องค์ประกอบทางเคมีและความเป็นพิษต่อเซลล์มะเร็งจากราก
นมสวรรค์ *Clerodendrum paniculatum* เปลือกต้นลำไยป่า *Walsura trichostemon* และ
เถากวาวเครือแดง *Butea superba*

นายเกียรติภูมิ พลตรี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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CHEMICAL CONSTITUENTS AND CYTOTOXICITY FROM ROOTS OF *Clerodendrum paniculatum*, THE STEM BARK OF *Walsura trichostemon* AND TUBER OF *Butea superba*

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A Dissertation Submitted in Partial Fulfillment of the Requirements
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Thesis Title	CHEMICAL CONSTITUENTS AND CYTOTOXICITY FROM ROOTS OF *Clerodendrum paniculatum*, THE STEM BARK OF *Walsura trichostemon* AND TUBER OF *Butea superba*
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Field of Study	Biotechnology
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เกียรติภูมิ พลตรี : องค์ประกอบทางเคมีและความเป็นพิษต่อเซลล์มะเร็งจากรากนมสวรรค์ *Clerodendrum paniculatum* เปลือกต้นลำไยป่า *Walsura trichostemon* และเถากวาวเครือแดง *Butea superba* (CHEMICAL CONSTITUENTS AND CYTOTOXICITY FROM ROOTS OF *Clerodendrum paniculatum*, THE STEM BARK OF *Walsura trichostemon* AND TUBER OF *Butea superba*) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: สันติ ทิพยางค์, 85 หน้า.

การศึกษาองค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพจากสิ่งสกัดไดคลอโรมีเทนจากรากของนมสวรรค์ *Clerodendrum paniculatum* พบสารที่มีรายงานแล้ว 6 สาร คือ β-sitosterol (1.1), lupeol (1.2), oleanolic aldehyde acetate (1.3), สารผสมระหว่าง stigmasta-4,25-diene-3-one (1.4) และ (22*E*)-stigmasta-4,22,25-trien-3-one (1.5) และ (3β)-stigmasta-4,22,25-trien-3ol (1.6). จากการทดสอบฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด KB และ HeLa ของสารทั้งหมดที่แยกได้ พบว่า สาร 1.3 มีฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด KB ได้อย่างมีนัยสำคัญที่ค่า IC_{50} 9.58 µg/mL ในขณะที่สาร 1.6 มีฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด KB ได้ปานกลางที่ค่า IC_{50} 13.14 µg/mL เป็นการรายงานองค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพเป็นครั้งแรกจากพืชชนิดนี้

การแยกสารจากสิ่งสกัดเอทิลแอซีเตตและอะซีโตนจากเปลือกของต้นลำไยป่า *Walsura trichostemon* สามารถแยกสารใหม่ได้ 1 ที่มีชื่อว่า trichostomonoate (2.1) พร้อมกับสารที่เคยมีรายงานแล้ว 8 ชนิด คือ mangstenone F (2.2), desmethoxy kaunugin (2.3), grandifolinolenenone (2.4), cholest-4-en-6β-ol-3-one (2.5), sapelin E acetate (2.6), α-mangostin (2.7) 11α,20-dihydroxydammar-24-ene-3-one (2.8) และ kaempferol (2.9) จากนั้นได้นำสารบางส่วนที่แยกได้มาทดสอบเซลล์มะเร็งชนิด KB, HeLa, COLO 205, HepG2 และ LLC พบว่า สารใหม่ที่แยกได้ (2.1) มีฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด HeLa ได้ดี ที่ค่า IC_{50} 3.8 µg/mL และยังมีฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด KB, COLO 205 และ LLC ได้ปานกลาง ที่ค่า IC_{50} 4.4, 5.3 and 5.5 µg/mL ตามลำดับ

ในส่วนของการแยกสิ่งสกัดไดคลอโรมีเทน อะซีโตนและเมทานอลจากเถาของต้นกวาวเครือแดง *Butea superba* ได้สารที่เคยมีรายงานแล้ว 7 สาร คือ β-sitosterol (1.1), 8-O-methylretusin (3.1), (6αR,11αR)-medicarpin (3.2), formononetin (3.3), hexacosanoic acid 2,3-dihydroxypropyl hexacosanoate (3.4), ononin (3.5) และ daidzein-7-*O*-glucoside (3.6). จากนั้นนำสารที่แยกได้ทั้งหมดมาทดสอบเซลล์มะเร็งชนิด KB, HeLa S-3, MCF-7, HepG-2 และ Vero พบว่าสาร 3.6 มีฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด KB และ HepG2 ได้ปานกลาง ที่ค่า IC_{50} 5.32 and 10.13 µg/mL ตามลำดับ สาร 1.1, 3.1, 3.2, 3.5 และ 3.6 เป็นการรายงานเป็นครั้งแรกในพืชชนิดนี้

สาขาวิชา เทคโนโลยีชีวภาพ ลายมือชื่อนิสิต .....

ปีการศึกษา 2557 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

5273803223 : MAJOR BIOTECHNOLOGY
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KIETTIPUM PHONTREE: CHEMICAL CONSTITUENTS AND CYTOTOXICITY FROM ROOTS OF *Clerodendrum paniculatum*, THE STEM BARK OF *Walsura trichostemon* AND TUBER OF *Butea superba*. ADVISOR: ASSOC. PROF. SANTI TIP-PYANG, Ph.D., 85 pp.

The phytochemical investigation of the CH_2Cl_2 crud extract from the roots of *C. paniculatum* Linn. afforded six known compounds, β-sitosterol (1.1), lupeol (1.2), oleanolic aldehyde acetate (1.3), the mixture of stigmasta-4,25-diene-3-one (1.4) and (22*E*)-stigmasta-4,22,25-trien-3-one (1.5) and (3β)-stigmasta-4,22,25-trien-3ol (1.6). All isolated compounds were tested for their cytotoxicity against KB and HeLa cell lines, oleanolic aldehyde acetate (1.3) showed significant cytotoxic activity against the KB cell line with IC_{50} value of 9.58 µg/mL. On the other hand, (3β)-stigmasta-4,22,25-trien-3ol (1.6) also exhibited moderate cytotoxic activity against the KB cell line with IC_{50} value of 13.14 µg/mL. To the best of our knowledge, this is the first report on chemical constituents and biological activity from this plant.

The various chromatographic techniques of EtOAc and acetone crude extracts from the bark of *W. trichostemon* Miq. led to the isolation of a new tirucallane, trichostomonoate (2.1), along with eight known compounds, mangstenone F (2.2), desmethoxy kaunugin (2.3), grandifolinolenenone (2.4), cholest-4-en-6β-ol-3-one (2.5), sapelin E acetate (2.6), α-mangostin (2.7) 11α,20-dihydroxydammar-24-ene-3-one (2.8) and kaempferol (2.9). The new compound (2.1) displayed good potent cytotoxicity against only HeLa cells (IC_{50} 3.8 µg/mL) in the *in vitro* tumor cell panel and showed moderate cytotoxicity against KB, COLO 205 and LLC cells (IC_{50} 4.4, 5.3 and 5.5 µg/mL, respectively).

The isolation and purification of the CH_2Cl_2, acetone and MeOH extracts from the tuber of *B. superba* Roxb. yielded the isolation of seven known compounds, β-sitosterol (1.1), 8-O-methylretusin (3.1), (6αR,11αR)-medicarpin (3.2), formononetin (3.3), 2,3-dihydroxypropyl hexacosanoate (3.4), ononin (3.5) and daidzein-7-*O*-glucoside (3.6). Compound 3.6 displayed moderate cytotoxicity against KB and HepG-2 (IC_{50} 5.32 and 10.13 µg/mL, respectively). In addition, compounds 1.1, 3.1, 3.2, 3.5 and 3.6 were reported for the first time for this plant.

Field of Study: Biotechnology Student's Signature .....

Academic Year: 2014 Advisor's Signature .....

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LISTS OF ABBREVIATIONS

δ	chemical shift
δ_C	chemical shift of carbon
δ_H	chemical shift of proton
CC	column chromatography
^{13}C NMR	carbon-13 nuclear magnetic resonance
COSY	correlated spectroscopy
IC_{50}	concentration that required for 50% inhibition *in vitro*
J	coupling constant
Acetone-d_6	deuterated acetone
$CDCl_3$	deuterated chloroform
CD_3OD	deuterated methanol
CH_2Cl_2	dichloromethane
DMSO	dimethyl sulfoxide
d	doublet (NMR)
dd	doublet of doublet (NMR)
ESIMS	electrospray ionization mass spectrometry
EtOAc	ethyl acetate
HMBC	heteronuclear multiple bond correlation experiment
HSQC	heteronuclear single quantum correlation
HRESIMS	high resolution electrospray ionization mass spectrometry
Hz	hertz
HeLa cell	human cervical carcinoma
KB cell	human epidermoid carcinoma
MCF-7	human breast adenocarcinoma
Hep G2	human hepatocellular carcinoma
COLO 205	human colorectal adenocarcinoma
LLC	mouse Lewis lung carcinoma
kg	kilogram

l	liter
MeOH	methanol
μg	microgram
μl	microliter
M	mole per liter (molar)
MW	molecular weight
μM	micromolar
mg	milligram
ml	milliliter
mM	millimolar
m/z	mass per charge
Vero cell	normal cell derived from the kidney of an African green monkey
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser enhancement spectroscopy
s	singlet
TLC	thin layer chromatography
UV	ultraviolet
2D NMR	two dimensional nuclear magnetic resonance
VLC	vacuum liquid chromatography

CHAPTER I
INTRODUCTION

1.1 Research Background and rationale

There are many causes of deaths in Thailand: the most common conventional causes of death are accident, cardiovascular disease and cancer. Moreover, diabetes mellitus, HIV, malarial and Alzheimer's diseases are still more prevalent.

Cancer or malignant neoplasm is an inclusive group of various diseases. In cancer, cells divide and grow uncontrollably forming malignant tumors and invading nearby parts of body. The cancer may also spread to more distant parts of the body through the lymphatic system or bloodstream. However, not all tumors are cancerous. Benign tumors do not grow uncontrollably, do not invade neighboring tissues, and do not spread throughout the body. There are over 200 different known cancers that afflict humans [1]. Determination of causes of cancer is complex. Many factors are known to increase the risk of cancer, including tobacco, radiation, obesity, lack of physical activity, certain infections, and environmental pollutants. These can directly damage genes or combine with existing genetic faults within cells to cause the disease. For example, approximately five to ten percent of cancers are entirely hereditary. Cancer can be detected in a number of ways, including the presence of certain signs, symptoms, screening tests, or medical imaging. Once a possible cancer is detected and diagnosed by microscopic method of a tissue sample. Cancer is usually treated with chemotherapy, radiation therapy and surgery. The chances of surviving the disease vary greatly by the type and by the location of the cancer and the extent of disease at the beginning of treatment. While cancer can affect people of all ages it generally increases with age.

A chemical compound or substance which is produced by plants, fungi, bacteria, protozoans, and animals in response to external stimuli such as nutritional changes, infection and competition [2] that usually has a pharmacological or biological activity for use in pharmaceutics is called a natural product. A natural product can be prepared by total synthesis. Natural products may be extracted from tissues of terrestrial plants, marine organisms or microorganisms. A crude extract from any one of these sources typically contains novel, structurally diverse chemical compounds, which the natural environment is a rich source of. These compounds are important in the treatment of life-threatening conditions. Biological and geographical diversity have more influence on chemical diversity in nature.

Some bioactive compounds are developed by synthesizing a lead compound originally obtained from a natural source. This has an advantage in that they are extremely novel compounds but this complexity also makes many lead compounds' synthesis difficult because most biologically active natural product compounds are secondary metabolites with very complex structures. Therefore, some bioactive compounds usually have to be extracted from their natural source.

Lead compounds such as alkaloids, morphine, tubocurarine, paclitaxel, nicotine and muscarine were always produced by plants. Several lead compounds are useful drugs in themselves and others have been the basis for synthetic drugs. Plants provide several complex structures which are difficult to synthesize in laboratories. Clinically useful drugs which have been isolated from plants include the anticancer agent such as taxol found in paclitaxel from the yew tree, and the antimalarial agent artemisinin from *Artemisia annua*. In general, natural compound types can be classified as terpenoids, alkaloids, flavonoids and polyphenols [3].

In the ancient times, traditional peoples or human civilization found out traditional medicine by experimenting with various berries, leaves, roots, animal parts

or minerals. For example, the therapeutic properties in Ancient Egypt of the opium poppy which has morphine as an active component and the active principle, reserpine in the snakeroot plant was well regarded in India, but some preparations may have been dangerous.

In modern time, many medicinal plants have many therapeutic properties such as reducing high blood pressure, cholesterol reduction, deterring heart and liver disease, and have also been one of the major sources of medicines. Medicinal plants especially have pharmacological activities and they are used as therapeutic drugs or herbal medicines. There are several publications that have reported some tropical plants which have many bioactive activities such as potent antimicrobial, antiparasitic antimalarial and cytotoxic activities [3-6]. In addition, some of them also have a potential anticancer activity in the prevention of cancer and the management of infectious and chronic diseases. The contribution of plant-derived drugs is also significant and much interest has been investigated on the wide diversity of medicinal plants to modern drug development.

1.1.1 Botanical aspect and distribution of *Clerodendrum* species and their biological activities

Kingdom : Plantae

Order : Lamiales

Family : Verbenaceae

Subfamily : Teucrioideae

Genus : *Clerodendrum*

The genus *Clerodendrum* belongs to the family Verbenaceae consisting of about 30 species. It is mainly distributed in Thailand, India and China [7]. This genus was first described by Linnacus in 1753 based on the species

Clerodendrum infortunatum from India and later Adanson changed the Latinized form "*Clerodendrum*" to its Greek form "*Clerodendron*" in 1763. After almost two centuries, Moldenke readopted the Latinized word "*Clerodendrum*" in 1942 which is now commonly used by taxonomists for classification and description of this genus. Many species of this genus have a long history of medical use in traditional medicine. Some of which have been reported to possess many biological activities. In Thai traditional medicine, the leaves and roots of *C. petasites* are used for the treatment of fever, inflammation and skin disease as well as asthma [8-10].

The roots of *C. serratum* are used clinically for the treatment of bronchitis, asthma, fever, blood diseases, tumors, inflammations, burning sensation, epilepsy, malaria, ulcers and wounds [11]. The leaves, branches and roots of *C. bungei* have been widely used in folk medicine for treatment of carbuncle-abscesses, hemorrhoids, eczema, hypertension, dizziness and rheumatism [12, 13]. Furthermore, the dry leaves and twigs of *C. trichotomum* have been used for the treatment of hypertension and rheumatism [14, 15].

C. paniculatum Linn. is commonly known as glorybower, bagflower and bleeding-heart. It is perennial woody shrub, up to 3-8 ft in height with blunt quadrangular stems. Young roots are smooth or finely longitudinally striated. Mature roots are hard, woody and cylindrical in shape with 1-1.5 cm in diameter, 2-3 cm in length and are pale brown externally and yellowish brown internally. The bark is acrid, thin, breaks with short fracture and easily separated from a broad wood showing marked medullary rays and concentric growth rings. This plant grows in moist deciduous forest. In Thai traditional medicine, roots of this plant are used to treat abscess. To the best of our knowledge, this plant was no report on chemical constituents and biological activity.

1.1.2 Chemical constituents from *Clerodendrum* genus

The chemical constituents and biological activities of this genus was investigated by many researchers and the compounds which isolated from this genus are shown as follows.

C. petasites, locally known as "Thao yaai mom" is prepared as a tea or an alcoholic extract and used to treat asthma. The dried aerial parts of this plant were found to be flavonoid hispidulin [9].

C. trichotomum Thunb. is a deciduous shrub widely distributed in Korea, Japan, China and northern of the Philippines. The stems of this plant have been isolated to be seven abietane diterpenoids along with three anthraquinones and one neolignan [16].

C. bungei, whose English name is rose glory bower, is a small shrub indigenous in China. Two new diterpenoids along with three known ketones were isolated from the roots of this plant [17].

C. inerme is a shrub distributed in Thailand, Malaysia and China. The aerial part of this plant had been isolated and found to be two megastigmane glucosides and an iridoid glucoside [9, 18].

C. grayi Munir is a sub canopy rainforest tree endemic to the northern part of Queensland, Australia. The cyanogenic diglycoside lucumin and monoglucoside prunasin were isolated from the leaves of this plant [19].

C. mandarinorum Diels is a Chinese medicinal plant known as "Hai Tang". The crude drug has a good reputation in the treatment of infantile paralysis and apoplexy. Three new abietane derivatives (mandarones A, B and C) had been isolated from the stems of this plant [20].

C. colebrookianum Walp. is distributed widely in the south and south east Asia. It mainly grows in the moist and waste place of the western and southern regions of Yunnan province, China. The chemical investigation of this plant led to the isolation of five new steroids named colebrins A and B, together with two known compounds, clerosterol and daucosterol [21].

C. quadriloculare Merr, an endemic Philippine medicinal plant, is locally known as “Bagauak”. It is a small tree that thrives in several provinces of the Luzon island. The leaves are ethnomedically used to treat wounds and ulcer and employed in tonic baths. The chemical constituents had been purified to be clerosterol [22].

C. wildii is an African medicinal plant which shows antifungal and molluscicidal properties. Triterpenoid saponin, named Mi-saponin A had been isolated from the roots of this plant [23].

Table 1.1 Chemical constituents of *Clerodendrum* genus

Compounds	Sources and plant parts	References
Hispidulin	*C. petasites,* aerial parts	[9]
Sugiol	*C. trichotomum*, stems	[16]
Teuvincenone F	*C. trichotomum*, stems	[16]
Teuvincenone A	*C. trichotomum*, stems	[16]
Uncinatone	*C. trichotomum*, stems	[16]
Cyrtophyllone B	*C. trichotomum*, stems	[16]
Emodin	*C. trichotomum*, stems	[16]
Sammangaosides A	*C. inerme*, aerial parts	[18]
Sammangaosides B	*C. inerme*, aerial parts	[18]
Sammangaoside C	*C. inerme*, aerial parts	[18]
Mandarone A	*C. mandarinorum,* stems	[20]
Mandarone B	*C. mandarinorum,* stems	[20]
Colebrin A	*C. colebrookianum*, aerial parts	[21]
22-Dehydroclerosterol	*C. quadriloculare*, leaves	[22]

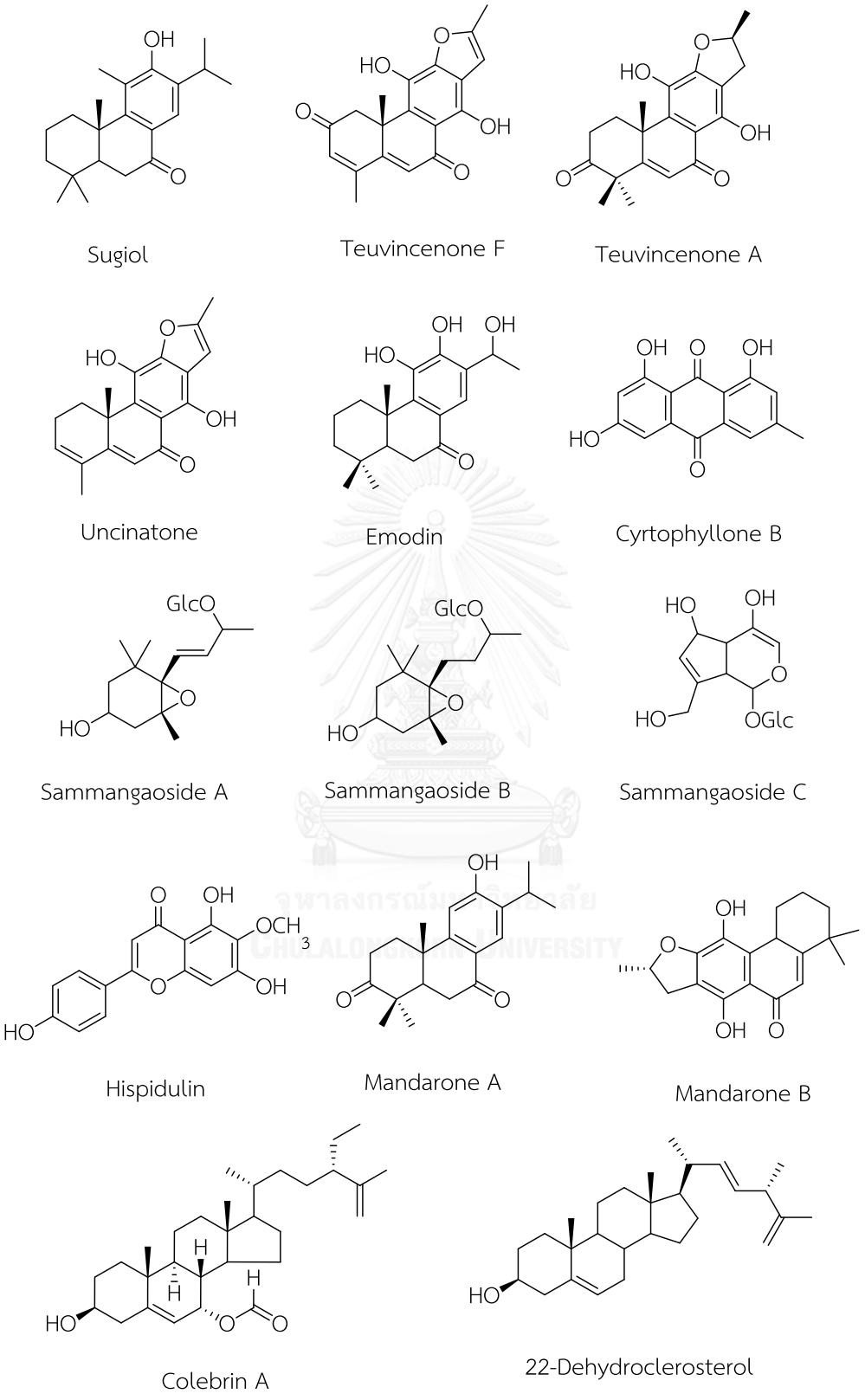


Figure 1.1 Isolated compounds from *Clerodendrum* genus

Figure 1.2 The flowers and roots of *C. paniculatum*

1.1.3 Botanical aspect and distribution of *Walsura* species and their biological activities

Kingdom : Plantae

Order : Sapindales

Family : Meliaceae

Genus : *Walsura*

The genus *Walsura* Roxb. (Meliaceae) is mainly distributed in India, China and Indonesia, and is comprised between 30-40 species. *Walsura trichostemon* Miq. is an evergreen tree, locally known as “Lamyai Pa” in Thailand. In Thai traditional medicine, this plant is used to treat tendon disabilities, as a staunch, for cleaning wounds, and as a treatment for hemorrhoids [24]. This plant is a perennial plant, up to 90 ft in height with bole to 1.2 ft diameter. The outer bark is dark reddish brown scaly while the inner bark is pinkish red. Young twigs are pale brown, finely sericeous, lenticellate and leaves are 0.6 ft long.

1.1.4 Chemical constituents from *Walsura* genus

Chemical and pharmacological studies of the plants from this genus have been very active in the past decade. The compounds which isolated from this genus are shown as follows.

W. trifoliata is an evergreen tree distributed widely in the tropical areas of Asia, such as China, India, Malaysia and Indonesia. The plant bark is used as astringent to treat diarrhea and other diseases. The chemical investigation of this plant led to the isolation of two novel classes of apo-tirucallane triterpenoids [25].

W. yunnanensis is mainly distributed in Asia. The MeOH extract of this plant has been used as antifeedant and antimalarial activities. The twigs of this plant have been isolated to be three tetranortriterpenoids [26].

W. cochinchinensis, a widespread species in Vietnam, is also found in the southern area of China such as Guangxi, Guangdong, Yunnan and Hainan. This plant shows the variety of biological properties such as antifeedant, antibacterial, antioxidative and antimalarial activities. Two rearranged limonoids were isolated from the leaves and twigs of this plant [27].

W. piscidia is a native plant of India. This plant is used in Indian traditional medicine. The leaves of this plant had been isolated and found to be a triterpenoid [28].

W. chrysogyne is a small tree 15-20 ft in high distributed in Sri lanka, Malaysia, Indonesia and New Guinea. Ten known terpenes were isolated from the leaves while six new limonoids were found from the bark of this plant [29].

W. robusta Roxb. Is a perennial plant, up to 80 ft in height and well-known multipurpose medicinal plant. This plant is naturally distributed in subtropical regions such as southern China, India and Indonesia. The extracts of the leaves and twigs of this plant is used as an insecticide. A new carotene sesquiterpene had been isolated from the leaves of this plant [30].

W. pinnata grows naturally in southern China, Indochina and Malaysia. The plant grows up to 120 ft. height. The bark is smooth and pale. The cyclohexane named pinnatane A was extracted from the bark of this plant [31].

W. trichostemon, this plant first investigates from our group. A new trichostemonate was isolated from the roots of this plant [32].

Table 1.2 Chemical constituents of *Walsura* genus

Compounds	Sources and plant parts	References
Piscidinone A	*W. trifoliata*, leaves	[25]
Piscidinone B	*W. trifoliata*, leaves	[25]
Anthothecol	*W. yunnanensis*, stems	[26]
Walsucochinoid A	*W. cochinchinensis*, leaves and twigs	[27]
Walsucochinoid B	*W. cochinchinensis*, leaves and twigs	[27]
Piscidinol-C	*W. piscidia*, leaves	[28]
Walsogyne B	*W. chrysogyne*, bark	[29]
Walsogyne C	*W. chrysogyne*, bark	[29]
Trichostemonate	*W. trichostemon,* roots	[32]

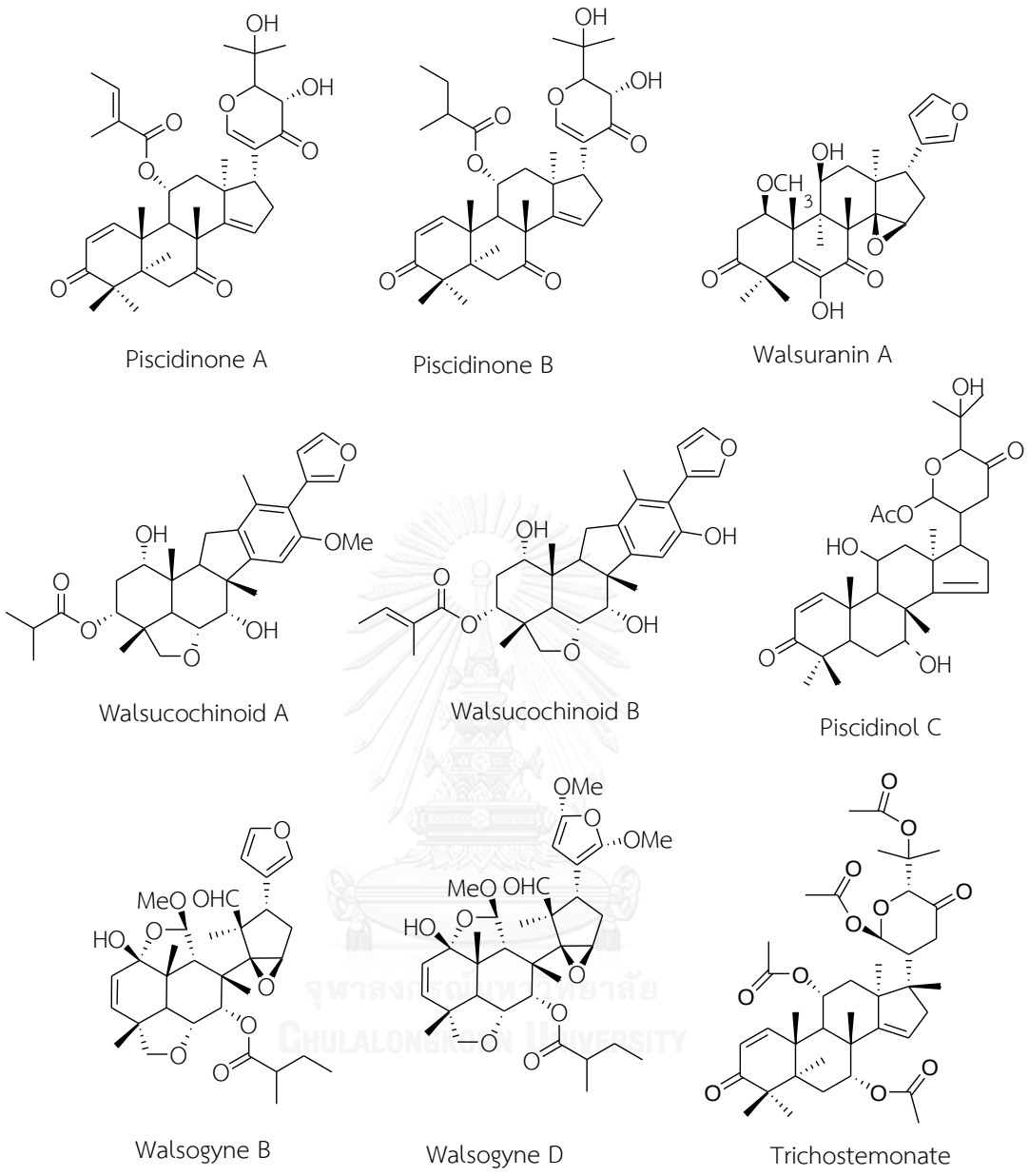


Figure 1.3 Isolated compounds from *Walsura* genus


Figure 1.4 The flowers and stem of *W. trichostemon*

1.1.5 Botanical aspect and distribution of *Butea* species and their biological activities

Kingdom : Plantae

Order : Fabales

Family : Fabaceae

Subfamily : Faboideae

Genus : *Butea*

Butea is a genus of flowering plants belonging to the family Fabaceae and consisting of 2 species. This genus includes *B. monosperma* and *B. superba* widely distributed in India, Malaysia and Thailand. The roots of *B. monosperma* are useful for the treatment of night blindness and other eye diseases. The stem bark of this plant displays antifungal activity, which is due to the presence of an active constituent (-)-medicarpin. The leaves of this plant exhibit ocular anti-inflammatory activity and antimicrobial activity. An extract from the flower of this plant is also used in India for the treatment of liver disorders [33].

B. superba (Kwao Krua Dang in Thai) is an herb in the family Fabaceae which is abundantly distributed in Thai deciduous forests. This plant is a climbing plant which found in the mountainous regional regions such as Northern and Northeastern part of Thailand. The flowers are usually range of shade of a yellowish

orange color. The lengthy root base of the plant is buried underneath the soil, exactly like the root of the yam. The long shape tubers usually are annually enlarged. The long underground tuberous root accumulates many phytochemicals which can be very beneficial human health [34]. This plant is also known as a male sexual enhancement herb, may act primarily by increasing the relaxation capacity of the corpus cavernosum smooth muscle via cAMP phosphodiesterase inhibition and may also affect the brain, activating the improvement of the emotional sexual reaction [35].

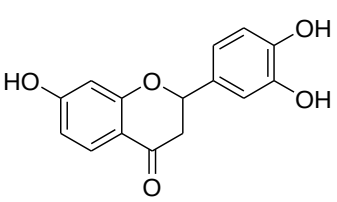
1.1.6 Chemical constituents from *Butea* genus

B. monosperma is popularly known as flame of the forest. This plant is reported to have medicinal potential in various ancient literatures. It is used for the treatment of anticancer, anti-obesity and peripheral pain. This plant is also known for hepatoprotective and antidiabiatic potential. The flowers of this plant had been isolated and found to be flavonoids, triterpenes and sterols [36].

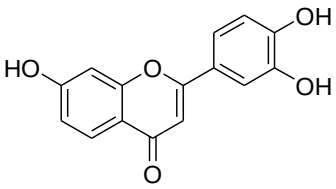
B. superba, the stems of this plant contains flavonoids, isoflavonones and flavonol glycosides [37].

Table 1.3 Chemical constituents of *Butea* genus

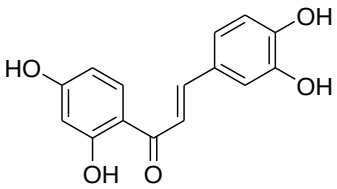
Compounds	Sources and plant parts	References
Butin	*B. monosperma, flowers*	[36]
7,3',4'-Trihydroxyflavone	*B. monosperma, flowers*	[36]
Butein	*B. monosperma, flowers*	[36]
Monospermoside	*B. monosperma, flowers*	[36]
Formononetin	*B. monosperma, flowers*	[36]
7-hydroxy-6,4'-dimethoxyisoflavone	*B. superba,* stems	[37]
5,4'-dihydroxy-7-methoxy-isoflavone	*B. superba,* stems	[37]



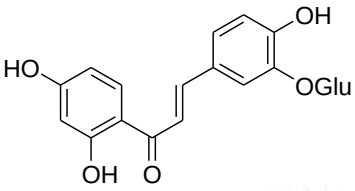
Butin



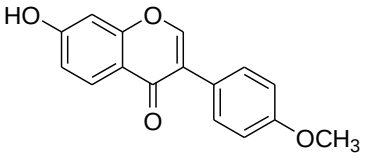
7,3',4'-Trihydroxyflavone



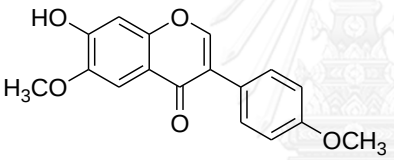
Butein



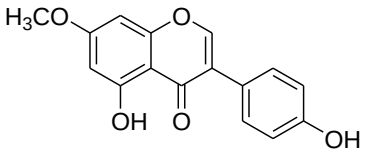
Monospermoside



Formononetin



7-Hydroxy-6,4'-dimethoxyisoflavone



5,4'-Dihydroxy-7-methoxy-isoflavone

Figure 1.5 Isolated compounds from *Butea* genus

Figure 1.6 The flowers and tubers of *B. superba*

1.2 Objectives

The objectives in this investigation are as follows:

1. To isolate and purify the compounds from the roots of *C. paniculatum* Linn, the stem bark of *W. trichostemon* Miq. and the tuber of *B. superba* Roxb.

2. To identify the chemical structures of all isolated compounds.

3. To evaluate the cytotoxicity against, KB, HeLa, MCF-7, COLO 205, HepG-2, LLC cell lines and Vero cell of all isolated compounds.

CHAPTER II

EXPERIMENTAL

2.1 Plant material

2.1.1 *C. paniculatum* Linn.

The roots of this plant were collected from Khon Kaen province in September 2011 and identified by Dr. Suttira Khumkratok, a botanist at the Walairukhavej Botanical Research Institute, Mahasarakham University and a specimen retained as a reference (Khumkratok no 1-13).

2.1.2 *W. trichostemon* Miq.

The stem bark of *W. trichostemon* was collected at Mahasarakham Province, Thailand in December 2012. The plant material was identified by Dr. Suttira Khumkratok, Walairukhavej Botanical Research Institute, Mahasarakham University and a specimen retained as a reference (Khumkratok no. 2-10).

2.1.3 *B. superba* Roxb.

The tuber of this plant was collected at Lampang Province, Thailand in June 2012. The plant material was identified by Dr. Suttira Khumkratok, Walairukhavej Botanical Research Institute, Mahasarakham University and a specimen retained as a reference (Khumkratok no. 1-12).

2.2 General experimental procedures

NMR spectra were recorded on a Bruker 400 AVANCE spectrometer and a Varian model Mercury^{+} 400 spectrometer operated at 400 MHz for ^{13}C NMR. The chemical shifts were reported in ppm and using TMS as the internal standard. Melting points were determined on a Fisher-Johns Melting Point apparatus. Adsorbents such as Dianion HP-20 (Sigma-Aldrich), silica gel (60 Merck cat. No. 7730, 7734 and 7749)

were used for CC, preparative TLC and radial chromatography (chromatotron model 7924T, Harrison Research). Merck silica gel $60F_{254}$ plates were used for TLC HRESIMS were obtained using a Bruker MICROTOF model mass spectrometer. UV-visible absorption spectra were taken on a UV-2550 (SHIMADZU) UV-Vis spectrometer (Shimadzu, Kyoto, Japan).

2.3 Extraction and purification

2.3.1 *C. paniculatum* Linn.

The dried roots of *C. paniculatum* Linn. (5.13 kg) were extracted in a soxhlet extracting apparatus with CH_2Cl_2 at room temperature. The solvent was evaporated in vacuo to give CH_2Cl_2 extract (23.37 g). This extract was chromatographed by vacuum liquid chromatography (VLC) on silica gel (Merk 7730), eluted with the gradient systems of hexane, CH_2Cl_2, EtOAc and MeOH to provide 8 fractions (C1-C8). Fraction C2 was subjected to silica gel column chromatography (CC) and eluted with hexane-CH_2Cl_2 (0.2:0.8) to afford *β*-sitosterol (2.82 g) (**1.1**). Fraction C3 was subjected to silica gel CC (hexane-CH_2Cl_2, 0.3:0.7) to afford lupeol (1.38 g) (**1.2**). Fraction C4 was further subjected to silica gel CC (hexane-CH_2Cl_2, 0.6:0.4) to yield oleanolic aldehyde acetate (0.73 g) (**1.3**). Fraction C6 was fractionated over silica gel CC, and eluted with gradient mixtures of hexane and EtOAc with increasing polarity followed by chromatotron to obtain stigmasta-4,25-dien-3-one (0.62 g) (**1.4**) and (22*E*)-stigmasta-4,22,25-trien-3-one (0.59 g) (**1.5**). Fraction C7 was subjected to silica gel (CH_2Cl_2-EtOAc, 0.7:0.3) and Sephadex LH-20 to yield (3*β*)-stigmasta-4, 22, 25-trien-3ol (0.68 g) (**1.6**).

The extraction and purification of all isolated compounds from the CH_2Cl_2 extract of the roots of *C. paniculatum* was briefly summarized in Figure 2.1.

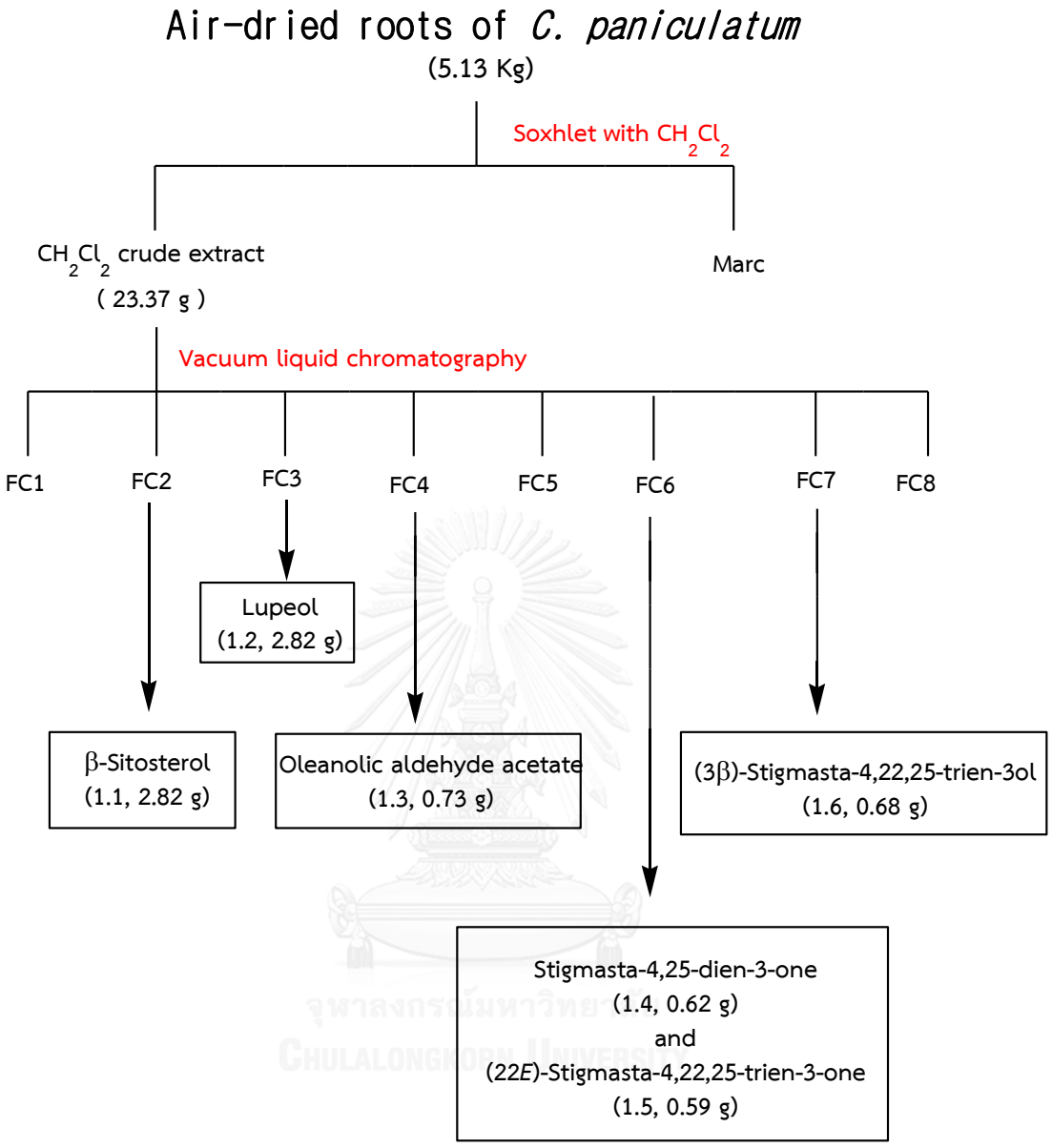


Figure 2.1 Extraction procedure of *C. paniculatum* roots

2.3.2 *W. trichostemon* Miq

The air-dried and powdered stem bark of *W. trichostemon* (4.23 kg.) was extracted with EtOAc and acetone at room temperature for one week (each 5L x 3). The EtOAc and acetone extracts were concentrated under reduced pressure to give a dark-brown gum of EtOAc (195.58 g) and acetone (103.10 g) crude extracts. The EtOAc crude extract (195.58 g) was purified by open CC eluting with 20:80, 50:50 and 100:0 EtOAc-hexane to give 8 major fractions (A-H). Fraction C (7.9 g) was separated by CC over silica gel, eluting with 20:80 EtOAc-hexane to furnish 11α, 20-dihydroxydammar-24-ene-3-one (10.36 mg) (**2.8**). Fraction D (9.5 g) was separated by CC over silica gel eluting with 20:80 and 40:60 EtOAc-hexane to yield 10 sub-fractions (D1-D10) and then sub-fraction D5 was purified by chromatotron using an isocratic system (50:50 EtOAc-hexane) as eluent to give sapelin E acetate (8.46 mg) (**2.6**) and α-mangostin (7.15 mg) (**2.7**). Fraction E (12.2 g) was subjected to CC over silica gel eluting with 30:70 EtOAc-CH_2Cl_2 to afford 5 sub-fractions (E1-E5). Sub-fraction E3 (1.8 g) was purified by preparative TLC eluted with a hexane-CH_2Cl_2-MeOH (20:70:10) gradient system to furnish grandifolinolenenone (7.85 mg) (**2.4**). Fraction G (9.95 g) was separated by CC over silica gel, eluting with 80:20 EtOAc-hexane to furnish kaempferol (8.76 mg) (**2.9**) and mangstenone F (3.28 mg) (**2.2**). Finally, the acetone crude extract (103.15 g) was fractionated by open silica gel CC to obtain 6 major fractions (I-N). Fraction J was separated by CC over silica gel using 20:80, 50:50 and 100:0 EtOAc-CH_2Cl_2 as eluent to yield 7 sub-fractions (J1-J7). Then, sub-fraction J3 was purified by preparative TLC eluting with hexane-CH_2Cl_2-MeOH (10:80:10) to give trichostemonoate (7.58 mg) (**2.1**). Fraction K was further subjected to silica gel CC (EtOAc : MeOH, 0.7:0.3) and Sephadex LH-20 to afford cholest-4-en-6β-ol-3-one (4.08 mg) (**2.5**). Fraction M (1.08 g) was purified by preparative TLC eluted with hexane-CH_2Cl_2-MeOH (10:60:30) gradient system to furnish desmethoxy kaunugin (2.95 mg) (**2.3**).

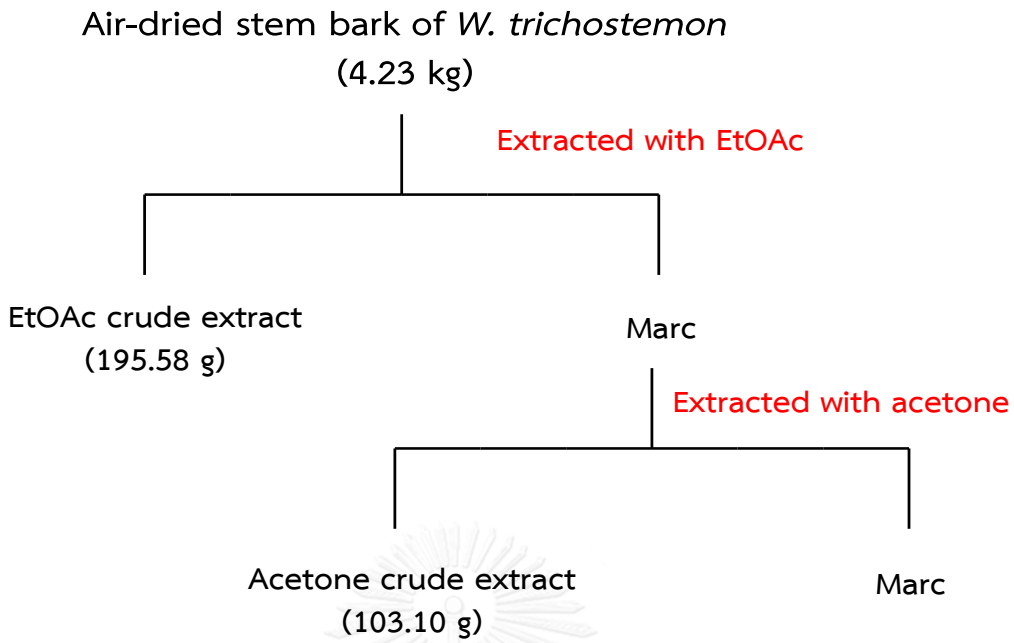


Figure 2.2 Extraction procedure of *W. trichostemon* stem bark

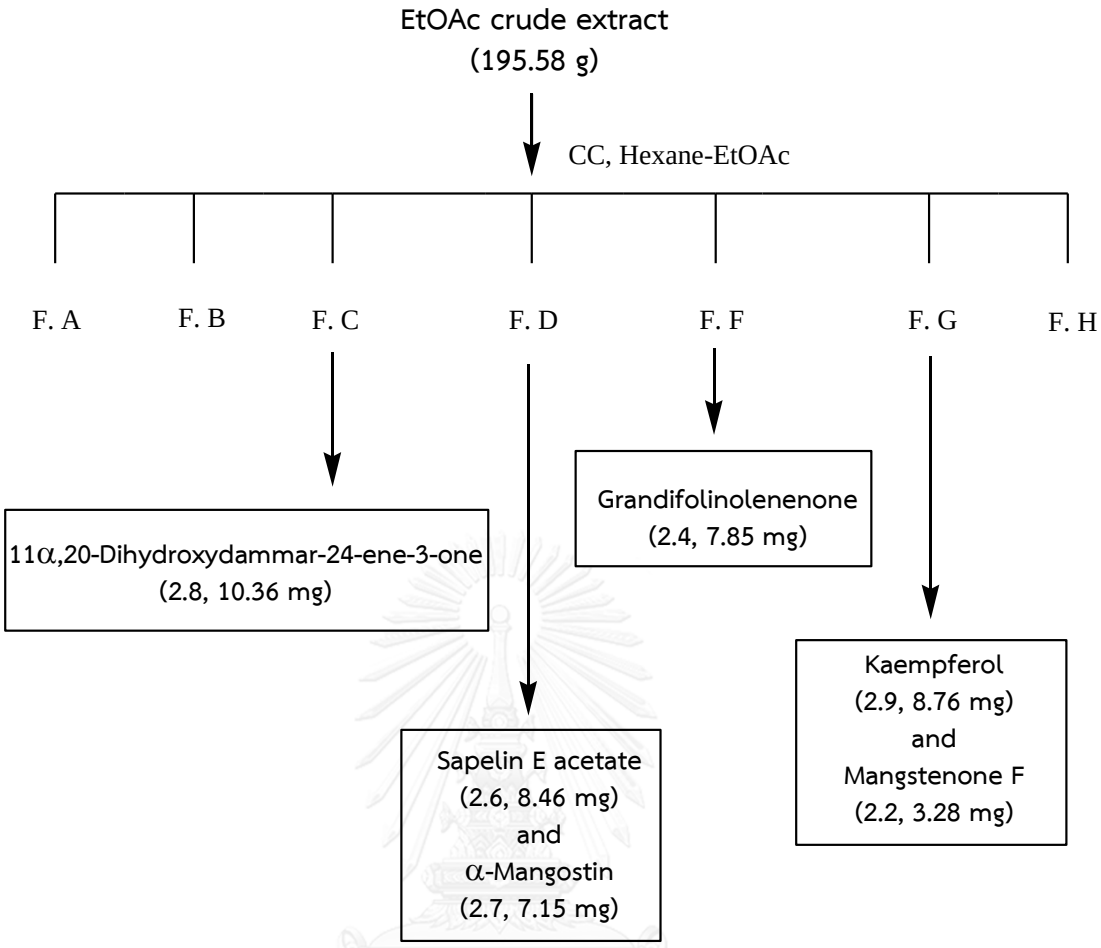


Figure 2.3 Isolation procedure of the EtOAc extract from *W. trichostemon* stem bark

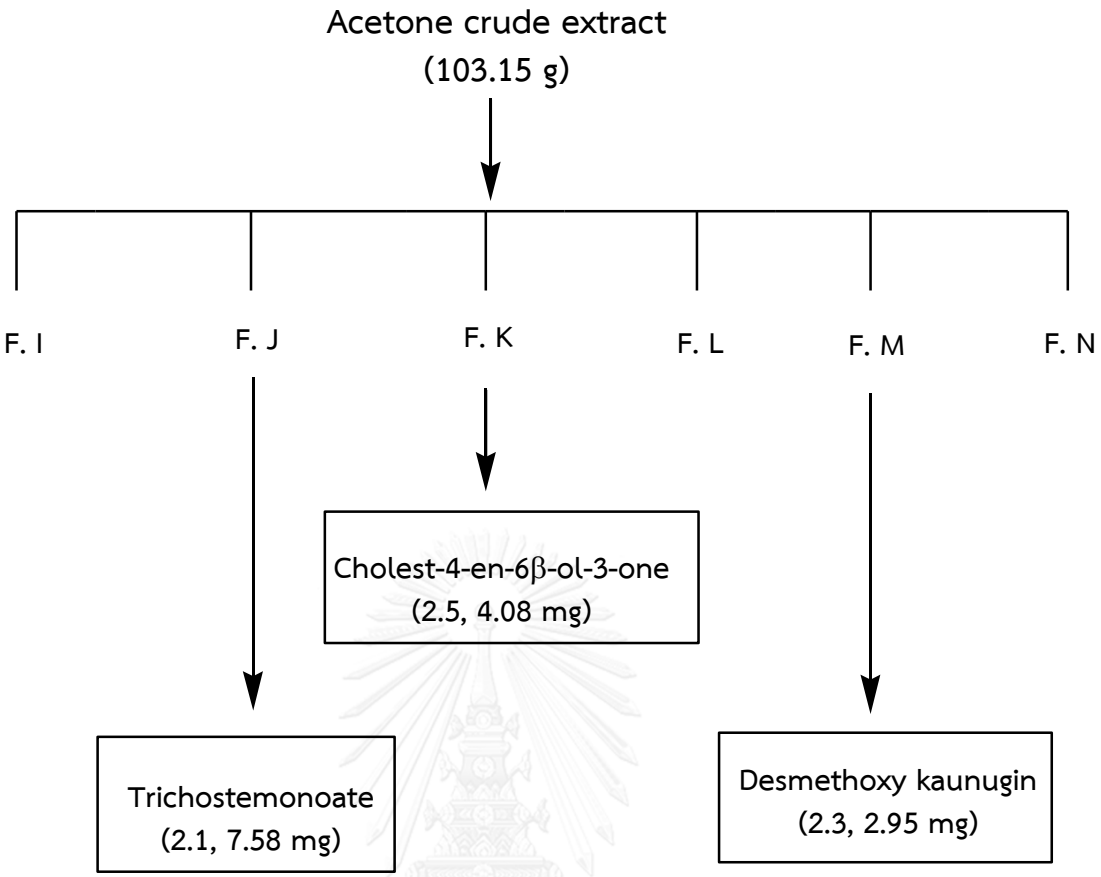


Figure 2.4 Isolation procedure of acetone extract from *W. trichostemon* stem bark

2.3.3 *B. sperba* Roxb.

The air-dried and powdered tuber of *B. sperba* (2.40 kg.) was extracted with CH_2Cl_2 and MeOH at room temperature for one week (each 5L x 2). The CH_2Cl_2 and MeOH extracts were concentrated under reduced pressure to give a brown gum of CH_2Cl_2 (78.54 g), acetone (65.59 g) and MeOH (87.10 g) crude extracts. The CH_2Cl_2 crude extract was chromatographed by vacuum liquid chromatography (VLC) on silica gel (Merk 7730), eluted with the gradient systems of hexane, CH_2Cl_2, EtOAc and MeOH to provide 7 fractions (A-G). Fraction C was purified by chromatotron using an isocratic system (30:70 EtOAc-hexane) as eluent to give (6αR, 11αR)-medicarpin (12.63

mg) (**3.2**) and 8-*O*-methylretusin (9.25 mg) (**3.1**). Fraction D (1.95 g) was purified by preparative TLC eluted with a hexane-CH_2Cl_2-MeOH (25:70:5) gradient system to furnish β-sitosterol (7.94 mg) (**1.1**). Fraction F was purified by open CC eluting with 50:50, 40:60, 30:70 and 100:0 hexane-CH_2Cl_2 to give 6 major sub-fractions (F1-F7). Fraction F3 (8.69 g) was separated by CC over silica gel, eluting with 40:60 and 30:70 Hexane-CH_2Cl_2 to yield 2,3-dihydroxypropyl hexacosanoate (3.45 mg) (**3.4**). Fraction G was purified by chromatotron using an isocratic system (50:50 EtOAc-hexane) as eluent to afford formononetin (12.63 mg) (**3.3**).

The acetone crude extract was purified by open CC eluting with, 10:90 and 15:85 MeOH-CH_2Cl_2 to give 6 major fractions (A-F). Fraction C (7.28 g) was subjected to CC over silica gel eluting with 15:85 MeOH-CH_2Cl_2 to afford 6 sub-fractions (C1-C6). Sub-fraction C4 (1.09 g) was purified by preparative TLC eluted with hexane-CH_2Cl_2-MeOH (5:80:15) gradient system to furnish ononin (6.95 mg) (**3.5**).

The methanolic extract was dissolved in water and the aqueous layer of *B. superba* was subjected to Diaion HP-20 column and successively eluted with water and MeOH. The MeOH extract from dissolved in water (25.46 g) was subjected to silica gel CC, using EtOAc-MeOH-H_2O (9:1:0, 40:10:1 and 70:30:3, respectively) to afford 5 major fractions (M-Q). Fraction N was purified using flash column chromatography eluted with 15:85 MeOH-CH_2Cl_2 to yield daidzein-7-*O*-glucoside (9.25 mg) (**3.6**).

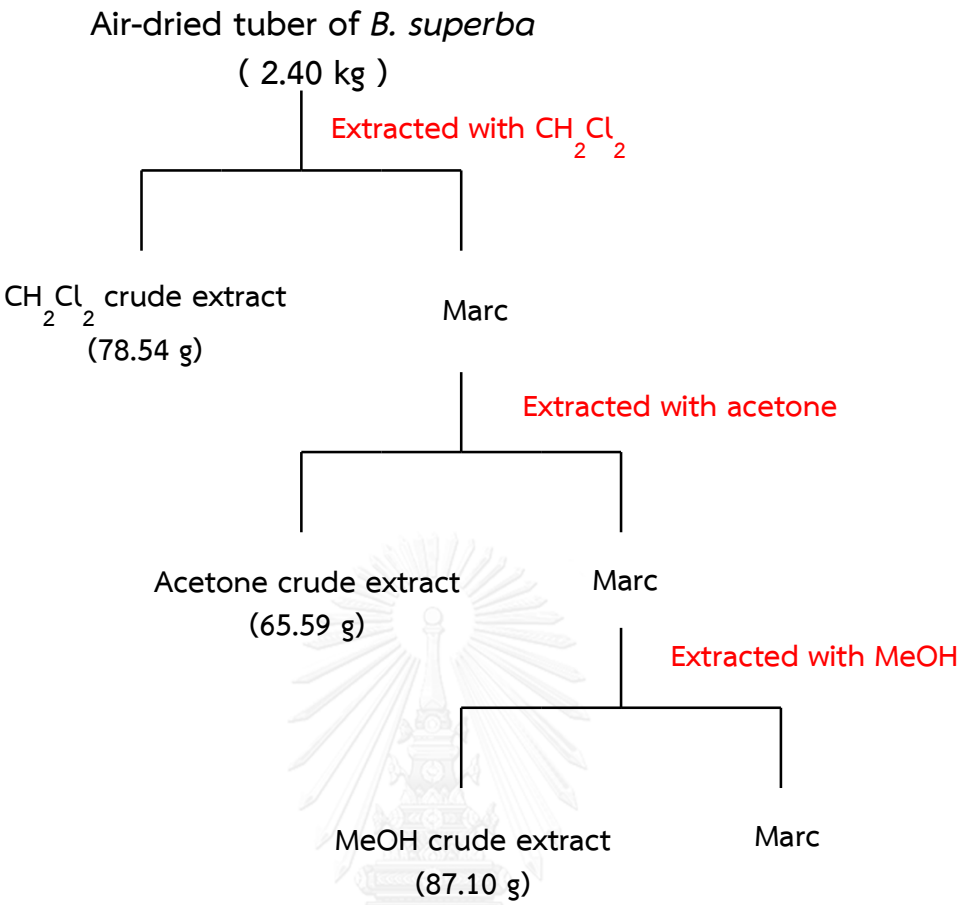


Figure 2.5 Extraction procedure of *B. superba* tuber

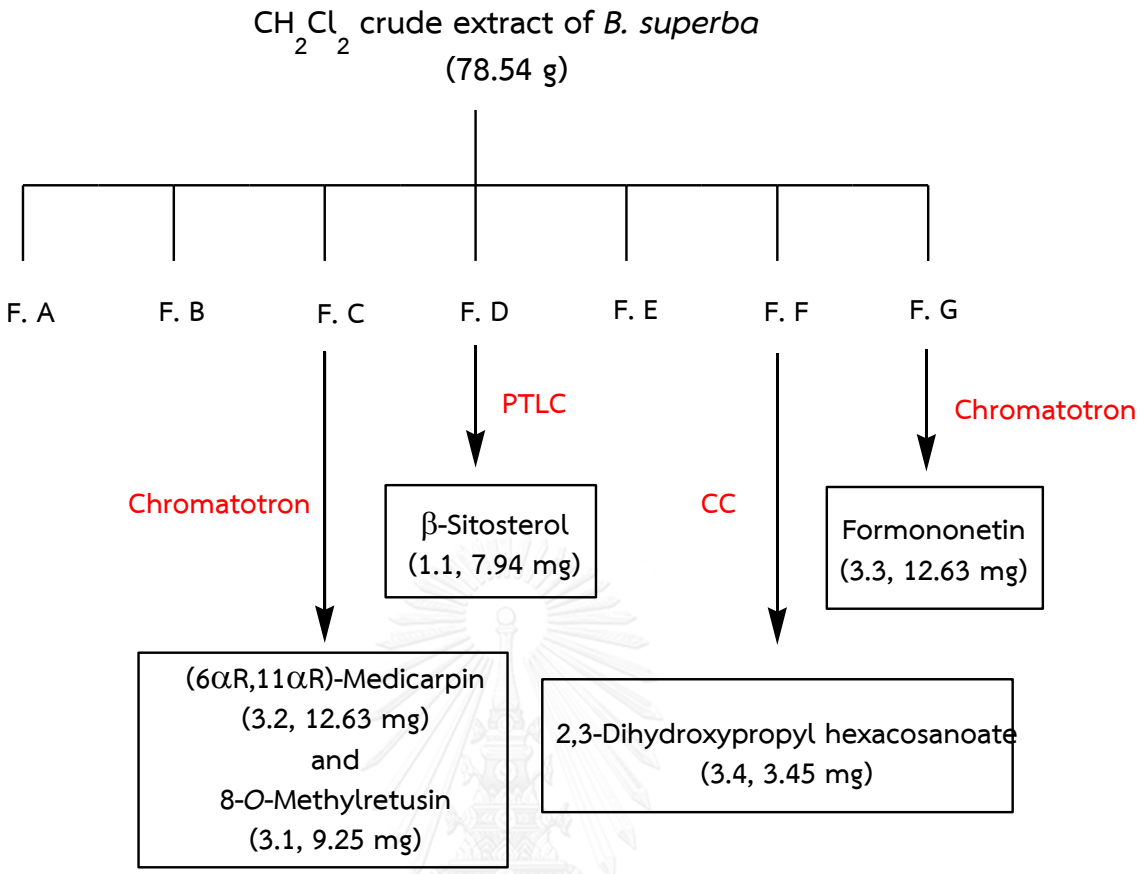


Figure 2.6 Isolation procedure of the CH_2Cl_2 crude extract from *B. superba* tuber

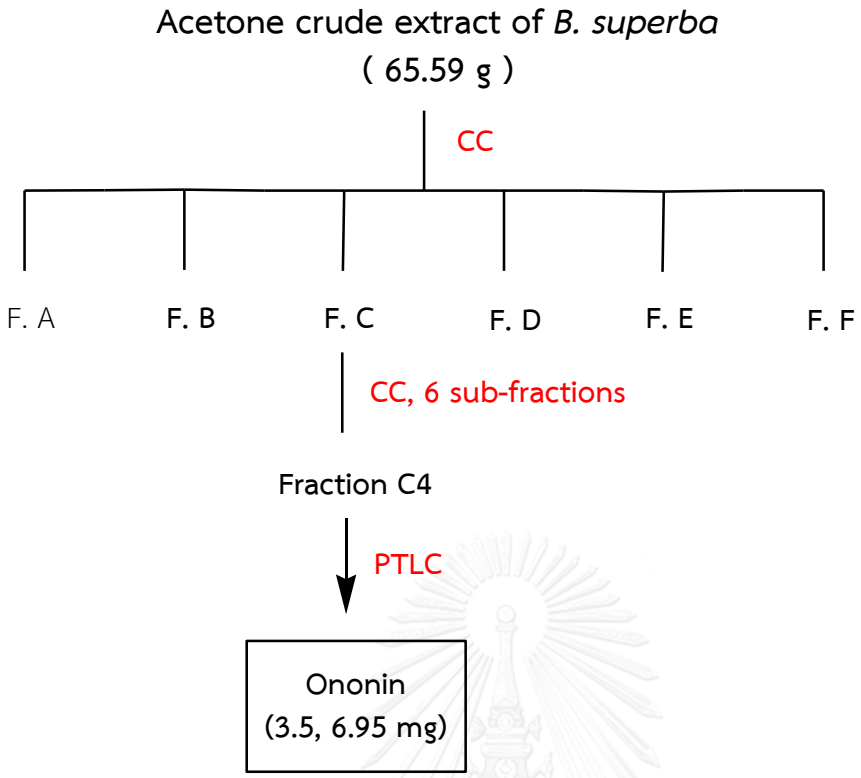


Figure 2.7 Isolation procedure of the acetone crude extract from *B. superba* tuber

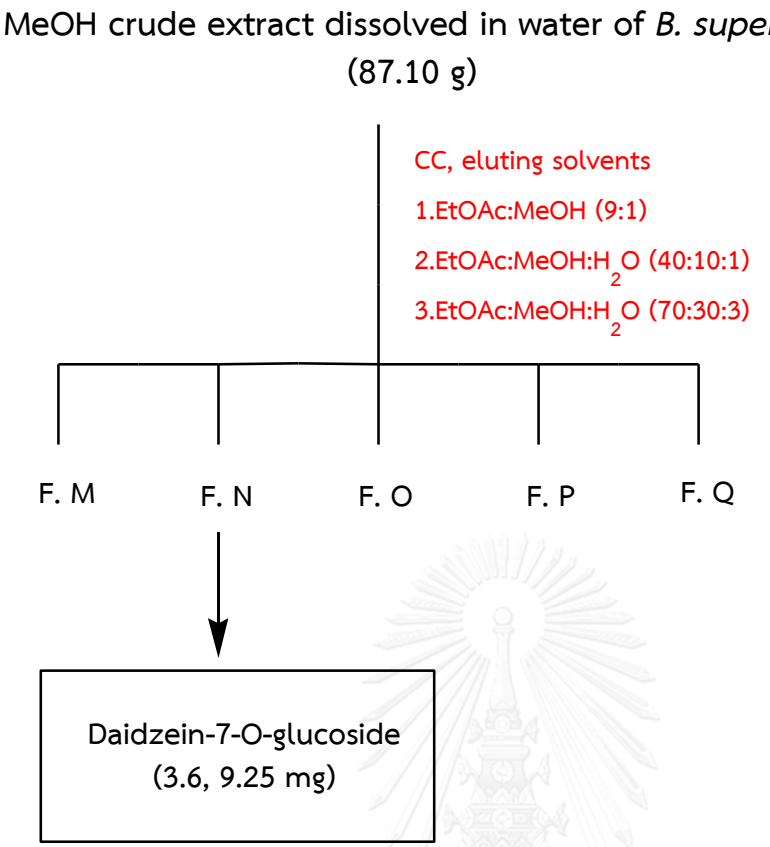


Figure 2.8 Isolation procedure of the MeOH crude extract from *B. superba* tuber

2.4 Cytotoxicity assay

The isolated compounds were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma) and HeLa-S-3 (human cervical carcinoma). Some compounds were tested with COLO 205 (human colorectal adenocarcinoma), MCF-7 (human breast adenocarcinoma), HepG2 (human hepatocellular carcinoma), LLC (mouse Lewis lung carcinoma) cell lines and Vero cell (normal cell derived from the kidney of an African green monkey) employing the colorimetric method. Adriamycin and doxorubicin were used as the reference substances, which exhibits activity against these cell lines. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Sigma Chemical Co., USA) was dissolved in saline to make a 5 mg/mL stock solution. Cancer cells (3×10^3 cells) suspended in 100 μg/wells of MEM medium containing 10% fetal calf serum (FCS, Gibco BRL, Life Technologies, NY, USA) were seeded onto a 96-well culture plate (Costar, Corning Incorporated, NY 14831, USA). After 24 h pre-incubation at 37°C in a humidified atmosphere of 5% CO_2/95% air to allow cellular attachment, various concentrations of test solution (10 μL/well) were added and these were then incubated for 48 h under the above conditions. At the end of the incubation, 10 μL of tetrazolium reagent was added into each well followed by further incubation at 37°C for 4 h. The supernatant was decanted, and DMSO (100 μL/well) was added to allow formosan solubilization. The optical density (OD) of each well was detected using a microplate reader at 550 nm and for correction at 595 nm. Each determination represented the average mean of 6 replicates. The 50% inhibition concentration (IC_{50} value) was determined by curve fitting [38]. This assay was kindly performed by Natural Products Research Section, Research Division, National Cancer Institute, Thailand.

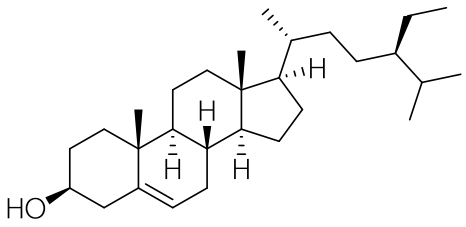
CHAPTER III

RESULTS AND DISCUSSION

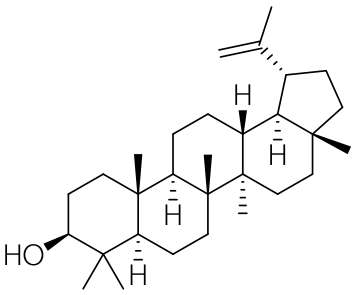
3.1 Extraction and isolation of *C. paniculatum* Linn.

The dried roots of *C. paniculatum* Linn. (5.13 kg) were extracted with CH_2Cl_2 at room temperature. The solvent was evaporated in vacuo to give CH_2Cl_2 extract (23.37g). This extract was chromatographed on silica gel column eluted with the gradient systems of Hexane, CH_2Cl_2, EtOAc and MeOH. Then, the compounds were separated and purified with various chromatrographic methods such as open column chromatrography, preparative thin layer chromatography, chromatotron and Sephadex LH-20 to afforded compounds 1-6. The six compounds were found to be identical with *β*-sitosterol (**1.1**), lupeol (**1.2**), oleanolic aldehyde acetate (**1.3**), stigmasta-4,25-dien-3-one (**1.4**), (22*E*)-stigmasta-4,22,25-trien-3-one (**1.5**) and (3*β*)-stigmasta-4,22,25-trien-3ol (**1.6**). The isolated compounds were identified by a combination of spectroscopic methods (^{1}H NMR, ^{13}C NMR and MS) as well as compared with the literature and also compared with an authentic sample.

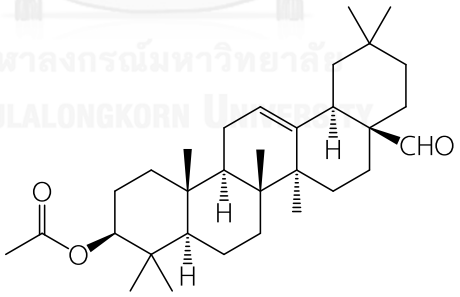
3.1.1 Properties and structure elucidation of isolated compounds from *C. paniculatum* Linn.



***β*-Sitosterol** (**1.1**) [39]: colorless needles. ESI-MS *m/z* 415[M +H]$^{+}$. This compound is identical to an authentic sample.

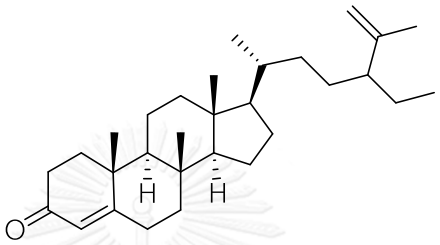


Lupeol (**1.2**) [40]: colorless needles : ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ_H, ppm, *J*/Hz) 3.16 (1H, s, H-3), 0.66 (1H, d, *J* = 9.1 Hz, H-5), 2.35 (1H, d, *J* = 9.1 Hz, H-19), 0.94 (1H, s, H-23), 0.73 (1H, s, H-24), 0.80 (1H, s, H-25), 1.00 (1H, s, H-26), 0.92 (1H, s, H-27), 1.65 (1H, s, H-29), 4.55 (1H, brs, H-30), 4.65 (1H, brs, H-30). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ_C): 38.7 (C-1), 27.4 (C-2), 79.0 (C-3), 38.8 (C-4), 55.3 (C-5), 18.3 (C-6), 34.3 (C-7), 50.4 (C-9), 37.1 (C-10), 20.9 (C-11), 25.1 (C-12), 38.0 (C-13), 42.8 (C-14), 27.4 (C-15), 35.6 (C-16), 43.0 (C-17), 48.0 (C-18), 48.3 (C-19), 150.9 (C-20), 29.7 (C-21), 40.0 (C-22), 28.0 (C-23), 15.3 (C-24), 15.9 (C-25), 16.1 (C-26), 14.5 (C-27), 18.0 (C-28), 19.3 (C-29), 109.3 (C-30).

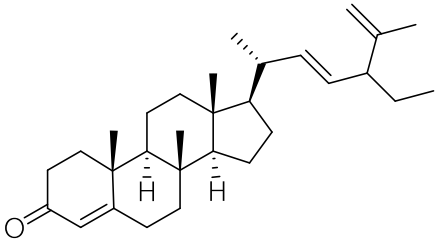


Oleanolic aldehyde acetate (**1.3**) [41] : white crystal : ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ_H, ppm, *J*/Hz) 9.42 (1H, s, C<u>H</u>O), 2.07 (3H, s, OCOC<u>H</u>$_3$), 1.89 (1H, d, *J* = 8.0 Hz, H-2), 4.50 (1H, t, *J* = 8.0 Hz, H-3), 1.46 (1H, dd, *J* = 16.0, 8.0 Mz, H-9), 1.98 (1H, dt, *J* = 16.0, 4.0 Mz, H-11), 5.36 (1H, d, *J* = 16.0 Hz, H-12), 2.66 (1H, d, *J* = 12.0 Hz, H-18), 1.89 (1H, d, *J* = 8.0 Hz, H-22), 0.90 (1H, s, H-23), 0.87 (1H, s, H-24), 0.94 (1H, s, H-25), 0.74 (1H, s, H-26), 1.16 (1H, s, H-27), 0.94 (1H, s, H-29), 0.93 (1H, s, H-30). ^{13}C NMR

spectrum (125 MHz, $CDCl_3$, δ_C): 28.1 (C-1), 23.4 (C-2), 80.9 (C-3), 33.2 (C-4), 49.5 (C-5), 17.0 (C-6), 30.6 (C-7), 41.4 (C-8), 45.6 (C-9), 30.6 (C-10), 25.5 (C-11), 123.2 (C-12), 143.0 (C-13), 45.6 (C-14), 33.0 (C-15), 26.7 (C-16), 55.3 (C-17), 32.7 (C-18), 38.2 (C-19), 23.5 (C-20), 38.2 (C-21), 21.4 (C-22), 21.3 (C-23), 21.3 (C-24), 22.1 (C-25), 18.2 (C-26), 16.7 (C-27), 207.5 (C-28), 27.8 (C-29) 27.8 (C-30), 15.4 ($OCO\underline{C}H_3$).

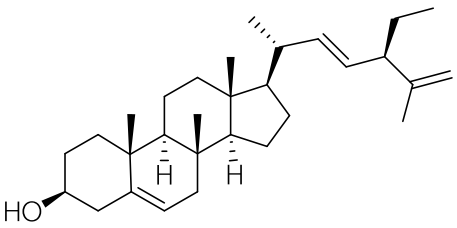


Stigmasta-4,25-dien-3-one (**1.4**) [42] : white powder : ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ_H, ppm, *J*/Hz) 5.66 (1H, s, H-4), 5.12-5.16 (1H, m, H 22/23), 1.63 (1H, s, CH_3-26). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ_C, ppm): 35.7 (C-1), 34.0 (C-2), 199.6 (C-3), 123.8 (C-4), 171.6 (C-5), 32.9 (C-6), 32.1 (C-7), 35.5 (C-8), 53.8 (C-9), 38.6 (C-10), 21.0 (C-11), 39.5 (C-12), 42.3 (C-13), 56.0 (C-14), 24.2 (C-15), 26.5 (C-16), 55.9 (C-17), 11.9 (C-18), 17.4 (C-19), 35.6 (C-20), 20.7 (C-21), 33.6 (C-22), 29.4 (C-23), 49.5 (C-24), 148.5 (C-25), 17.8 (C-26), 111.4 (C-27), 26.5 (C-28), 12.1 (C-29).



(22*E*)-Stigmasta-4,22,25-trien-3-one (**1.5**) [42] : white powder : ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ_H, ppm, *J*/Hz) 5.66 (1H, s, H-4), 5.12-5.16 (1H, m, H22/23), 4.63-4.66 (1H, m, H-27), 1.57 (1H, s, CH_3-26). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ_C): 35.7 (C-1), 33.6 (C-2), 199.6 (C-3), 123.8 (C-4), 171.6 (C-5), 32.9 (C-6), 32.1 (C-7), 35.6 (C-

8), 53.8 (C-9), 38.6 (C-10), 21.0 (C-11), 39.6 (C-12), 42.3 (C-13), 56.0 (C-14), 24.2 (C-15), 28.1 (C-16), 55.9 (C-17), 12.0 (C-18), 17.4 (C-19), 40.4 (C-20), 20.7 (C-21), 137.0 (C-22), 130.2 (C-23), 52.0 (C-24), 148.5 (C-25), 20.7 (C-26), 109.6 (C-27), 26.5 (C-28), 12.1 (C-29).



(3*β*)-stigmasta-4,22,25-trien-3ol (**1.6**) [42] : yellowish oil : ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ_H, ppm, *J*/Hz) 3.58 (1H, s, H-3), 5.29 (1H, d, *J* = 8.0 Hz, H-6), 5.11-5.20 (1H, m, H-22/23), 1.58 (1H, s, H-26), 4.62-4.63 (1H, m, H-27). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ_C): 36.3 (C-1), 30.7 (C-2), 70.8 (C-3), 41.3 (C-4), 139.8 (C-5), 120.7 (C-6), 30.9 (C-7), 30.9 (C-8), 49.2 (C-9), 35.5 (C-10), 20.1 (C-11), 38.7 (C-12), 41.3 (C-13), 55.8 (C-14), 23.4 (C-15), 27.7 (C-16), 54.9 (C-17), 11.1 (C-18), 18.4 (C-19), 39.2 (C-20), 19.8 (C-21), 136.2 (C-22), 129.1 (C-23), 51.0 (C-24), 147.6 (C-25), 19.2 (C-26), 108.5 (C-27), 24.7 (C-28), 11.1 (C-29).

3.1.2 Cytotoxicity against HeLa and KB cell lines of isolated compounds

The cytotoxicity against HeLa and KB cell lines of isolated compounds were determined using MTT colorimetric assays and the results were shown in Table 3.1

Table 3.1 *In vitro* cytotoxicity of isolated compounds against cancer cell lines

Isolated compounds	IC_{50}(μg/ml)[a]	
	KB cell line	HeLa cell line
β-Sitosterol (**1.1**)	26.63	45.37
Lupeol (**1.2**)	28.75	36.87
Oleanolic aldehyde acetate (**1.3**)	9.58	31.43
Stigmasta-4,25-dien-3-one (**1.4**)	46.35	40.63
(22*E*)-Stigmasta-4,22,25-trien-3-one (**1.5**)	46.35	40.63
(3*β*)-Stigmasta-4,22,25-trien-3ol (**1.6**)	13.14	28.52
Adriamycin	0.018	0.018

[a]inactive at > 100 μg/mL.(IC_{50} ≤ 4 = good activity, 4 < IC_{50} ≤ 15 = moderate activity, 15 < IC_{50} ≤ 30 = weak activity,IC_{50} > 30 = inactive)

All isolated compounds were tested for their cytotoxicity against KB and HeLa cell lines, oleanolic aldehyde acetate (**1.3**) showed significant cytotoxic activity against the KB cell line with IC_{50} value of 9.58 μg/mL. On the other hand, (3*β*)-stigmasta-4,22,25-trien-3ol (**1.6**) also exhibited moderate cytotoxic activity against the KB cell line with IC_{50} value of 13.14 μg/mL. The standard agent (adriamycin) showed the IC_{50} value of 0.018 μg/mL to both cell lines.

3.2 Extraction and isolation of *W. trichostemon* Miq

The air-dried and powdered stem bark of *W. trichostemon* (4.23 kg.) was extracted with EtOAc and acetone at room temperature for one week (each 5L x 3). The EtOAc and acetone extracts were concentrated under reduced pressure to give a dark-brown gum of EtOAc (195.58 g) and acetone (103.10 g) crude extracts. The EtOAc and acetone extracts were similarly chromatographed on silica gel VLC, rechromatographed on CC over silica gel and followed by chromatotron, to obtain a new natural product named trichostemonoate (**2.1**) along with eight known compounds, mangostenone F (**2.2**), desmethoxy kaunugin (**2.3**), grandifolinolenenone (**2.4**), Cholest-4-en-6β-ol-3-one (**2.5**), sapelin E acetate (**2.6**), α-mangostin (**2.7**), 11α,20-dihydroxydammar-24-ene-3-one (**2.8**) and kaempferol (**2.9**). The isolated compounds were identified by a combination of spectroscopic methods (^{1}H NMR, ^{13}C NMR and MS) as well as compared with the literature and also compared with an authentic sample.

3.2.1 Properties and structure elucidation of isolated compounds from *W. trichostemon* Miq.

Trichostemonoate (2.1)

Yellowish gum

$[\alpha]_{D}^{20}$ +32.0 (c 0.10, $CHCl_3$).

UV/Vis λ_{max} ($CHCl_3$) nm (log ε): 227 (3.02), 220 (2.49).

^{1}H and ^{13}C NMR: Table 3.2

HRESIMS: *m/z* [M+ Na^+] calcd for $C_{32}H_{48}O_6Na$: 551.3349; found: 551.32925

Table 3.2 ^{1}H and ^{13}C NMR data of 1 in $CDCl_3$

No.	δ_H (ppm), *J* (Hz)	δ_C (ppm)	No.	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1α	1.46, m	32.1	16	2.31, m	29.3
1β	1.64, m		17	2.63, dd (18.8, 10.0)	40.5
2α	1.70, m	22.4	18	0.90, s	19.8
2β	1.88, m		19	0.89, s	14.5
3	4.59, brs	78.9	20	1.90, m	39.6
4	-	36.1	21	0.75, d (6.8)	11.6
5	2.48, s	61.2	22	4.19, brs	78.5
6		200.4	23	-	200.8
7	5.70, d (3.5)[a]	125.4	24	6.08, brs	118.1
8	-	169.4	25	-	160.3
9	2.78, m	49.7	26	1.97, s	27.8
10	-	43.7	27	2.22, s	21.3
11α	1.52, m	28.2	28	1.21, s	25.9
11β	2.32, m		29	1.20, s	21.5
12	3.97, dd (11.5, 8.5)	73.6	30	1.16, s	27.8
13	-	47.6	3-OAc		170.4
14	-	52.9		20.7, s	21.6
11β	2.32, m		29	1.20, s	21.5
12	3.97, dd (11.5, 8.5)	73.6	30	1.16, s	27.8
15	1.59, m	34.1			

[a] H-7 is coupled to H-9.

Trichostemonoate (**2.1**) is a tirucallane triterpenoid and showed a pseudomolecular ion at *m/z* 551.3293 ($[M+Na]^+$), corresponding to a molecular formula of $C_{32}H_{48}O_6Na$. The ^{1}H NMR spectrum displayed the resonances of two olefinic protons [δ_H 5.70 (d, *J* = 3.5 Hz) and 6.08 (brs)], five methylene protons, and nine methyl groups comprised of eight methyl protons [δ_H 0.75, 0.89, 0.90. 1.16, 1.20, 1.21, 1.97 and 2.22] and one acetyl methyl proton [δ_H 2.07 (s)]. The ^{13}C NMR spectrum showed 32 distinct carbon resonances that were assigned to three carbonyls, two sp^2 quaternary carbons, four sp^3 quaternary carbons, two sp^2

methines, seven sp^3 methines, five sp^3 methylenes, and nine methyl centers, as shown in Table 3.2. The full analysis of both the 1D and 2D NMR spectra (Figure 3.1B) showed that **2.1** contained a tetracyclic system, which was essentially the same as that of trichostemonol [32], except for the presence of a 3-OAc instead of a 3-OH resonance. The occurrence of acetyl groups substituted at C-3 (δ_C 78.9) was determined by the HMBC correlation network from H-3 (δ_H 4.59 (brs)) to the acetyl carbonyl center at δ_C 170.4. In addition, the relative configuration of **2.1** was defined by analysis of its NOESY spectrum. The NOESY spectrum showed the correlation networks of CH_3-28/CH_3-19, CH_3-19/CH_3-30 and CH_3-30/H-17 of the tetracyclic core structure indicating that all were β-oriented. On the other hand, the observation of the correlations of H-3/H-1α/H-2α indicated that these protons were cofacial and assigned to the α-position consistent with the previously reported analogue. Thus, the chemical structure of **2.1** was concluded to be a new tirucallane triterpenoid, to which we assign the name of trichostemonoate.

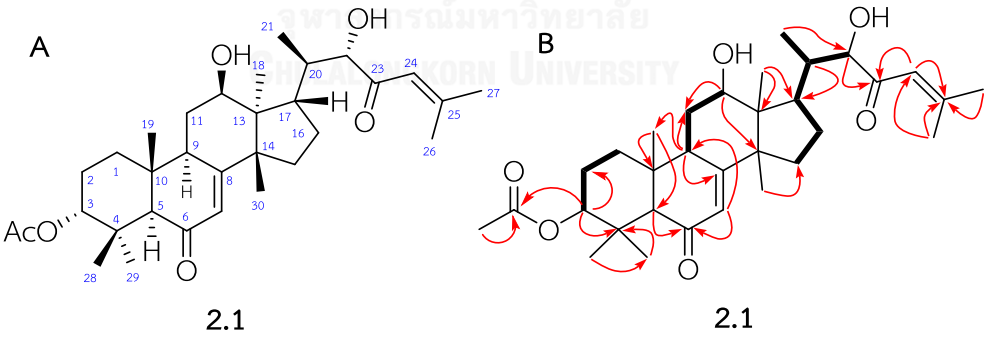
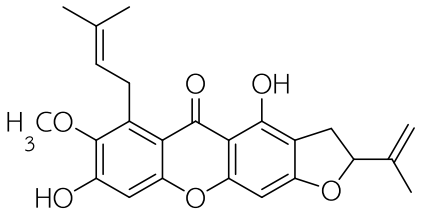
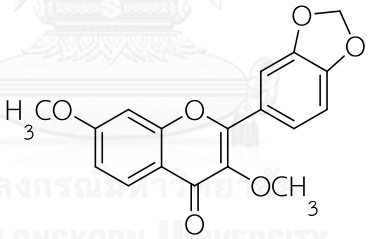


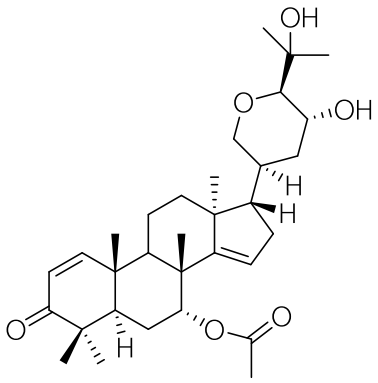
Figure 3.1 (A) Structure of **2.1** from the stem bark of *W. trichostemon*, and (B) Selected HMBC (arrows) and COSY (bold lines) correlations of **2.1**



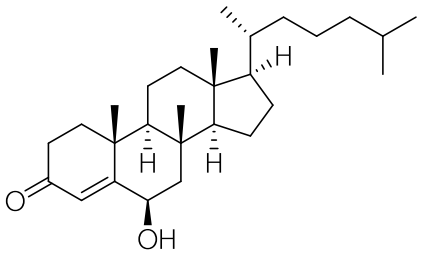
Mangostenone F (**2.2**) [43]: yellowish powder : ^{1}H-NMR (400 MHz, $CDCl_3$ δ_H, ppm, *J*/Hz) : 1.70 (3H, s, H-15), 1.68 (3H, s, H-19). 3.37 (1H, d, *J*=14.5 Hz, H-11a), 3.39 (1H, d, *J* = 8.0 Hz, H-11b), 3.74 (3H, s, OCH_3-7), 4.01 (2H, s, H-16), 4.03 (1H, m, H-12), 5.14 (1H, brs, H-14a), 5.28 (1H, brs, H-14b), 6.22 (1H, m, H-17), 6.18 (1H, s, H-4), 6.75 (1H, s, H-5), 13.47 (1H, s, 1-OH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ : 161.8 (C-1), 110.5 (C-2), 161.8 (C-3), 94.6 (C-4), 155.3 (C-4a), 101.8 (C-5), 155.3 (C-5a), 160.7 (C-6), 142.8 (C-7), 132.3 (C-8), 110.5 (C-8a), 182.2 (C=O, C-9), 101.9 (C-9a), 29.9 (C-10), 30.9 (C-11), 62.8 (C-12), 154.7 (C-13), 110.5 (C-14), 18.0 (C-15, 19), 26.8 (C-16), 129.0 (C-17), 137.3 (C-18), 26.7 (C-20), 62.1 (7-OCH_3).



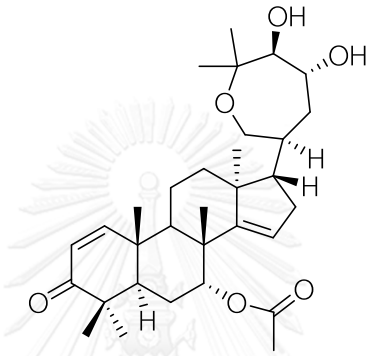
Desmethoxy kaunugin (**2.3**) [44] : white crystal : ^{1}H-NMR (400 MHz, $CDCl_3$) δ : 3.63 (3H, s, 3-OMe) 3.87 (3H, m, 7-OMe), 6.17 (2H, s, -O-CH_2-O-), 6.23 (1H, d, *J* =8.0 Hz, H-8), 6.49 (1H, d, *J* = 1.8 Hz, H-3'), 6.91 (1H, dd, *J* =8.0, 8.2 Hz, H-6), 7.79 (1H, d, *J* =8.8 Hz, H-6'), 7.45 (1H, d, *J* = 8.2 Hz, H-2'), 8.22 (1H, d, *J* = 1.8 Hz, H-5). ^{13}C-NMR (100 MHz, $CDCl_3$) **:** 156.7 (C-2), 141.3 (C-3), 173.2 (C-4), 126.9 (C-5), 113.9 (C- 6), 157.1 (C-7), 99.8 (C-8), 163.8 (C-9), 117.8 (C-10), 123.9 (C-1'), 122.8 (C-2'), 107.7 (C-3'), 148.8 (C-4'), 147.2 (C-5'), 108.0 (C-6'), 59.8 (3-OMe), 54.8 (7-OMe), 100.9 (-0-CH,-0-).



Grandifoliolenone (**2.4**) [45] : amorphous solid : ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H, *J*/Hz) : 7.09 (1H, d, *J* = 10.0 Hz, H-1), 5.79 (1H, d, *J* = 8.0 Hz, H-2), 5.27 (1H, d, *J* = 4.0 Hz, H-15), 5.16 (1H, d, *J* = 4.0 H-7), 3.91 (1H, d, *J* = 12.0 Hz, H-21b), 3.81 (1H, dd, *J* = 10.0, 4.8 Hz, H-23), 3.42 (1H, s, H-21a), 2.85 (1H, d, *J* = 8.0 Hz, H-24), 2.29 (1H, dd, *J* = 4.0, 8.0 Hz, H-16b), 2.26 (1H, dd, *J* = 5.2, 11.7 Hz, H-9), 2.16 (1H, dd, *J* = 2.1,1 2.7 Hz, H-5), 2.04 (1H, m, H-22b), 1.99 (1H, m, H-17), 1.97 (1H, m, H-16a), 1.96 (3H, s, OAc), 1.93 (1H, m, H-6b), 1.92 (1H, m, H-12 a), 1.90 (1H, m, H-11 a), 1.85 (1H, m, H-20), 1.74 (1H, d, *J* = 8.0 Hz, H-6a), 1.63 (1H, m, H-11b), 1.67 (1H, m, H-12b), 1.53 (1H, dd, *J* = 11.7, 10.8 Hz, H-22a), 1.26 (3H, s, H3-27), 1.29 (3H, s, H_3-26), 1.19 (3H, s, H_3-30), 1.19 (3H, s, H3-19), 1.09 (6H, m, H3-28, H3-29), 0.99 (3H, s, H3-18). ^{13}C-NMR (100 MHz, $CDCl_3$, **δ,** ppm) : 202.6 (C, C-3) 170.3 (C, O<u>C</u>OCH_3), 158.3 (C, C-14), 157.1 (CH, C-1), 125.5 (CH, C-2), 119.6 (CH, C-15), 86.6 (CH, C-24), 74.9 (CH, C-7), 70.9 (C, C-25), 70.1 (CH_2, C-21), 64.4 (CH, C-23), 52.5 (CH, C-17), 46.2 (C, C-13), 46.1 (CH, C-5), 44.5 (C, C-4), 42.9 (C, C-8), 38.7 (C, C-10), 36.3 (CH, C-9), 36.2 (CH_2, C-22), 35.9 (CH, C-20), 34.9 (CH_2, C-12), 34.9 (CH_2, C-16), 28.6 (CH_3, C-27), 27.3 (CH_3, C-30), 27.1 (CH_3, C-28), 24.1 (CH_3, C-26), 23.9 (CH_2, C-6), 21.2 (2C, CH_3, C-29, OCO<u>C</u>H_3), 20.3 (CH_3, C-18), 19.0 (CH_3, C-19), 16.9 (CH_2, C-11).

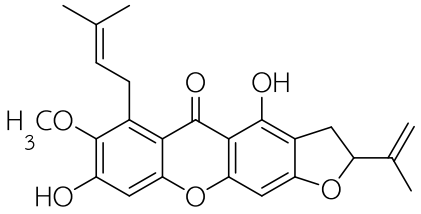


Cholest-4-en-6β-ol-3-one (**2.5**) [46] : yellow solid. ESI-MS *m/z* 415[M +H]$^{+}$. This compound is identical to an authentic sample.

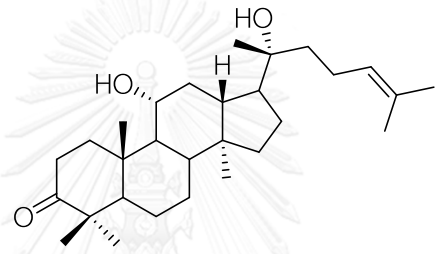


Sapelin E acetate (**2.6**) [47] **:** amorphous solid. ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H, ppm *J*/Hz) : 7.09 (1H, d, *J*= 8.0 Hz, H1), 5.78 (1H, d, *J* = 12.0 Hz, H-2), 5.30 (1H, d, *J* = 2.2 Hz, H-15), 5.22 (1H, s-like, H-7), 3.81 (1H, td, *J* = 9.0, 2.9 Hz, H-23), 3.61 (1H, dd, *J* = 12.9, 1.7 Hz, H-21a), 3.56 (1H, s, H-21b), 3.41 (1H, d, *J* = 8.0 Hz, H-24), 2.27 (1H, s, H-16b), 2.18 (1H, dd, *J* = 13.1, 5.4 Hz, H-9), 2.14 (1H, d, *J* = 14.5 Hz, H-5), 1.98 (1H, m, H-16a), 1.88 (1H, m, H-22a), 1.84 (3H, s, OAc), 1.92 (1H, m, H-6b), 1.89 (3H, m, H-11a , H-17, H-20), 1.83 (1H, m, H-12a), 1.74 (1H, d, *J* = 12.9, 2.6 Hz, H-6a), 1.72 (1H, m, H-11b), 1.62 (1H, m, H-12b), 1.61 (1H, m, H-22b), 1.26 (3H, s, H-27), 1.19 (3H, m, H-30), 1.14 (6H, s, H-19, H-26), 1.10 (6H, s, H-28, H-29), 1.00 (3H, s, H-18). ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm) : 203.5 (C, C-3), 158.3 (C, O<u>C</u>OCH_3), 158.2 (C, C-14), 157.4 (CH, C-1), 125.5 (CH, C-2),119.1 (CH, C-15), 80.8 (CH, C-24), 76.7 (C, C-25), 74.7 (CH, C-7), 68.2 (CH, C-23), 64.3 (CH_2, C-21), 54.3 (CH, C-17), 46.2 (C, C-13), 46.1 (CH, C-5), 44.2 (C, C-4), 42.5 (C, C-8), 39.9 (C, C-10), 38.6 (CH, C-9), 37.9 (CH_2, C-22), 36.4 (CH, C-20), 35.0 (CH_2, C-16), 34.1 (CH_2, C-12), 27.4 (CH_3, C-30), 27.0 (CH_3, C-28), 26.3 (CH_3, C-27), 23.8 (CH_2, C-

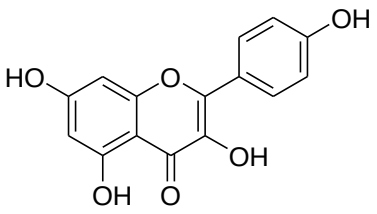
6), 22.4 (CH_3, C-26), 21.2 (CH_3, C-29), 21.3 (CH_3, $OCO\underline{C}H_3$), 19.9 (CH_3, C-18),19.1 (CH_3, C-19), 16.7 (CH_2, C-11).



α-Mangostin (**2.7**) [43] : yellowish needle. ESI-MS *m/z* 415$[M+H]^+$. This compound is identical to an authentic sample.



11α,20-dihydroxydammar-24-ene-3-one (**2.8**) [48] : amorphous solid : ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm, *J*/Hz : δ 5.46 (1H, dd, *J* =7.0, 8.0 Hz, H-24), 4.19 (OH), 3.36 (1H, dt, *J* =5 Hz, 10 Hz, H-11). ^{13}C-NMR 100 MHz, $CDCl_3$:, δ , ppm 217.7 (C-1), 132.1 (C-2), 124.8 (C-3), 74.8 (C-4), 70.7 (C-5), 55.4 (C-6), 53.4 (C-7), 51.6 (C-8), 49.4 (C-9), 48.1 (C-10), 47.4 (C-11), 39.8 (C-12), 39.7 (C-13), 31.0 (C-14), 34.1 (C-15), 34.3 (C-16), 34.2 (C-17), 31.5 (C-18), 31.2 (C-19), 27.2 (C-20), 26.7 (C-21), 26.5 (C-22), 25.8 (C-23), 22.4 (C-24), 21.2 (C-25), 19.8 (C-26), 17.9 (C-27), 16.8 (C-28), 16.0 (C-29), 14.8 (C-30).



Kaempferol (**2.9**) : white powder [41] : ESI-MS *m/z* 415$[M+H]^+$. This compound is identical to an authentic sample.

3.2.2 Cytotoxicity against cell lines of isolated compounds

Some isolated compounds were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma), HeLa (human cervical carcinoma), COLO 205 (human colorectal adenocarcinoma), HepG2 (human hepatocellular carcinoma) and LLC (Mouse Lewis lung carcinoma) cell lines employing the colorimetric method. Adriamycin was used as the reference substance.

Table 3.3 *In vitro* cytotoxicity of isolated compounds against cancer cell lines

Compounds	Anti-proliferation activity (IC_{50}; μg/mL)[a]				
	KB	HeLa	COLO 205	HepG2	LLC
2.1	4.4	3.8	5.3	16.2	5.5
2.4	41.2	24.1	13.6	20.4	22.5
2.6	22.3	18.9	64.0	>100	>100
2.7	13.5	19.7	6.6	19.5	5.1
2.8	2.0	1.9	3.7	17.4	3.2
Adriamycin	< 0.03	< 0.03	<0.03	0.31	<0.03

[a]inactive at > 100 μg/mL.(IC_{50} ≤ 4 = good activity, 4 < IC_{50} ≤ 15 = moderate activity, 15 < IC_{50} ≤ 30 = weak activity, IC_{50} > 30 = inactive).

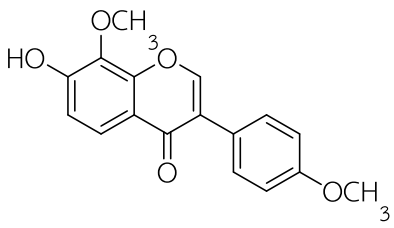
The isolated compounds were evaluated for their cytotoxicity against five cancer cell lines (Table 3.3). The new compound (**2.1**) displayed good potent cytotoxicity against only HeLa cells (IC_{50} 3.8 μg/mL) in the in *vitro* tumor cell panel and showed moderate cytotoxicity against KB, COLO 205 and LLC cells (IC_{50} 4.4, 5.3 and 5.5 μg/mL, respectively). This new compound was also more potent on both KB and HeLa cells than trichostemonol. On the other hand, compound **2.8** exhibited the most cytotoxicity toward KB, HeLa, COLO 205 and LLC cells (IC_{50} 2.0, 1.9, 3.7 and 3.2 μg/mL, respectively), while compound **2.4** displayed moderate cytotoxicity toward only COLO 205 cells (IC_{50} 13.6 μg/mL). In addition, compound **2.7** displayed

moderate cytotoxicity against KB, COLO 205 and LLC cells (IC_{50} 13.5, 6.6 and 5.1 μg/mL, respectively).

3.3 Extraction and isolation of *B. superba* Roxb.

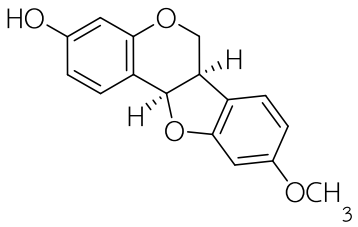
The air-dried and powdered tuber of *B. sperba* (2.40 kg) was extracted with CH_2Cl_2 and MeOH at room temperature for one week (each 5L x 2). The CH_2Cl_2 and MeOH extracts were concentrated under reduced pressure to give a brown gum of CH_2Cl_2 (78.54 g), acetone (65.59 g) and MeOH (87.10 g) crude extracts. The CH_2Cl_2 crude extract was chromatographed by vacuum liquid chromatography (VLC) on silica gel (Merk 7730), eluted with the gradient systems of hexane, CH_2Cl_2, EtOAc and MeOH to furnish β-sitosterol (**1.1**), 8-*O*-methylretusin (**3.1**), (6αR,11αR)-medicarpin (**3.2**), formononetin (**3.3**) and hexacosanoic acid 2,3-dihydroxy-propyl ester (**3.4**). The acetone crude extract was purified by open CC eluting with, 10:90 and 15:85 MeOH-CH_2Cl_2 to furnish ononin (**3.5**). The methanolic extract was dissolved in water and the aqueous layer of *B.superba* was subjected to Diaion HP-20 column and successively eluted with water and MeOH. The MeOH extract from dissolved in water (25.46 g) was subjected to silica gel CC, using EtOAc-MeOH-H_2O (9:1:0, 40:10:1 and 70:30:3, respectively) to afford daidzein-7-*O*-glucoside (**3.6**).

3.3.1 Properties and structure elucidation of isolated compounds from *B. superba*

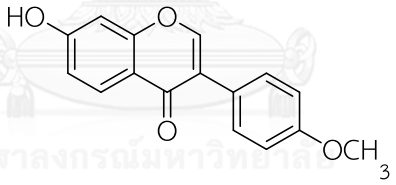


8-*O*-methylretusin (**3.1**) [49] : white solid, ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H, ppm *J*/Hz) : 3.76 (3H, *s*, C_4–OMe), 3.870 (3H, *s*, C_8–OMe), 7.00 (2H, d, *J* = 8.8 Hz, H-

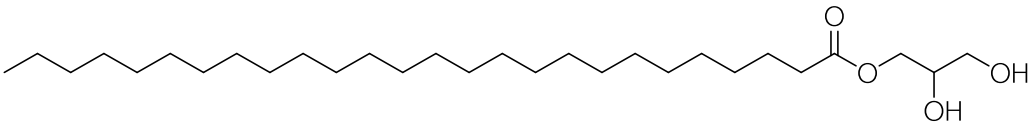
3′,5′), 7.14 (1H, d, *J* = 8.0 Hz, H-6), 7.29 (2H, d, *J* = 8.8 Hz, H-2′,6′), 7.46 (1H, d, *J* = 8.0 Hz, H-5), 8.39 (1H, *s*, H-2), 10.86 (1H, s, C_7–OH).



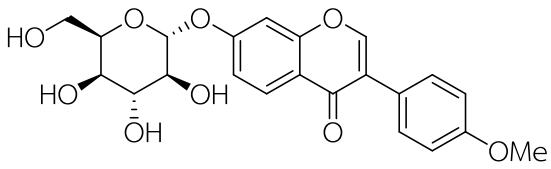
(6αR,11αR)-medicarpin (**3.2**) [50] **:** white solid; , ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H, ppm *J*/Hz) : 3.52 (1H, s, H-6a), 3.63 (1H, d, *J* = 10.8 Hz, H-6β), 3.75 (3H, s, OCH_3-9), 4.41 (1H, d, *J* = 8.0 Hz, H-6α), 5.35 (1H, d, *J* = 6.6 Hz, H-11a), 6.29 (1H, d, *J* = 2.4 Hz, H-4), 6.45 (2H, d, *J* = 6.6 Hz, H-8, H-10), 6.32 (1H, d, *J* = 6.6 Hz, H-2).



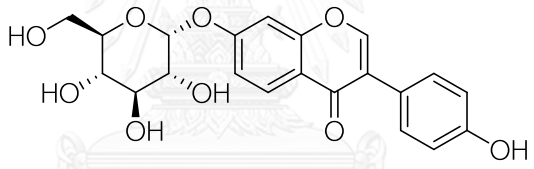
Formononetin (3.3) [36] **:** white powder**,** ESI-MS *m/z* 415[M +H]$^+$. This compound is identical to an authentic sample.



2,3-Dihydroxypropyl hexacosanoate (3.4) : yellowish oil, ESI-MS *m/z* 415[M+H]$^+$. This compound is identical to an authentic sample.



Ononin (**3.5**) [51] : white needles : ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H, ppm *J*/Hz) : 8.13 (1H, s, H-2), 8.02 (1H, s, H-5), 7.39 (2H, d, *J* = 8.0, H-2′, 6′), 7.16 (1H, m, H-8), 7.14 (1H, dd, *J* = 9.0, 8.0 Hz, H-6), 6.91 (2H, d, *J* = 10.8 Hz, H-3′, 5′), 5.16 (1H, d, *J* = 4.8, Glu), 3.96 (3H, s, OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, **δ,** ppm) : 163.5 (C-4), 161.2 (C-7), 159.3 (C-4′), 155.2 (C-9), 159.3 (C-2), 131.4 (C-2′, 6′), 128.3 (C-5), 125.3 (C-3), 120.3 (C-1′), 117.1 (C-10), 114.3 (C-6), 112.8 (C-3′, 5′), 103.2 (C-8), 101.9 (C-1″), 77.9 (C-3″), 74.9 (C-5″), 74.9 (C-2″), 71.3 (C-4″), 61.3 (C-6″), 55.8 (OCH3).



Daidzein-7-*O*-glucoside (**3.6**) [52] **:** white needles ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H, ppm *J*/Hz) : 3.31-3.94 (12H, *m*, H-2′, 3′, 4′, 5′, 6′), 4.20, 5.10 (1H, d, *J* = 8.0 Hz, H-1′), 8.20 (1H, *s*, H-2), 6.84 (2H, *d*, *J* = 8.6 Hz, H-13, 15), 7.21 (1H, *d*, *J* = 2.0 Hz, H-6), 7.25 (1H, d, *J* = 8.6 Hz, H-8), 7.39 (2H, d, *J* = 6.4 Hz, H-12, 16), 8.14 (1H, d, *J* = 8.0 Hz, H-5); ^{13}C-NMR (100 MHz, $CDCl_3$, **δ,** ppm) : 155.0 (C-1), 153.4 (C-2), 128.3 (C-3), 174.9 (C-4), 128.3 (C-5), 116.3 (C-6), 161.8 (C-7), 105.0 (C-8), 157.6 (C-9), 117.1 (C-10), 123.8 (C-11), 131.4 (C-12), 115.0 (C-13), 157.2 (C-14), 117.1 (C-15), 131.2 (C-16), 99.8 (C-1'), 73.6 (C-2'), 76.9 (C-3'), 69.2 (C-4'), 76.8 (C-5'), 68.2 (C-6').

3.3.2 Cytotoxicity against cell lines of isolated compounds

Table 3.4 *In vitro* cytotoxicity of isolated compounds against cancer cell lines

Compounds	Anti-proliferation activity (IC_{50}; μg/mL)[a]				
	KB	HeLa	MCF-7	HepG-2	Vero
3.1	>100	>100	>100	>100	>100
3.2	>100	>100	>100	>100	>100
3.3	>100	>100	>100	>100	>100
3.4	>100	>100	>100	>100	>100
3.5	60.92	>100	82.30	>100	>100
3.6	5.32	17.30	16.32	10.13	20.44
Adriamycin	0.125	0.050	0.103	0.306	5.684

[a] inactive at > 100 μg/mL.(IC_{50} ≤ 4 = good activity, 4 < IC_{50} ≤ 15 = moderate activity, 15 < IC_{50} ≤ 30 = weak activity, IC_{50} > 30 = inactive).

The isolated compounds were evaluated for their cytotoxicity against four cancer cell lines (Table 3.4). Compound **3.6** displayed moderate cytotoxicity against KB and HepG-2 (IC_{50} 5.32 and 10.13 μg/mL, respectively), whereas, the other compounds showed inactive activity.

CHAPTER IV
CONCLUSION

The phytochemical investigation of the CH_2Cl_2 crude extract from the roots of *C. paniculatum* Linn. afforded six known compounds, *β*-sitosterol (**1.1**), lupeol (**1.2**), oleanolic aldehyde acetate (**1.3**), the mixture of stigmasta-4,25-diene-3-one (**1.4**) and (22*E*)-stigmasta-4,22,25-trien-3-one (**1.5**) and (3*β*)-stigmasta-4,22,25-trien-3ol (**1.6**). All isolated compounds were tested for their cytotoxicity against KB and HeLa cell lines, oleanolic aldehyde acetate (**1.3**) showed significant cytotoxic activity against the KB cell line with IC_{50} value of 9.58 μg/mL. On the other hand, (3*β*)-stigmasta-4,22,25-trien-3ol (**1.6**) also exhibited moderate cytotoxic activity against the KB cell line with IC_{50} value of 13.14 μg/mL. The standard agent (adriamycin) showed the IC_{50} value of 0.018 μg/mL to both cell lines. To the best of our knowledge, this is the first report on chemical constituents and biological activity from this plant.

The various chromatographic techniques of EtOAc and acetone crude extracts from the bark of *W. trichostemon* Miq. led to the isolation of a new tirucallane, trichostomonoate (**2.1**), along with eight known compounds, mangstenone F (**2.2**), desmethoxy kaunugin (**2.3**), grandifolinolenenone (**2.4**), cholest-4-en-6*β*-ol-3-one (**2.5**), sapelin E acetate (**2.6**), α-mangostin (**2.7**) 11α,20-dihydroxydammar-24-ene-3-one (**2.8**) and kaempferol (**2.9**). The new compound (**2.1**) displayed the most potent cytotoxicity against only HeLa cells (IC_{50} 3.8 μg/mL) in the *in vitro* tumor cell panel and showed moderate cytotoxicity against KB, COLO 205 and LLC cells (IC_{50} 4.4, 5.3 and 5.5 μg/mL, respectively). The new compound (**2.1**) was also more potent on both KB and HeLa cells than trichostemonol. On the other hand, grandifolinolenenone (**2.4**) exhibited good cytotoxicity toward KB, HeLa, COLO 205 and LLC cells (IC_{50} 2.0, 1.9, 3.7 and 3.2 μg/mL, respectively), while α-mangostin (**2.7**)

displayed moderate cytotoxicity toward only COLO 205 cells (IC_{50} 13.6 μg/mL). In addition, 11α,20-dihydroxydammar-24-ene-3-one (**2.8**) displayed moderate cytotoxicity against KB, COLO 205 and LLC cells (IC_{50} 13.5, 6.6 and 5.1 μg/mL, respectively).

The isolation and purification of the CH_2Cl_2, acetone and MeOH extracts from the tuber of *B. superba* yielded the isolation of seven known compounds, *β*-sitosterol (**1.1**), 8-*O*-methylretusin (**3.1**), (6αR,11αR)-medicarpin (**3.2**), formononetin (**3.3**), 2,3-dihydroxypropyl hexacosanoate (**3.4**), ononin (**3.5**) and daidzein-7-*O*-glucoside (**3.6**). Compound **3.6** displayed moderate cytotoxicity against KB and HepG-2 (IC_{50} 5.32 and 10.13 μg/mL, respectively). In addition, compounds **1.1**, **3.1**, **3.2**, **3.5** and **3.6** were reported for the first time for this plant.

The phytochemical investigation of the CH_2Cl_2 crude extract from the roots of *C. paniculatum*, EtOAC and acetone crude extracts from the stem bark of *W. trichostemon*, and CH_2Cl_2, acetone and MeOH of *B. superba* led to the isolation one new tirucallane, trichostemonoate (**2.1**) along with 20 known compounds. Furthermore, compounds **2.8** exhibited the most cytotoxicity toward KB, HeLa, COLO 205 and LLC cells (IC_{50} 2.0, 1.9, 3.7 and 3.2 μg/mL, respectively).

Future work may involve the synthesis of the isolated compounds for increasing the quality, quantity and biological activity which could be developed into the source of drugs. This thesis will be a benefit for the database to study the chemical constituents of other plants in these genera.

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APPENDIX

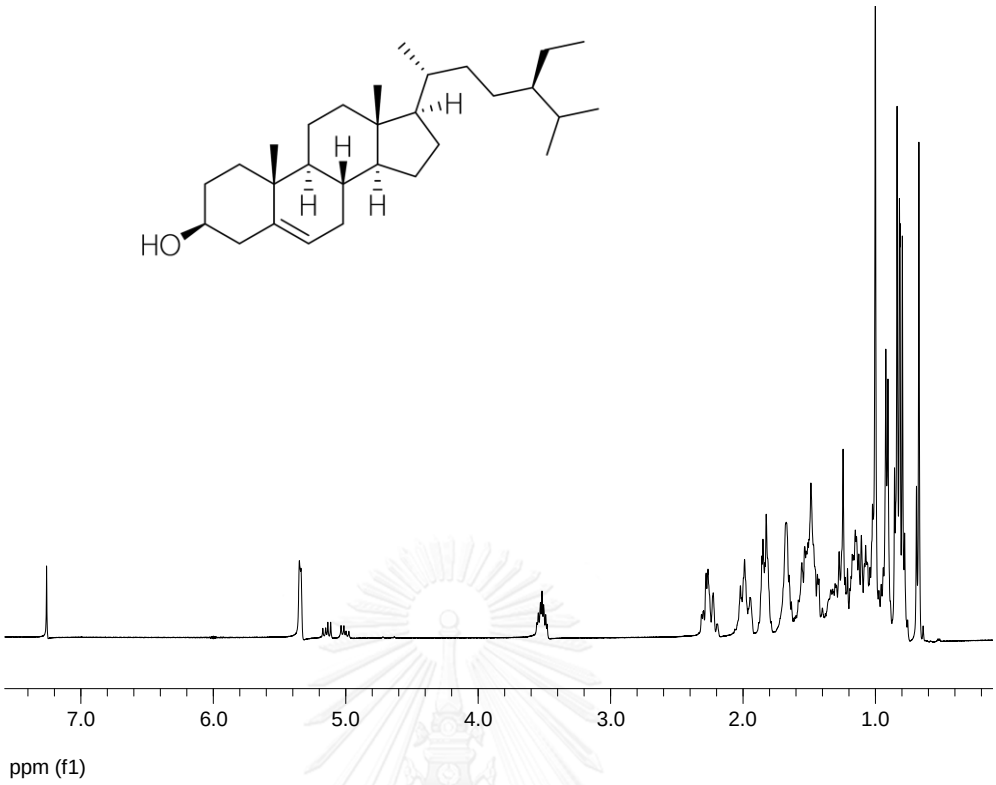

Figure A-1 ^{1}H NMR spectrum ($CDCl_3$) of 1.1

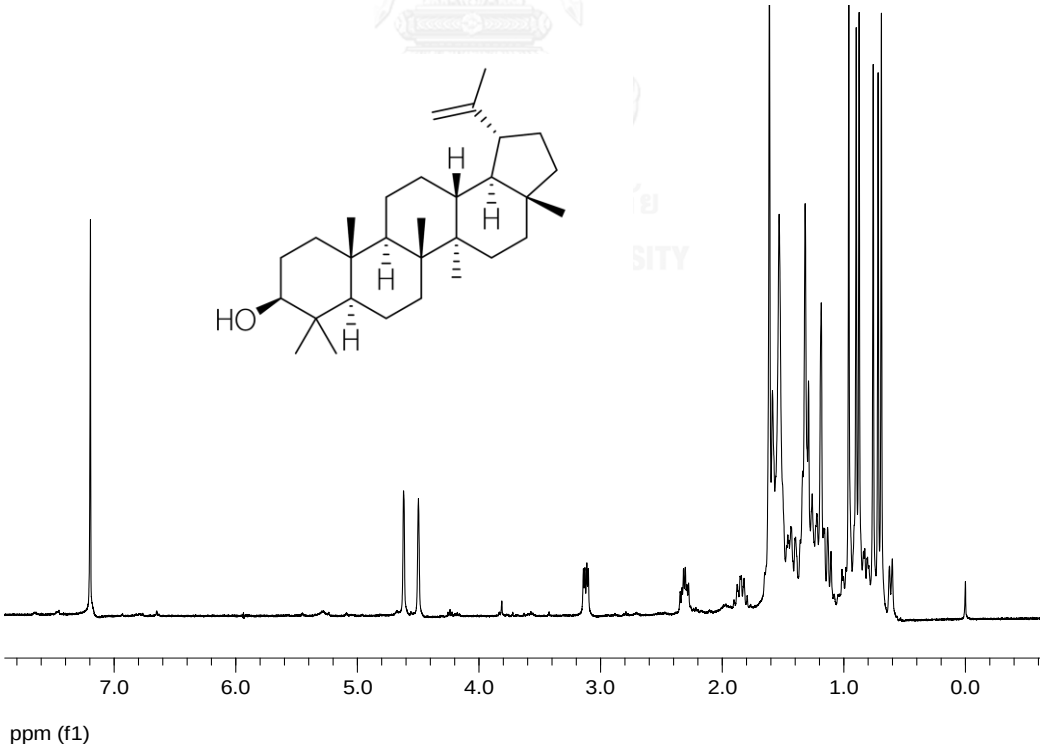

Figure A-2 ^{1}H NMR spectrum ($CDCl_3$) of 1.2

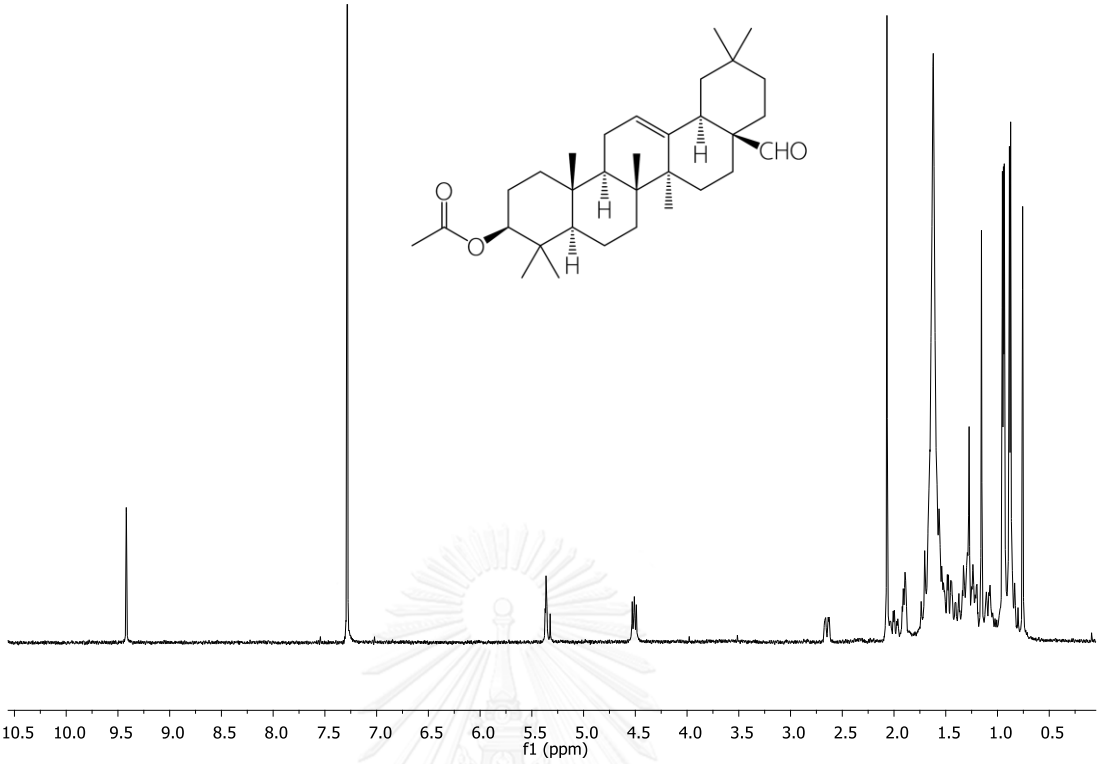

Figure A-3 ^{1}H NMR spectrum ($CDCl_3$) of 1.3

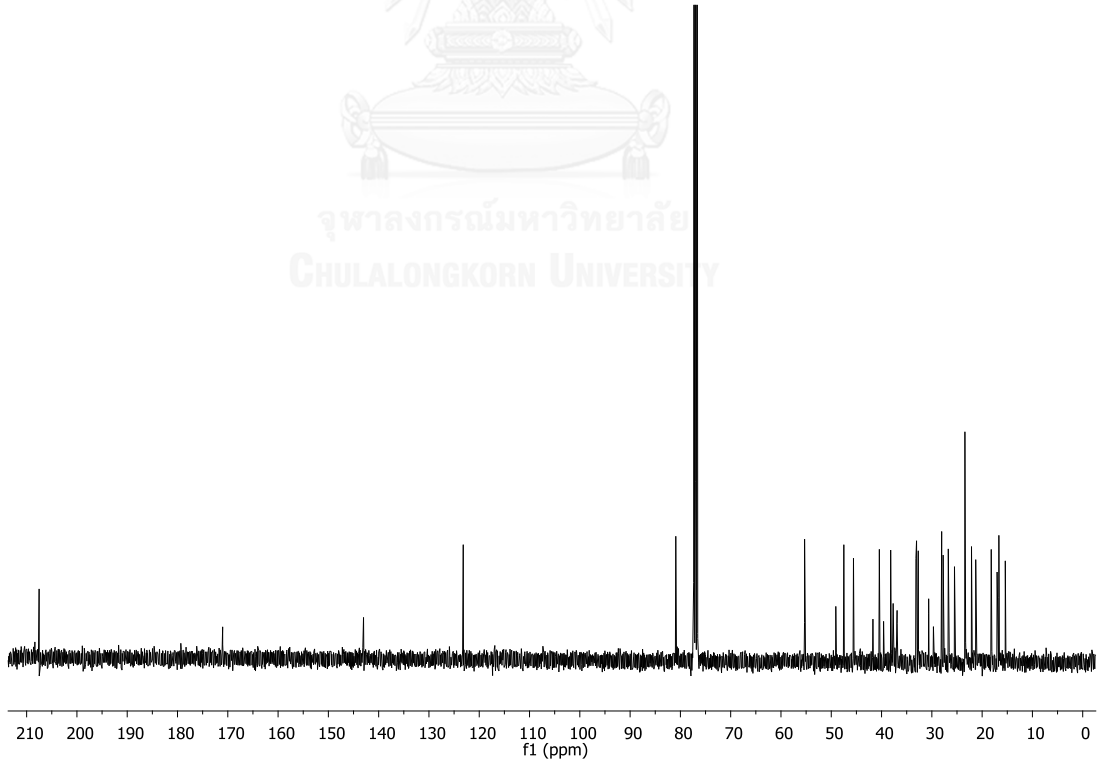

Figure A-4 ^{13}C NMR spectrum ($CDCl_3$) of 1.3

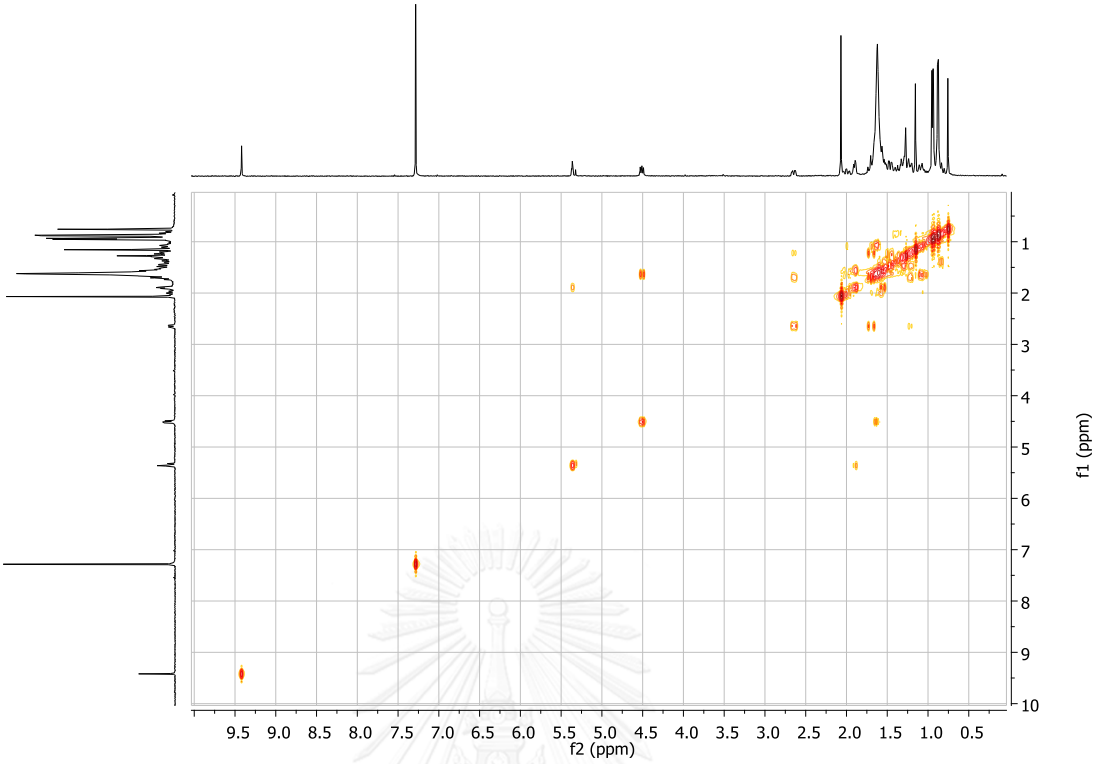

Figure A-5 COSY NMR spectrum ($CDCl_3$) of 1.3

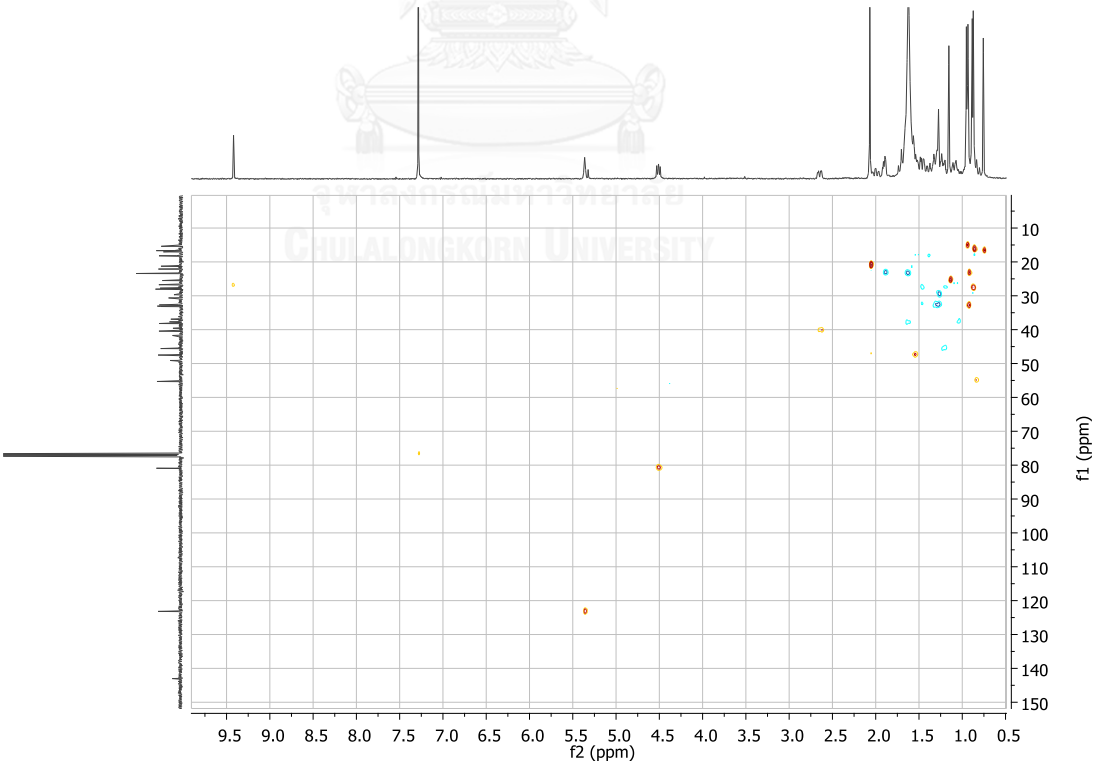

Figure A-6 HSQC NMR spectrum ($CDCl_3$) of 1.3

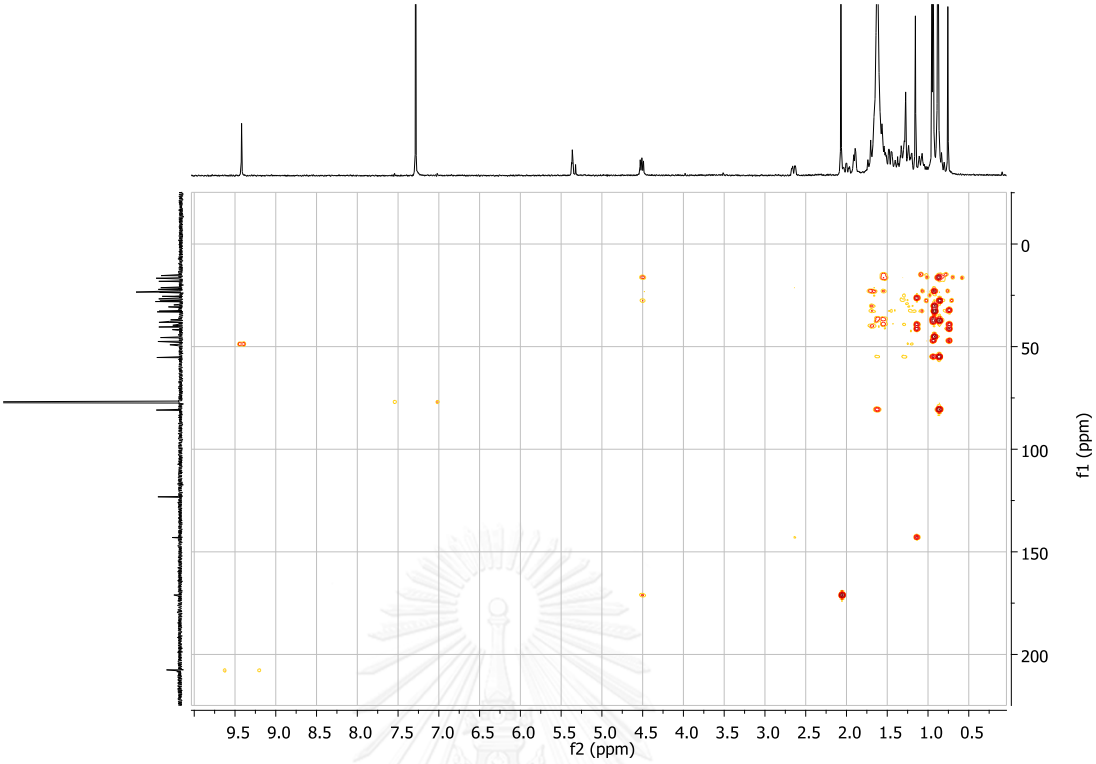

Figure A-7 HMBC NMR spectrum ($CDCl_3$) of 1.3

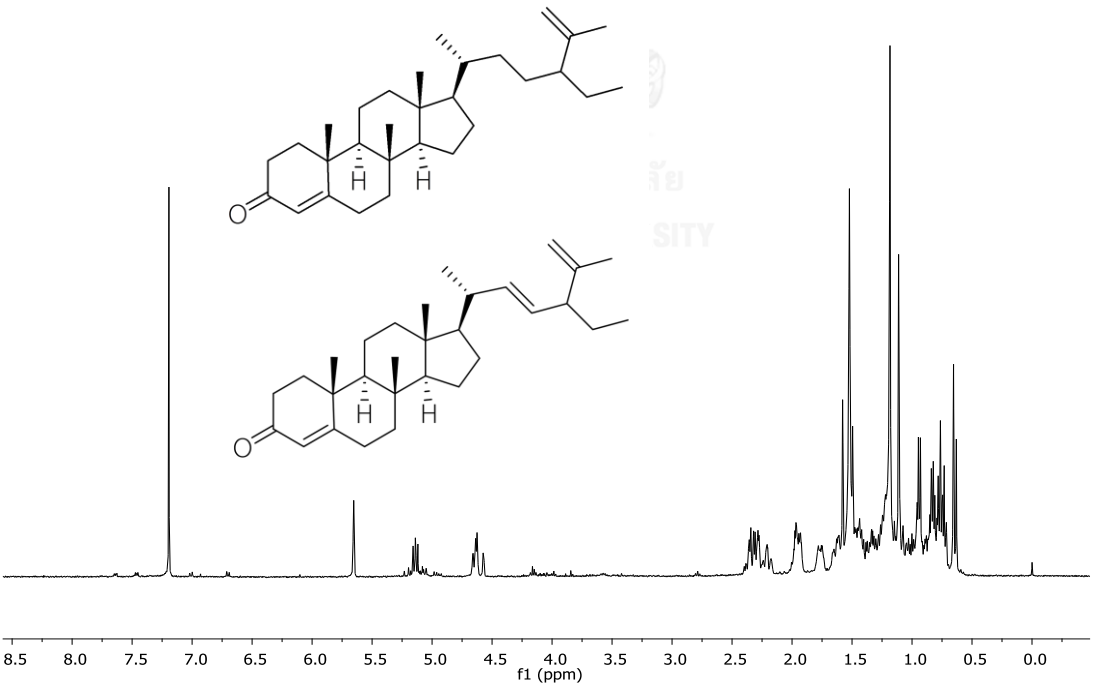

Figure A-8 ^{1}H NMR spectrum ($CDCl_3$) of a mixture of 1.4 and 1.5

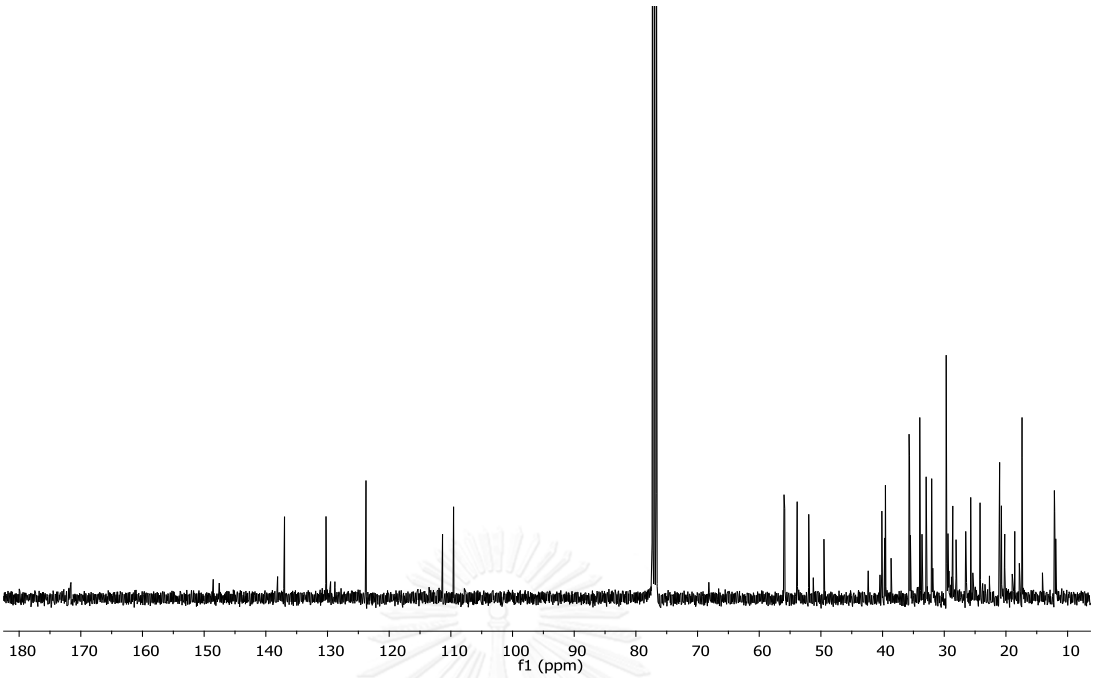

Figure A-9 ^{13}C NMR spectrum ($CDCl_3$) of a mixture of 1.4 and 1.5

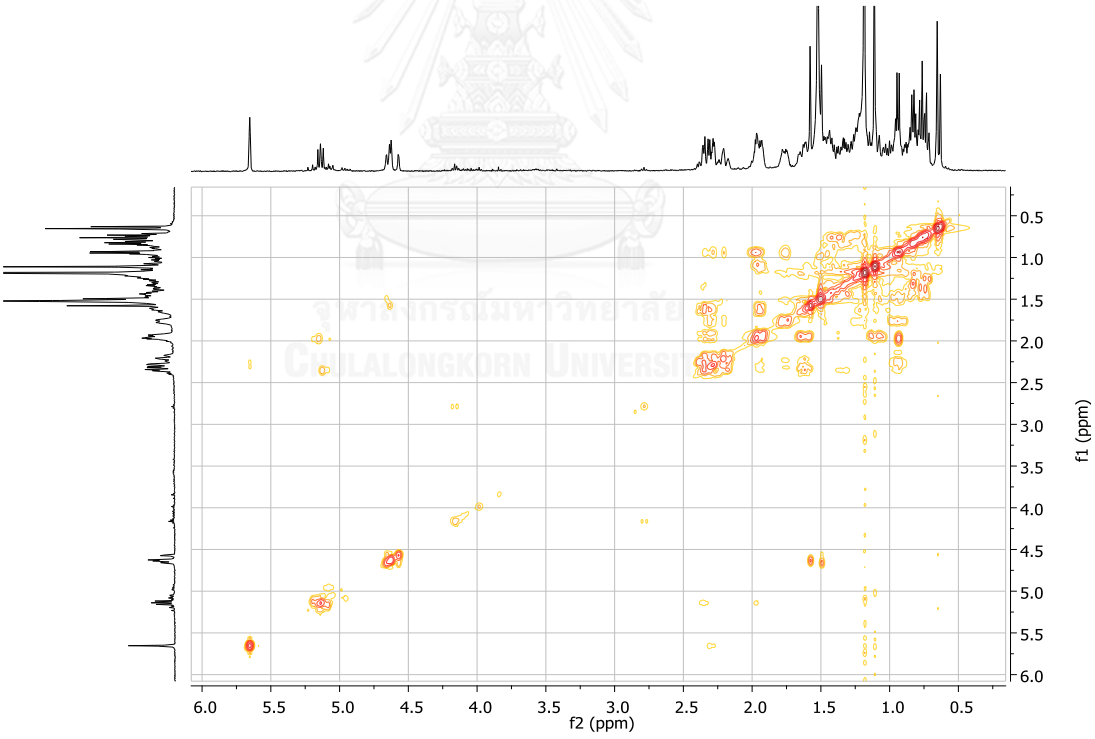

Figure A-10 COSY NMR spectrum ($CDCl_3$) of a mixture of 1.4 and 1.5

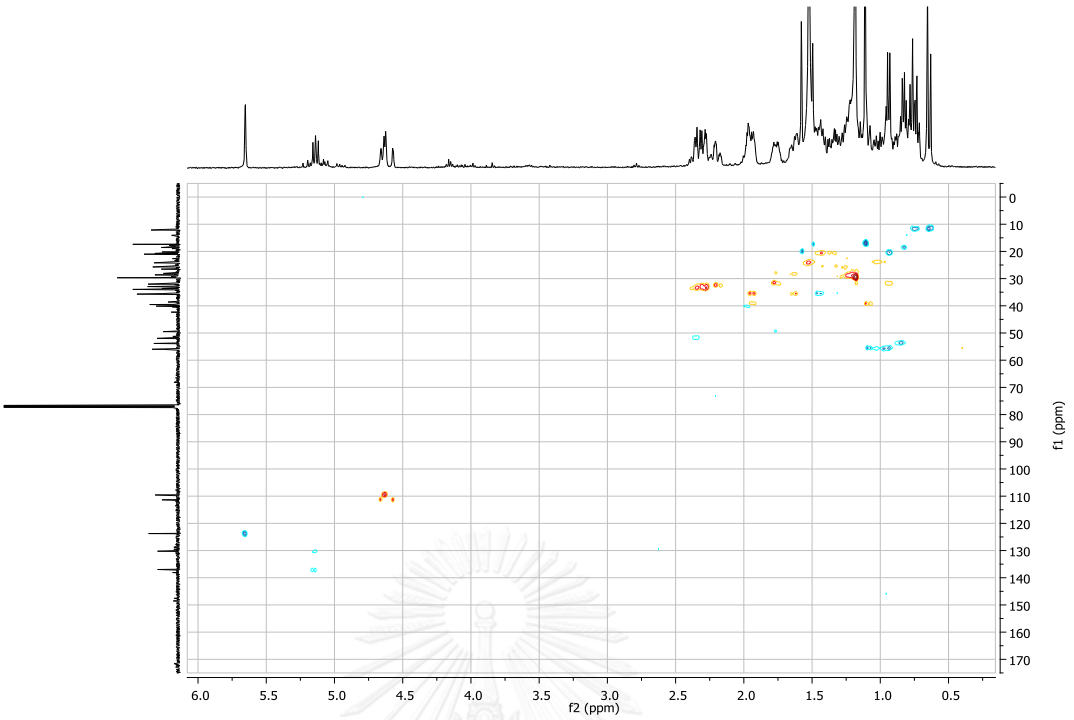

Figure A-11 HSQC NMR spectrum ($CDCl_3$) of a mixture of 1.4 and 1.5

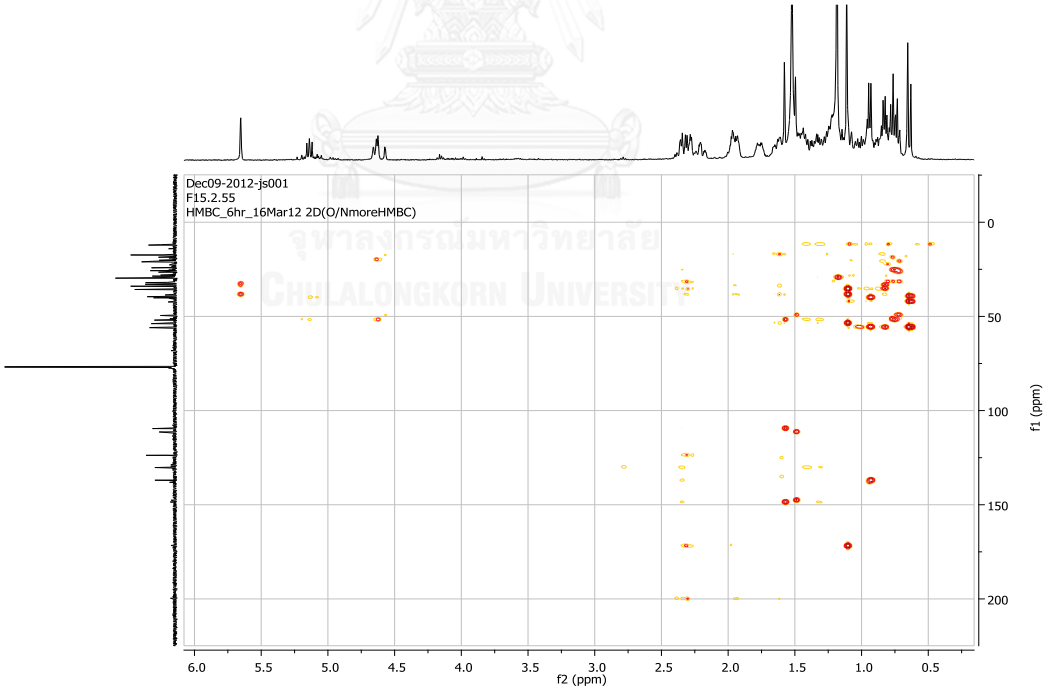

Figure A-12 HMBC NMR spectrum ($CDCl_3$) of a mixture of 1.4 and 1.5

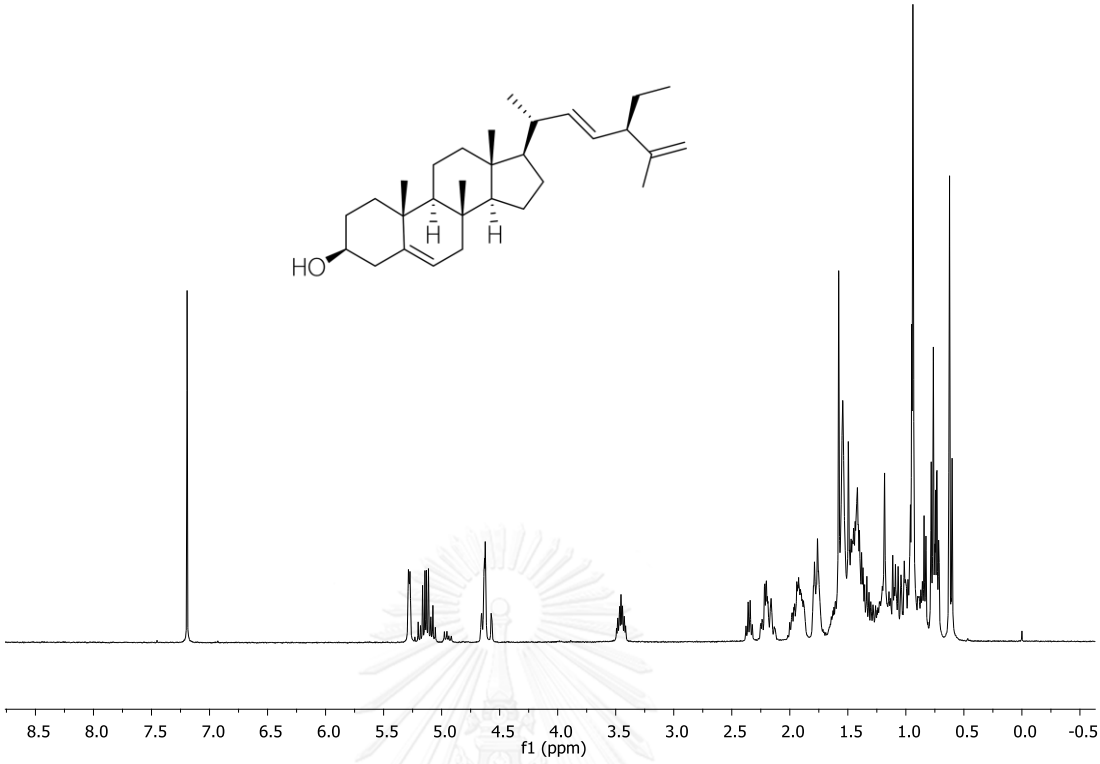

Figure A-13 ^{1}H NMR spectrum ($CDCl_3$) of 1.6

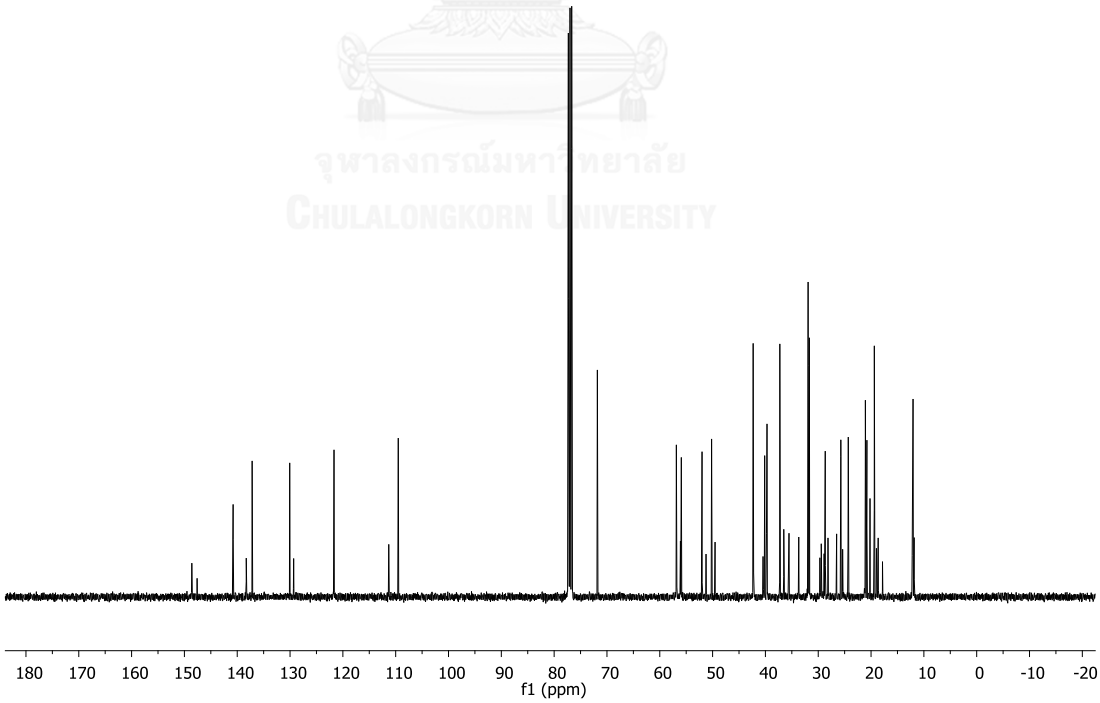

Figure A-14 ^{13}C NMR spectrum ($CDCl_3$) 1.6

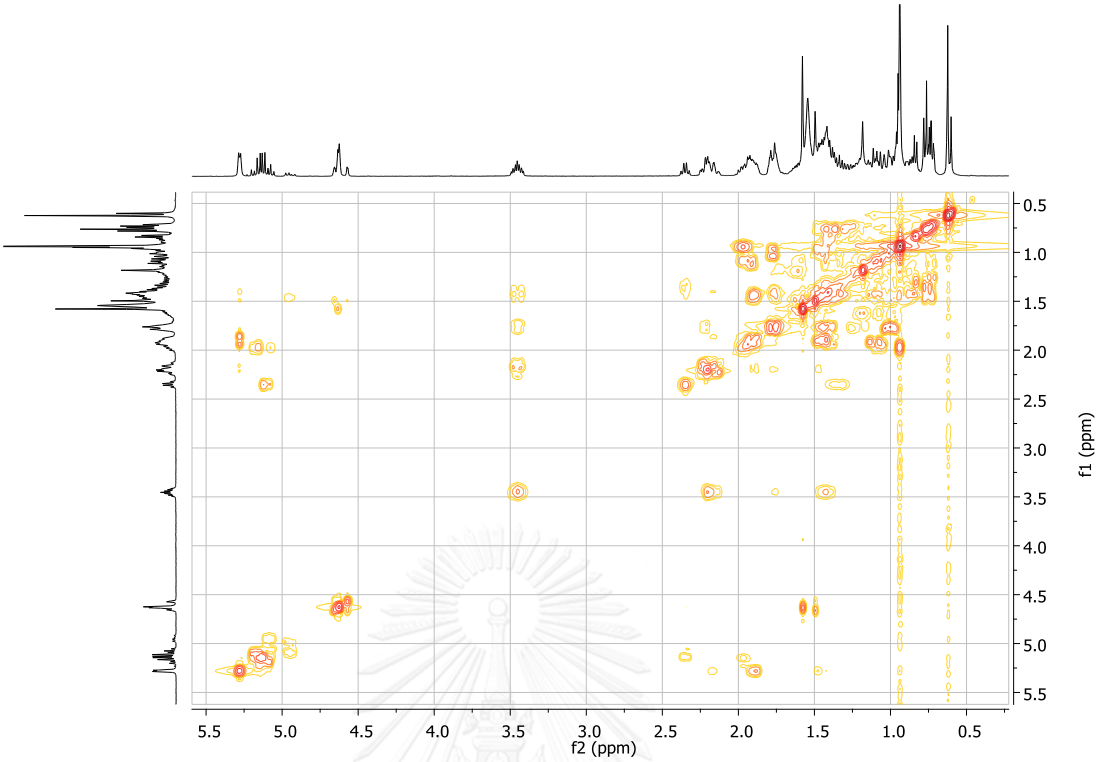

Figure A-15 COSY NMR spectrum ($CDCl_3$) of 1.6

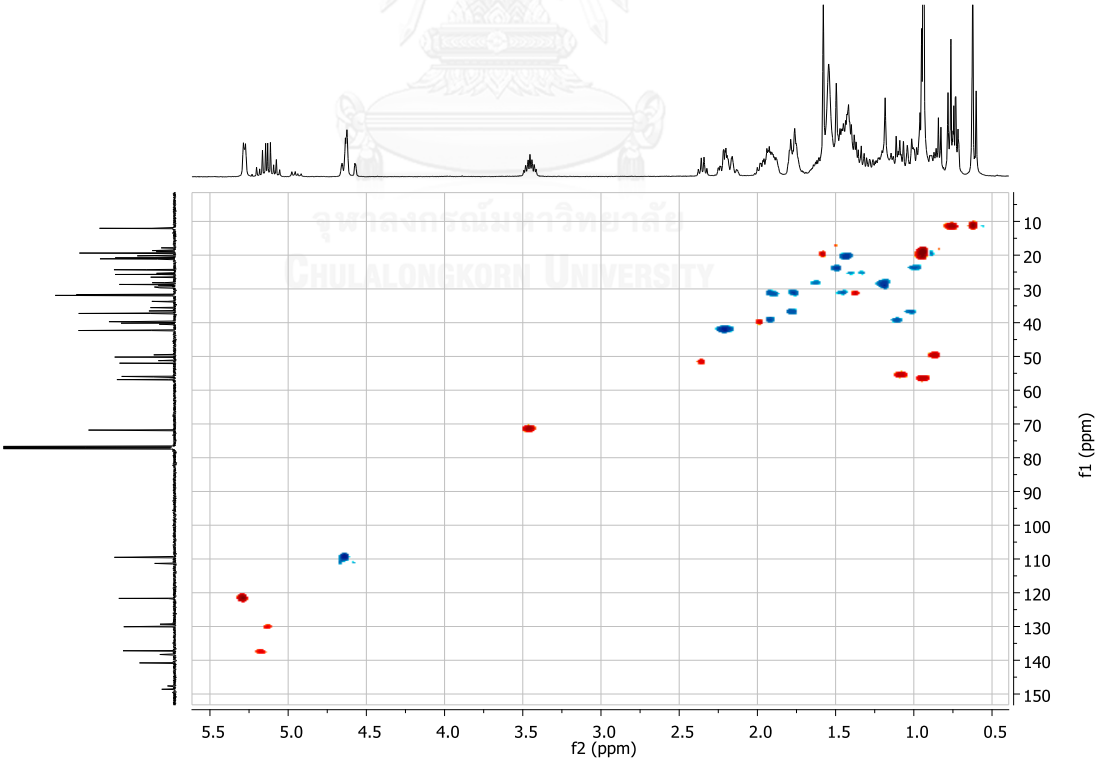

Figure A-16 HSQC NMR spectrum ($CDCl_3$) of 1.6

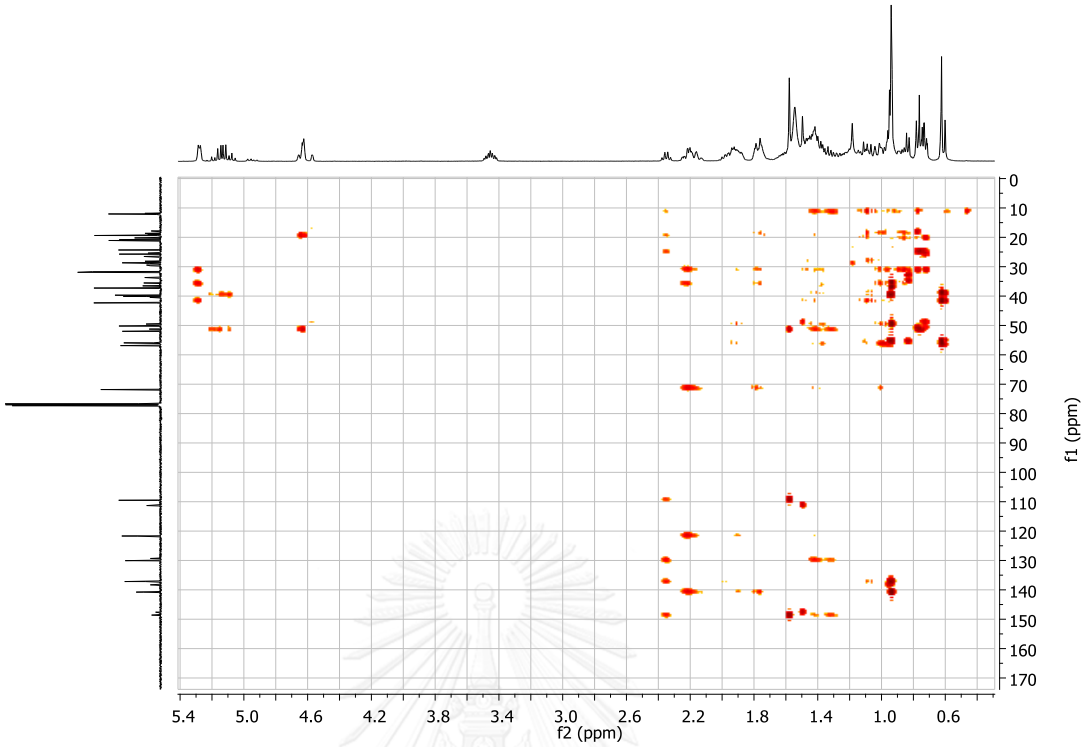

Figure A-17 HMBC NMR spectrum ($CDCl_3$) of 1.6

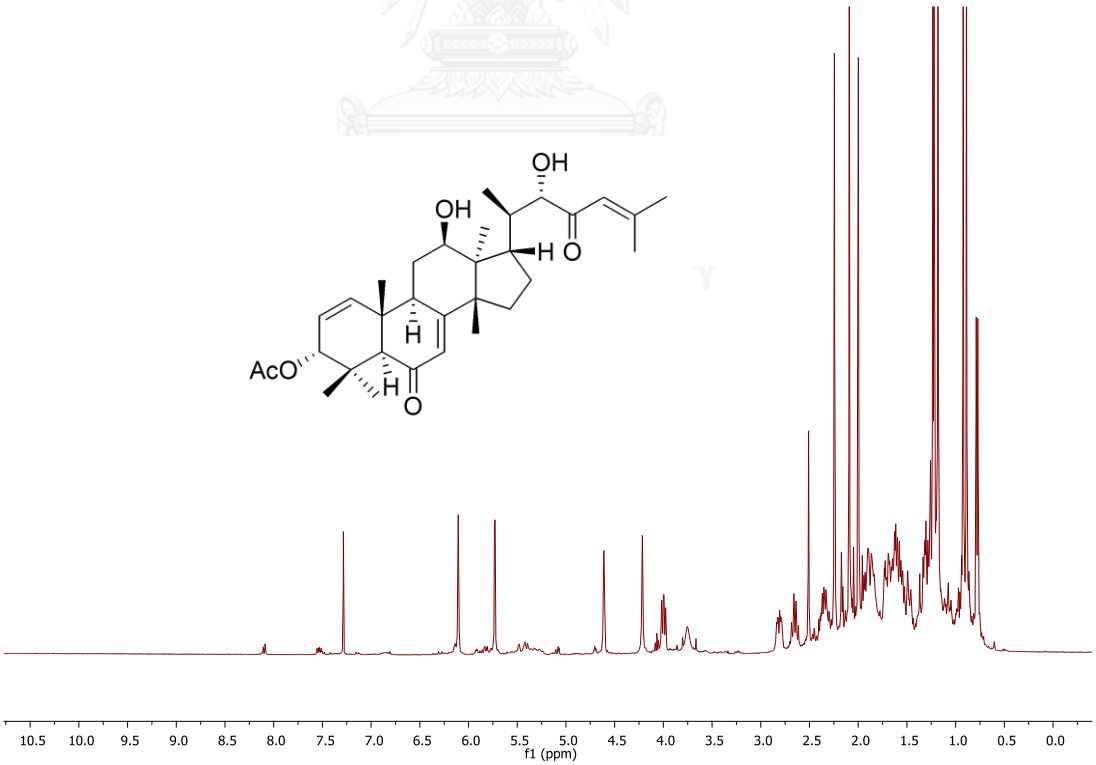

Figure A-18 ^{1}H NMR spectrum ($CDCl_3$) of 2.1

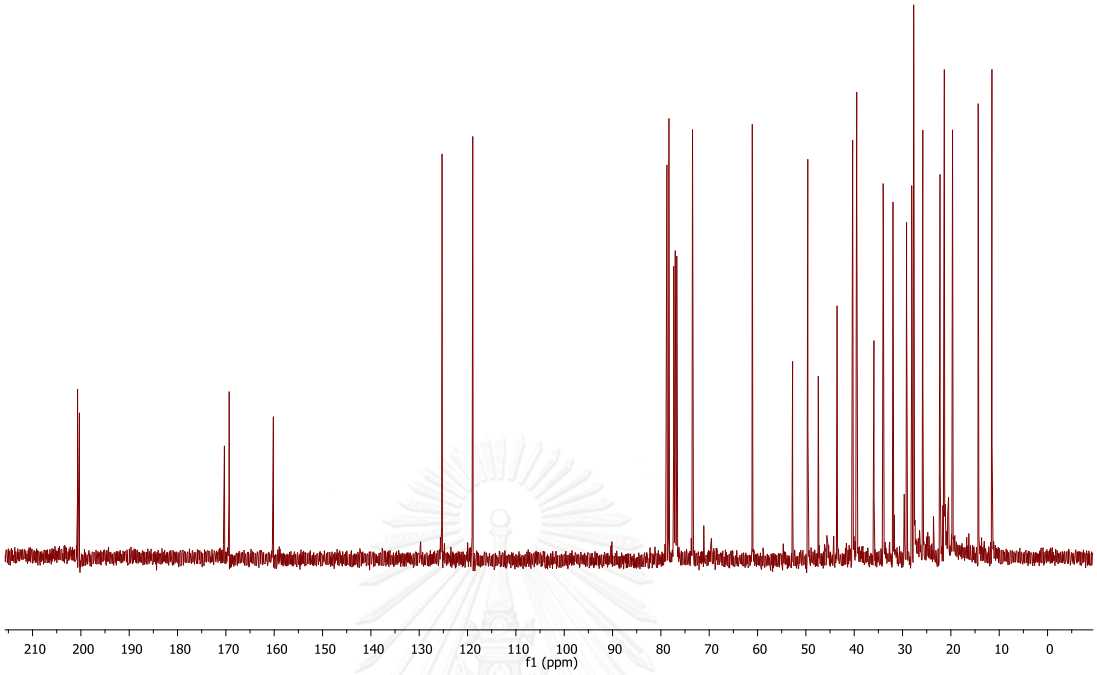

Figure A-19 ^{13}C NMR spectrum ($CDCl_3$) of 2.1

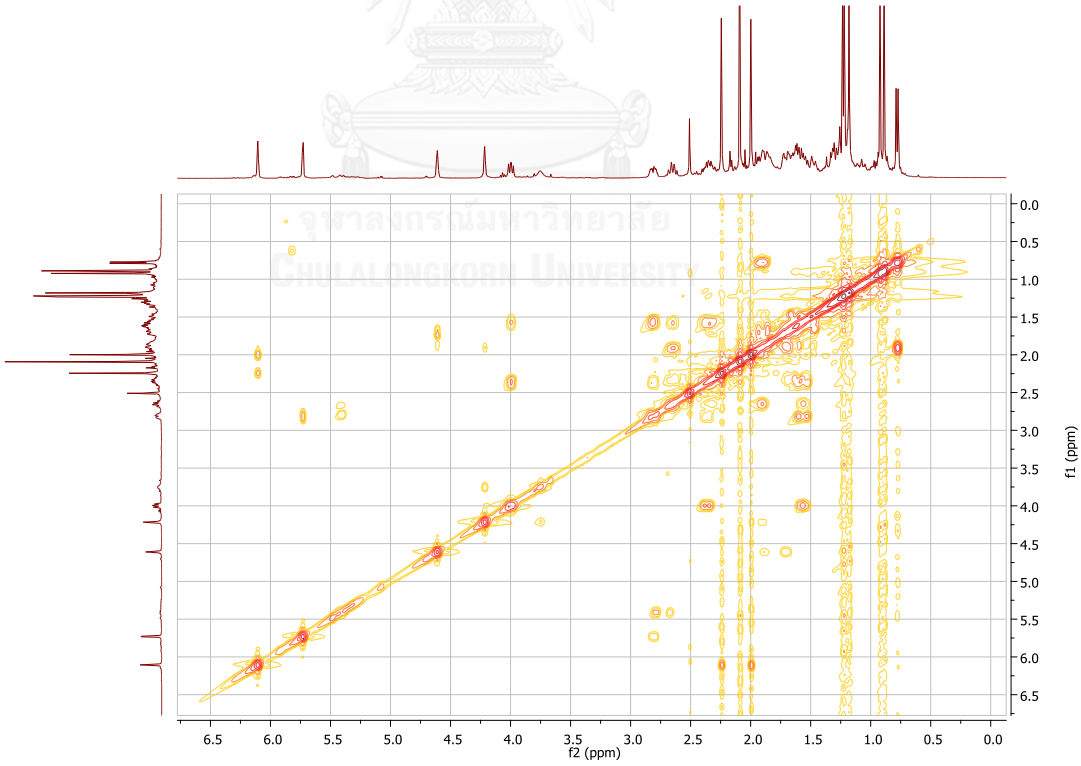

Figure A-20 COSY NMR spectrum ($CDCl_3$) of 2.1

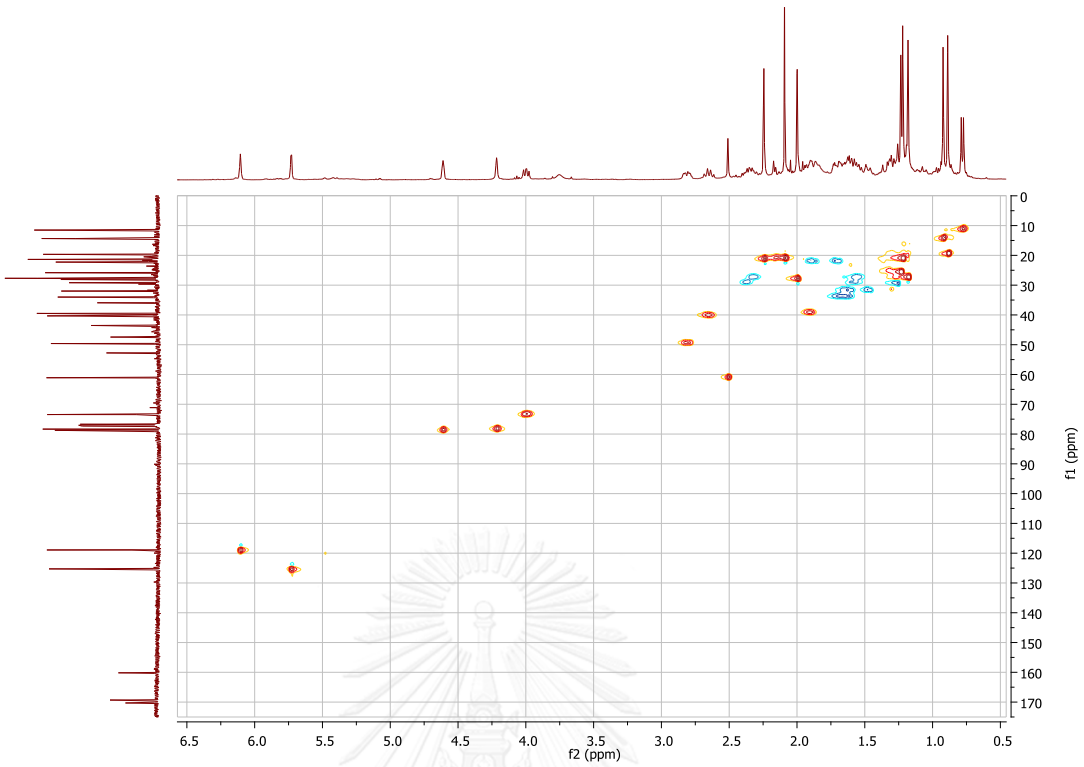

Figure A-21 HSQC NMR spectrum ($CDCl_3$) of 2.1

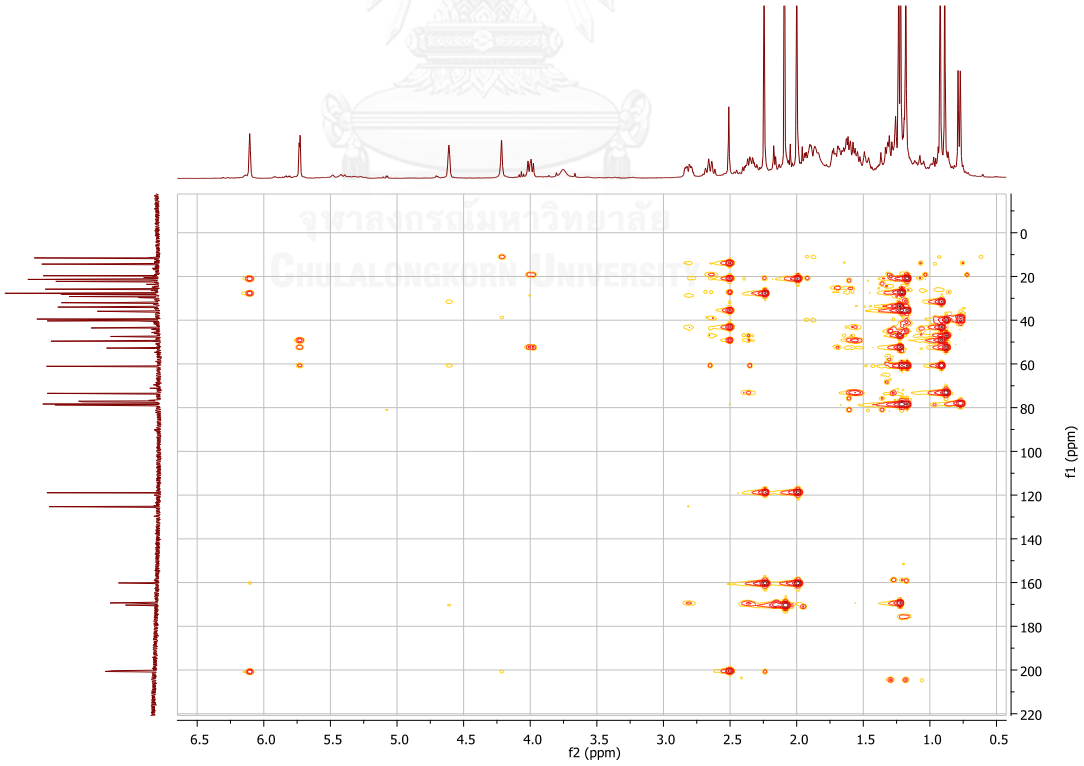

Figure A-22 HMBC NMR spectrum ($CDCl_3$) of 2.1

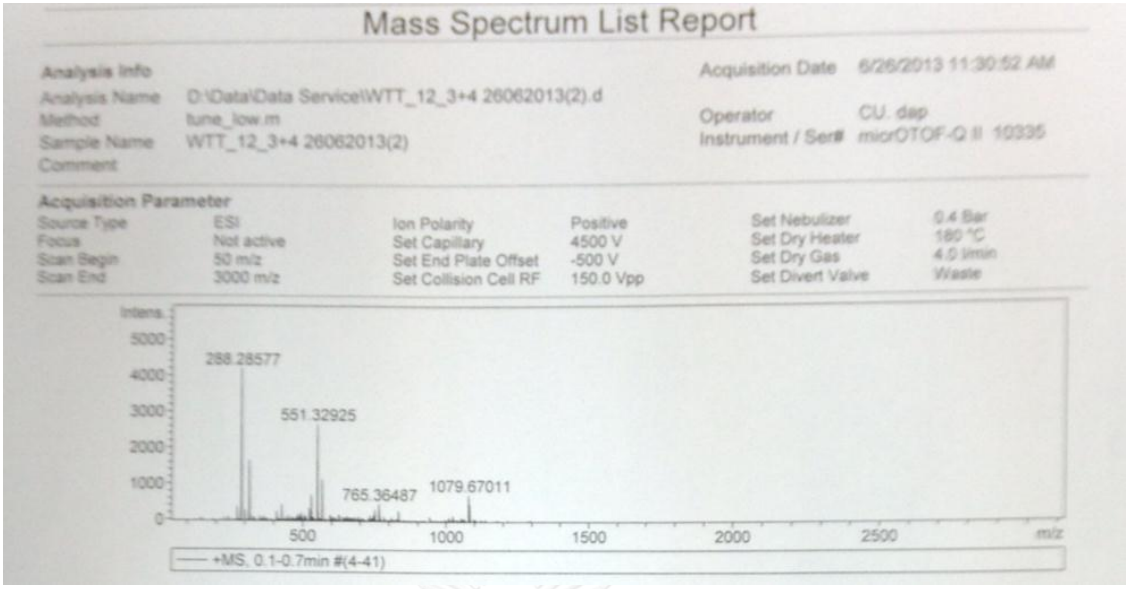

Figure A-23 HRESIMS spectrum of 2.1.

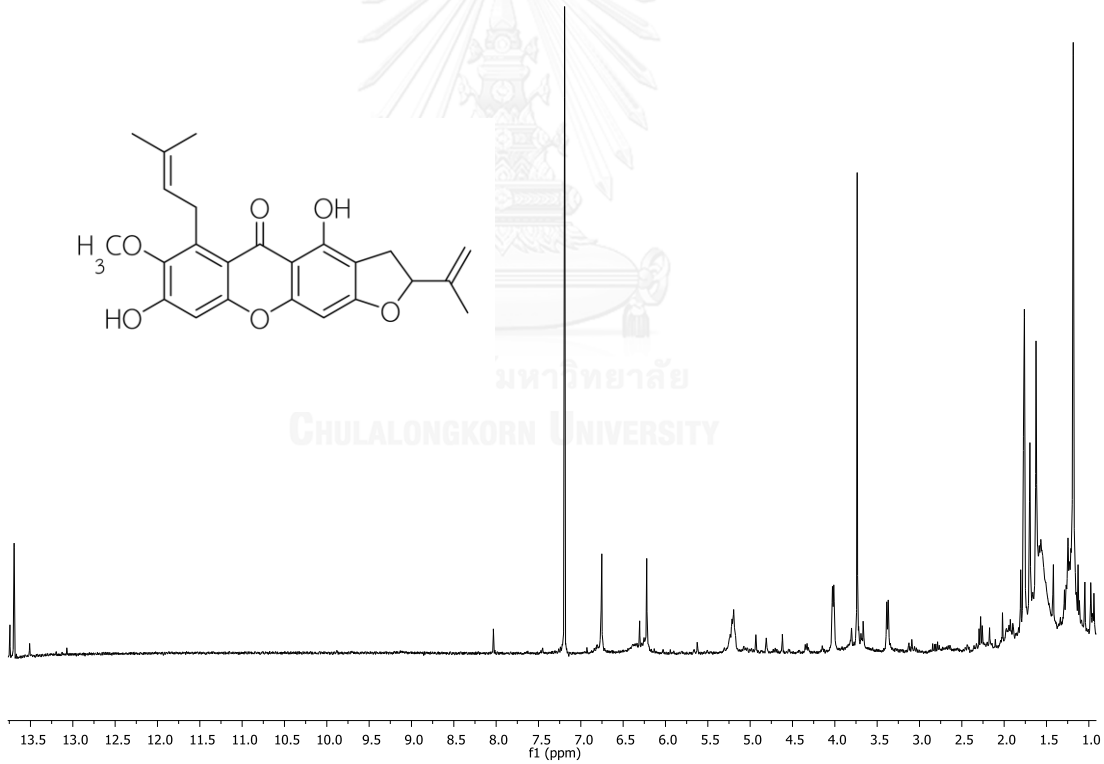

Figure A-24 ^{1}H NMR spectrum ($CDCl_3$) of 2.2

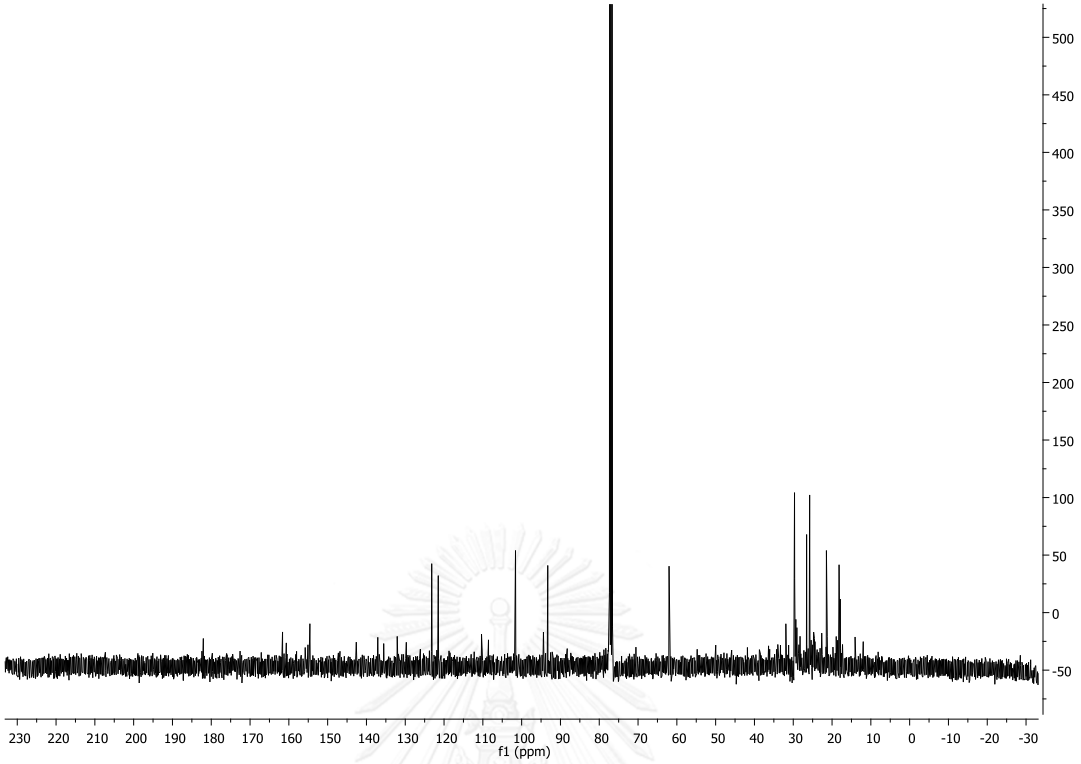

Figure A-25 ^{13}C NMR spectrum ($CDCl_3$) of 2.2

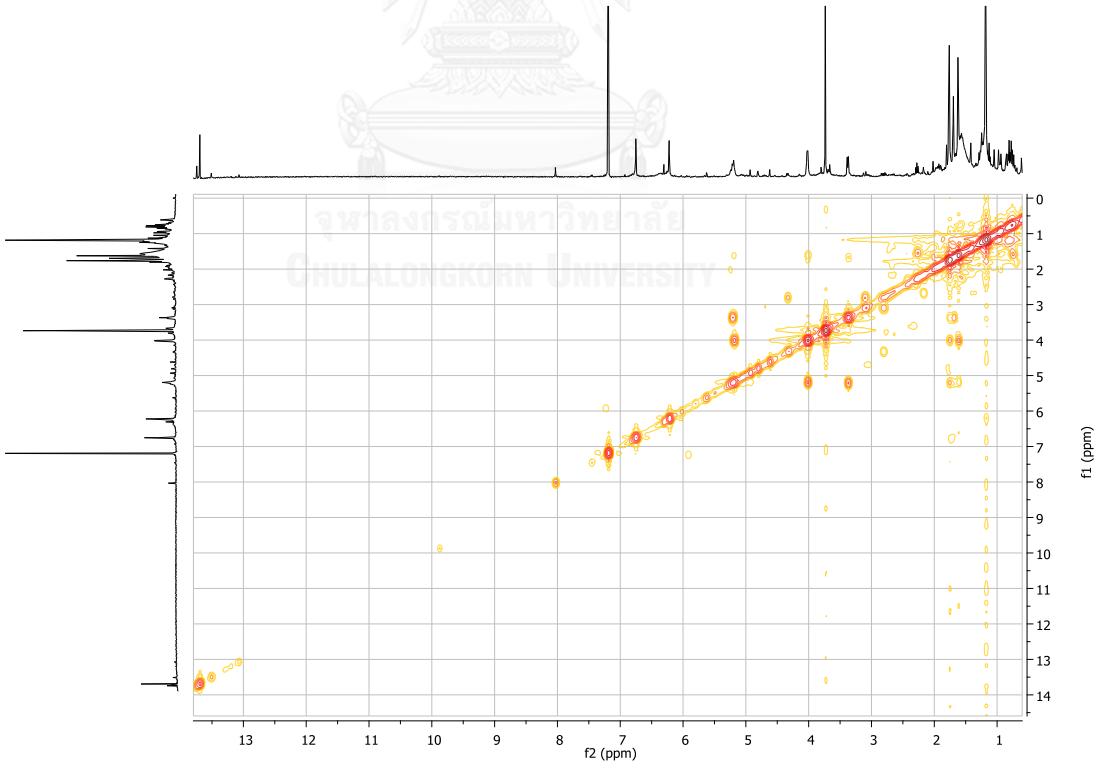

Figure A-26 COSY NMR spectrum ($CDCl_3$) of 2.2

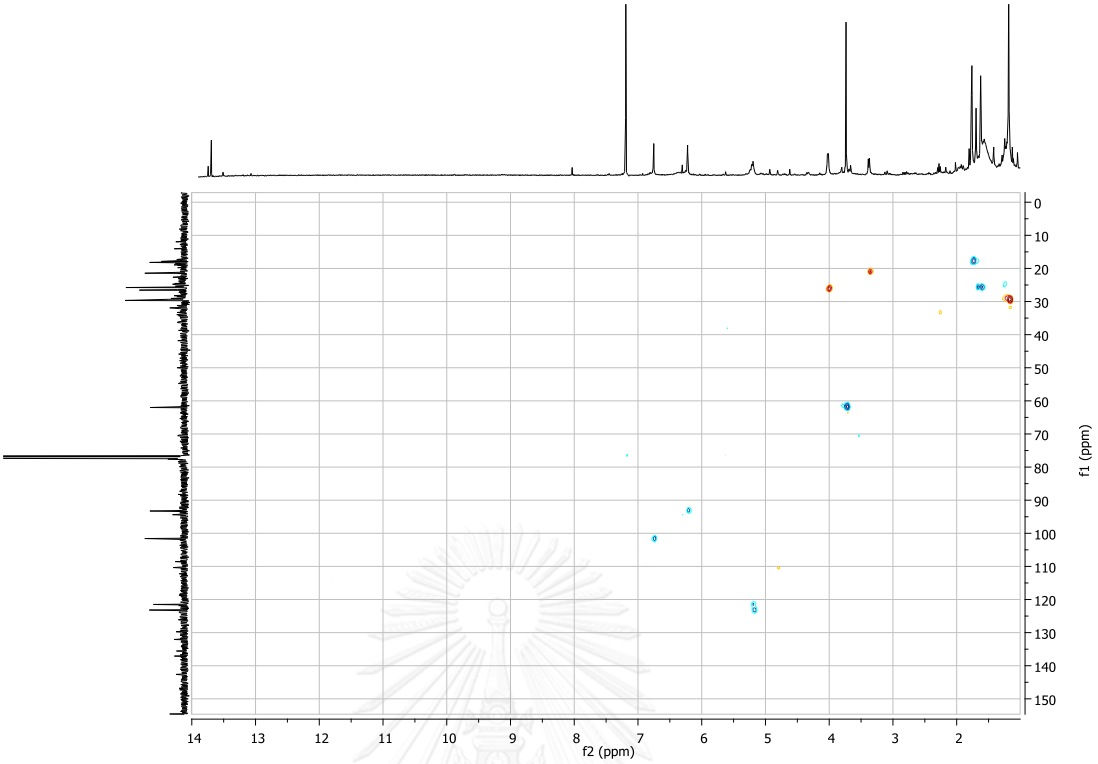

Figure A-27 HSQC NMR spectrum ($CDCl_3$) of 2.2

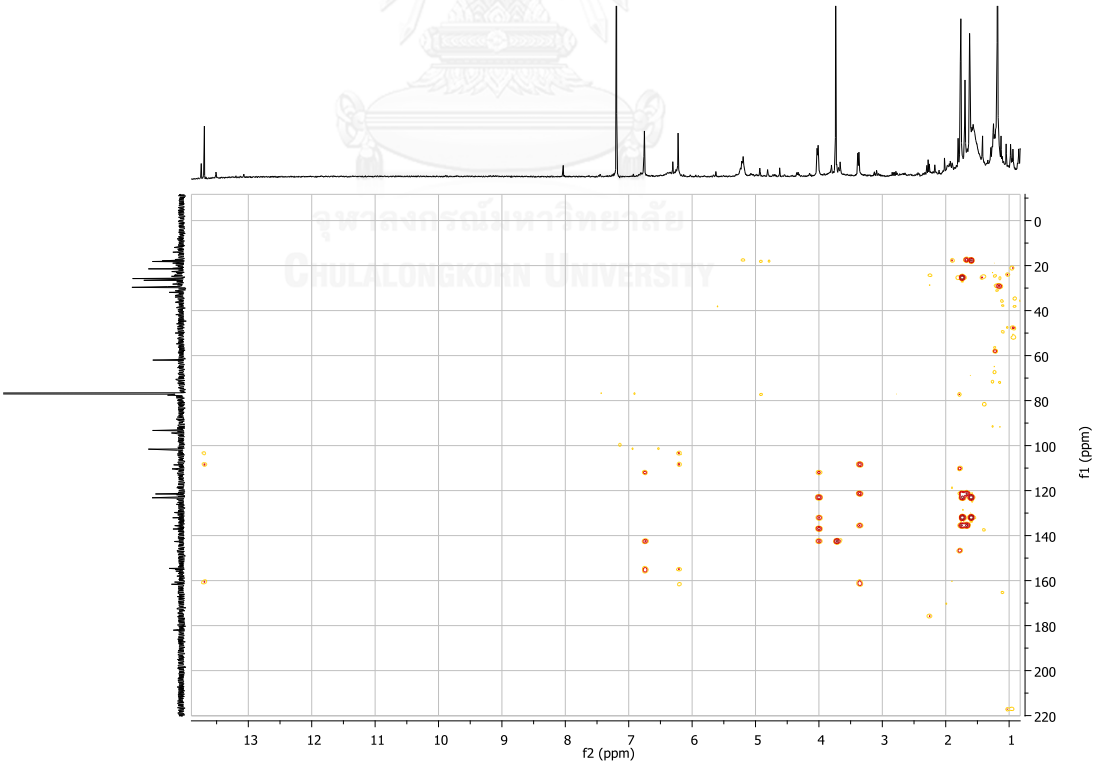

Figure A-28 HMBC NMR spectrum ($CDCl_3$) of 2.2

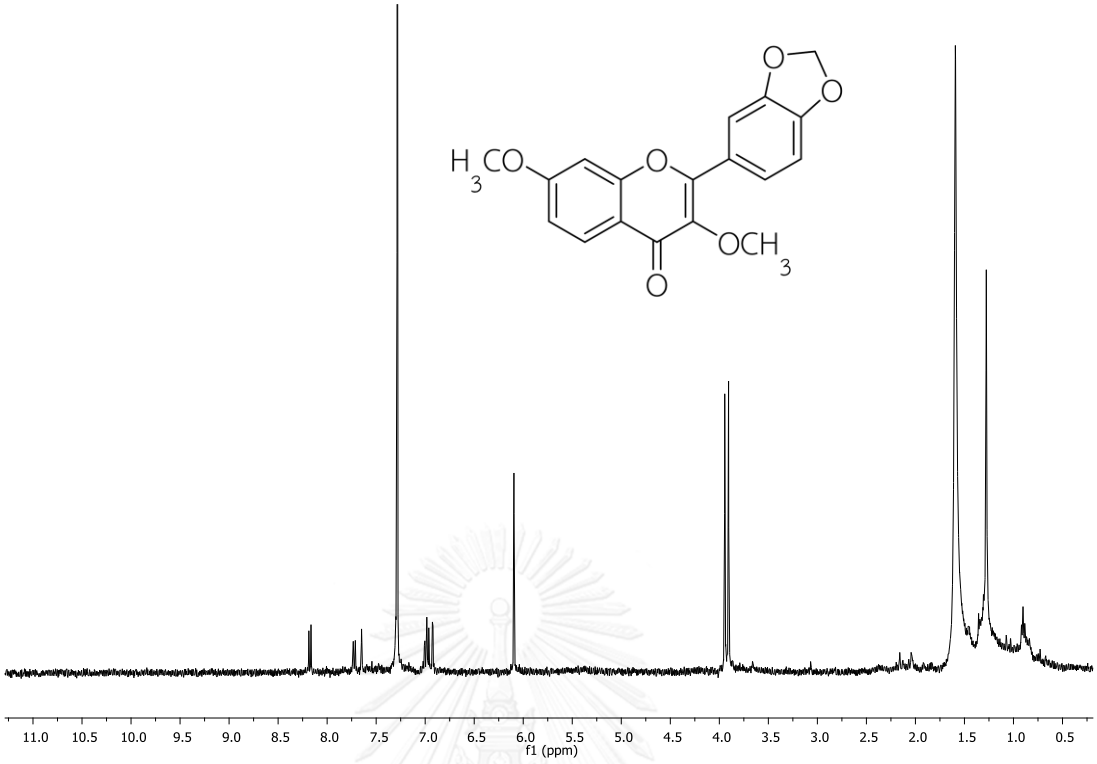

Figure A-29 ^{1}H NMR spectrum ($CDCl_3$) of 2.3

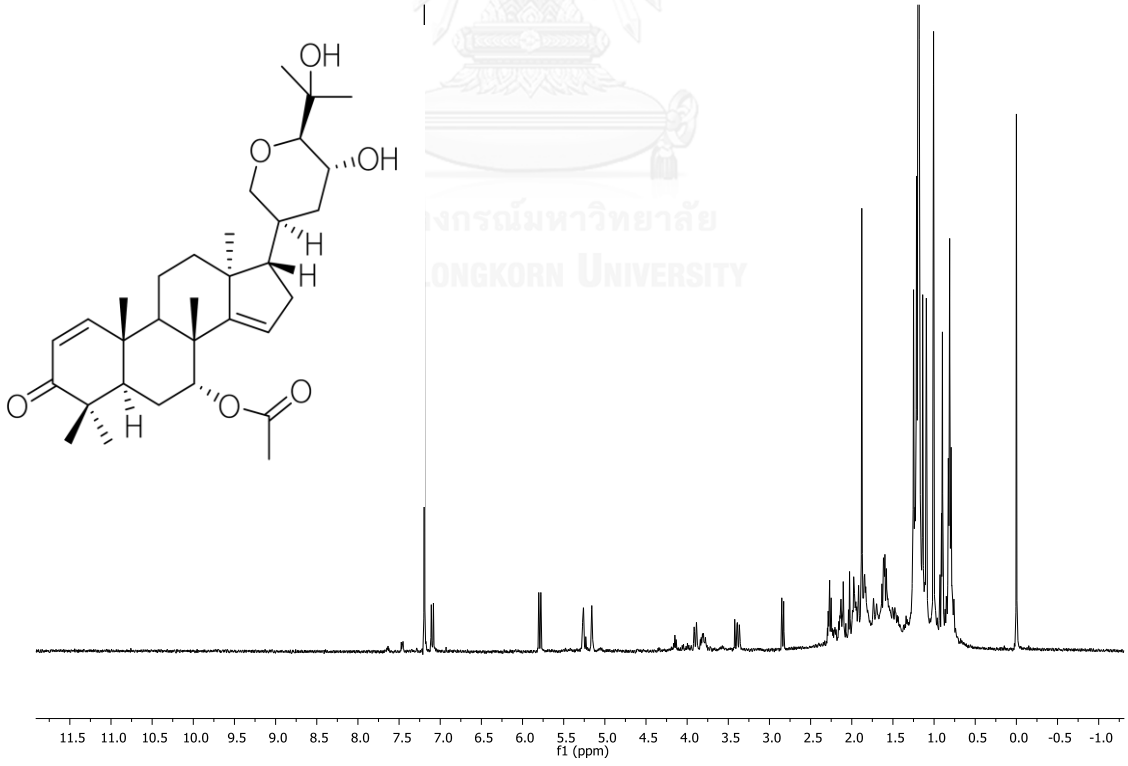

Figure A-30 ^{1}H NMR spectrum ($CDCl_3$) of 2.4

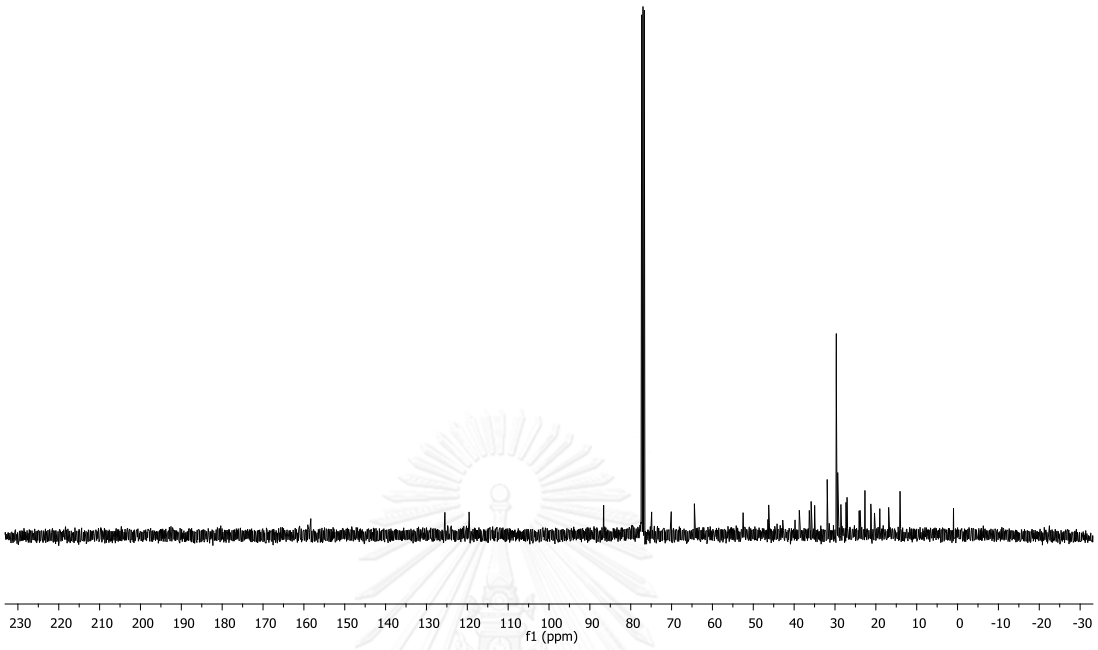

Figure A-31 ^{13}C NMR spectrum ($CDCl_3$) of 2.4

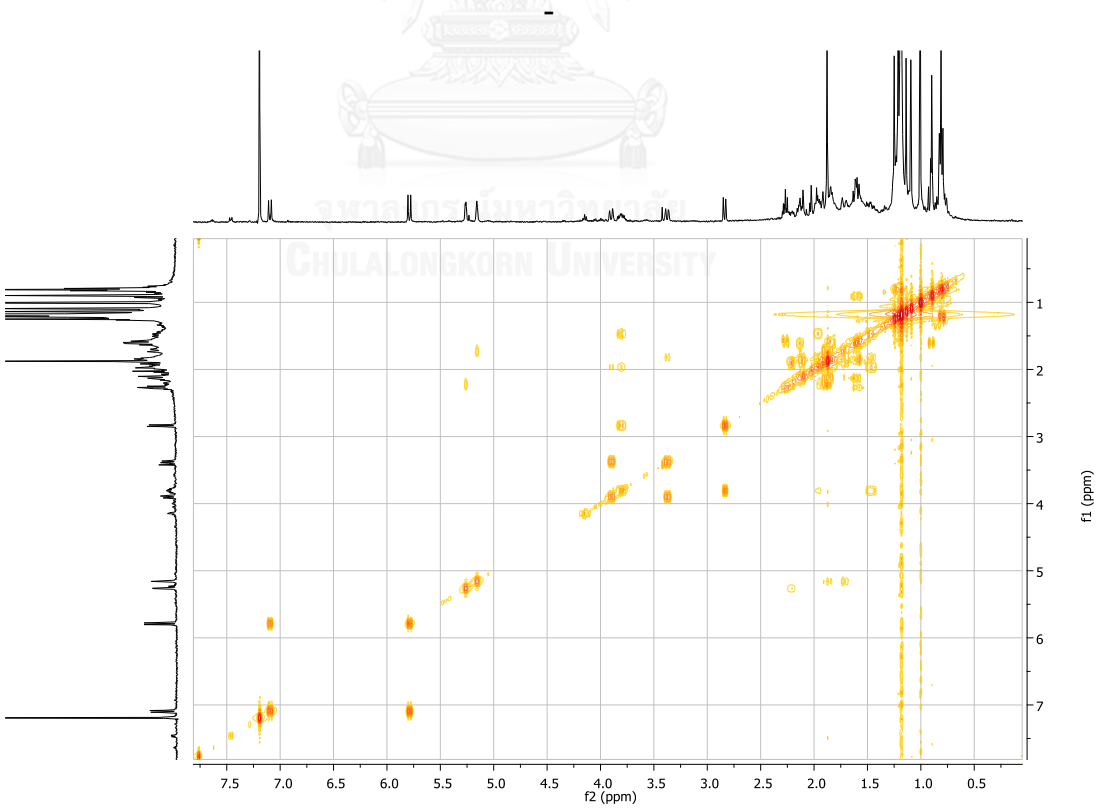

Figure A-32 COSY NMR spectrum ($CDCl_3$) of 2.4

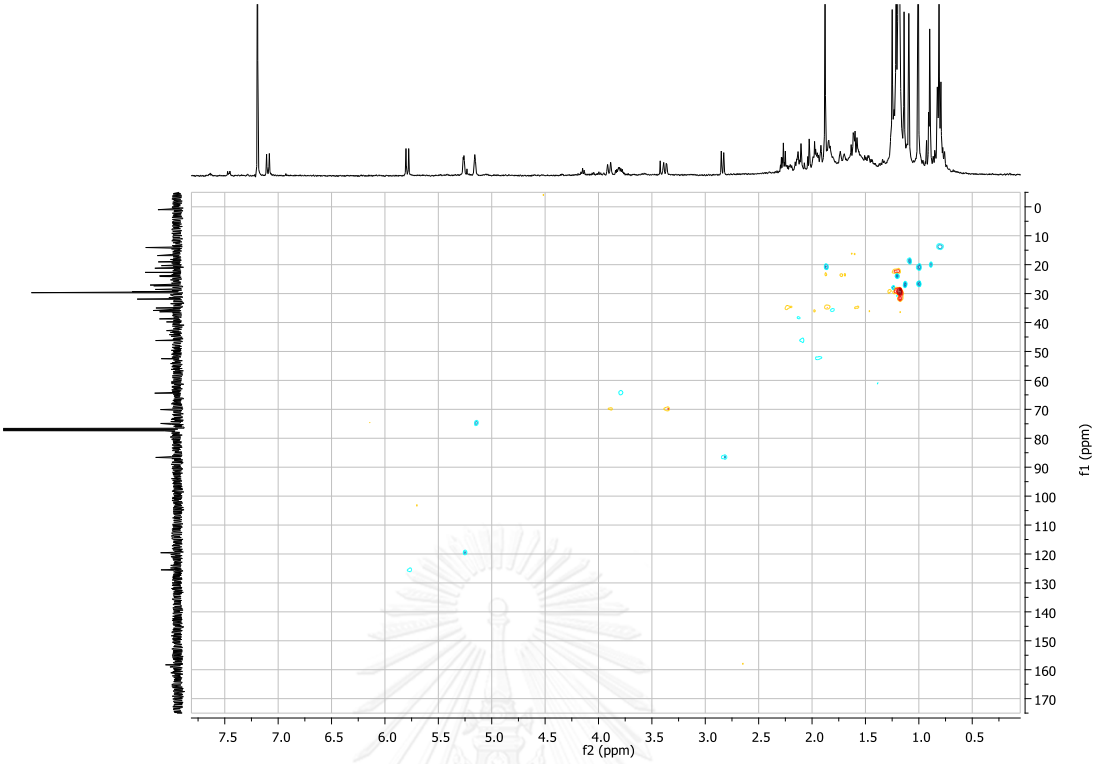

Figure A-33 HSQC NMR spectrum ($CDCl_3$) of 2.4

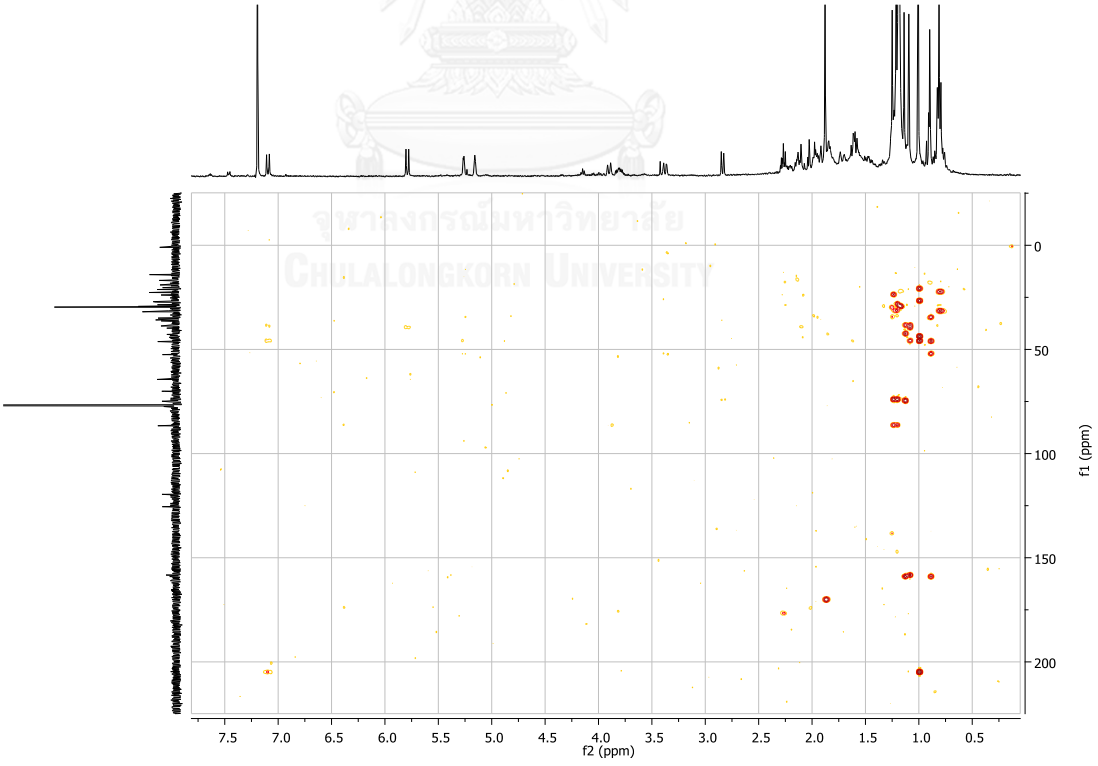

Figure A-34 HMBC NMR spectrum ($CDCl_3$) of 2.4

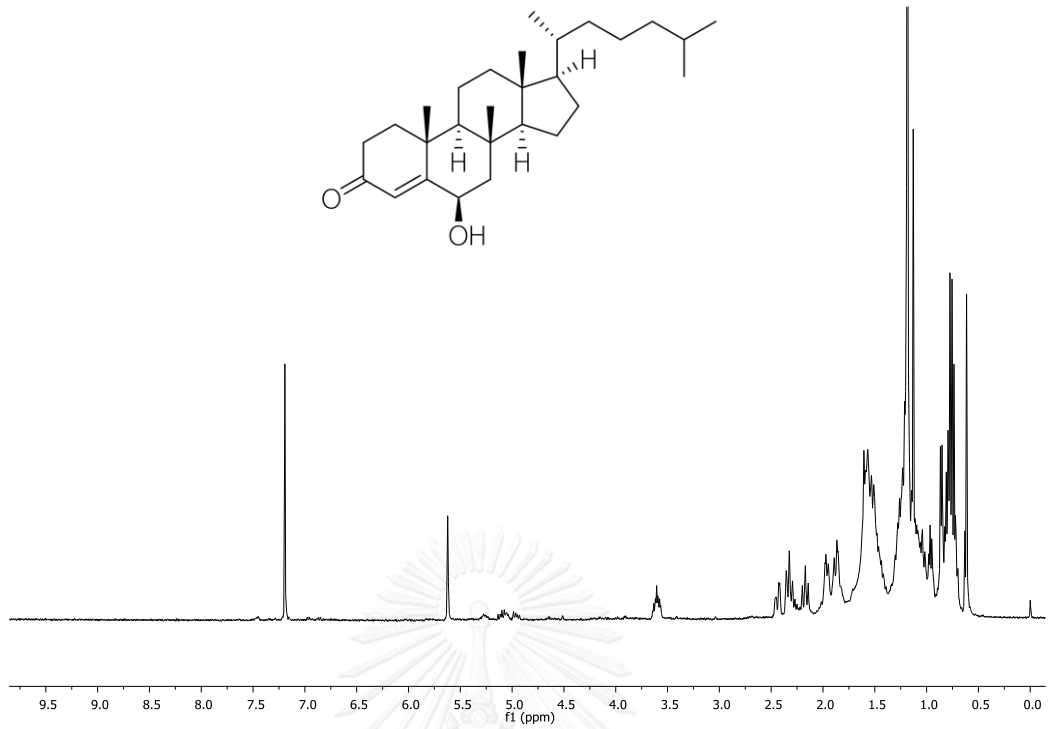

Figure A-35 ^{1}H NMR spectrum ($CDCl_3$) of 2.5

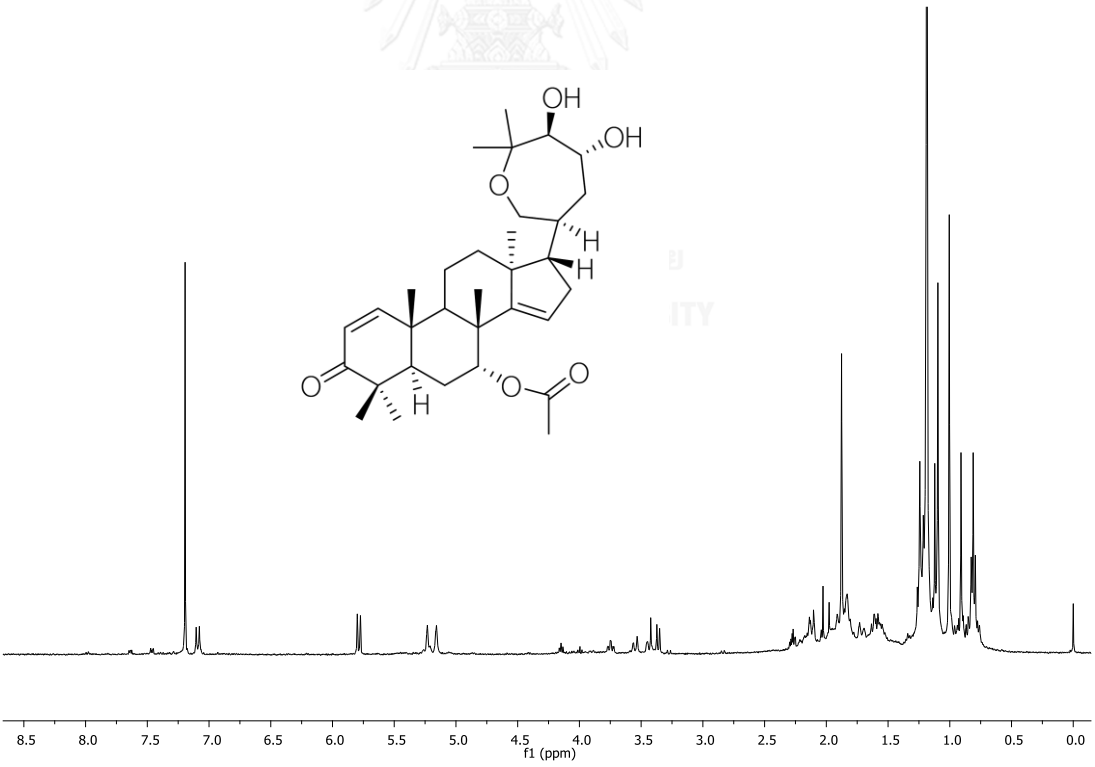

Figure A-36 ^{1}H NMR spectrum ($CDCl_3$) of 2.6

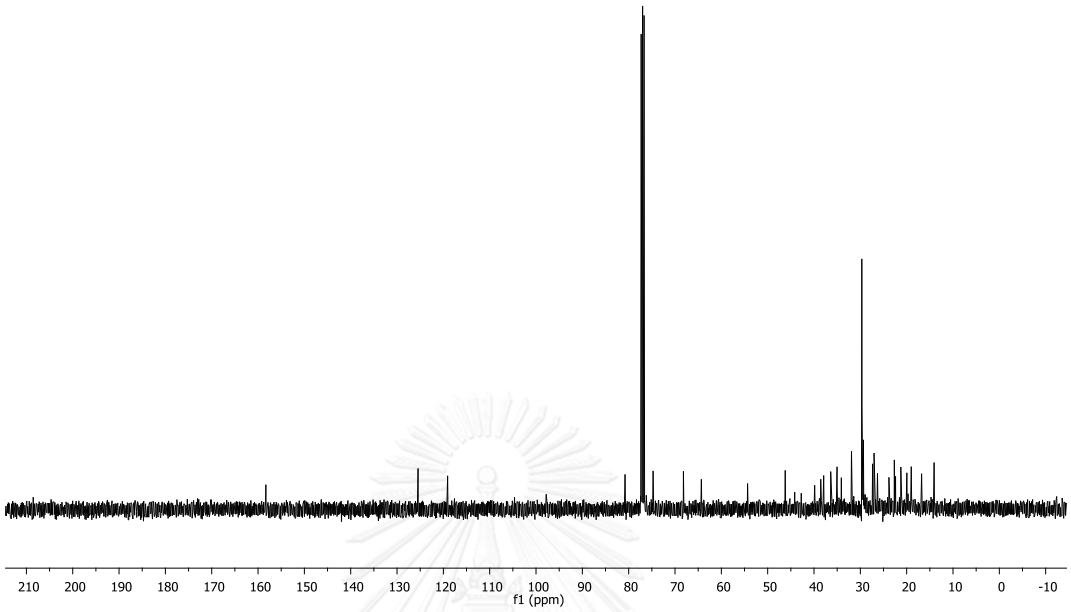

Figure A-37 ^{13}C NMR spectrum ($CDCl_3$) of 2.6

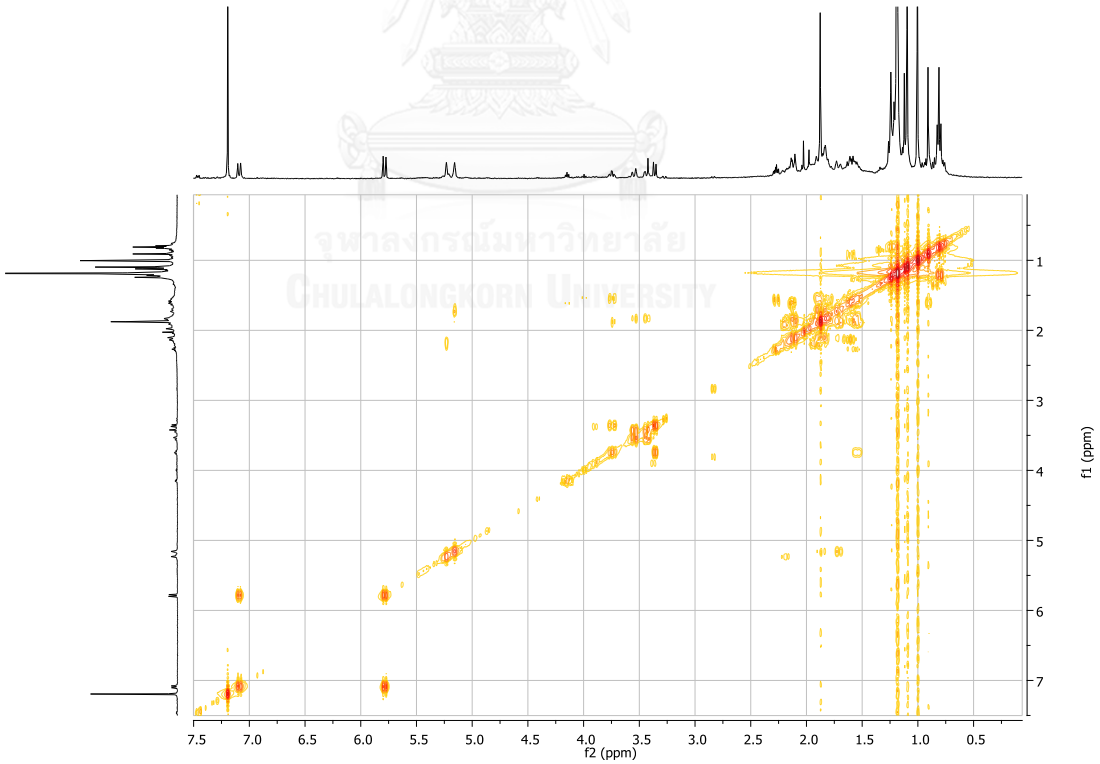

Figure A-38 COSY NMR spectrum ($CDCl_3$) of 2.6

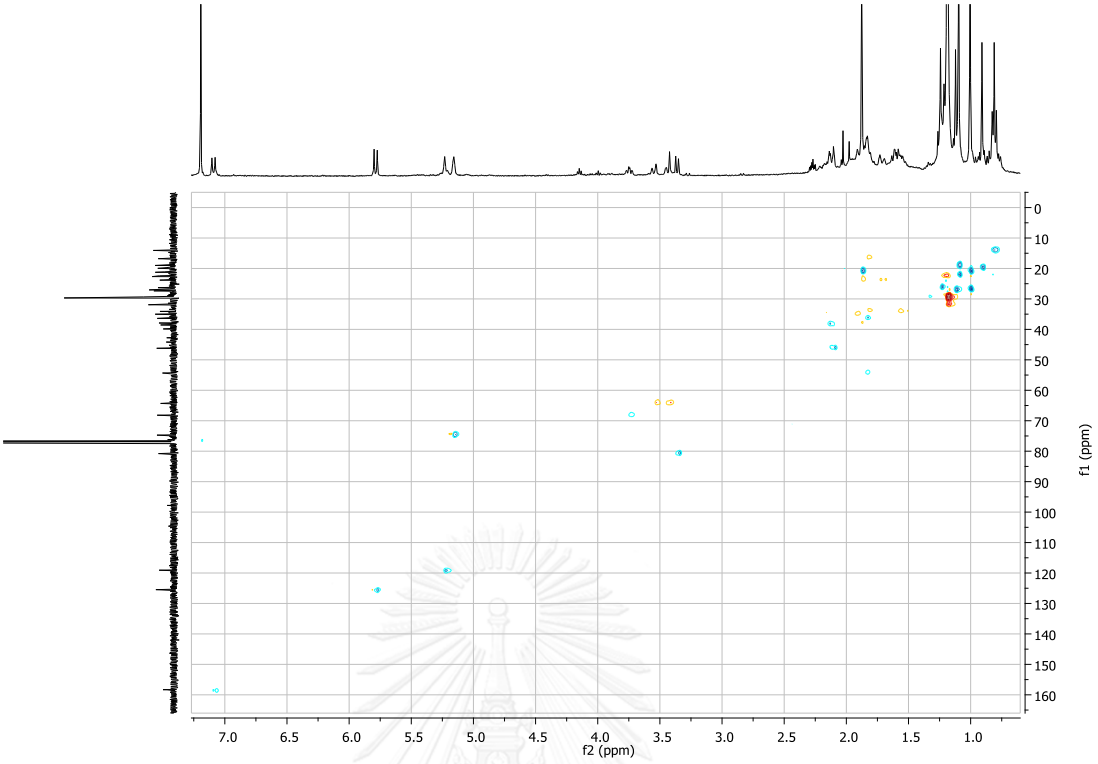

Figure A-39 HSQC NMR spectrum ($CDCl_3$) of 2.6

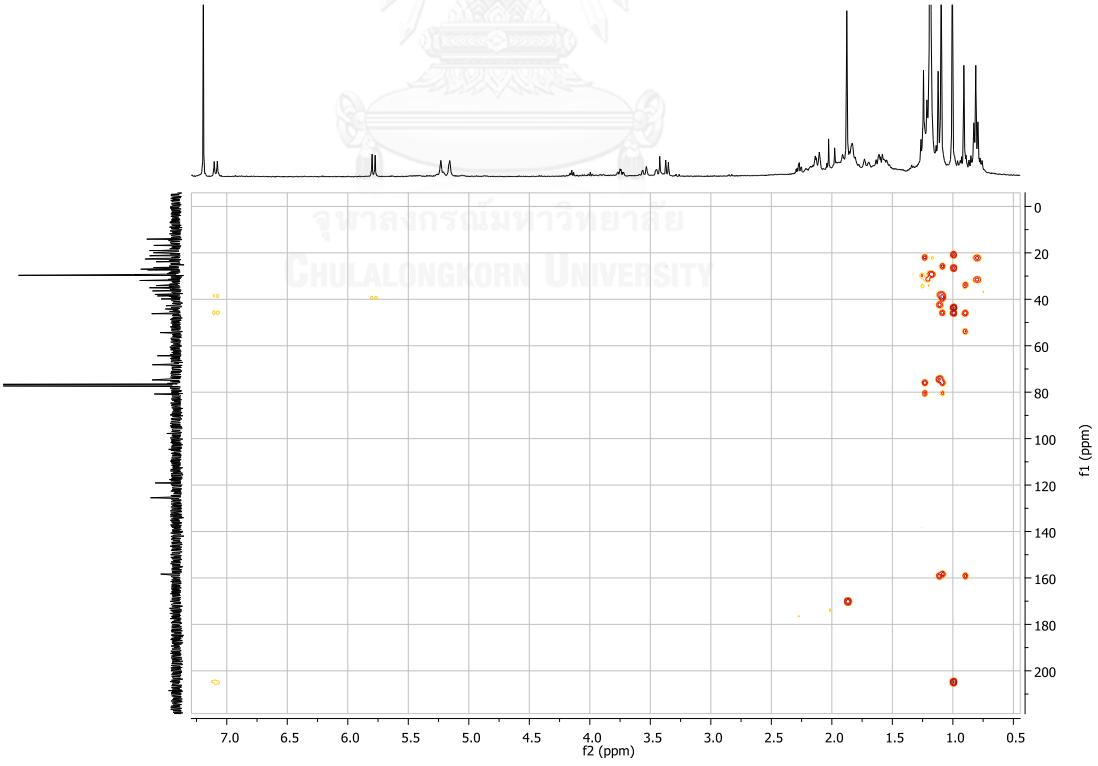

Figure A-40 HMBC NMR spectrum ($CDCl_3$) of 2.6

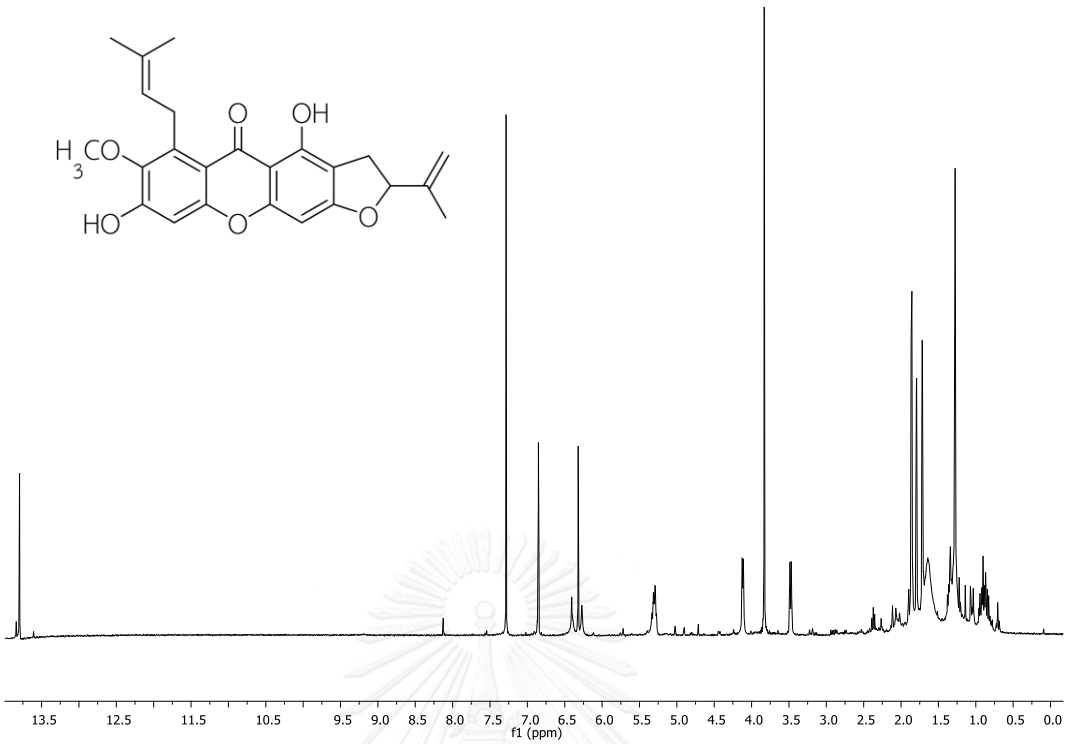

Figure A-41 ^{1}H NMR spectrum ($CDCl_3$) of 2.7

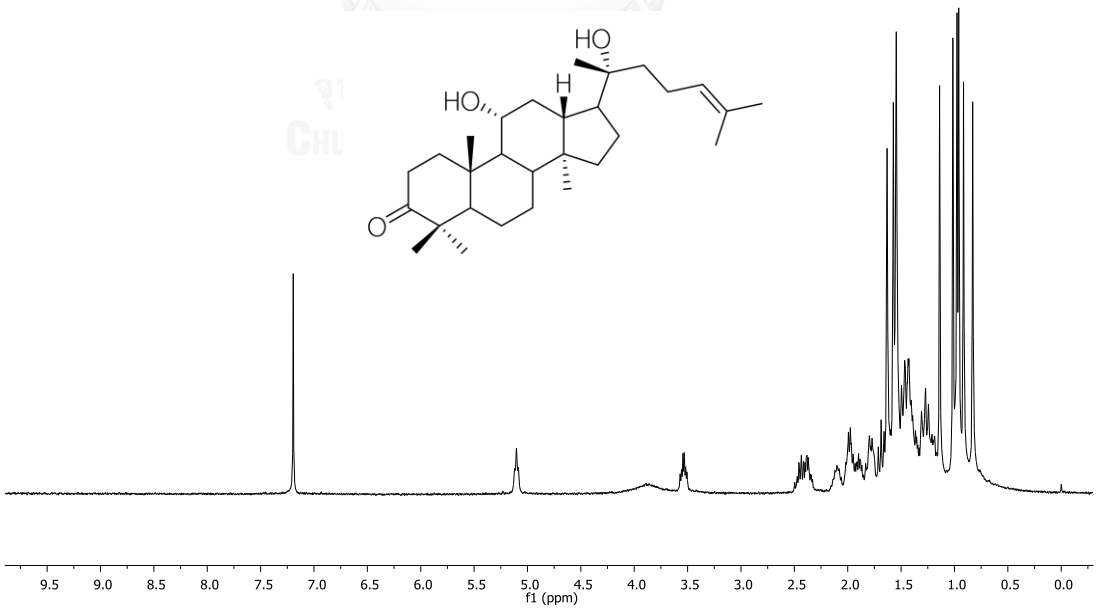

Figure A-42 ^{1}H NMR spectrum ($CDCl_3$) of 2.8

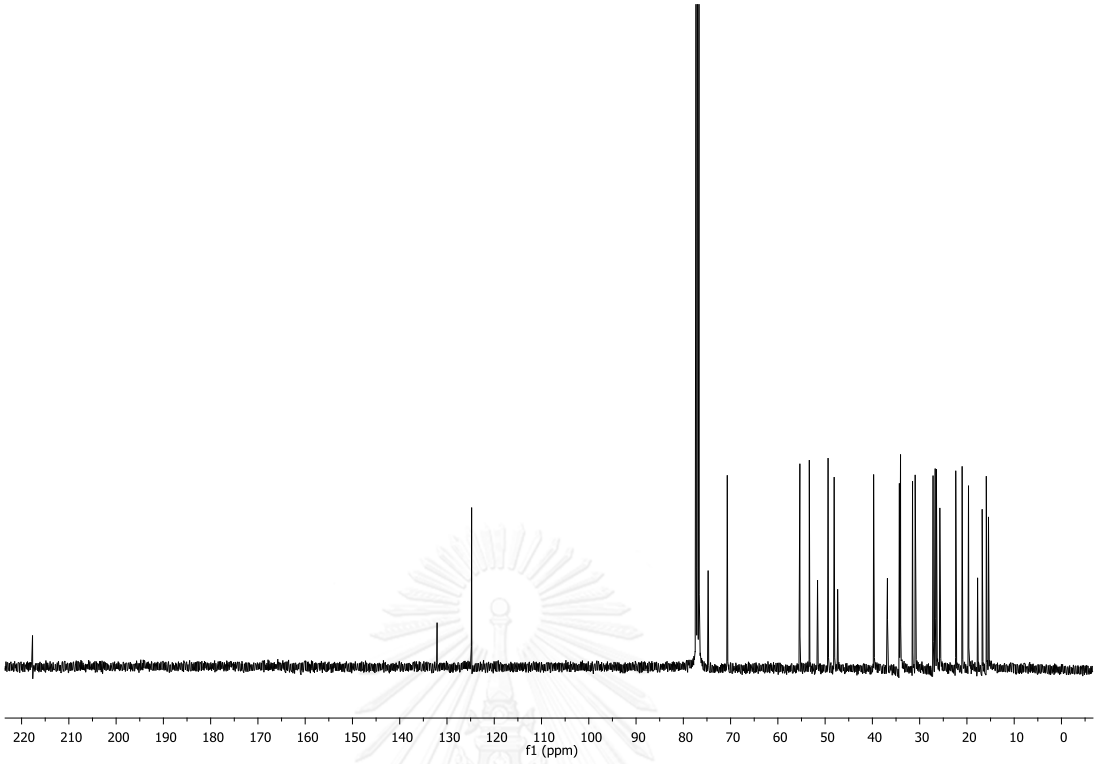

Figure A-43 ^{13}C NMR spectrum ($CDCl_3$) of 2.8

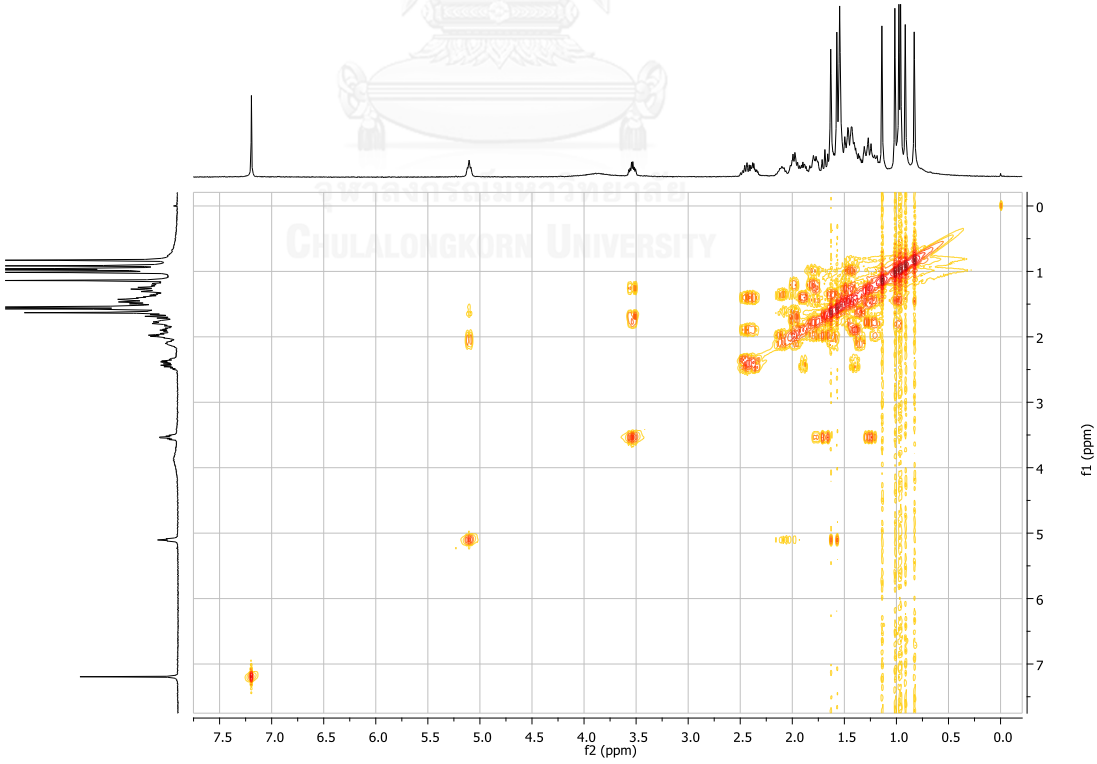

Figure A-44 COSY NMR spectrum ($CDCl_3$) of 2.8

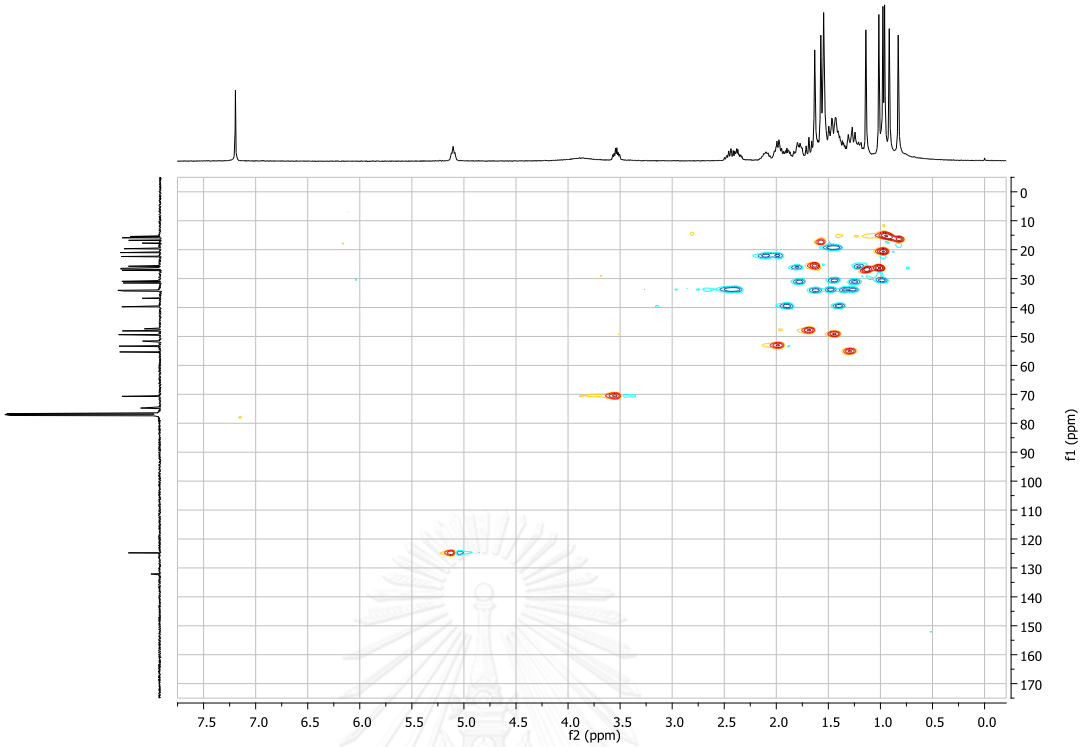

Figure A-45 HSQC NMR spectrum ($CDCl_3$) of 2.8

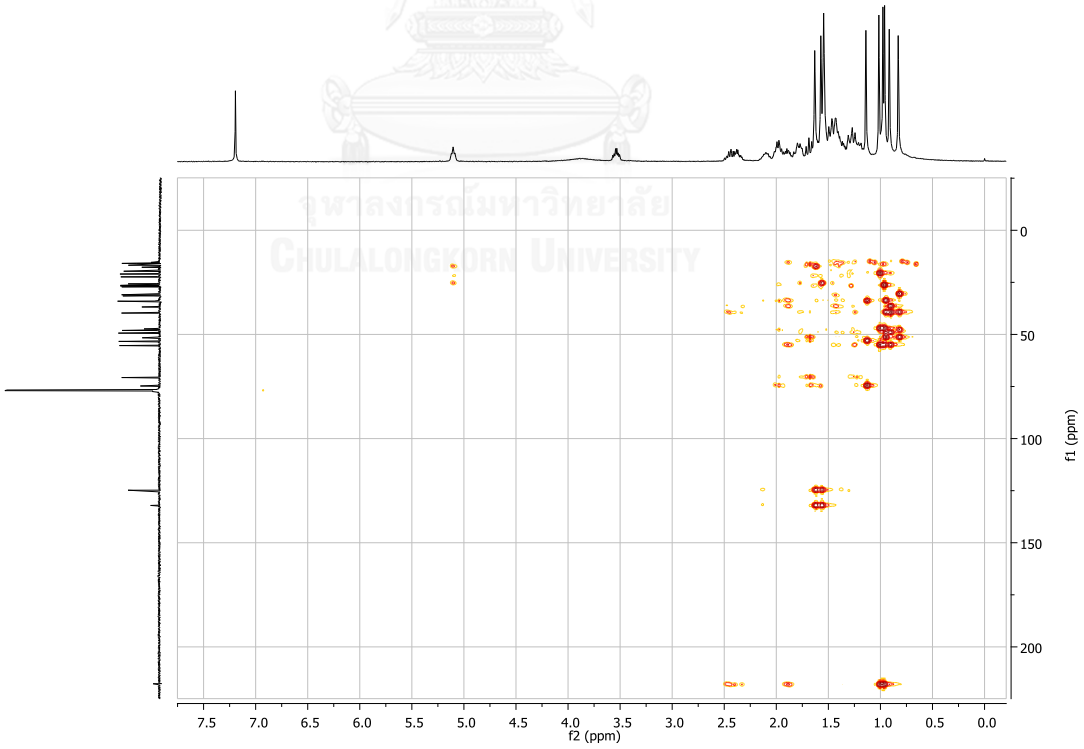

Figure A-46 HMBC NMR spectrum ($CDCl_3$) of 2.8

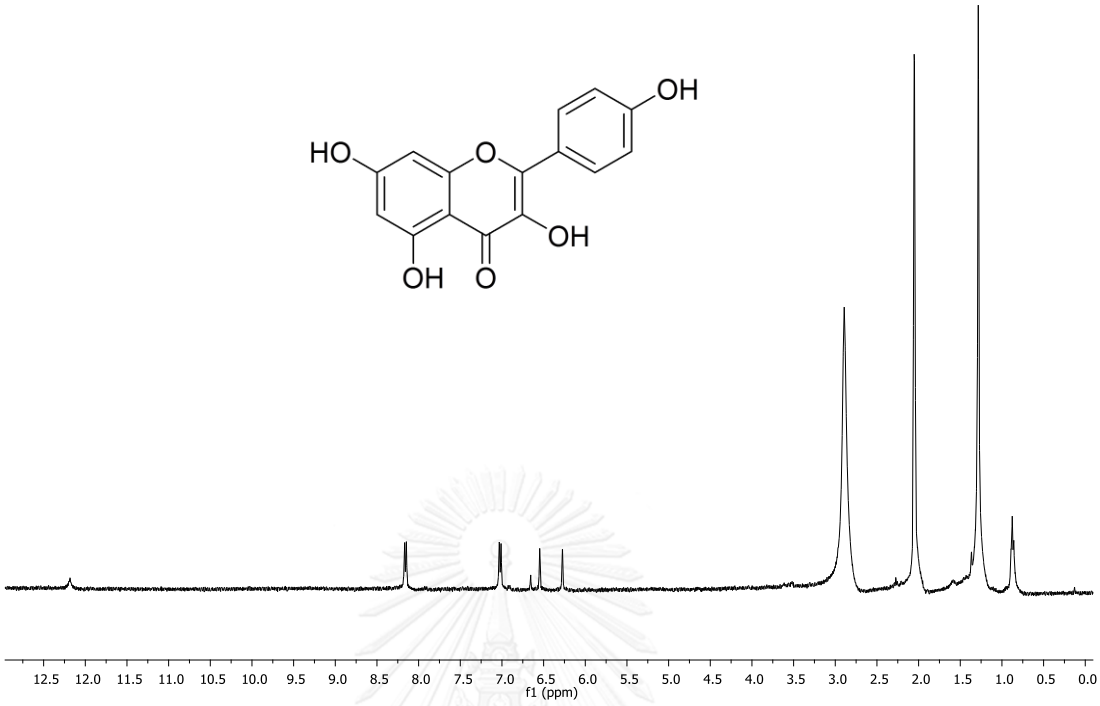

Figure A-47 ^{1}H NMR spectrum ($CDCl_3$) of 2.9

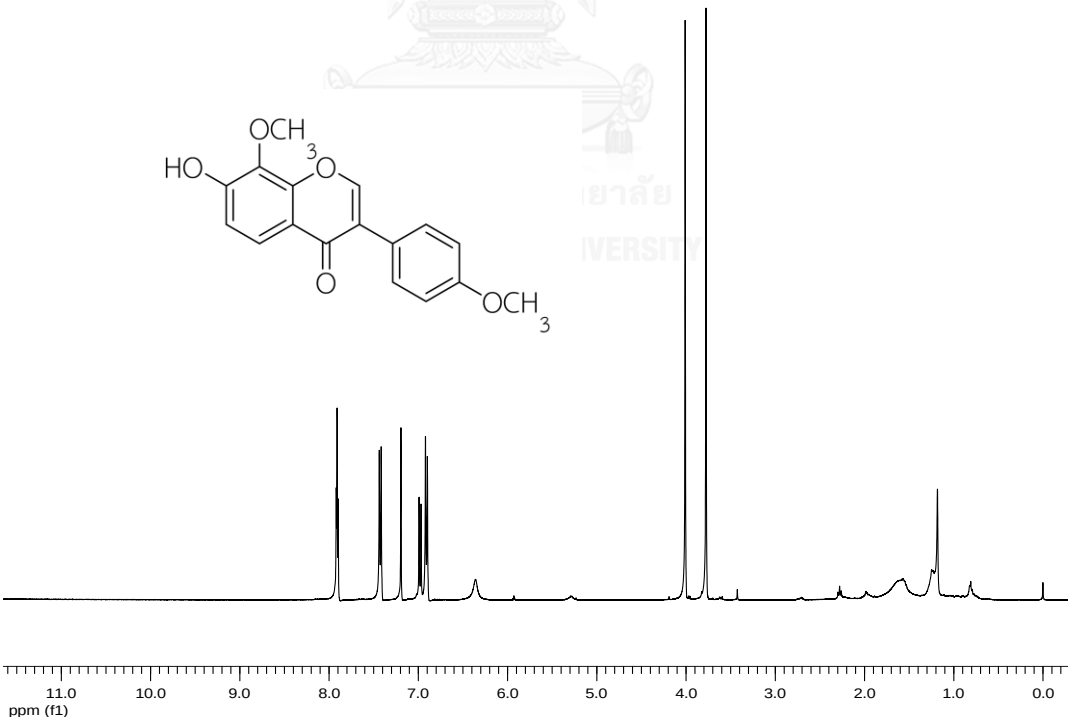

Figure A-48 ^{1}H NMR spectrum of 3.1 in $CDCl_3$

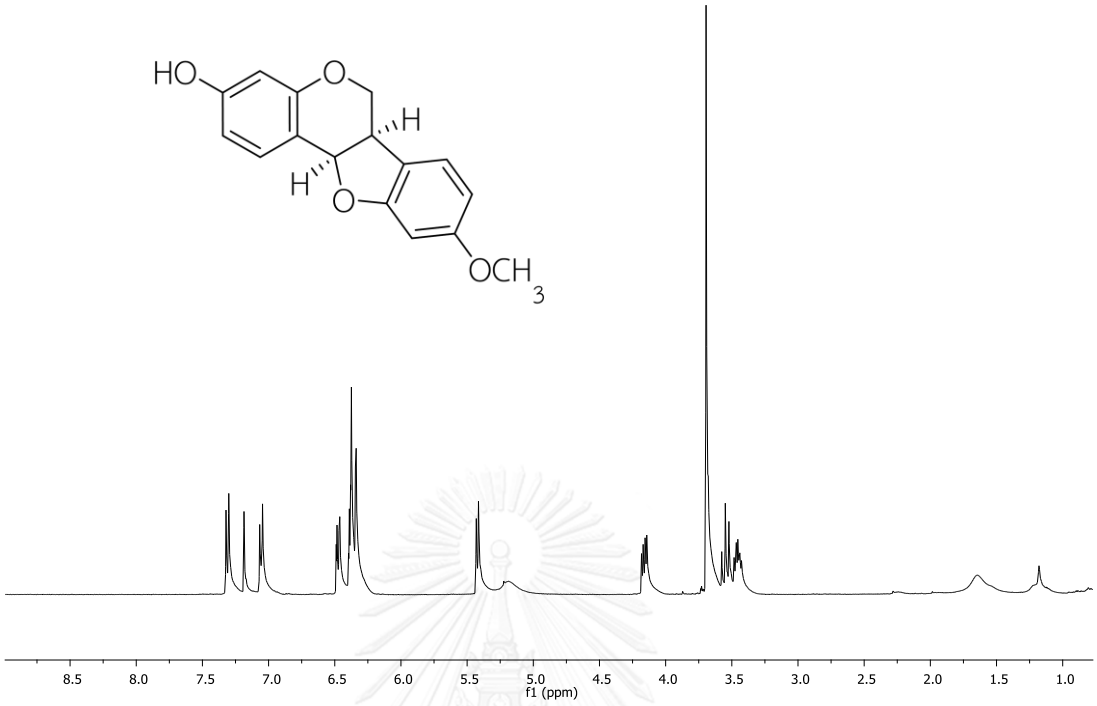

Figure A-49 ^{1}H NMR spectrum of 3.2 in $CDCl_3$

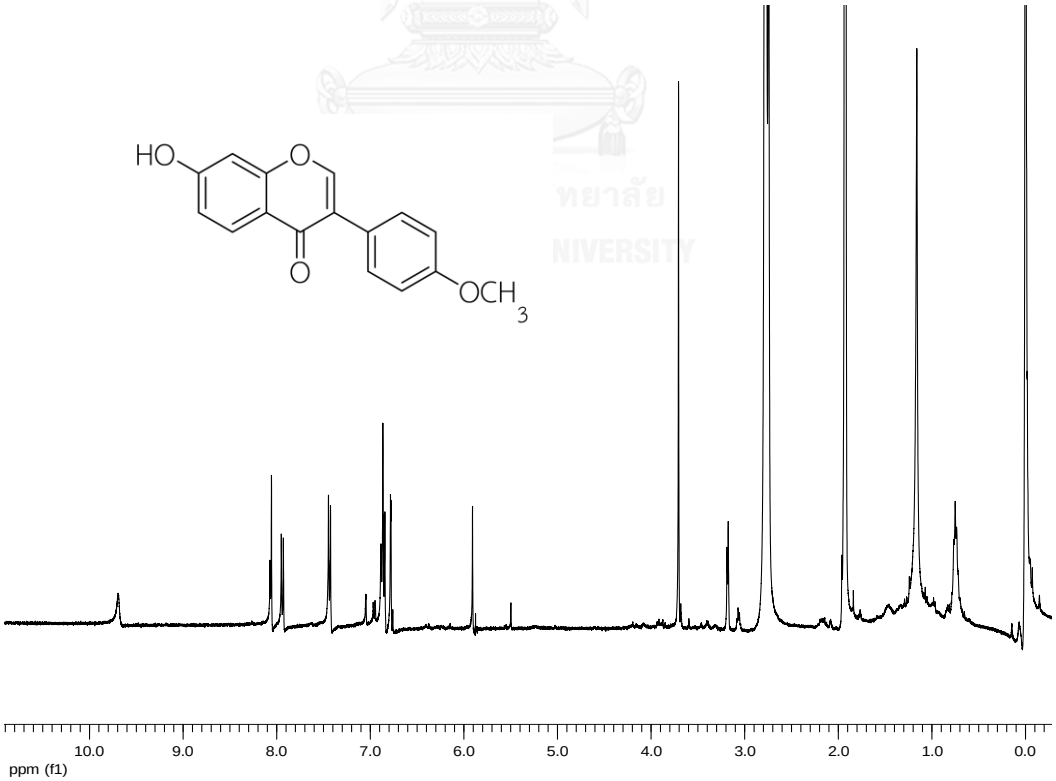

Figure A-50 ^{1}H NMR spectrum of 3.3 in $CDCl_3$

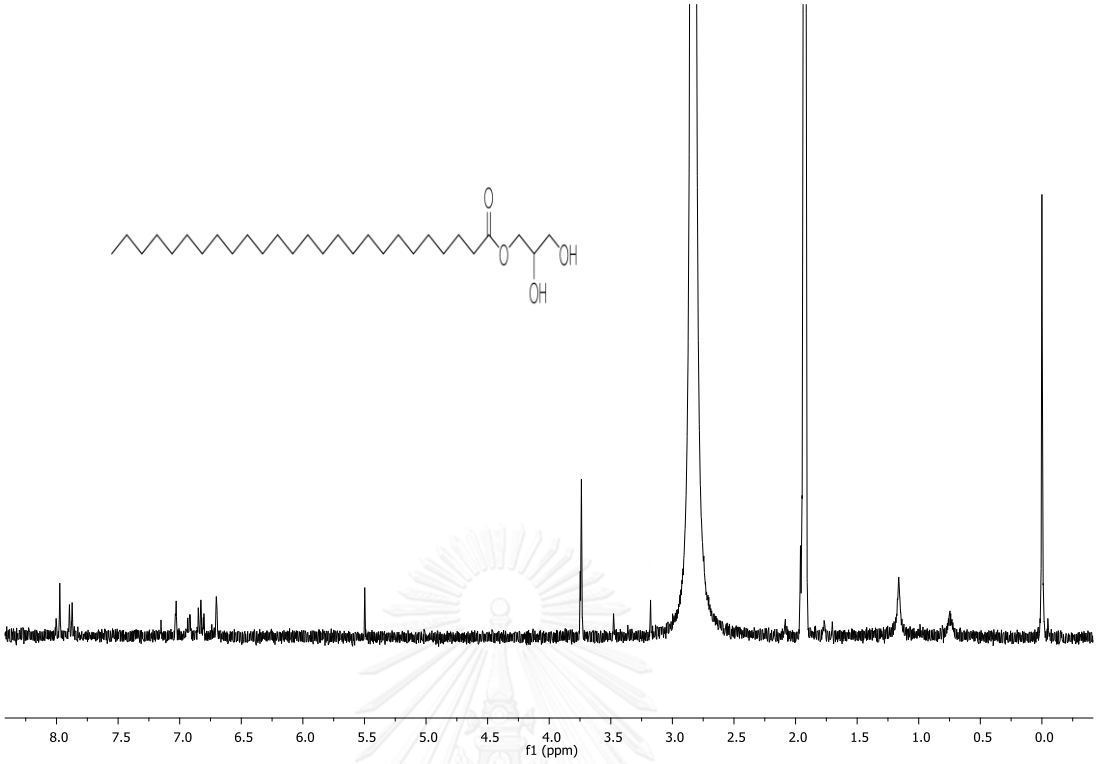

Figure A-51 ^{1}H NMR spectrum of 3.4 in $CDCl_3$

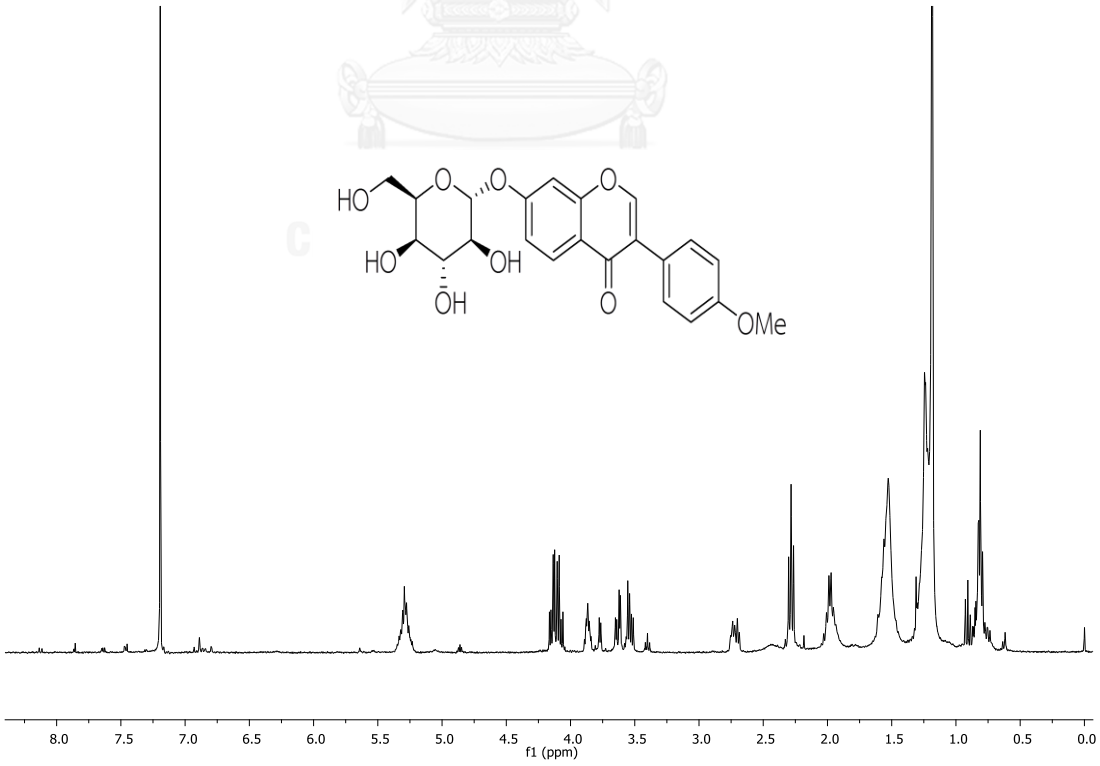

Figure A-52 ^{1}H NMR spectrum of 3.5 in $CDCl_3$

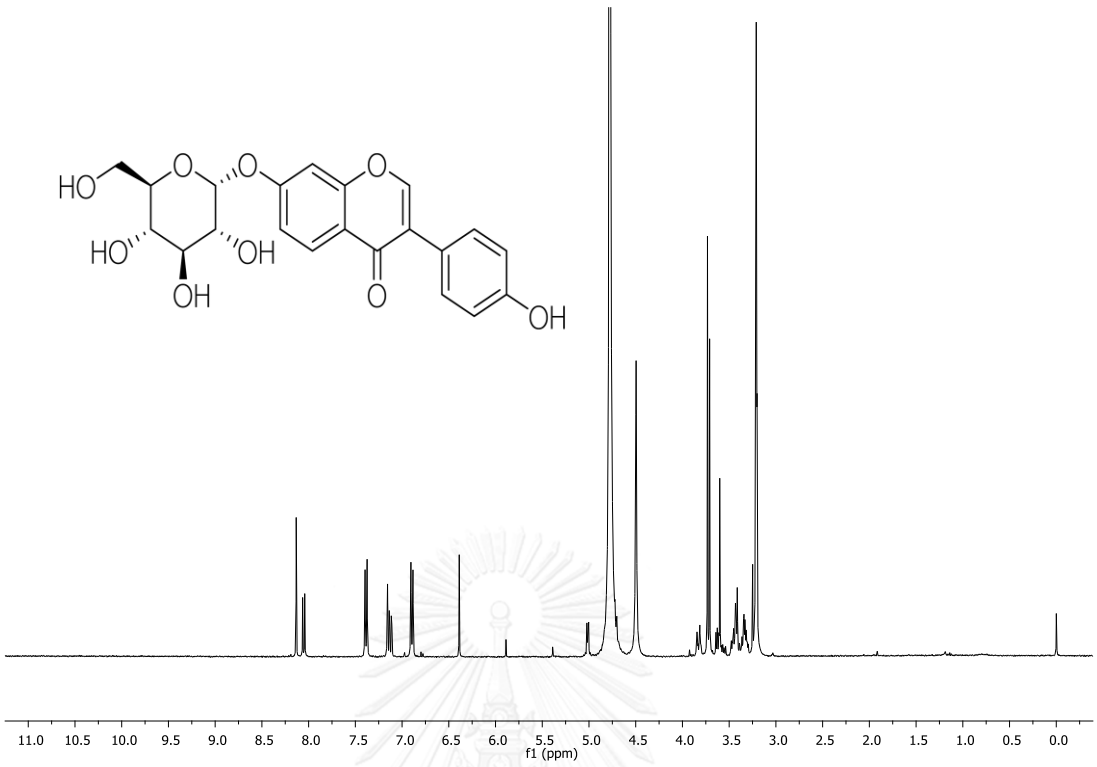

Figure A-53 ^{1}H NM R spectrum CD_3OD of 3.6

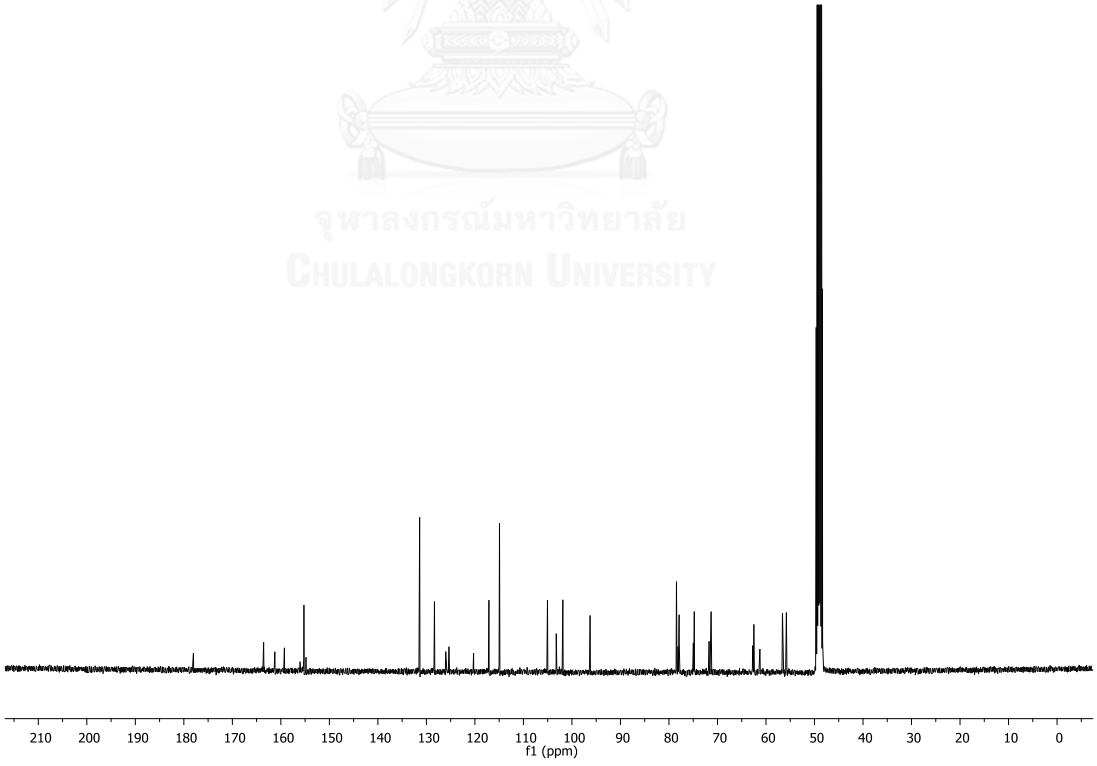

Figure A-54 ^{13}C NMR spectrum CD_3OD of 3.6

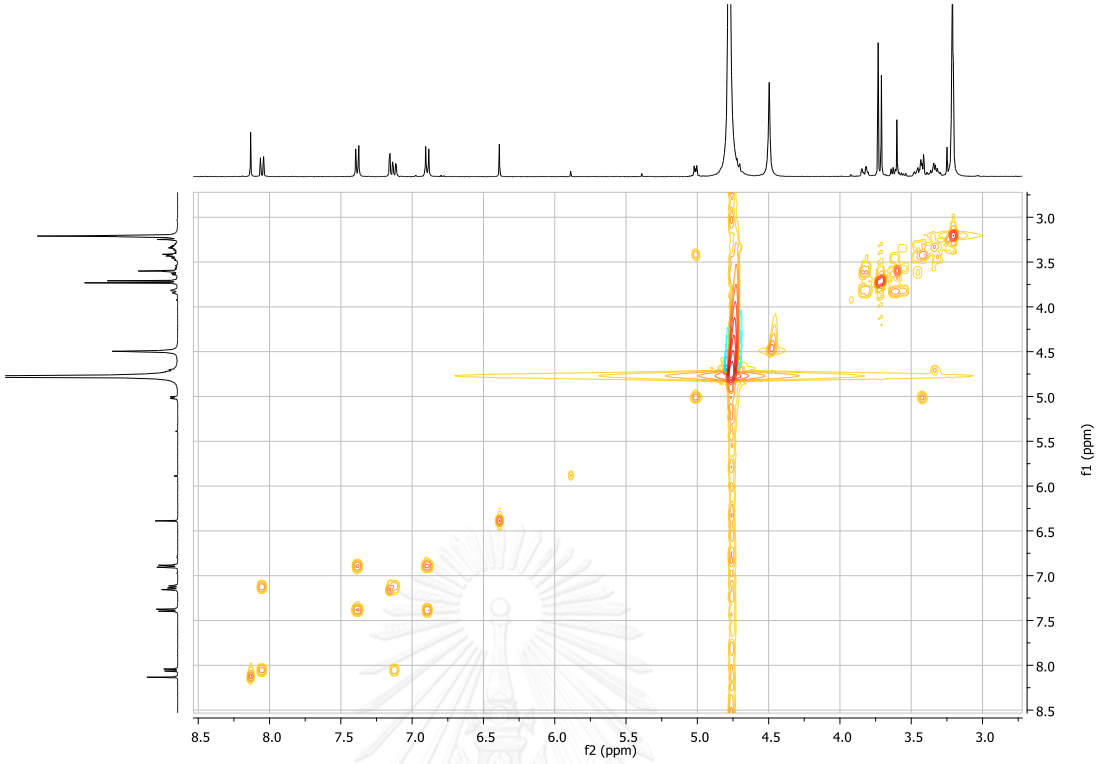

Figure A-55 COSY NMR spectrum CD_3OD of 3.6

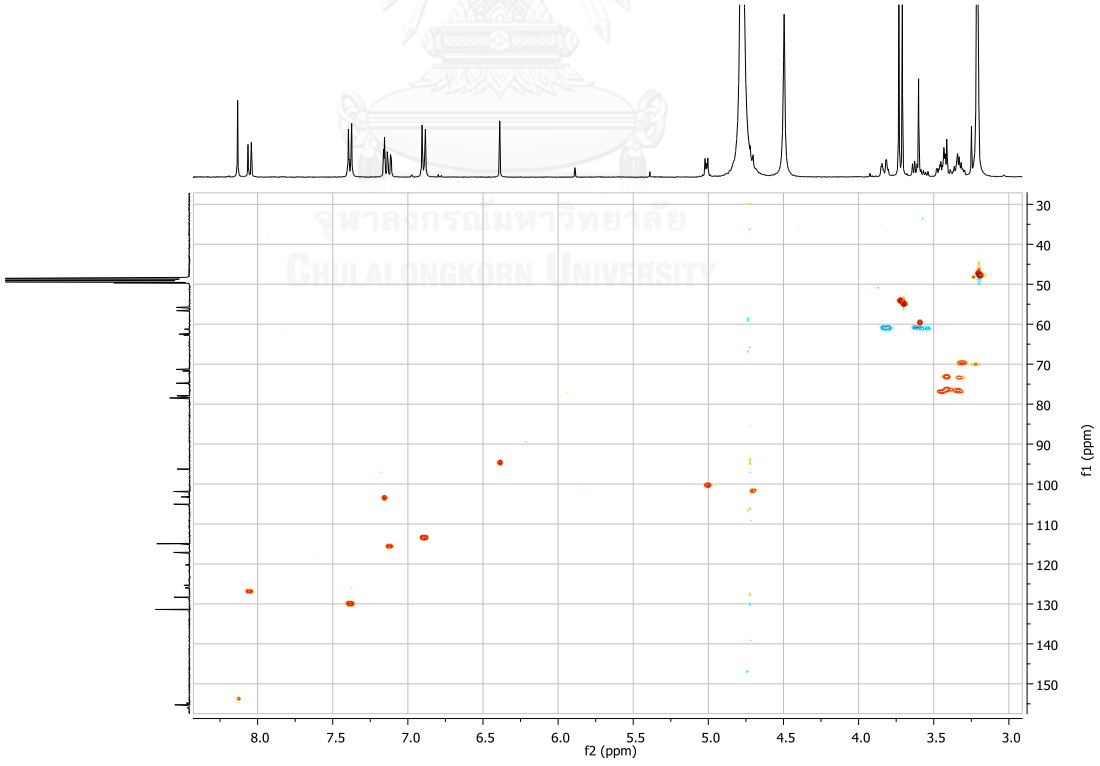

Figure A-56 HSQC NMR spectrum CD_3OD of 3.6

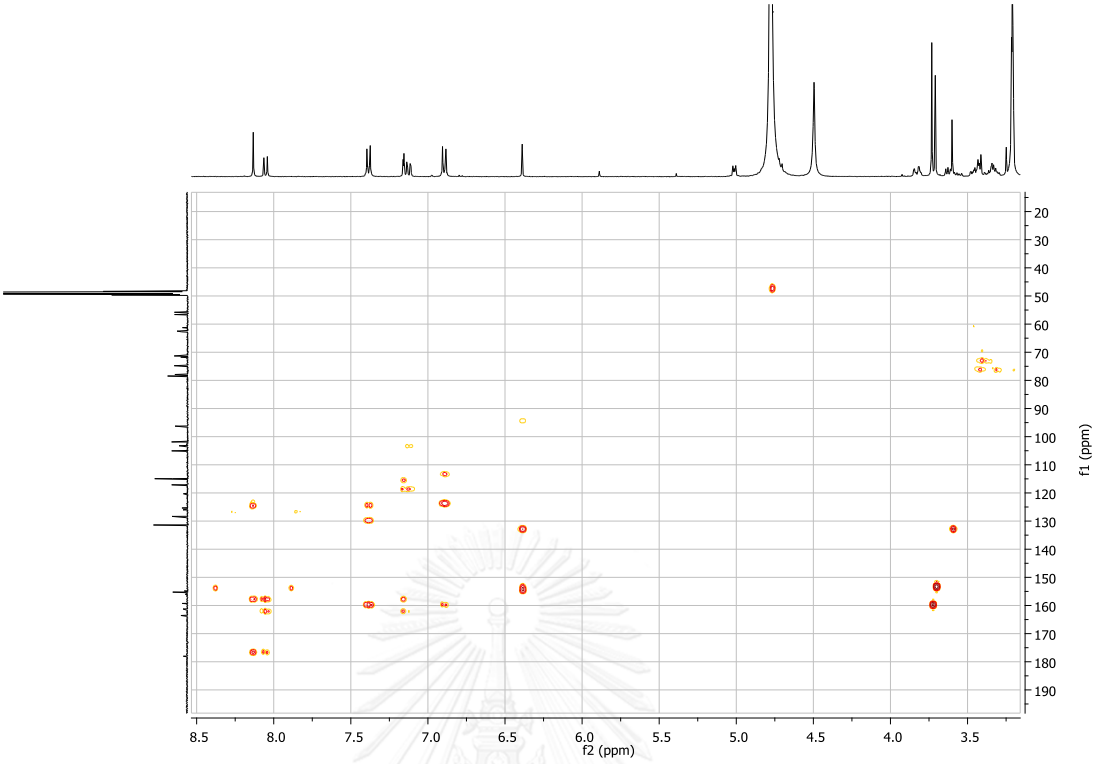

Figure A-57 HMBC NMR spectrum CD_3OD of 3.6

VITA

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