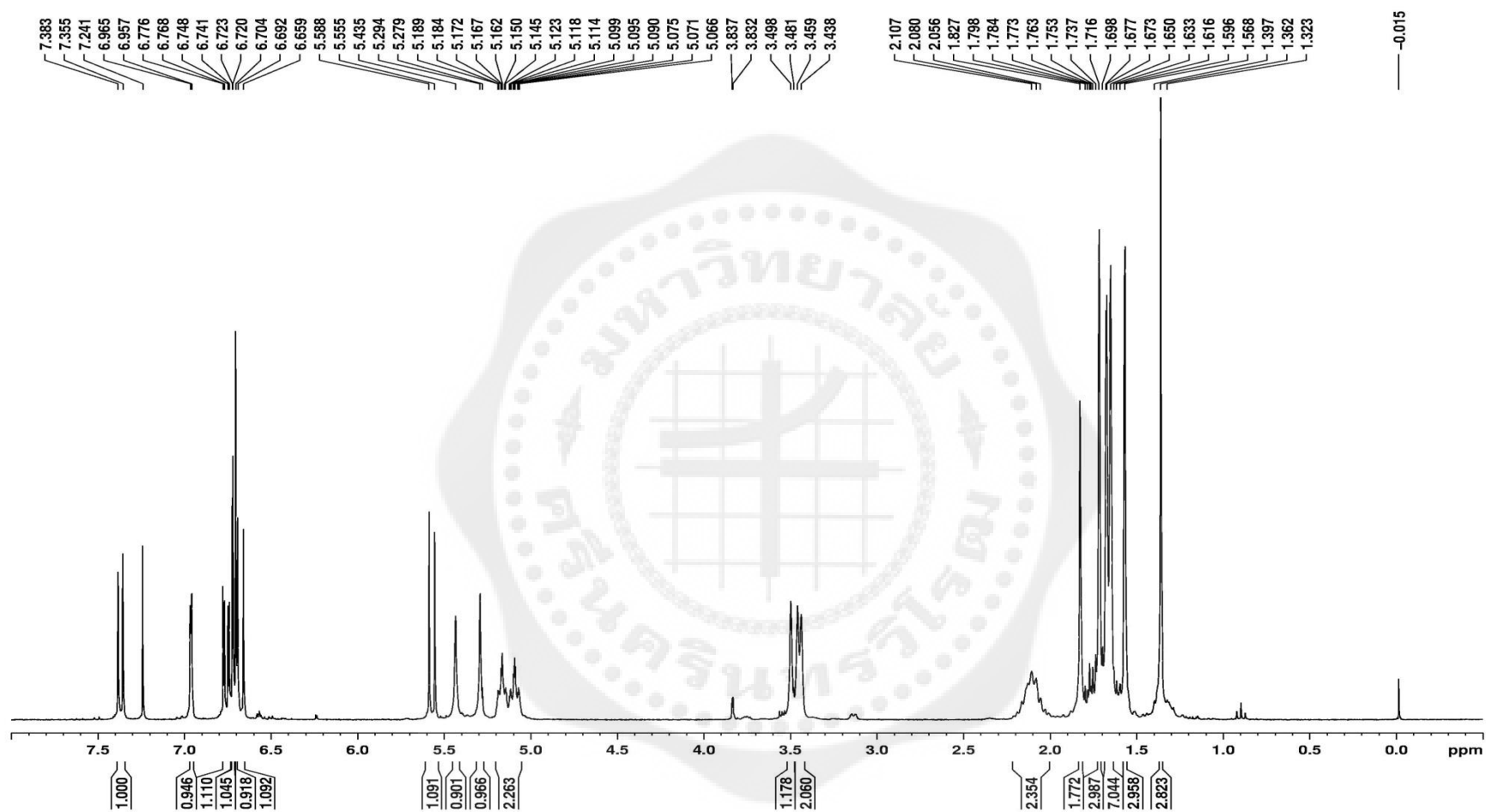


Figure 98 ^1H NMR spectrum of compound **E** (Artolakoochol, sss5975) in CDCl_3

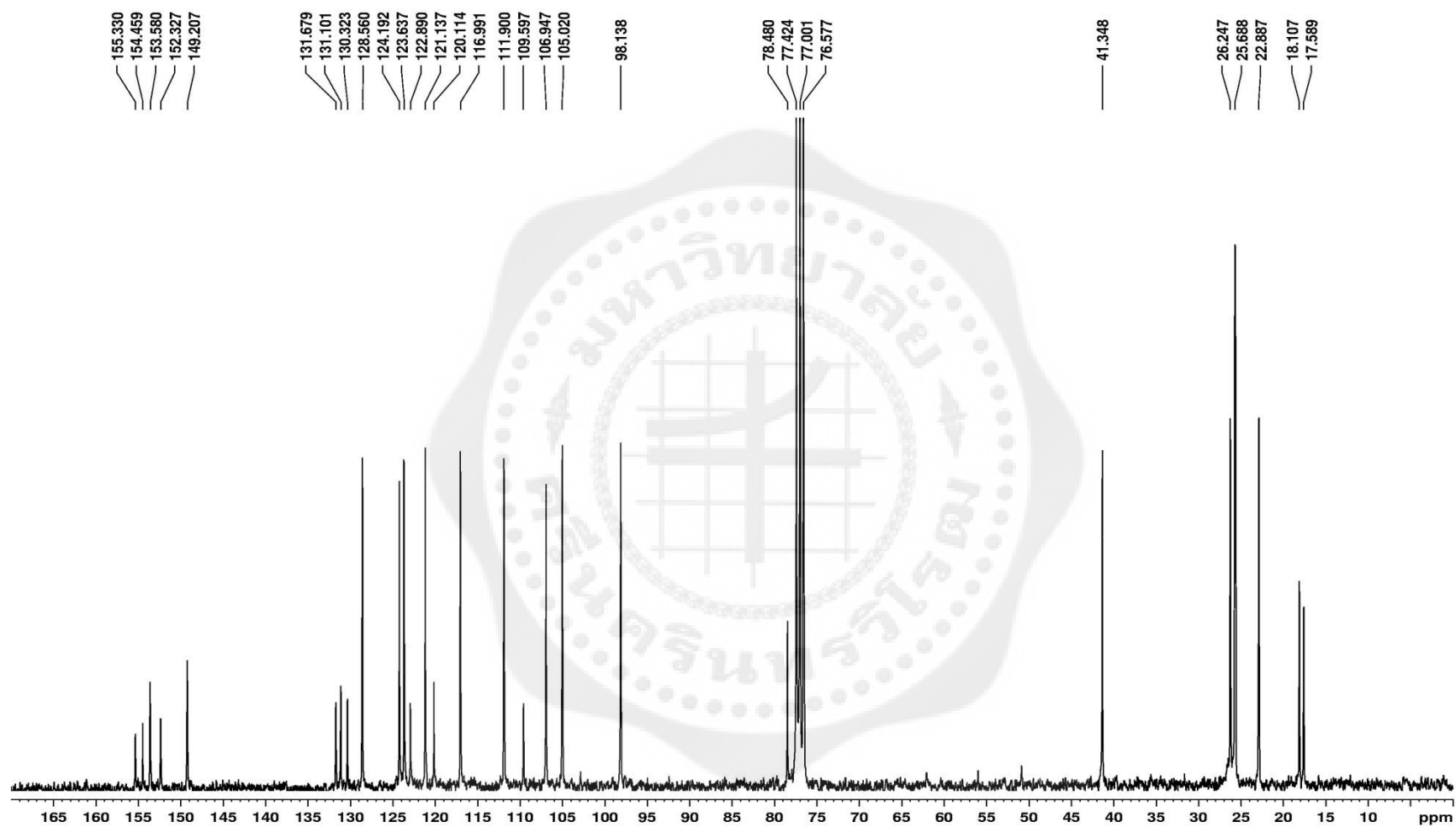


Figure 99 ^{13}C NMR spectrum of compound **E** (Artolakochole, sss5975) in CDCl_3

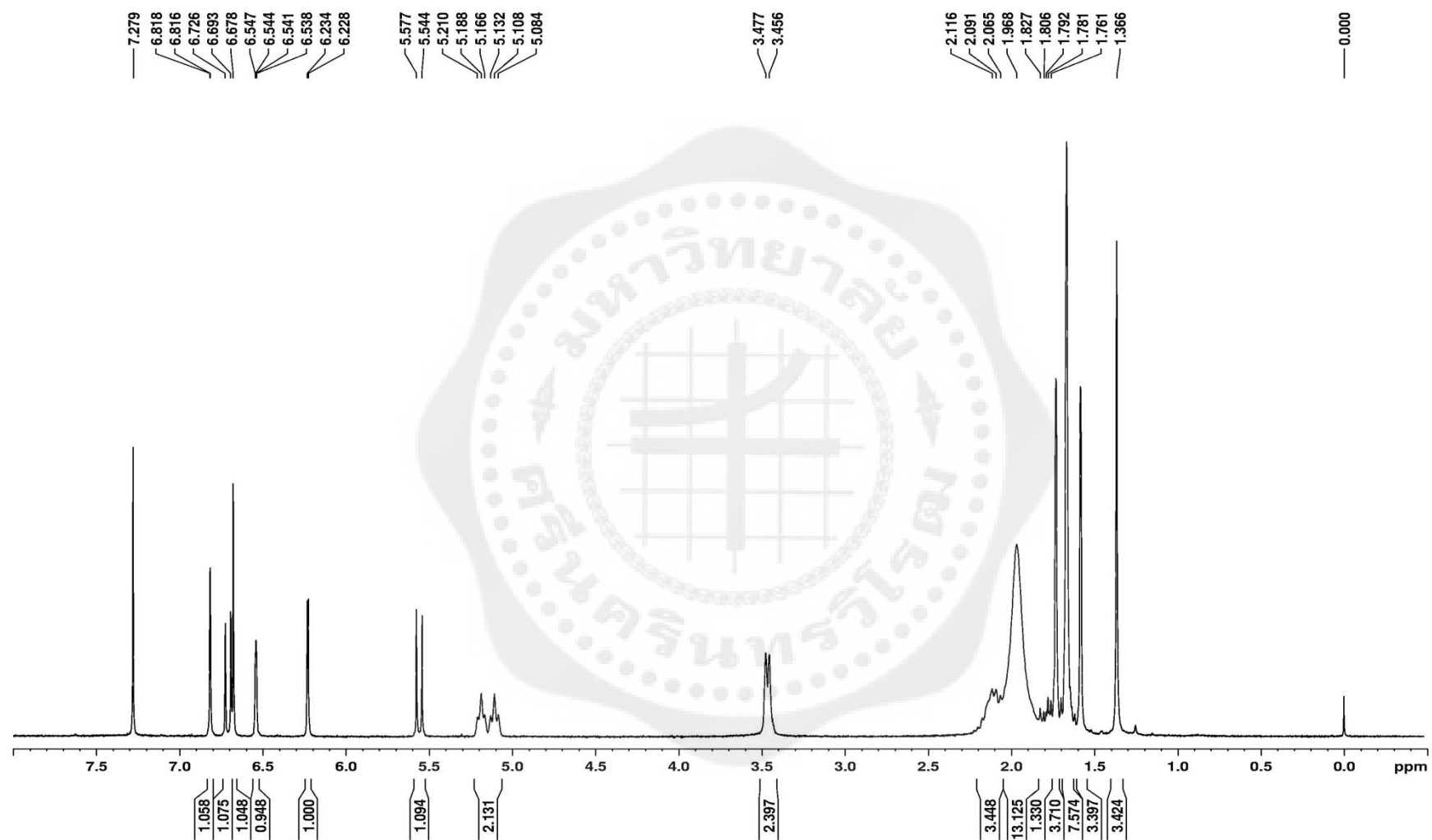


Figure 100 ^1H NMR spectrum of compound **F** (4-hydroxyartolakochole, sss5303) in $\text{CDCl}_3 + 2$ drops CD_3OD

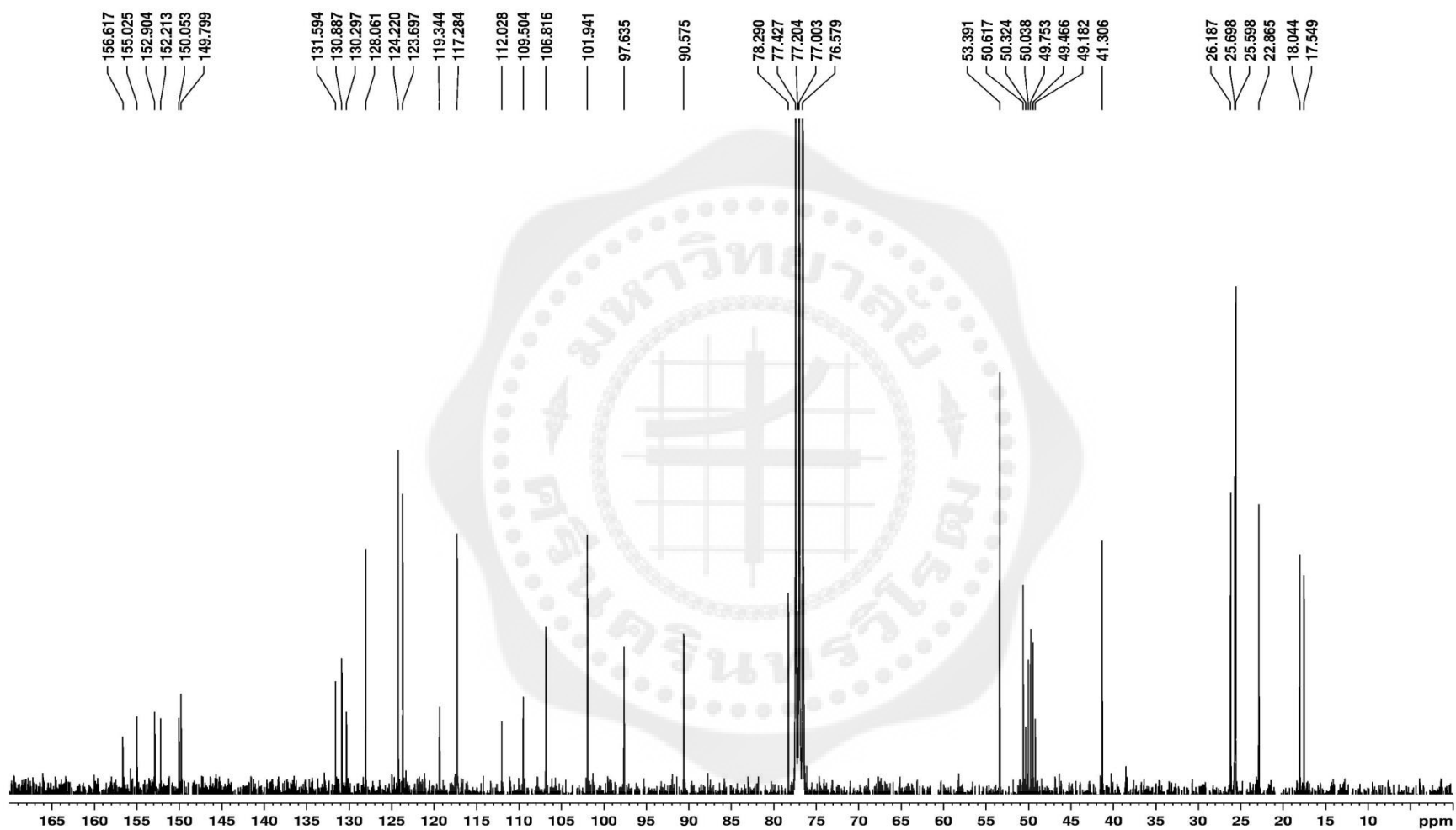


Figure 101 ^{13}C NMR spectrum of compound **F** (4-hydroxyartolakochole, sss5303) in CDCl_3 + 2 drops CD_3OD

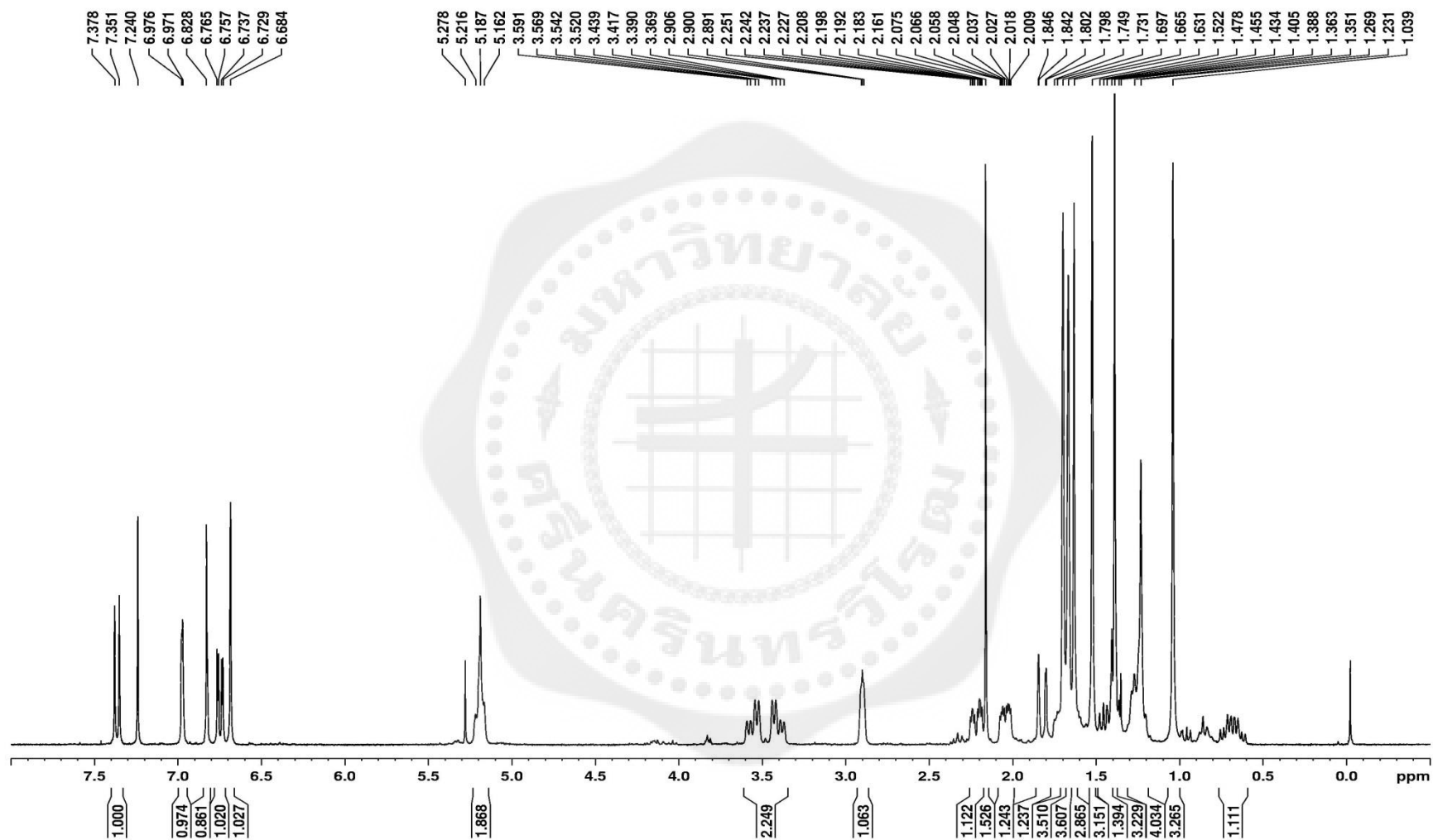


Figure 102 ^1H NMR spectrum of compound **C** (Cycloartolakoochol, sss5420) in CDCl_3

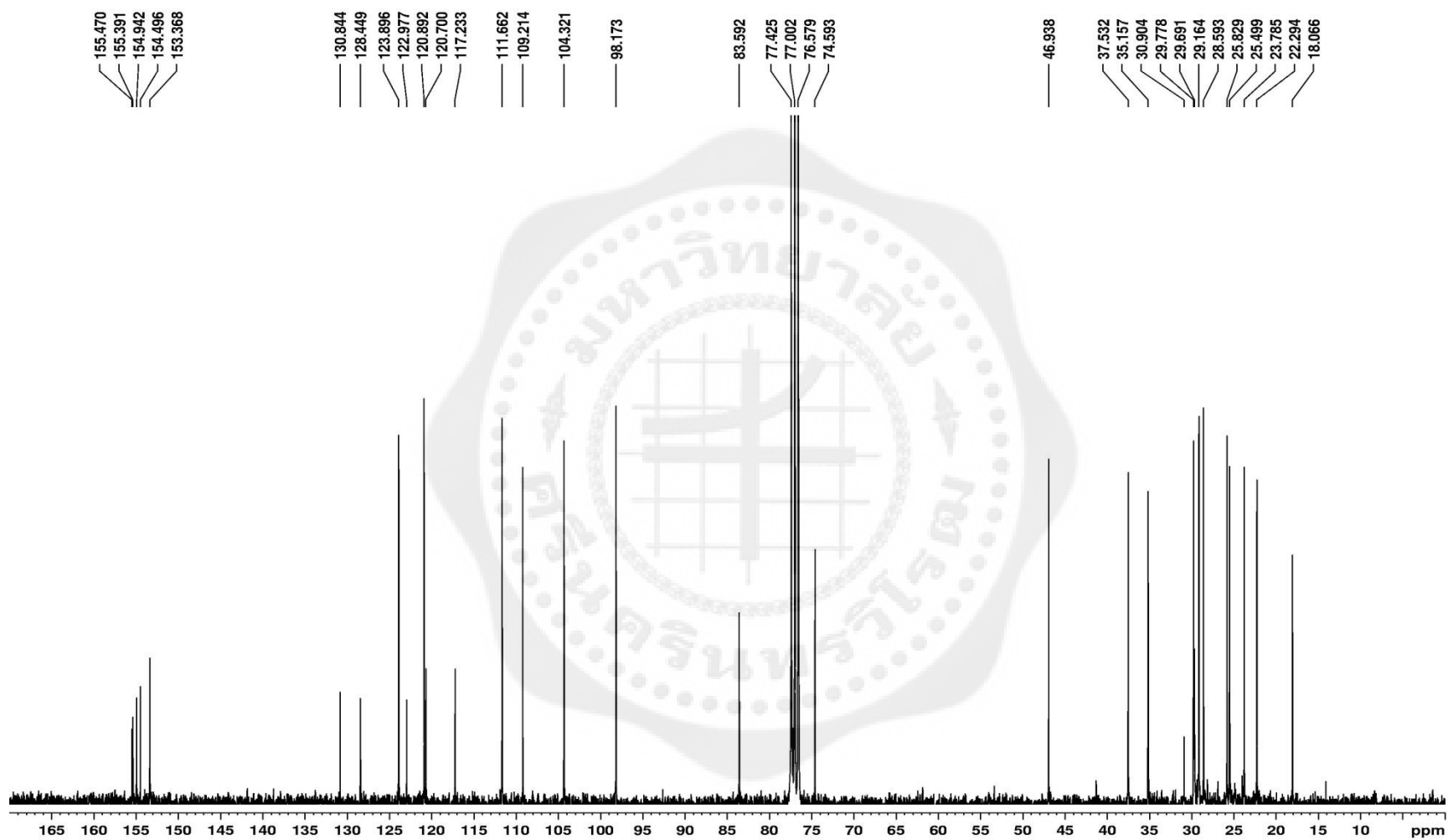


Figure 103 ¹³C NMR spectrum of compound **C** (Cycloartolakoochol, sss5420) in CDCl₃

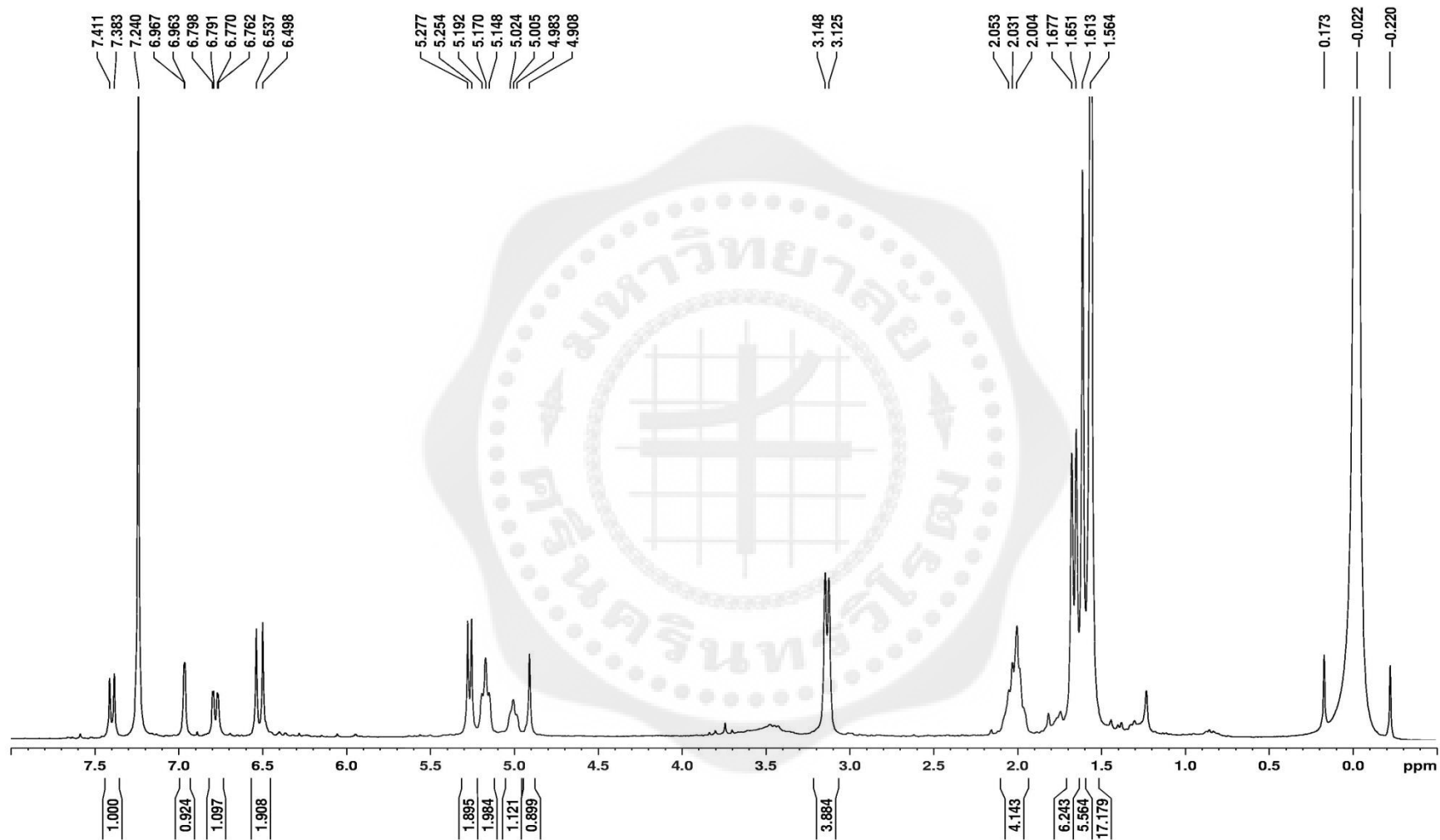


Figure 104 ¹H NMR of spectrum compound J (Lakoochin B, sss6049) in CDCl₃

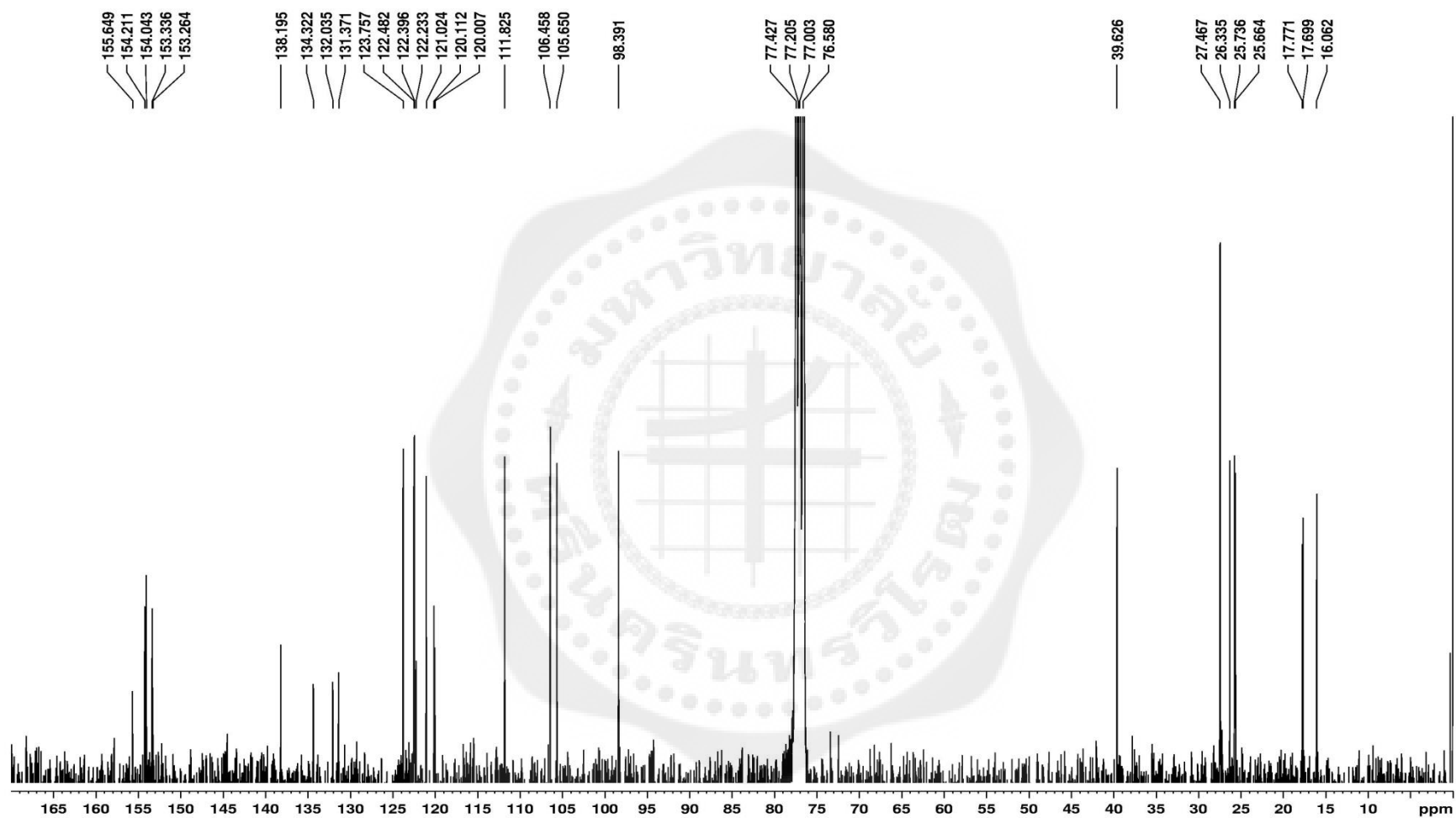


Figure 105 ^{13}C NMR spectrum compound **J** (Lakoochin B, sss6049) in CDCl_3

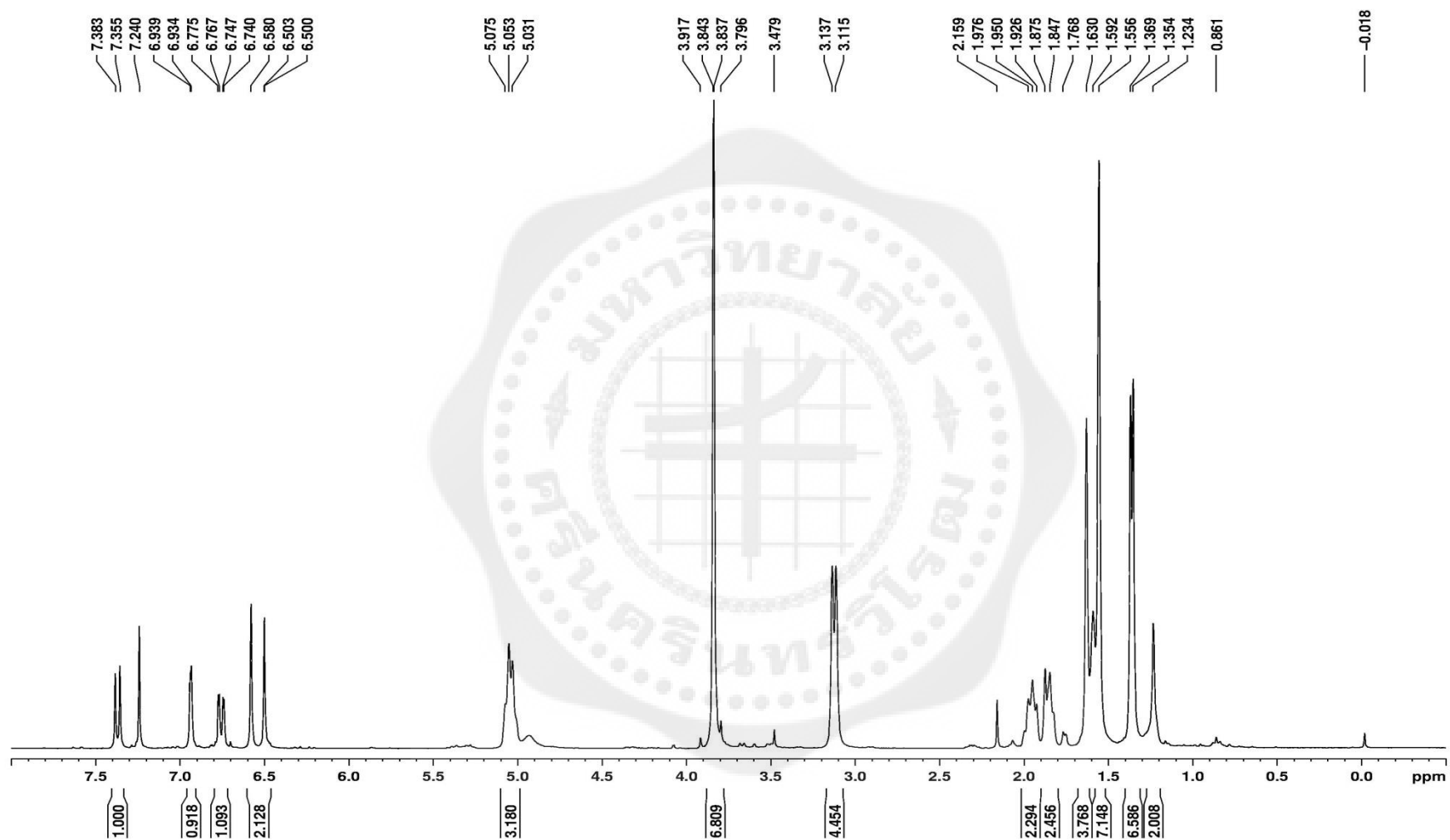


Figure 106 ¹H NMR spectrum of compound A (Lakoochin D, sss5311) in CDCl₃

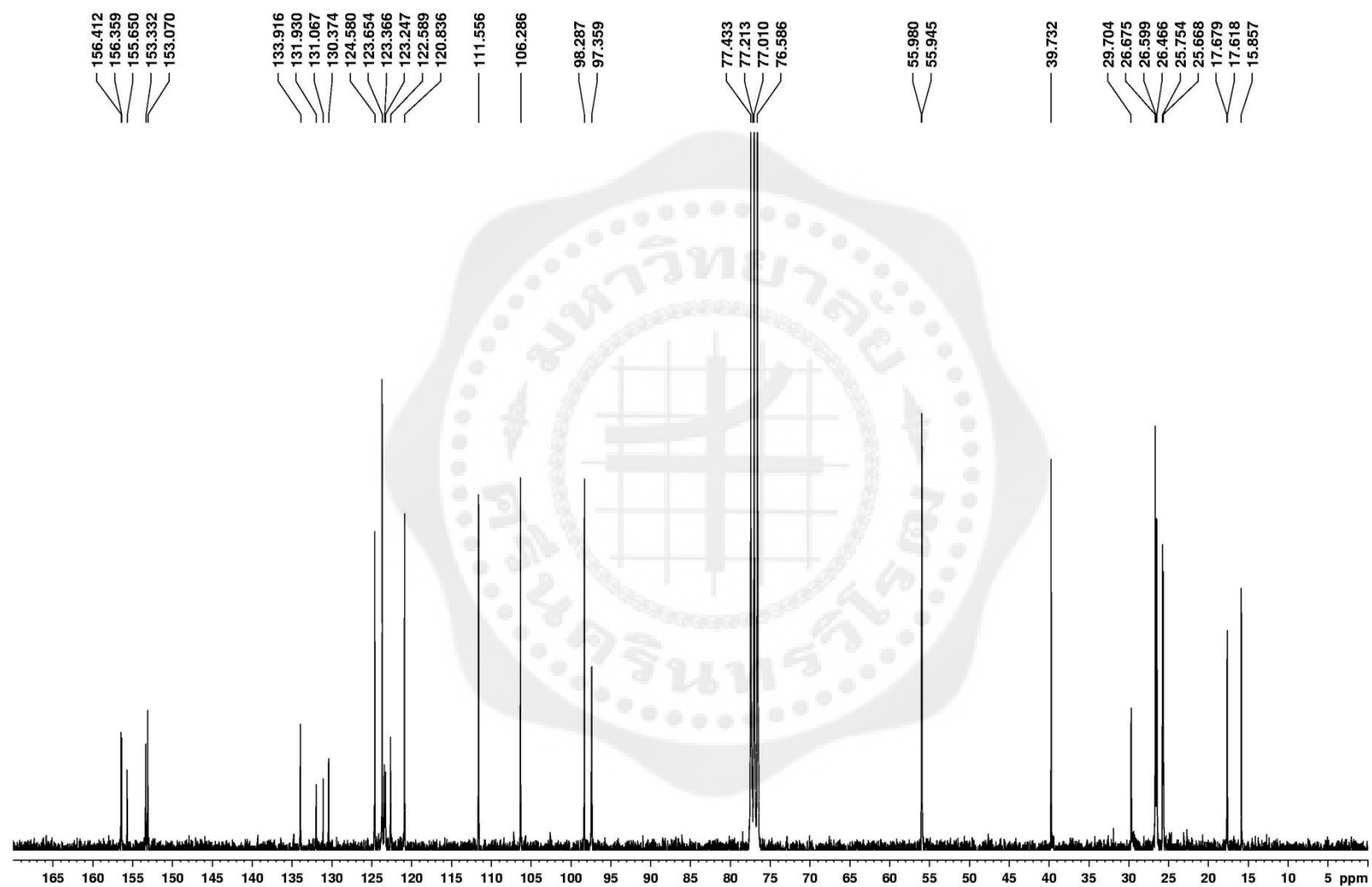


Figure 107 ¹³C NMR spectrum of compound **A** (Lakoochin D, sss5311) in CDCl₃

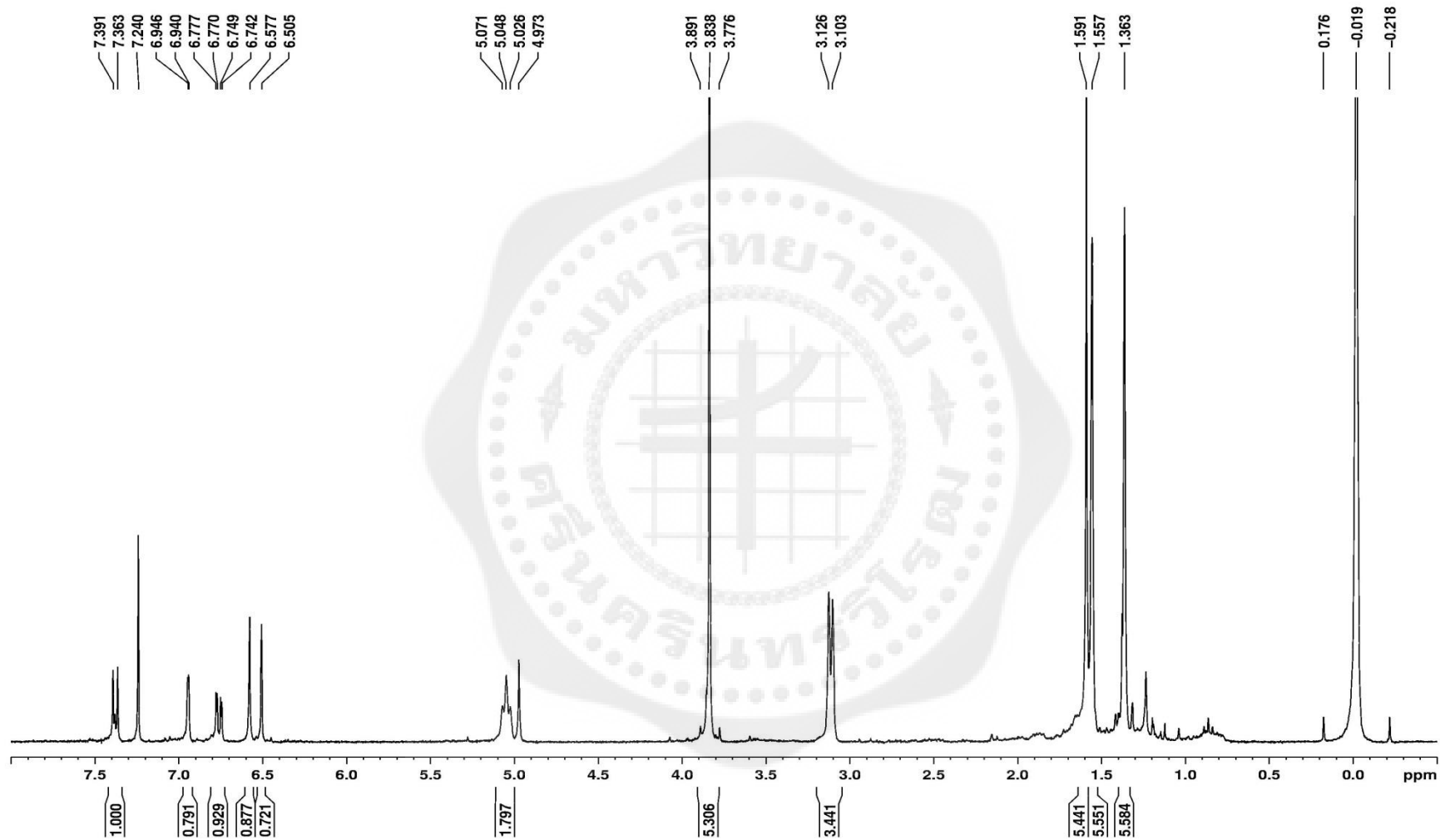


Figure 108 ¹H NMR spectrum of compound **B** (Lakoochin A, sss6096) in CDCl₃

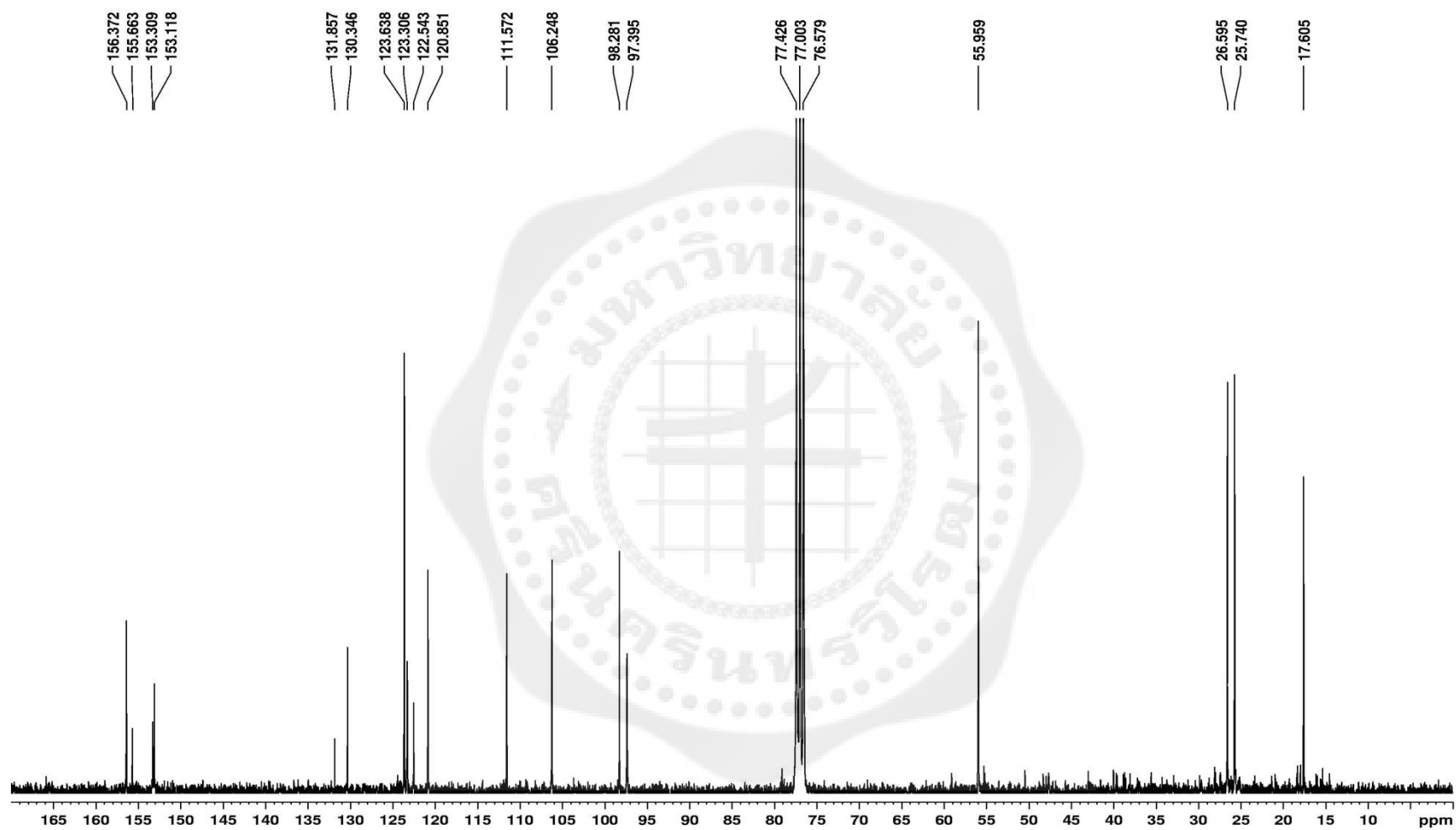


Figure 109 ^{13}C NMR spectrum of compound **B** (Lakoochin A, sss6096) in CDCl_3

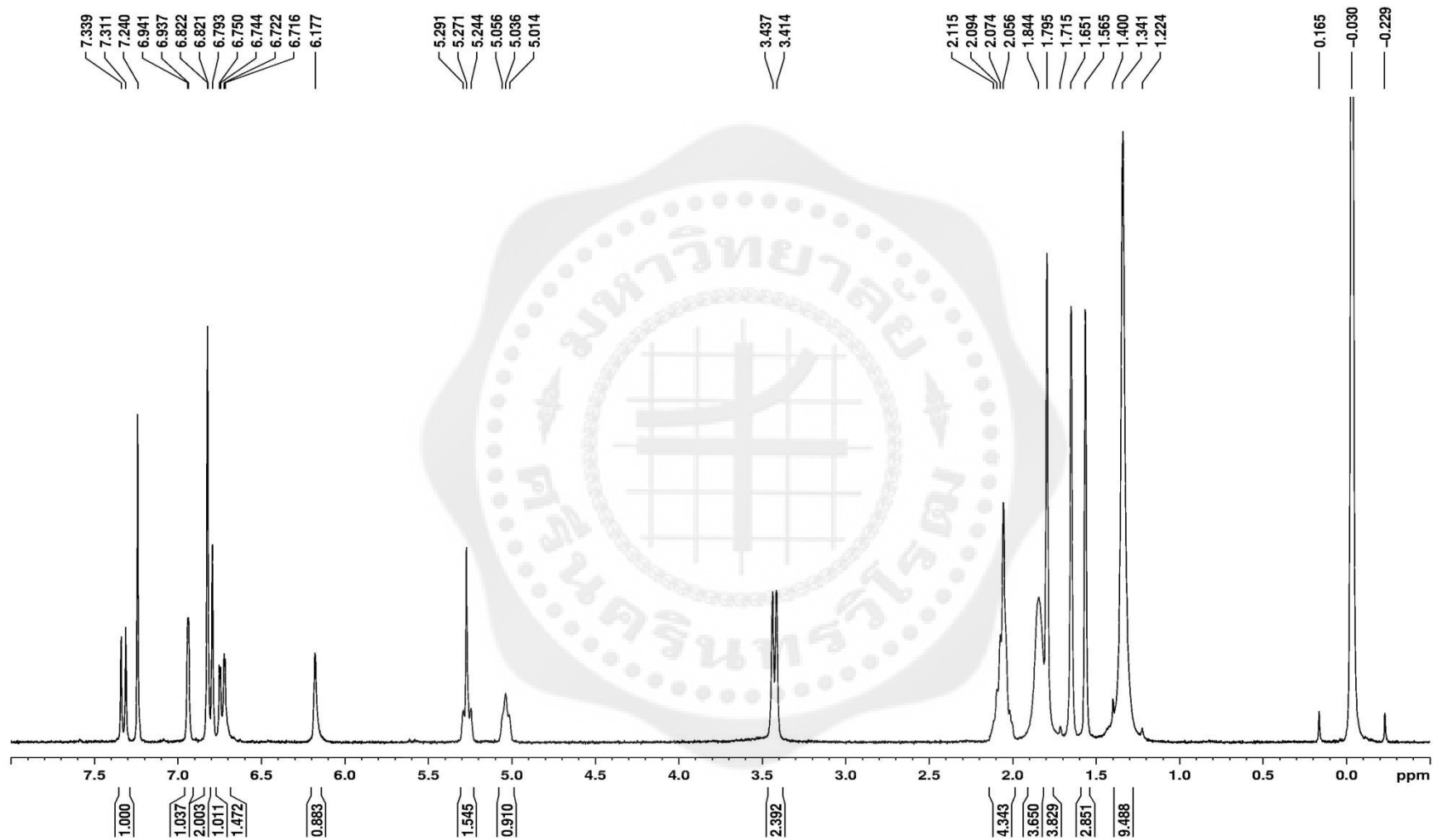


Figure 110 ¹H NMR spectrum of compound I (Albafuran B, sss5827) in CDCl₃ + 1 drop CD₃OD

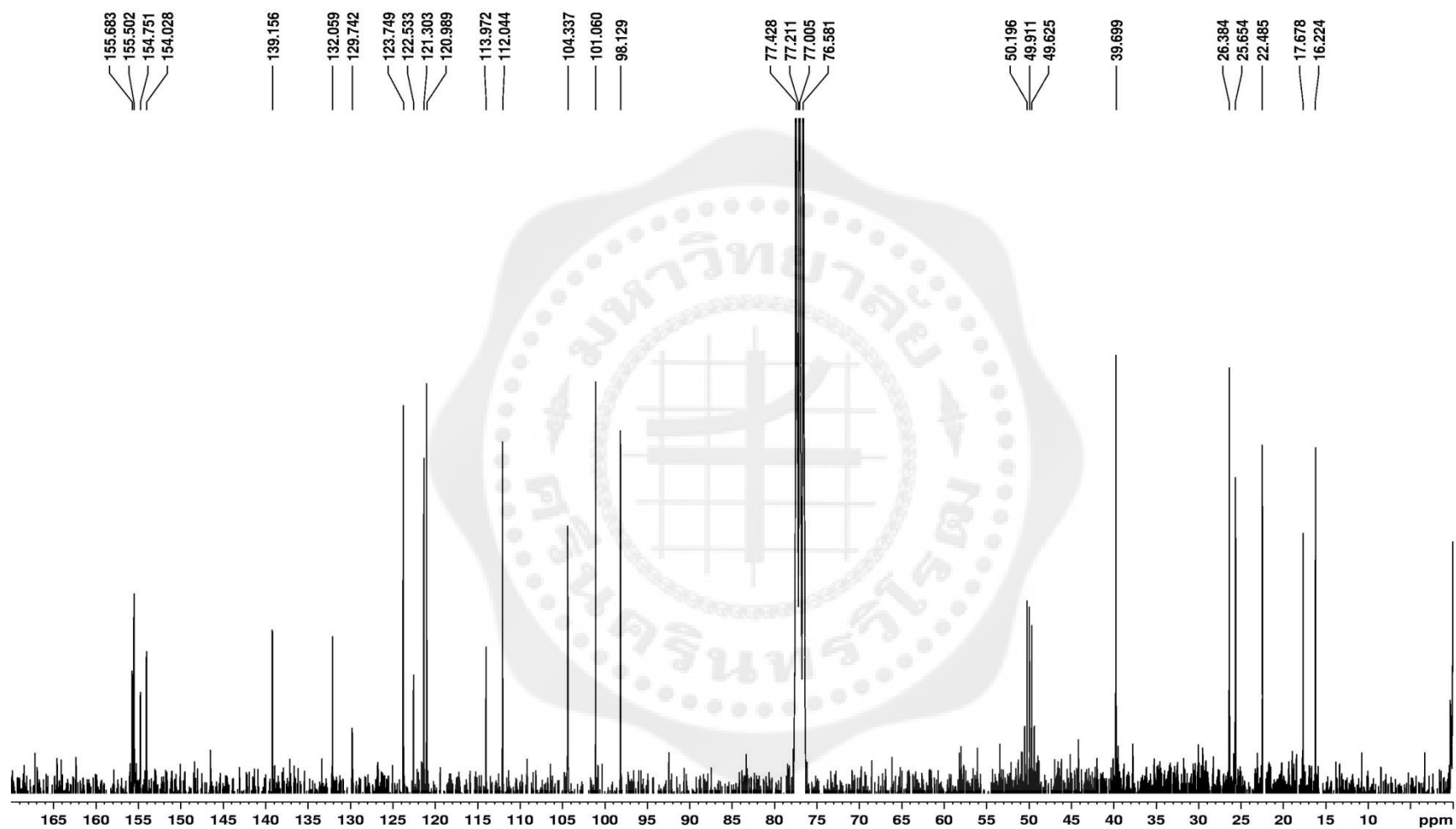


Figure 111 ¹³C NMR spectrum of compound I (Albafuran B, sss5827) in CDCl₃ + 1 drop CD₃OD

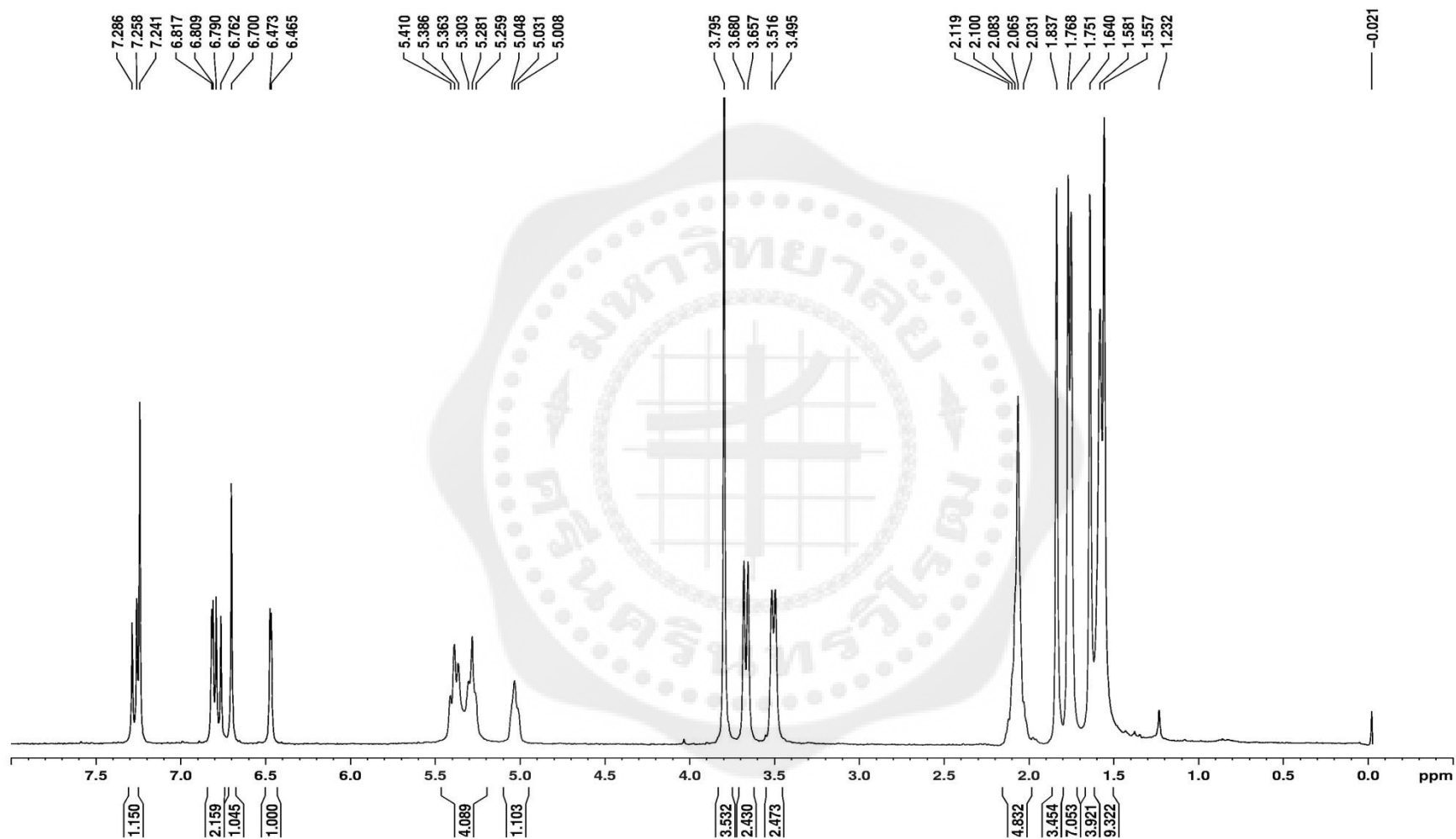


Figure 112 ¹H NMR spectrum of compound **D** (Lakoochin C, sss5286) in CDCl₃

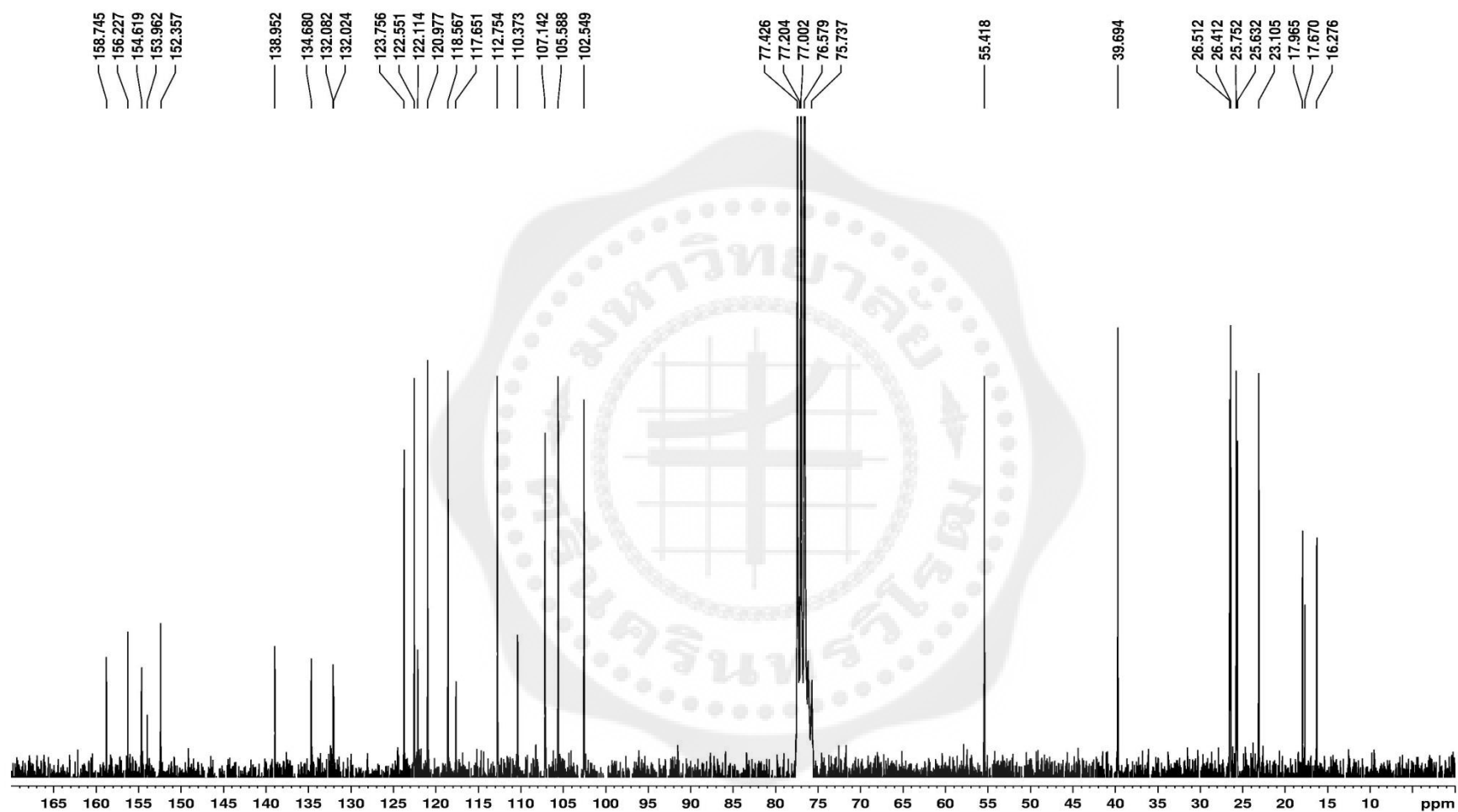


Figure 113 ¹³C NMR spectrum of compound **D** (Lakoochin C, sss5286) in CDCl₃

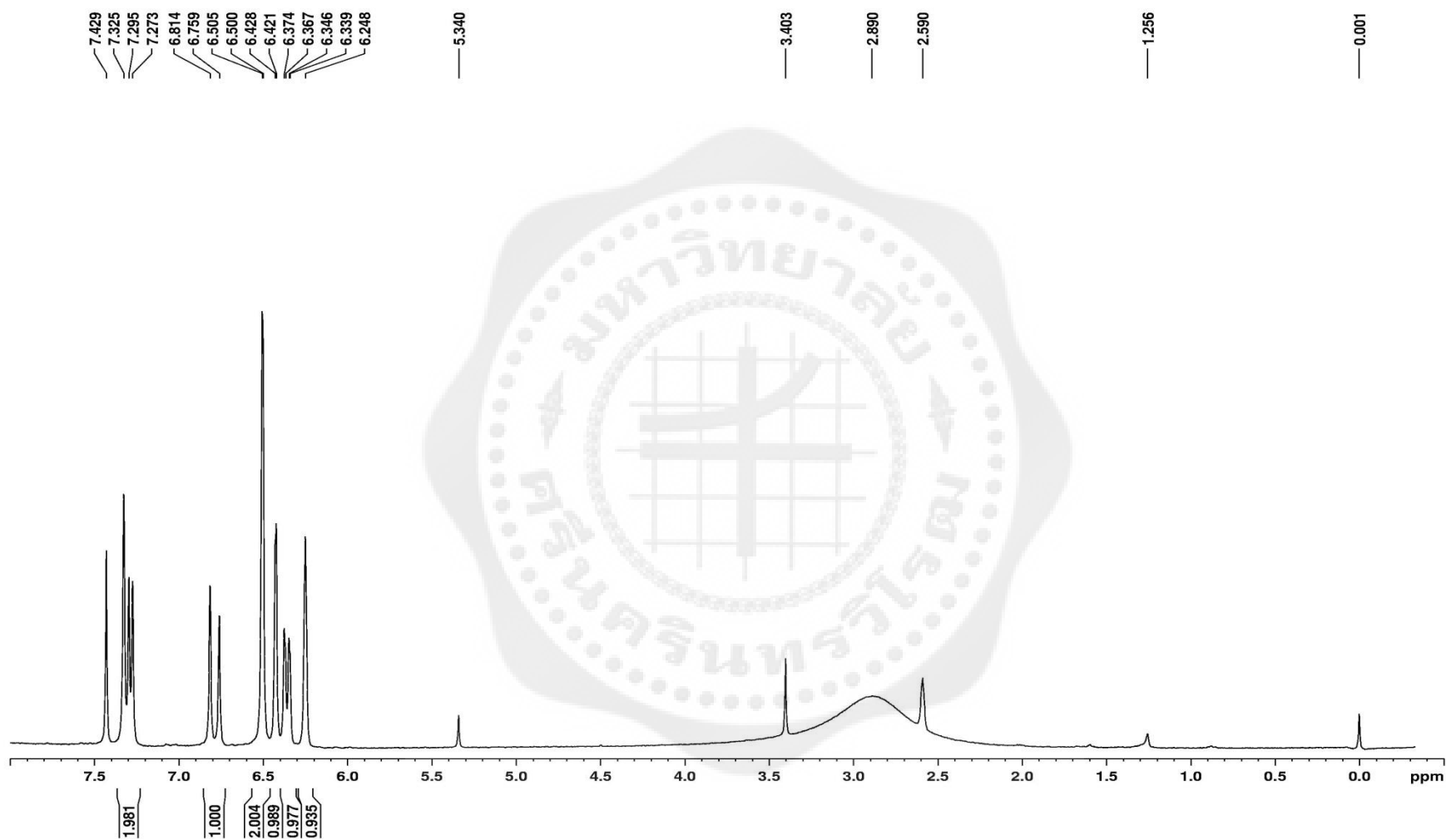


Figure 114 ^1H NMR of spectrum compound **G** (Oxyresveratrol, sss5745) in CDCl_3 + 7 drops $\text{DMSO}-d_6$

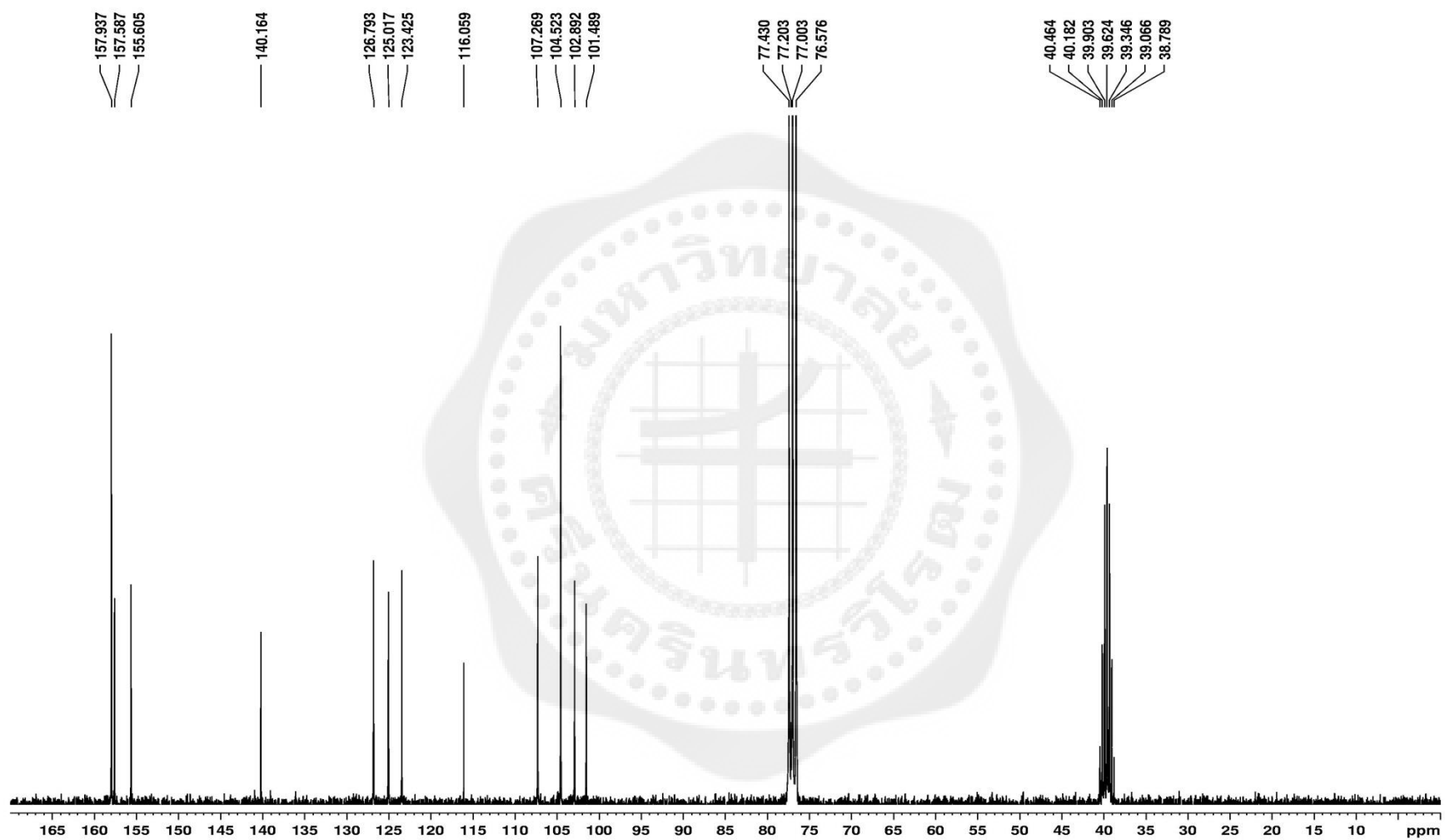


Figure 115 ¹³C NMR spectrum compound **G** (Oxyresveratrol, sss5745) in CDCl₃ + 7 drops DMSO-*d*₆

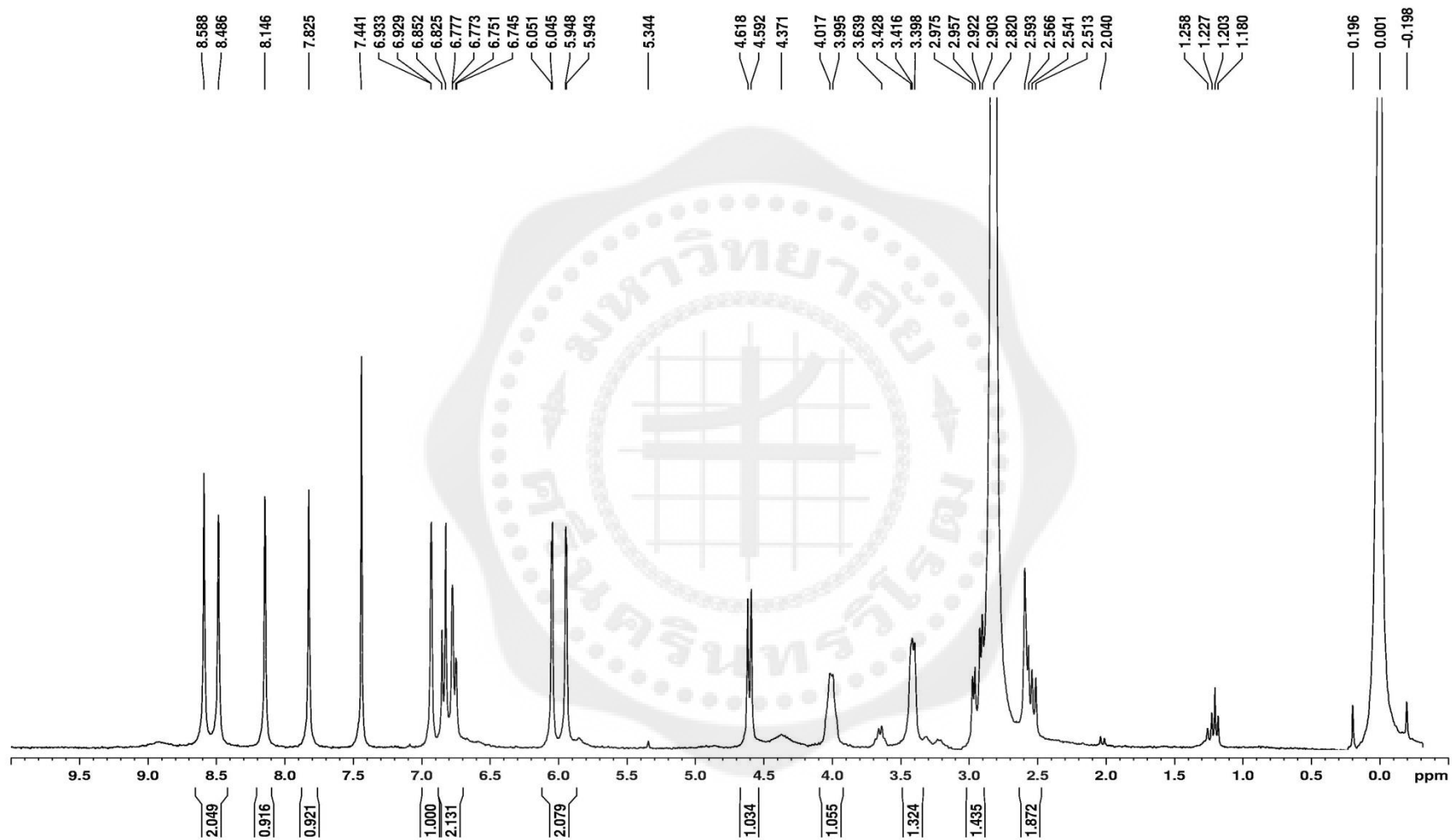


Figure 116 ¹H NMR spectrum of compound **H** ((+)-Catechin, sss6127) in CDCl₃ + 8 drops DMSO-*d*₆

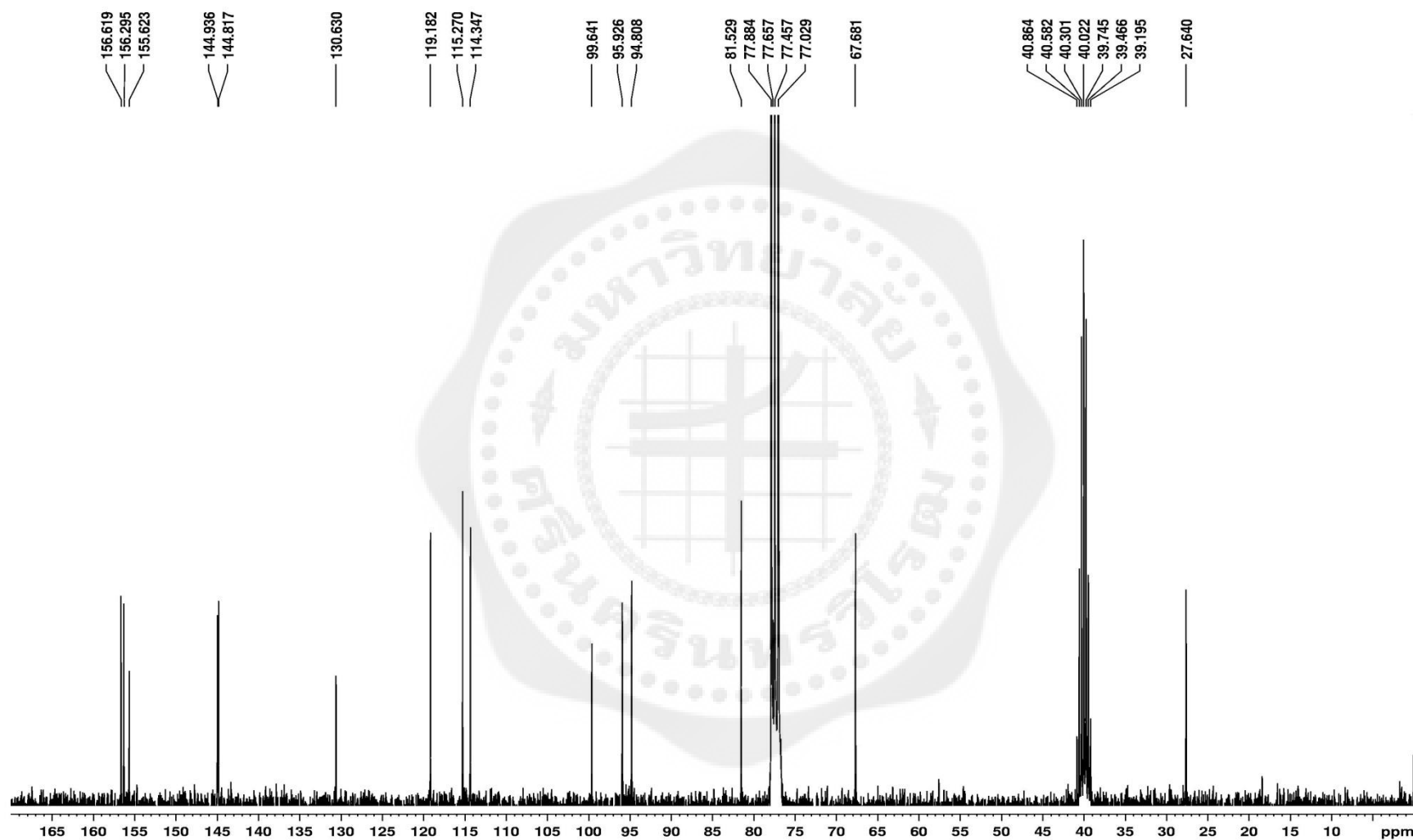


Figure 117 ¹³C NMR spectrum of compound **H** ((+)-Catechin, sss6127) in CDCl₃ + 8 drops DMSO-*d*₆

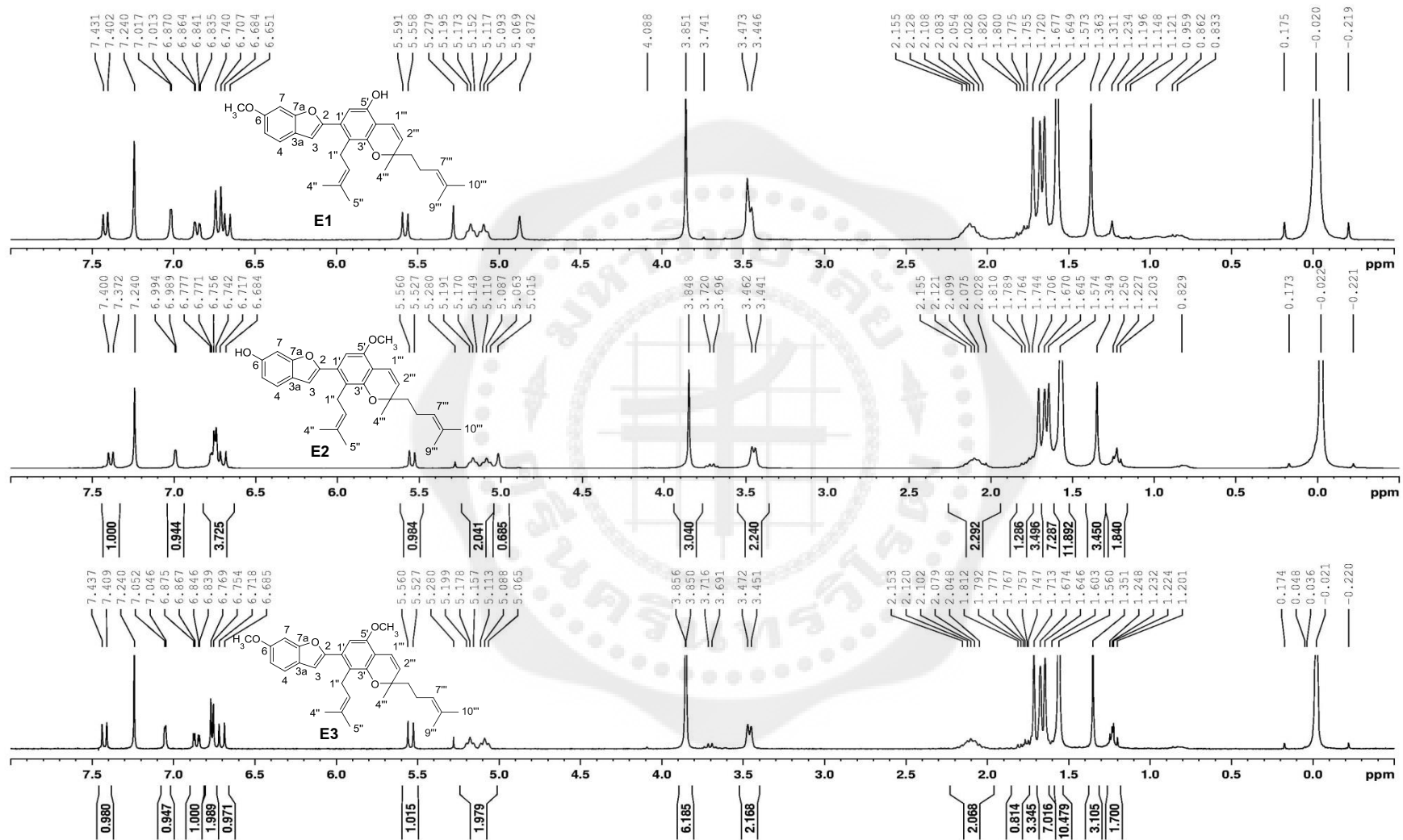


Figure 118 Comparison of ^1H NMR spectra of 6-O-methyl ether (**E1**), 5'-O-methyl ether (**E2**) and 6,5'-di-O-methyl ether (**E3**) in CDCl_3

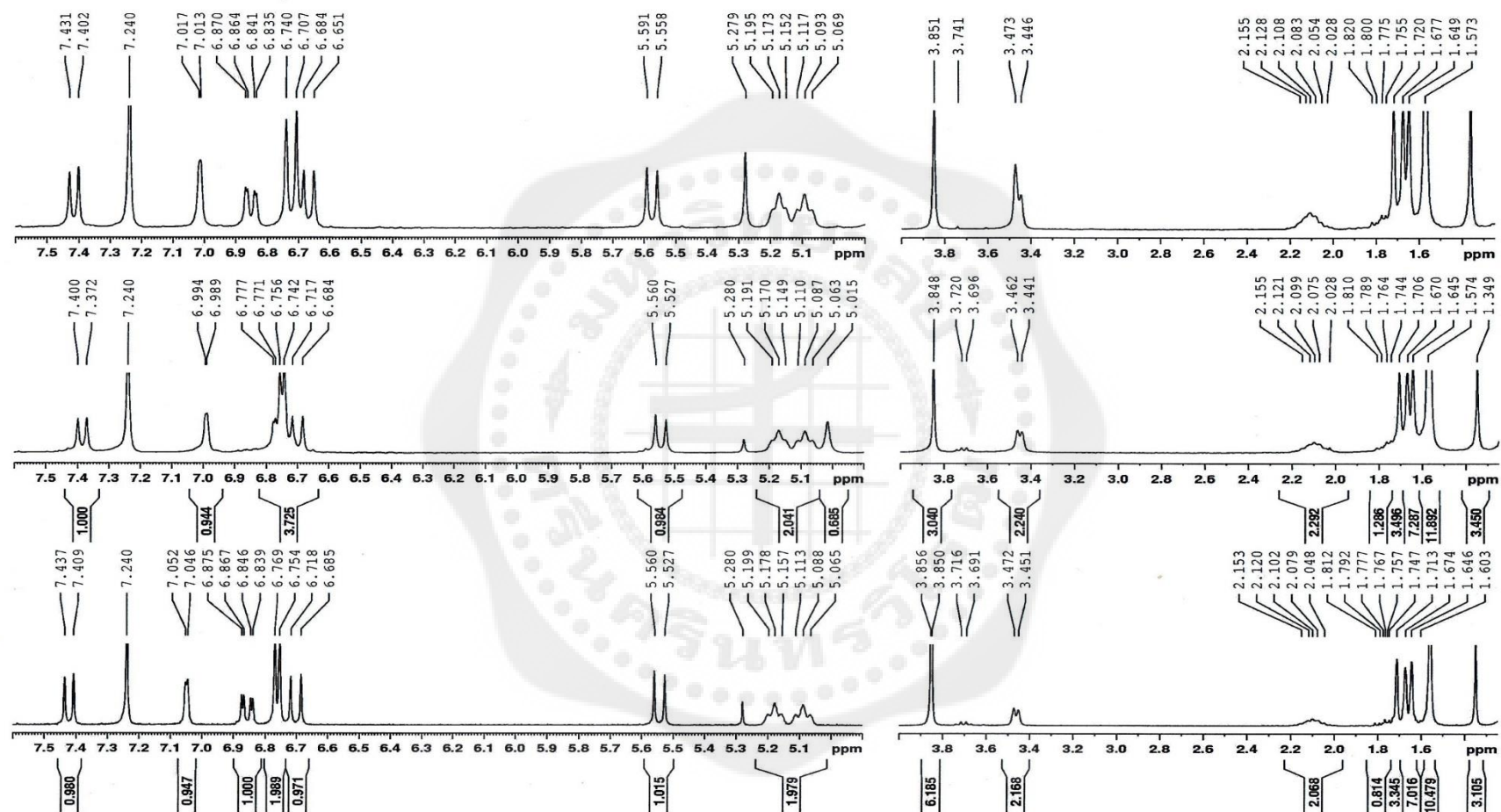


Figure 119 Expansion of ^1H NMR spectra of 6-O-methyl ether (**E1**), 5'-O-methyl ether (**E2**) and 6,5'-di-O-methyl ether (**E3**) in CDCl_3

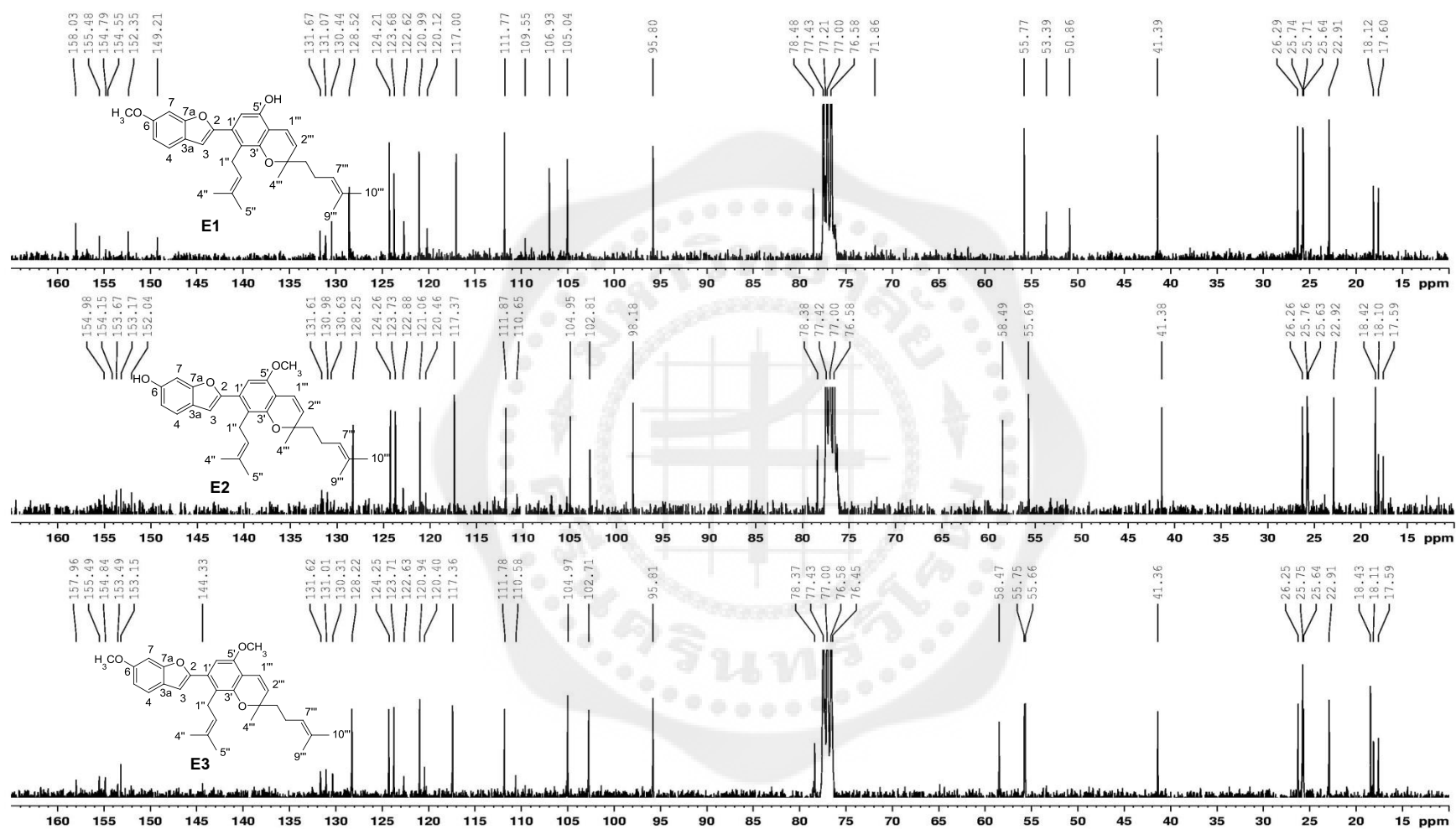


Figure 120 Comparison of ^{13}C NMR spectra of 6-O-methyl ether (**E1**), 5'-O-methyl ether (**E2**) and 6,5'-di-O-methyl ether (**E3**) in CDCl_3

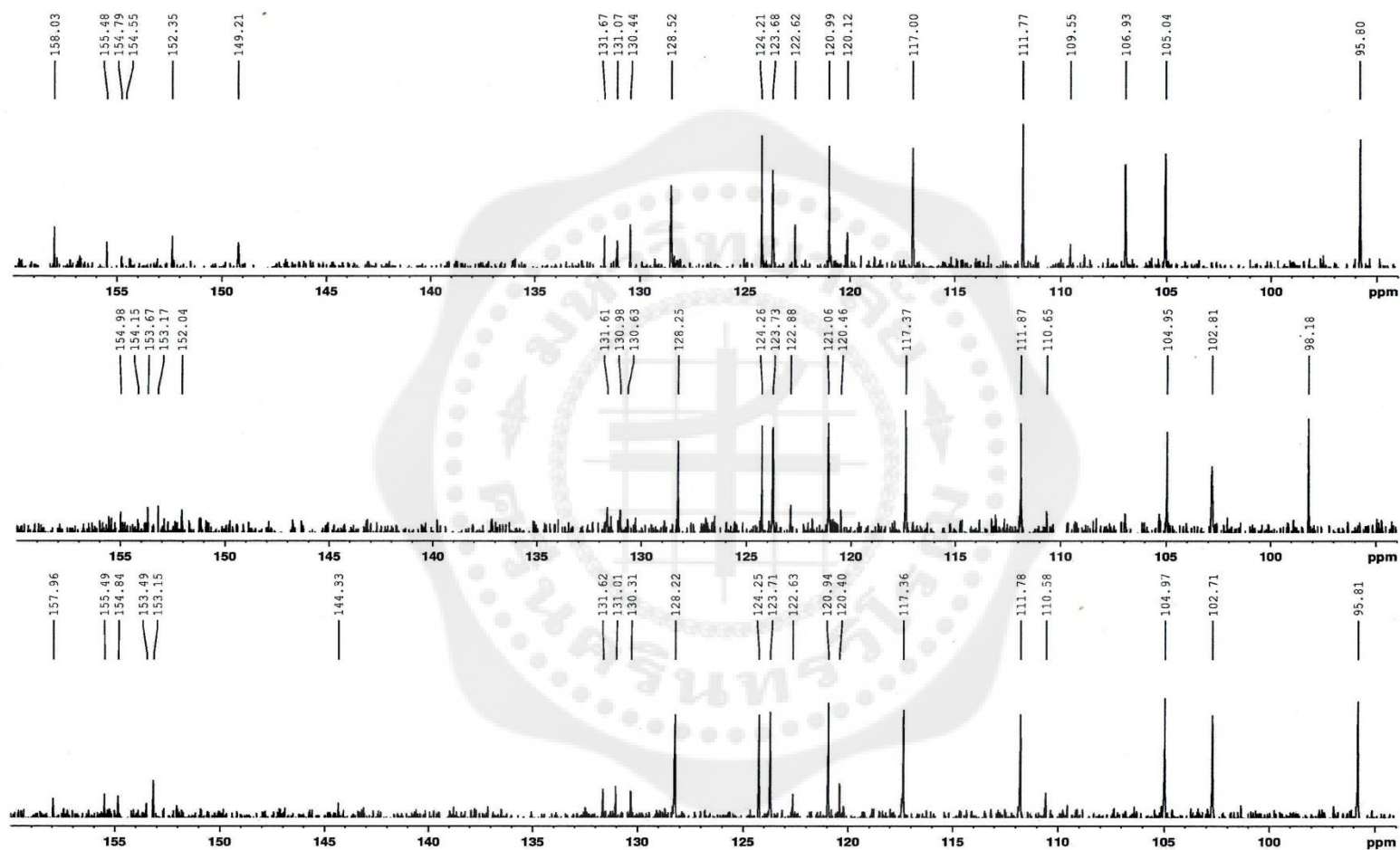


Figure 121 Expansion of ^{13}C NMR spectra of 6-O-methyl ether (**E1**), 5'-O-methyl ether (**E2**) and 6,5'-di-O-methyl ether (**E3**) in CDCl_3

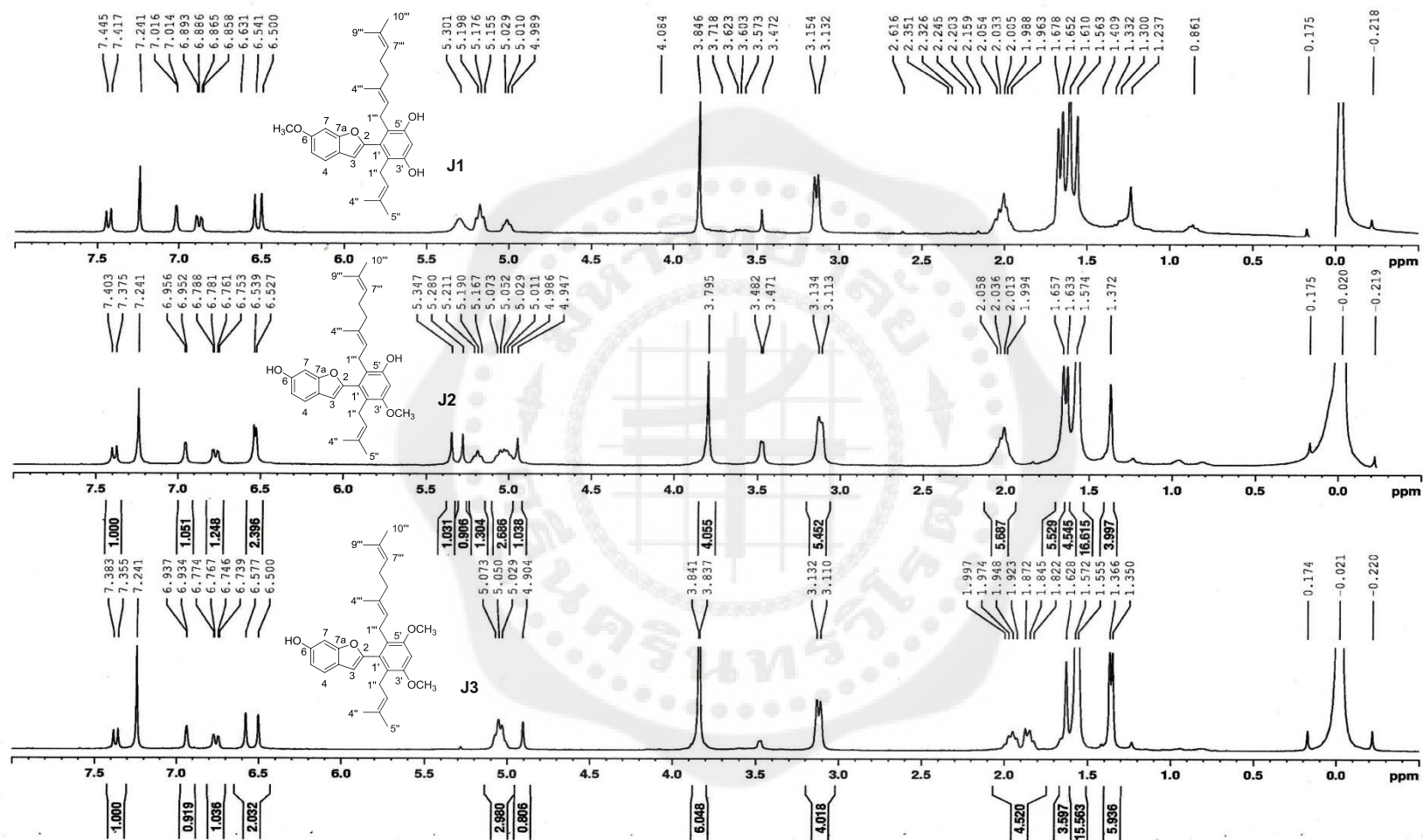


Figure 122 Comparison of ^1H NMR spectra of 6-O-methyl ether (**J1**), 3'-O-methyl ether (**J2**) and 3',5'-di-O-methyl ether (**J3**) in CDCl_3

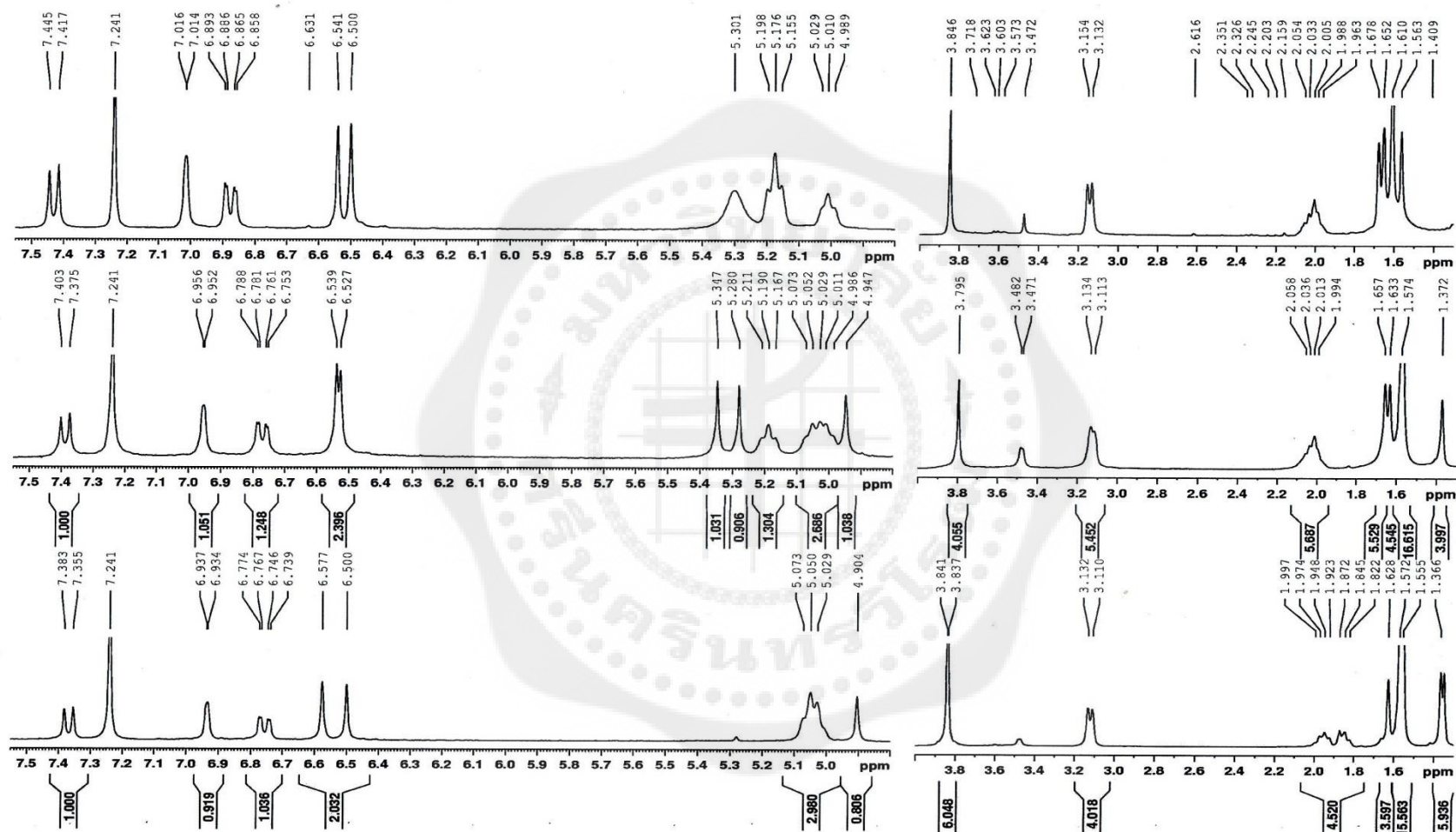


Figure 123 Expansion of ^1H NMR spectra of 6-O-methyl ether (**J1**), 3'-O-methyl ether (**J2**) and 3',5'-di-O-methyl ether (**J3**) in CDCl_3

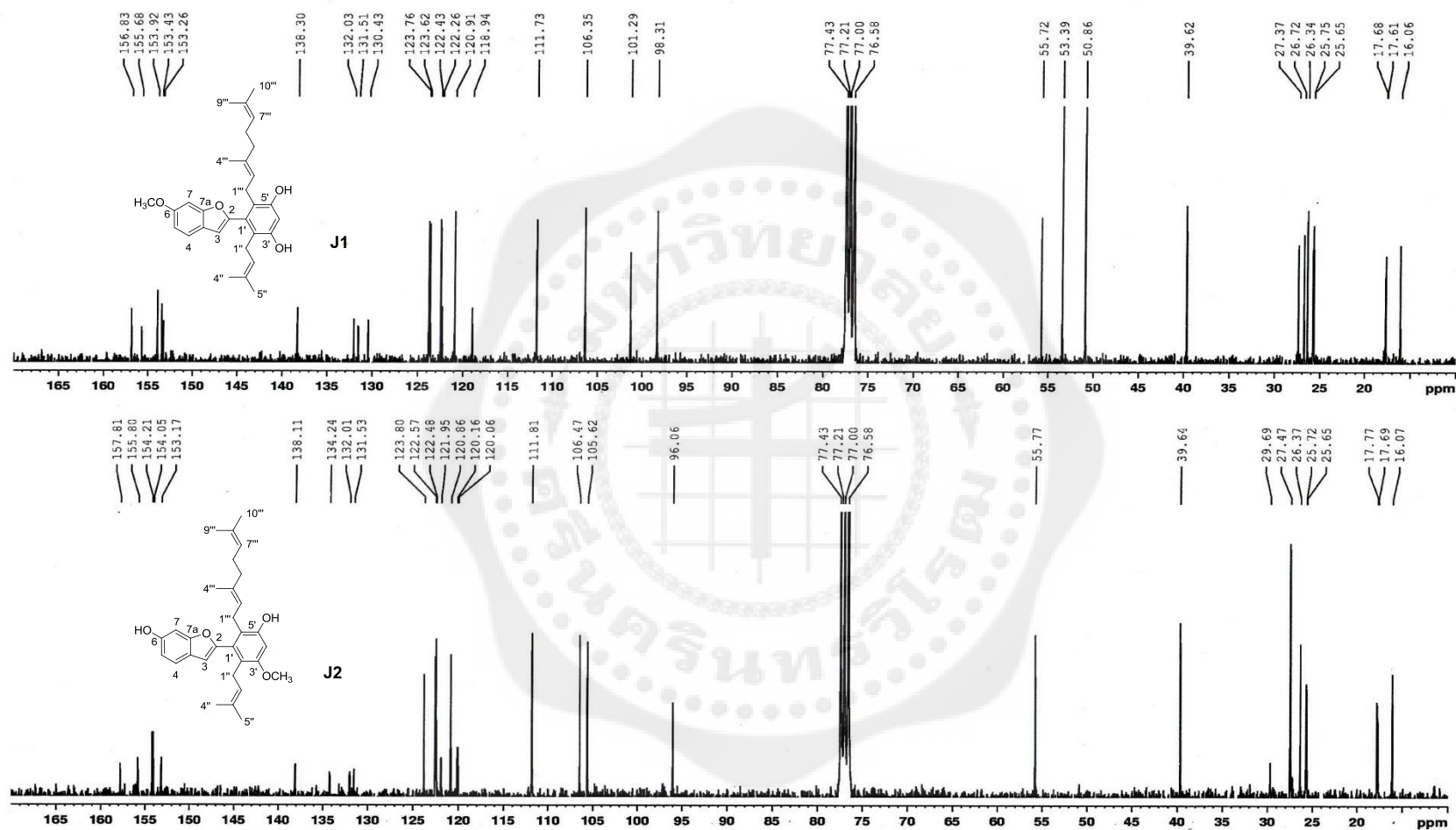


Figure 124 Comparison of ^{13}C NMR spectra of 6-O-methyl ether (**J1**) and 3'-O-methyl ether (**J2**) in CDCl_3

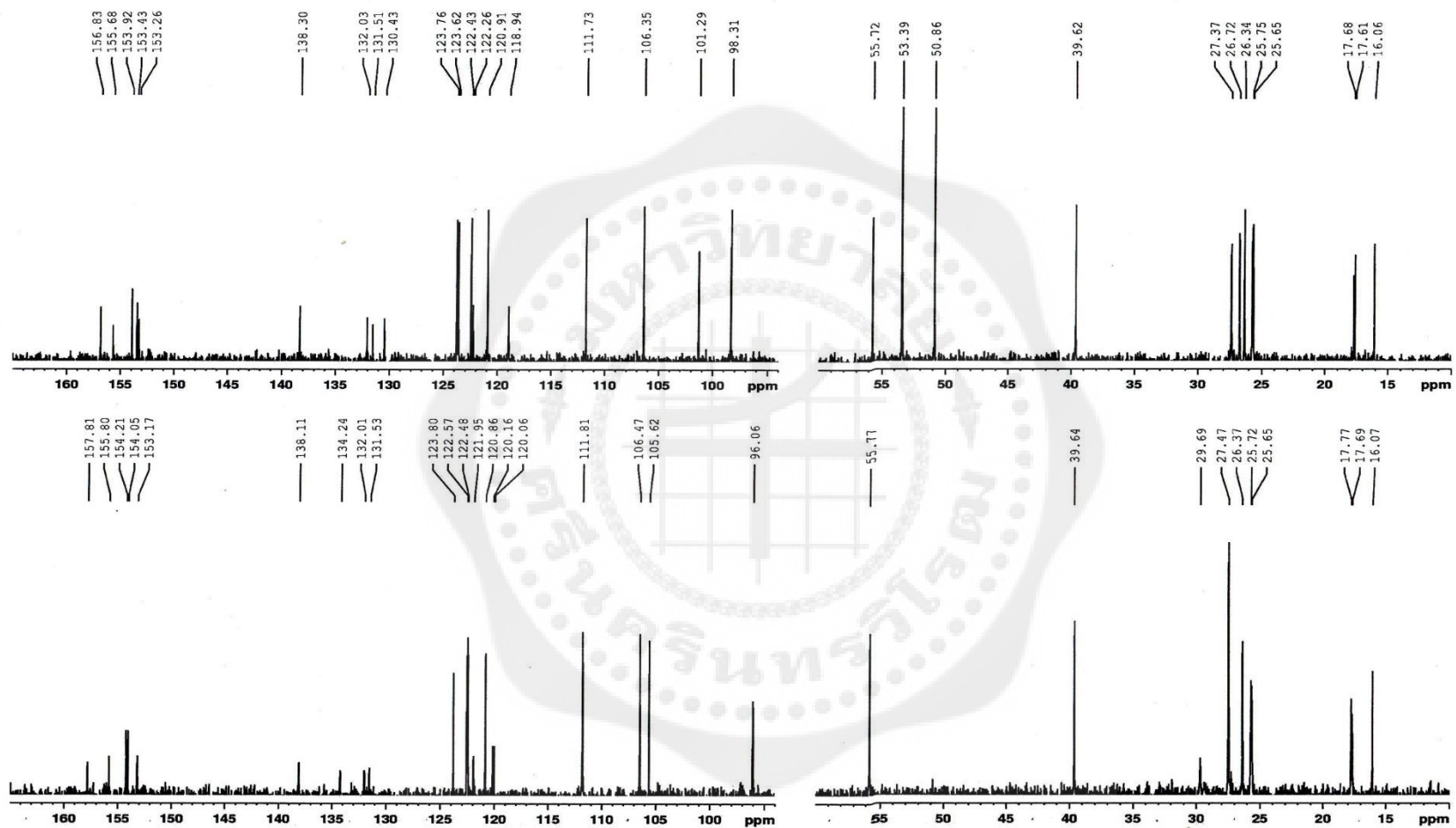


Figure 125 Expansion of ^{13}C NMR spectra of 3'-O-methyl ether (**J2**) and 6-O-methyl ether (**J1**) in CDCl_3

GLOSSARY



LIST OF ABBREVIATIONS AND SYMBOLS α

Alpha

 β

Beta

 ^{13}C NMR

13-Carbon Nuclear Magnetic Resonance Spectroscopy

CC

Column chromatography

J

Coupling constant

 $^{\circ}\text{C}$

Degree Celsius

 CDCl_3

Deuterated chloroform

 CH_2Cl_2

Dichloromethane

DMSO

Dimethylsulfoxide

dd

Doublet of doublet

LIST OF ABBREVIATIONS AND SYMBOLS (continued)**EtOAc**

Ethyl acetate

g

Gram

Hz

Hertz

IC₅₀

50% Inhibitory Concentration

MeOH

Methanol

QCC

Quick column chromatography

μg

Microgram

μm

Micromolar

μL

Microliter

mL

Mililiter

LIST OF ABBREVIATIONS AND SYMBOLS (continued)**MIC**

Minimum Inhibitory Concentration

m

Multiplet

NOESY

Nuclear Overhauser Effect Spectroscopy

 ^1H NMR

Proton Nuclear Magnetic Resonance Spectroscopy

 cm^{-1}

Reciprocal centimeter (unit of wave number)

s

Singlet

TLC

Thin Layer

UV

Ultraviolet

 λ_{max}

Wavelegth at maximal absorption

 ν_{max}

Wavenumber at maximal absorption



Vitae

Name: Umalee Namdaung
Date of Birth: December, 6 1980
Place of Birth: Bangkok
Address: 19/55 Khlongchun Sukhapiban road, Bangkapi Bangkok, Thailand
10240

Education Background:

1999 Bachelor of Science in Chemistry
Srinakharinwirot University, Bangkok, Thailand
2004 Master of Science in Applied Chemistry
Ramkhamhaeng University, Bangkok, Thailand

Pubilcation:

2006 Namdaung, U.; Aronnreck, N.; Suksamrarn, S.; Danwisetkanjana, K.;
Saenboonrueng, J.; Arjchomphu, W.; Suksamrarn, A. (2006).
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