องค์ประกอบทางเคมีจากเปลือกลำต้น *Erythrina stricta* และ *E. subumbrans* และความเป็นพิษต่อเซลล์มะเร็ง

.

นายพงศธร เปรมรัตนชัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเทคโนโลยีชีวภาพ
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2555

CHEMICAL CONSTITUENTS FROM THE STEM BARK OF *Erythrina stricta* AND *E. subumbrans* AND ANTICANCER ACTIVITY

Mr. Pongsathon Premratanachai

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Biotechnology
Faculty of Science
Chulalongkorn University
Academic Year 2012

Thesis Title	CHEMICAL CONSTITUENTS FROM THE STEM BARK OF *Erythrina stricta* AND *E. subumbrans* AND ANTICANCER ACTIVITY
By	Mr. Pongsathon Premratanachai
Field of Study	Biotechnology
Thesis Advisor	Associate Professor Santi Tip-pyang, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

…….. Dean of the Faculty of Science
(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

…….. Chairman
(Assistant Professor Warinthorn Chavasiri, Ph.D.)

…….……. Thesis Advisor
(Associate Professor Santi Tip-pyang, Ph.D.)

…….. Examiner
(Associate Professor Chanpen Chanchao, Ph.D.)

…….. External Examiner
(Withawat Mingvanish, Ph.D.)

พงศธร เปรมรัตนชัย : องค์ประกอบทางเคมีจากเปลือกลำต้น *Erythrina stricta* และ *E. subumbrans* และความเป็นพิษต่อเซลล์มะเร็ง (CHEMICAL CONSTITUENTS FROM THE STEM BARK OF *Erythrina stricta* AND *E.subumbrans A*ND ANTICANCER ACTIVITY) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ดร.สันติ ทิพยางค์, 47 หน้า.

จากการศึกษาองค์ประกอบทางเคมีและทดสอบความเป็นพิษต่อเซลล์มะเร็งจากสิ่งสกัดไดเฮกเซนและคลอโรมีเทนของเปลือกลำต้น *Erythrina stricta* สามารถแยกสารที่มีรายงานแล้ว 6 ชนิด ได้แก่ *p*-hydroxybenzoic acid (**1**), osajin (**2**), derrone (**3**), erythinasinate (**4**), และของผสมของ β-sitosterol (**5**) และ stigmasterol (**6**) ในการทดสอบความเป็นพิษต่อเซลล์มะเร็งชนิด KB, HeLa, MCF-7, HepG-2, Colo205, และ LLC พบว่า สาร **2** มีความเป็นพิษต่อเซลล์มะเร็งชนิด KB และ Colo205 ได้ปานกลาง โดยมีค่า IC_{50} เท่ากับ 14.5 และ 9.74 μg/mL ตามลำดับและมีความเป็นพิษต่อเซลล์มะเร็งชนิด HeLa, MCF-7, HepG-2 และ LLC ได้ต่ำ โดยมีค่า IC_{50} เท่ากับ 21.8, 16.9, 17.4, 16.3 μg/mL สาร **3** มีความเป็นพิษต่อเซลล์มะเร็งชนิด KB, HeLa, MCF-7, HepG-2, Colo205 และ LLC ได้ต่ำ โดยมีค่า IC_{50} เท่ากับ 15.8, 19.1, 17.3, 20.1, 15.2 และ 17.1 μg/mL ตามลำดับ ส่วนสารอื่นไม่มีความเป็นพิษต่อเซลล์มะเร็ง (IC_{50} >30.0 μg/mL)

ในการศึกษาองค์ประกอบทางเคมีและทดสอบความเป็นพิษต่อเซลล์มะเร็งจากสิ่งสกัดไดคลอโรมีเทนและอะซิโตนของเปลือกลำต้น *E. subumbrans* สามารถแยกสารที่มีรายงานแล้ว 6 ชนิด ได้แก่ *p*-hydroxybenzoic acid (**1**), erythinasinate (**4**), และของผสมของ β-sitosterol (**5**) และ stigmasterol (**6**), hexacosyl *trans*-ferulate (**7**) และ lupeol (**8**). ในการทดสอบความเป็นพิษต่อเซลล์มะเร็งชนิด KB, HeLa, MCF-7, HepG-2, Colo205 และ LLC พบว่า สาร **8** มีความเป็นพิษต่อเซลล์มะเร็งชนิด KB, HeLa, HepG-2, Colo205 และ LLC ได้ปานกลาง โดยมีค่า IC_{50} เท่ากับ 14.0, 13.9, 13.7, 7.97 และ 10.6 μg/mL ตามลำดับและมีความเป็นพิษต่อเซลล์มะเร็งชนิด MCF-7 ได้ต่ำ โดยมีค่า IC_{50} เท่ากับ 16.7 μg/mL ส่วนสารอื่นไม่มีความเป็นพิษต่อเซลล์มะเร็ง (IC_{50} >30.0 μg/mL)

สาขาวิชา เทคโนโลยีทางชีวภาพ ลายมือชื่อนิสิต .....

ปีการศึกษา 2555 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

5272433523: MAJOR BIOTECHNOLOGY
KEYWORDS: *Erythrina stricta* / *Erythrina subumbrans* / CYTOTOXICITY
PONGSATHON PREMRATANACHAI: CHEMICAL CONSTITUENTS FROM THE STEM BARK OF *Erythrina stricta* AND *E. subumbrans* AND THEIR CYTOTOXICITY THESIS ADVISOR: ASSOC. PROF. SANTI TIP-PYANG, 47 pp.

The investigation for chemical constituents and their biological activities from the Hexane and CH_2Cl_2 crude extracts of the stem barks of *Erythrina stricta* led to the isolation of six known compounds, *p*-hydroxybenzoic acid (**1**), osajin (**2**), derrone (**3**), erythinasinate (**4**), and a mixture of *β*-sitosterol (**5**) and stigmasterol (**6**). All isolated compounds were evaluated for their cytotoxicity on KB, HeLa, MCF-7, HepG-2, Colo205, and LLC cells. Compound **2** exhibited moderate cytotoxicity against both KB and Colo205 cells with IC_{50} values of 14.5 and 9.74 µg/mL and exhibited weak cytotoxicity against HeLa, MCF-7, HepG-2, and LLC cells with IC_{50} values of 21.8, 16.9, 17.4, and 16.3 µg/mL, respectively. Compound **3** exhibited weak activity against all the tested cells (KB, HeLa, MCF-7, HepG-2, Colo205, and LLC cells) with IC_{50} values of 15.8, 19.1, 17.3, 20.1, 15.2, and 17.1 µg/mL, respectively. Other compounds gave only very high IC_{50} values and were regarded as inactive to these cytotoxicity assays.

The investigation for chemical constituents and their biological activities from the CH_2Cl_2 and acetone crude extracts of the stem bark of *E. subumbrans* led to the isolation of six known compounds, *p*-hydroxybenzoic acid (**1**), erythinasinate (**4**), and a mixture of *β*-sitosterol (**5**) and stigmasterol (**6**), hexacosyl *trans*-ferulate (**7**), and lupeol (**8**). All isolated compounds were evaluated for their cytotoxicity on KB, HeLa, MCF-7, HepG-2, Colo205, and LLC cells. Compound **8** exhibited moderate cytotoxicity against most of the tested cells including KB, HeLa, HepG-2, Colo205, and LLC cells with IC_{50} values of 14.0, 13.9, 13.7, 7.97, and 10.6 µg/mL respectively, whereas it exhibited weak cytotoxicity against MCF-7 cells with IC_{50} value of 16.7 µg/mL. On the other hand, other compounds gave only very high IC_{50} values and were regarded as inactive to these cytotoxicity assays.

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

Academic Year : 2012 Advisor's Signature .....

ACKNOWLEDGEMENTS

I would like to express my deep gratitude to my advisor, Associate Professor Dr. Santi Tip-pyang for his assistance and encouragement in conducting this research.

I also gratefully acknowledge the members of my thesis committee, Assistant Professor Dr. Warinthorn Chavasiri, Associate Professor Dr. Chanpen Chanchao and Dr. Withawat Mingvanish for their discussions and guidance. The special thank you goes to Ms. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University for identifying plants and making the voucher specimen of plant material as well as providing the stem bark of *Erythrina stricta* and *E. subumbrans* in this research.

My appreciation is also expressed to thank Dr. Pongpan Siripong and the staffs of Natural Products Research Section, Research Division, National Cancer Institute for their testing on cytotoxicity against KB, HeLa, MCF-7, HepG-2, Colo205, and LLC cell lines.

I would like to express my gratitude to the Natural Products Research Unit, the Department of Chemistry, Faculty of Science, Chulalongkorn University for providing the chemicals and facilities throughout the course of study. A deep affectionate gratitude is acknowledged to my family for their understanding, encouragement and support throughout the education program. Furthermore, I would like to thank all of my friends in the laboratory for their friendship and help during the course of my graduate research.

Finally, I would like to thank Graduate school, Chulalongkorn University for the thesis grant, which is highly appreciated.

CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS

^{13}C NMR	carbon 13 nuclear magnetic resonance
^{1}H NMR	proton nuclear magnetic resonance
br s	broad singlet (NMR)
COSY	correlated spectroscopy
d	doublet (NMR)
dd	doublet of doublet (NMR)
g	gram (s)
HMBC	heteronuclear multiple bond correlation
HRESIMS	high resolution electrospray ionization mass spectrometry
HSQC	heteronuclear single quantum correlation
Hz	hertz
IC_{50}	concentration that is required for 50% inhibition in vitro
J	coupling constant
m	multiplet (NMR)
M	molar
MeOH	methanol
mg	milligram (s)
MHz	megahertz
mL	milliliter (s)
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser enhancement spectroscopy
q	quartet (NMR)
s	singlet (NMR)
t	triplet (NMR)
VLC	vacuum liquid chromatography
δ	chemical shift

δ_C	chemical shift of carbon
δ_H	chemical shift of proton
μ	micro
ε	molar extinction coefficient
λ_{max}	maximum wavelength
2D NMR	two dimentional nuclear magnetic resonance
$[\alpha]_D^{20}$	specific optical rotation

CHAPTER I

INTRODUCTION

In respond to external stimuli including nutritional changes, adaptation, and competition, plants undergoes metabolised and releases special chemical which could turn into natural products [1]. Human always rely on natural products from medicinal plants since ancient time. Written records the used for herbal plants since 5000 years ago to the Sumerians era, and archaeological records suggest even possibly earlier use. As the new technology develops, human could use plants in much more efficient way. Despite the thousand years of studies and usages, yet, many plants chemical and their biological activity is unknown to human [2].

While plants synthesize a wide variety of primary and secondary metabolites that is toxic to other plants in order to gain competitive advantage, it also synthesizes the same in order to blockage toxic from other plants. This is the reason why many plants have many antidotes that human could use. Researchers classified more than 122 compounds used in mainstream medicine which were found to more than 80% of the compounds that were used in the same way as traditional ethnomedical use. Therefore, learning traditional medicines will eventually leading the way to isolate potential future medicinal compound [3].

Wild diversity of plants has been investigated for conventional drugs developments throughout the world. Since the mortality rate from cancers have increased and become crisis, prevention to those diseases is the most focus on drugs development. With the high express primary and secondary metabolite system especially on tropical plants which are usually known to have many potential related to bioactive activities such as antithrombotic, antiflammatory, antigout, and anticancer activity, many new drugs have been found [4]. Many of the new found natural compounds from plants are usually focus by cytotoxicity activity test. In addition, many people who live with rural area trend to live longer than people who live in the city. Thus, many believe that there must be some clue to prolonger life in the natural

products. Many researches related to risk of using synthesis drugs have been published around the world. With little encouragement from many organization and campaign, people start to turn their interest into natural products and deny using chemical drugs. The natural products researches become much more wide spread than what have it ever been [5].

When cancerous cells are killed by the chemotherapy and, more healthy cells are killed in the process. Generally, the healthy cells die easier than the cancer cells. In fact, those chemicals can kill people faster than the cancer would have done. This is why most of the new research focuses on natural supplements that do a good job of killing cancerous cells, but don't cause harm to human body. The natural product is generally weaker but much safer. Over 60% of the current anticancer drugs have their origin in one way or another from natural sources. Nature continues to be the best source of biologically active and diverse drugs. Even though only few of the actual isolated compounds from nature could become clinically effective drugs in their own, these unique molecules can serve as a mixture, or models for the preparation of more efficacious advance drugs using the chemical methodology such as total or combinatorial synthesis, or manipulations of biosynthetic pathways [6].

1.1 Botanical aspect and distribution

Erythrina is a genus from Leguminose family, consisting of approximately 120 species or more throughout tropical and subtropical regions [7]. The generic name is derived from the Greek word "*erythros"* meaning "red" referring to the flower color of certain species. Most of the species from this genus have bright red color flowers, and are called flame trees by some native. *Erythrina* can presumable be found anywhere with the possible exception of extremely dry areas and very few species are limited to specific ecological zones [8]. Scientific research has showed that many *Erythrina* species are rich sources of bioactive alkaloids, and flavonoids compounds. Mostly are isoflavones, pterocarpans, flavanones, and isoflavanones [9-13]. Some compounds also show decent biological activity such as antimicrobials activity, antifungal activity, anti-inflammatory activity, anti-plasmodial activity, and antioxidant activity. Most of the species belong to this genus are used as traditional medicine among local folks. [14-19].

Erythrina stricta is a perennial plant with shed leaves, and flowery. It can reach to 10-20 m tall with thick bark, and have many crowns spread. It has bright red flowers lay out similar to bouquet in the end tip of small branches which is around 5-8 cm long. Each flower has 5 petals and 10 pollen. The fruit part is cylinder-like with little curve, size around 0.6-1 cm in diameter and 7-10 cm long. It usually has bronze color and contains many seed inside [20].

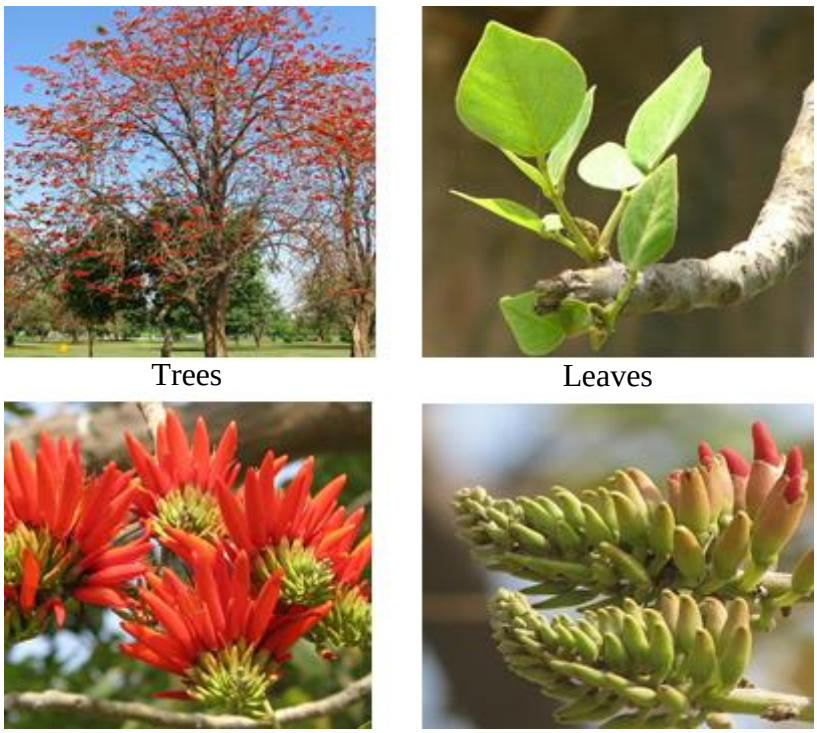


Figure 1.1 Trees, leaves, flowers, and buds of *E. stricta*.

Erythrina subumbrans is a deciduous, mediem-sized tree which can reach to 25 m tall while its trunk can reach up to 60 cm in diameter. The crown spreads and the bark is whitish. The trunk and branches are armed with stout while in cultivation, it is mostly unarmed. The leaves are arranged alternate and with three leaflets which are ovate-triangular-rhomboid. The inflorescence is racemose at the upper leat axils. It is 5-23 long and brownish-hairy. The pod is flat and curved around 10-15 cm long. The lower part is seedless and the upper part is thicker which has 1-5 dull-black seeded. The bark could be used in remedies for cough and poultice treatment [21].

Figure 1.2 Trees, buds, flowers, and leaves of *E. subumbrans.*

There are about 5 Erythrina species that are native to Thailand, most of them have common name start with “Thong” which means as “gold” in Thai language due to its bright color. for example:

E. fusca (ทองโหลง)

E. stricta (ทองเดือนห้า)

E. suberosa (ทองหลางใบมน)

E. subumbrans (ทองหลาง)

E. variegate (ทองบ้าน)

Most of the species shares common compounds that give rise to bright orange color pigment on the flowers, and usually contain mixture of *β*-sitosterol and stigmasterol on waxy parts of the plants. From previous study analysis, *E. fusca* and *E. suberosa* have been already been widely studied throughout the world. However, *E. stricta*, *E. subumbrans*, and *E. variegate* are consider much less well-known.

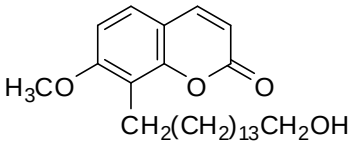
E. variegate *E. suberosa* *E. fusca*

Figure 1.3 *E. fusca*, *E. suberosa*, and *E. variegate.*

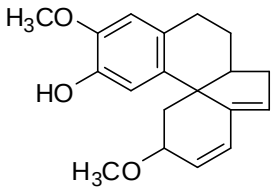
1.2 Previous researches

1.2.1 Previous researches of *E. Stricta*

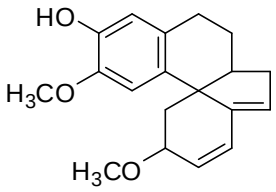
In 1981, the first compound isolation on record of the *E. stricta* has been done by Harkishan *et al*. The barks of the *E. stricta* has been extracted with petroleum ether, and then fractionated into various non-nitrogenous fractions which consist of n-alkanes, fatty ester, n-alkanols, alkyl ferulates, fatty acids, sitosterol, and stigmasterol. The ethanol extractive yielded a coumarin entity, identified as 7-methoxy-8-(15-hydroxypentadecyl)-coumarin, erysovine, and erysodine [22].



7-methoxy-8-(15-hydroxypentadecyl)-coumarin



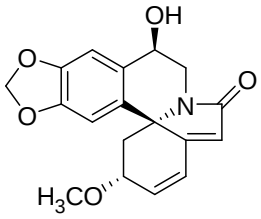
erysovine



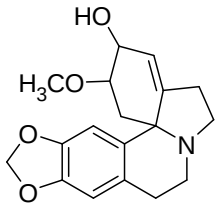
erysodine

Figure 1.4 Isolated compounds from bark of *E. stricta*

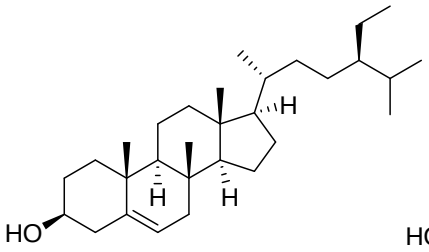
In 2007, Rukachaisirikul *et al* has investigated the hexane and CH_2Cl_2 extracts of *E. stricta* roots which yield four pterocarpans, one flavanone, two triterpenes, two alkaloids, two esters, and two steroids. The compounds were identified as erythrabyssin II, erystagallin A, erythrabissin-1, 5-hydroxysophoranone, sandwicensin, sophoradiol, soyasapogenoi B, 8-oxoerythrinine, erythratine, alkyl *trans*-ferulates, and a mixture of *β*-sitosterol and stigmasterol. In antiplasmodial activity test, 5-hydroxysophoranone exhibited the most potent result [23].



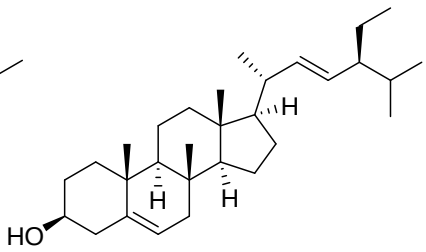
8-oxoerythrinine



erythratine

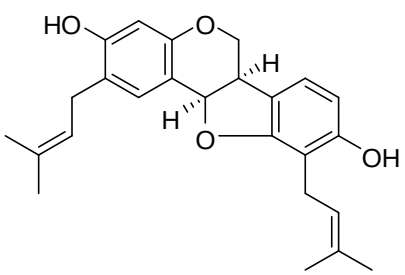


β-sitosterol

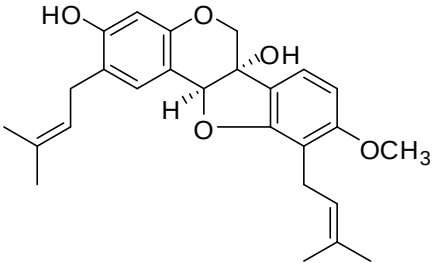


stigmasterol

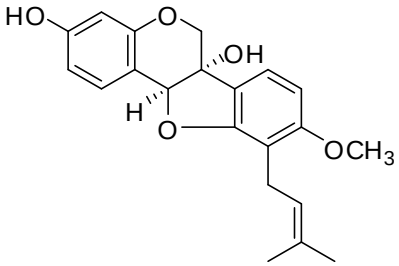
Figure 1.5 Isolated compounds from roots of *E. stricta*



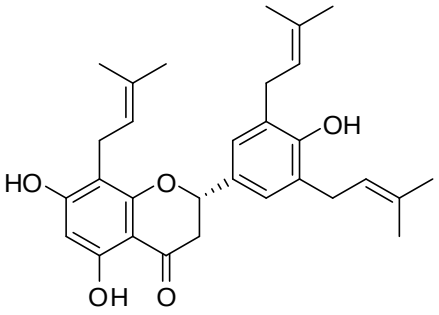
erythrabyssin II



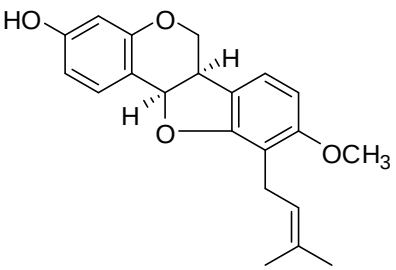
erystagallin A



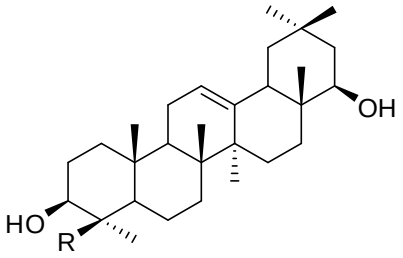
erythrabissin-1



5-hydroxysophoranone



sandwicensin



sophoradiol ; R = CH_3
soyasapogenoi B ; R = CH_2OH

Figure 1.5 Isolated compounds from roots of *E. stricta* (cont.)

In 2008, Umamaheswari et al has studied in *vitro* xanthine oxidase inhibitory activity of the various fractions of the hydromethanolic extract of the leaves of *E. stricta*. Among the factions tested, the chloroform fraction exhibited highest potency (IC_{50} 21.2±1.6 µg/ml) compare to the standard value from allppurinol (IC_{50} 6.1±0.3µg/ml). The graph shows that the mode of enzyme inhibition mechanism is mixed type. This suggests that the use of *E. stricta* for the treatment of gout could be attributed to its xanthine oxidase activity [24].

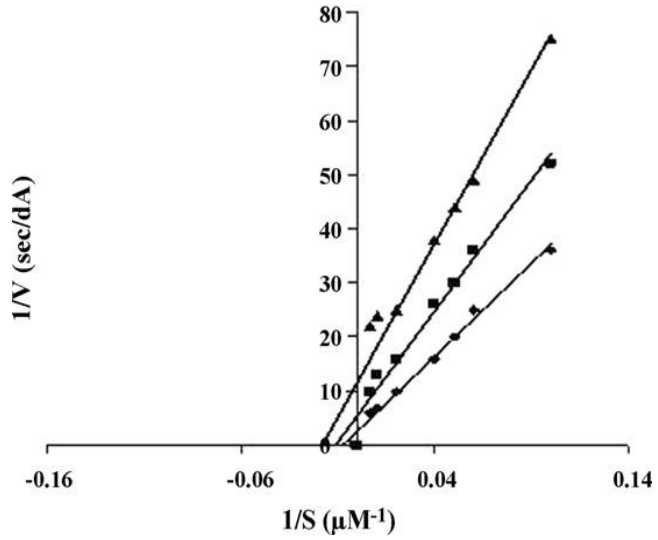
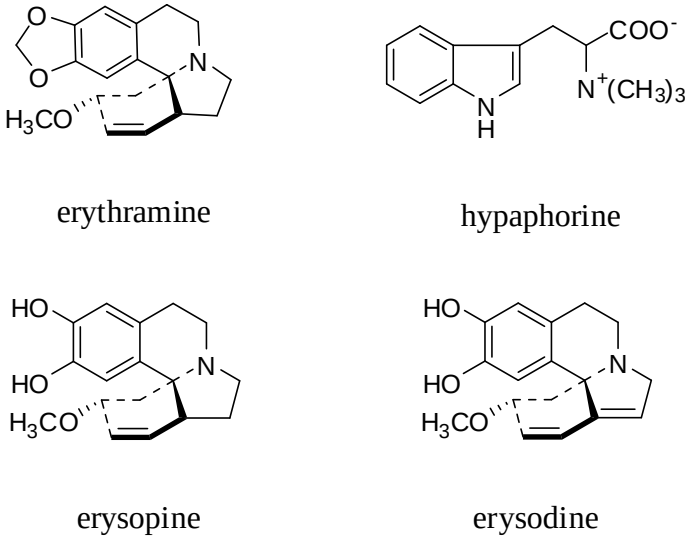


Figure 1.6 Lineweaver-Burk plot of inhibition of xanthine oxidase by chloroform fraction of *E. stricta* (■) and allopurinol (▲). The symbol (♦) represents negative control.

1.2.2 Previous researches of *E. subumbrans*

In 1939, Folkers *et al*. has extracted the seeds of *E. subumbrans* with petroleum ether, and isolated two new alkaloids, which are erythramineand hypaphorine [25]. In 1941, Folkers *et al*. continue his research with *E. subumbrans* and isolated two more alkaloids which are erysopine and erysodine [26].



erythramine hypaphorine

erysopine erysodine

Figure 1.7 Isolated compounds from seeds of *E. subumbrans*

In 2006, Rukachaisirikul et al. has extracted stems of *E.subumbrans* with hexane and dichloromethane. Erythrabissin I, erythrabyssin II, erycristagallin, erybraedin A, erybraedin B, phaseollin, erystagallin A, 5-hydroxysophoranone, glabrol, and erysubin F were isolated. Some of the compound has shown high activity against antibacterial test [27]. In the same year, the bark of *E.subumbrans* has been investigated by similar extraction, and three new compounds have been isolated, which are identified as 1-methoxyerythrabyssin II, (+)-10,11-dioxoepierythratidine, and (+)-10,11-dioxoerythratine [23].

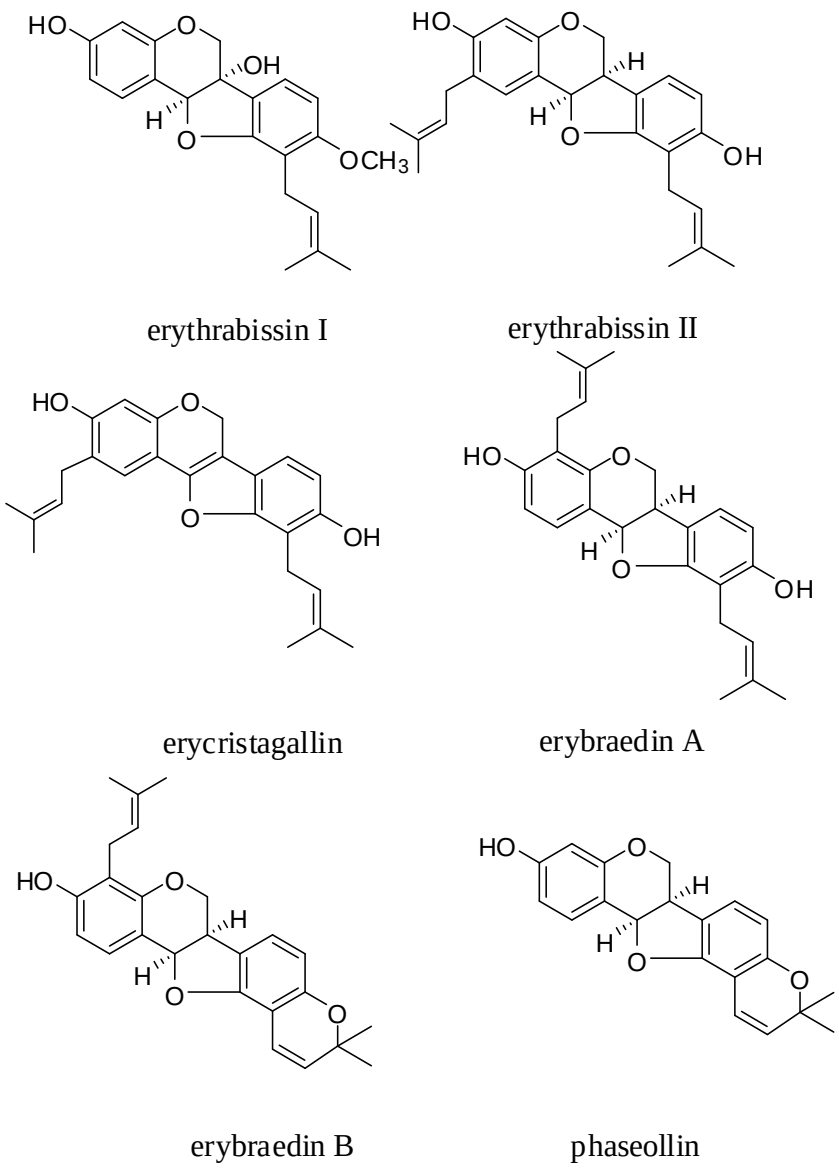
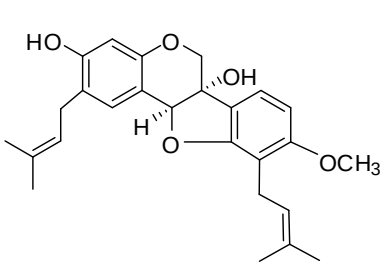
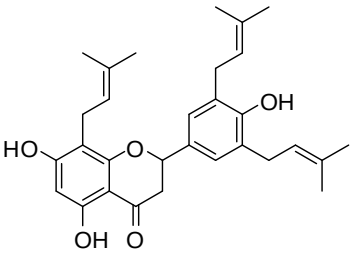


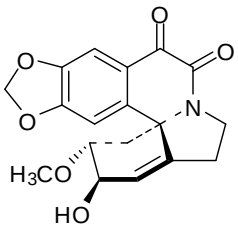
Figure 1.8 Isolated compounds from stems of *E. subumbrans*



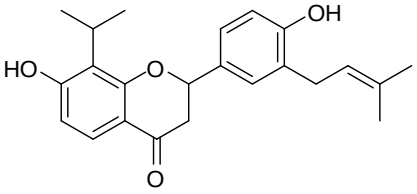
erystagallin A



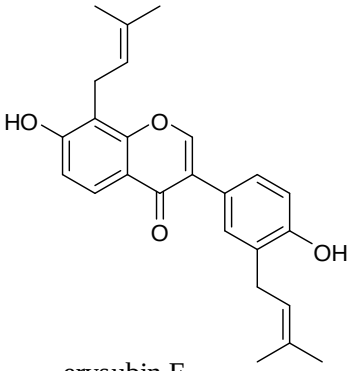
5-hydroxysophoranone



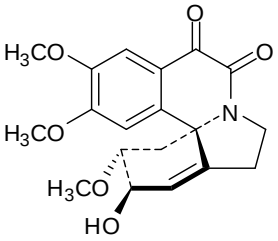
(+)-10,11-dioxoerythratine



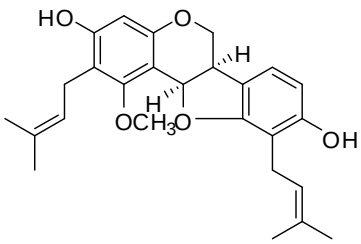
glabrol



erysubin F



(+)-10,11-dioxoepierythratidine



1-methoxyerythrabyssin II

Figure 1.8 Isolated compounds from stems of *E. subumbrans* (cont.)

1.3 Biological activity

1.3.1 Cytotoxicity against cancer cell lines

Using colorimetric reaction, MTT or MTS assay can measures the reducing potential of the cancer cells. MTS reagent is reduced to a colored formazan product by viable cells. A similar redox-based assay has also been developed using the fluorescent dye, resazurin. In order to monitor viability, new assay that use ATP content as marker has been developed [28]. The ATP-based assays also include bioluminescent assays in which ATP is the limiting reagent for the luciferase reaction. In addition, there are many more assay to determine cytotoxicity such as SRB assay, WST assay, and clonogenic assay [29].

From the literature review on the chemical constituents, there aren't many reports on the cytotoxicity and chemical constituents from *E. stricta* and *E. subumbrans* yet. Therefore, the stem bark of *E.* stricta and *E.* subumbrans are attracted for further investigation.

1.4 The objectives of the research:

The main objectives of this investigation are as follows:

1. To isolate and purify compounds from the stem bark of *E. stricta* and *E. subumbrans*.
2. To identify the chemical structures of all isolated compounds.
3. To evaluate the cytotoxicity against cancers cell lines of the isolated compounds

CHAPTER II

EXPERIMENTAL

2.1 General experimental procedures

NMR spectra were recorded with a Varian model Mercury^{+} 400 operated at 400 MHz for ^{1}H and 100 MHz for ^{13}C nuclei. Radical chromatography was performed on a Chromatotron (model 7924 T, Harrison Research) with a silica gel plate of 1 mm thickness. High resolution mass spectra were recorded on Micromass LCT or Bruker MICROTOF mass spectrometers. UV–visible absorption spectra were recorded on a UV-2552PC UV-Vis spectrometer (Shimadzu, Kyoto, Japan).

2.2 Plant material

The stem bark of *E. stricta* (Khumkratok no. 4-11) was collected from Lamphang province of Thailand in July 2011. The stem bark of *E. subumbrans* (Khumkratok no. 3-11) was collected from Sakonnakorn province of Thailand in July 2011. The plants materials were identified by Ms. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, and the specimens were retained as references.

2.3 Extraction and isolation

2.3.1 Extraction and isolation of *E. Stricta*

The air-dried stem bark of *E. stricta* (1.1 kg) was successively extracted in a Soxhlet with hexane and CH_2Cl_2 in order of increasing polarity. All solvents were evaporated to give the crude extract total of 18.6 g consist of hexane extract (8.9 g) and CH_2Cl_2 extract (9.7 g).

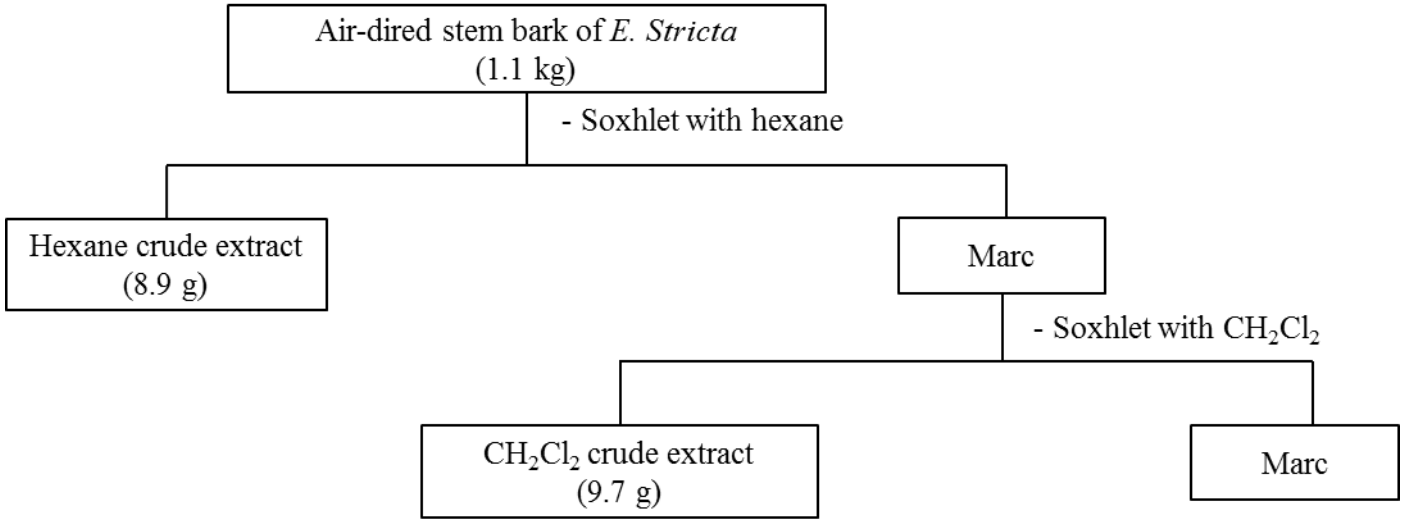
The hexane extract (8.9 g) of *E. stricta* stem bark was subjected to vacuum liquid chromatography (VLC) on silica gel (Merck Art 7730), using successive elution of hexane, CH_2Cl_2, and EtOAc with increasing polarity to provide seven fractions (FH1-FH7). Fraction FH4 was further purification by flash column (silica

gel Merck Art 7734; CH_2Cl_2-hexane, 50:50) to afford erythinasinate (**4**) (13.5 mg). Fraction FH6 was further fractionated by column chromatography to afford ten fractions (FH6.1-FH6.10). By purifying a combine fraction of FH6.2 and FH6.3 with another round of column chromatography, the mixture of *β*-sitosterol (**5**) and stigmasterol (**6**) were obtained (15.6 mg).

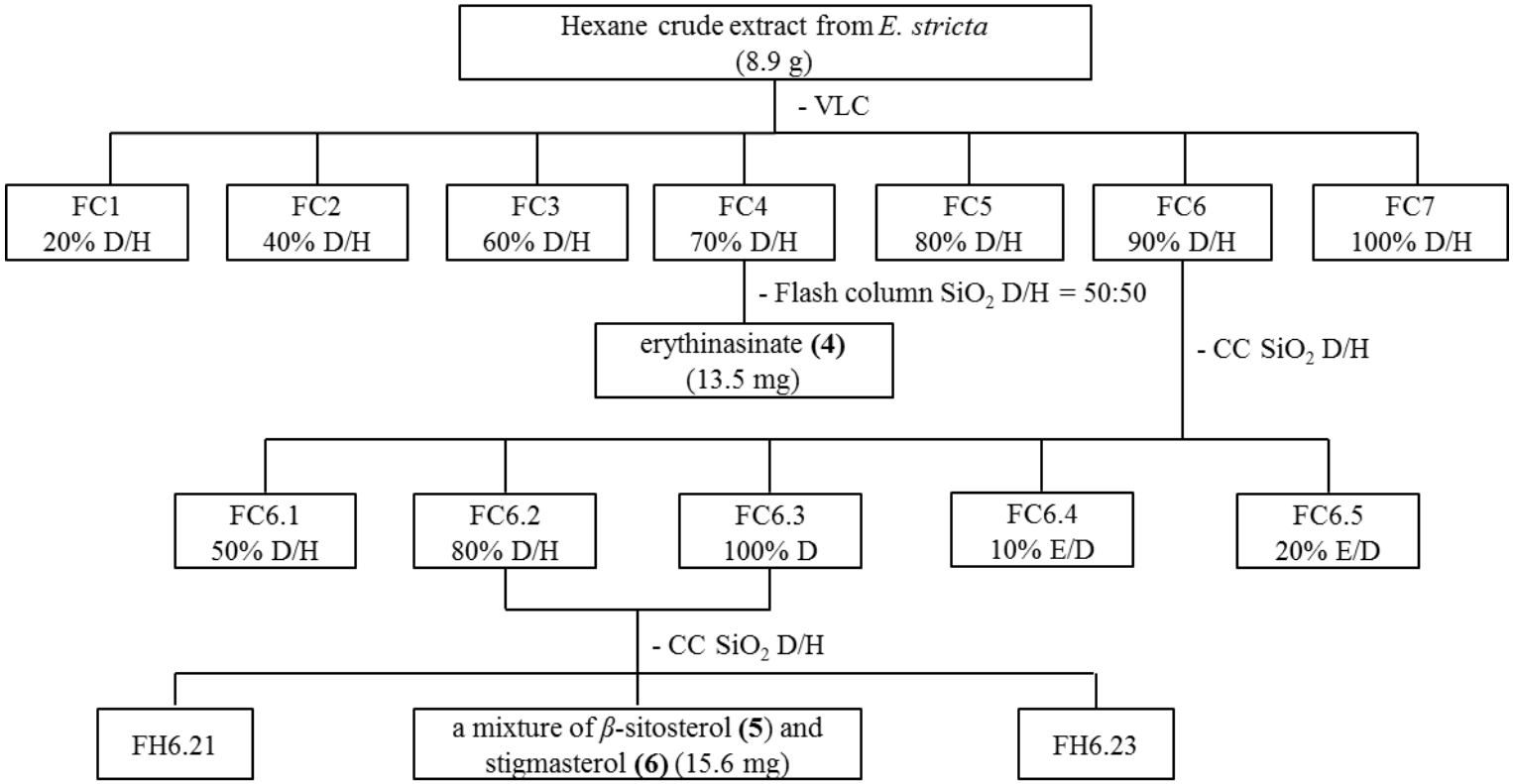
The CH_2Cl_2 extract (9.7 g) of *E. stricta* stem bark was similarly chromatographed on silica gel VLC, eluting with hexane, CH_2Cl_2, and EtOAc with increasing polarity to yield eight fractions (FC1-FC8). Fraction FC5 was further fractionated by column chromatography (silica gel; Hexane-CH_2Cl_2-MeOH gradient) into nine fractions (FC5.1-FC5.9). Fraction FC5.8 was then further fractionated by Sephadex LH-20 (using hexane-CH_2Cl_2-MeOH, 50:30:20) to give subfractions FC5.81, FC5.82 which is osajin (**2**) (5.7 mg), FC5.83, and FC5.84. Subfraction 5.81 was then purified by flash column (Hexane-CH_2Cl_2, 30:70) to get derrone (**3**) (7.4 mg), and subfraction FC5.83 was then purified by preparative thin layer chromatography (PTLC) (CH_2Cl_2-MeOH, 95:5) to obtain *p*-hydroxybenzoic acid (**1**) (3.9 mg).

The isolated compounds were then identified by means of various spectroscopic methods including MS and 1D NMR techniques together with comparison with the previous literature data.

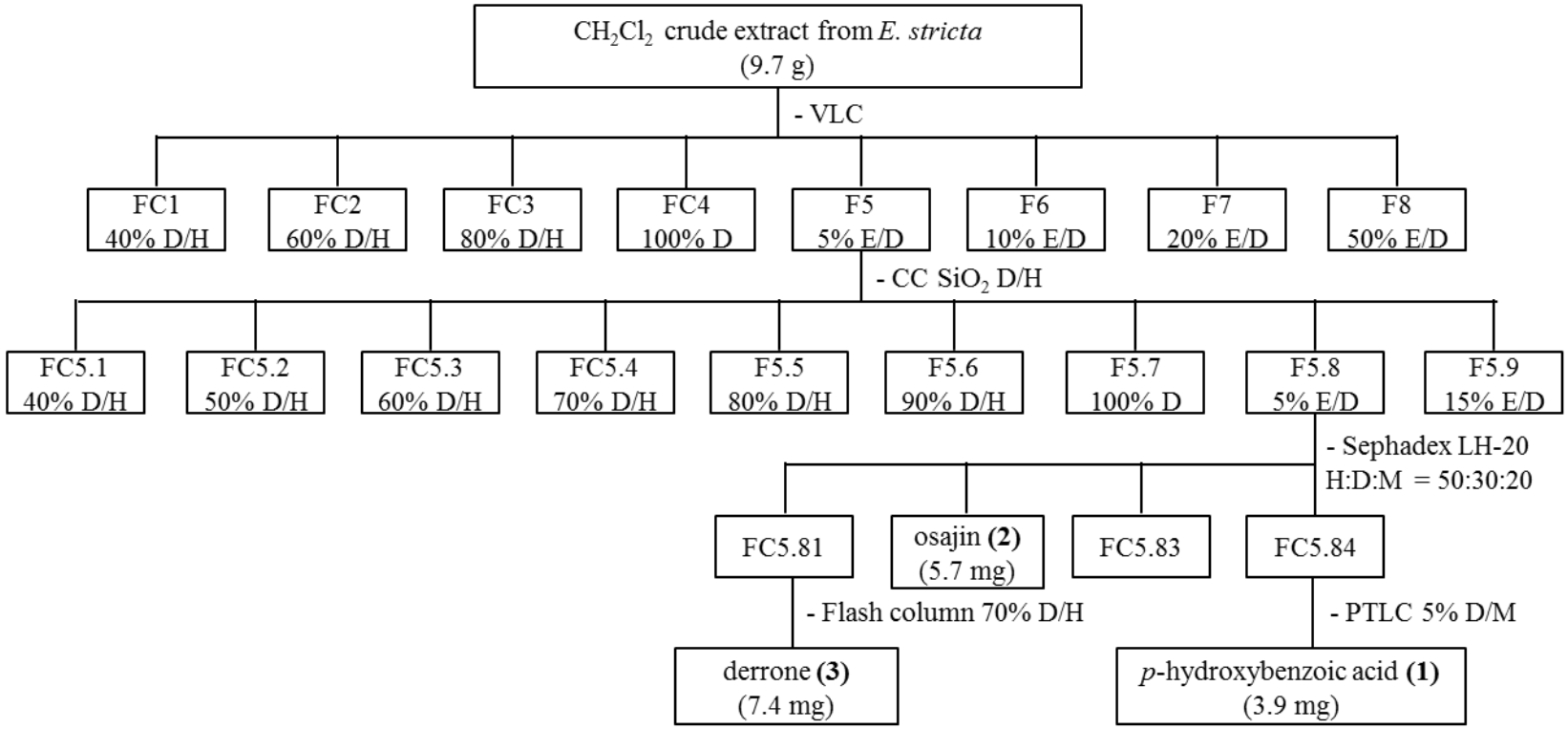
The isolation and purification of all isolated compounds from the stem bark of *E. stricta* were briefly summarized in Schemes 1.1 to 1.3.



Scheme 1.1 Extraction procedure of *E. stricta*



Scheme 1.2 Isolation procedure of the hexane crude extract from *E. stricta*



Scheme 1.3 Isolation procedure of the CH_2Cl_2 crude extract from *E. stricta*

2.3.2 Extraction and isolation of *E. Subumbrans*

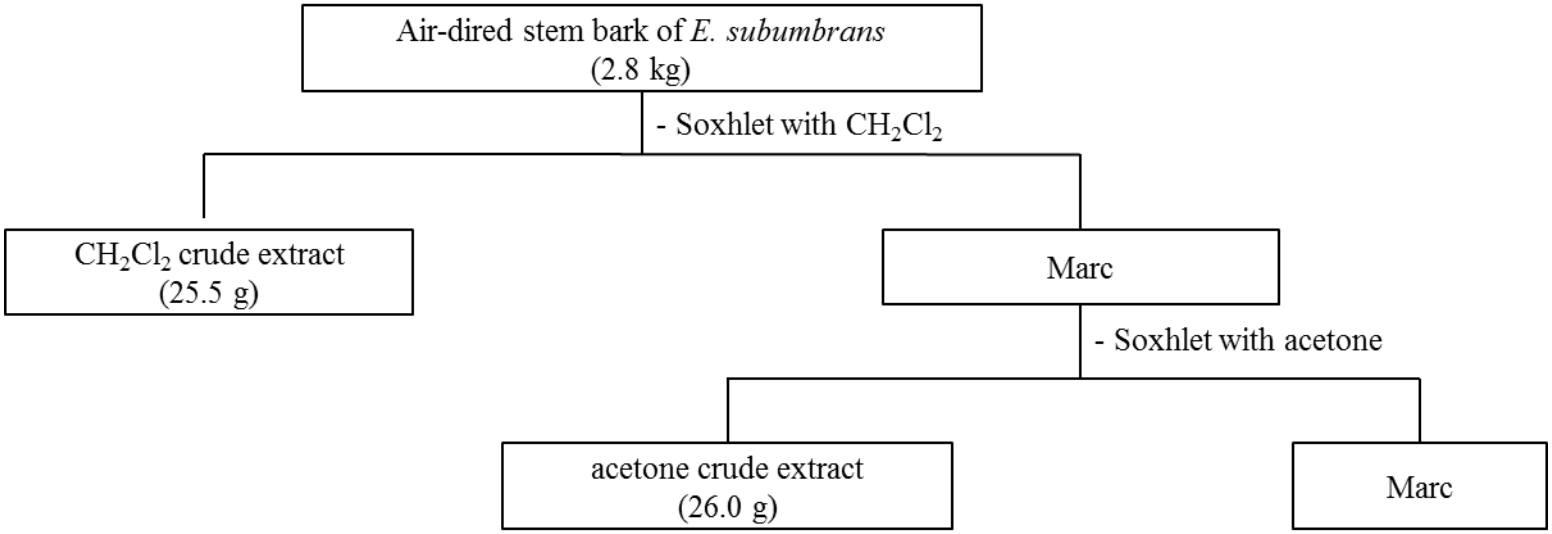
The air-dried stem bark of *E. subumbrans* (2.8 kg) was successively extracted in a Soxhlet with CH_2Cl_2 and acetone in order of increasing polarity. All solvents were evaporated to give the crude extract total of 18.6 g consist of CH_2Cl_2 extract (25.5 g) and acetone extract (26.0 g).

The CH_2Cl_2 extract (25.5 g) of *E. subumbrans* stem bark was fractionated by large column chromatography (silica gel; hexane-CH_2Cl_2-MeOH gradient) to yield five fractions (SC1-SC5). Fraction SC1 was purified with flash column chromatography method to give raise to erythinasinate (**4**) (10.0 mg). Fraction SC2 was further chromatographed to afford the mixture of *β*-sitosterol (**5**) and stigmasterol (**6**) (19.2 mg).

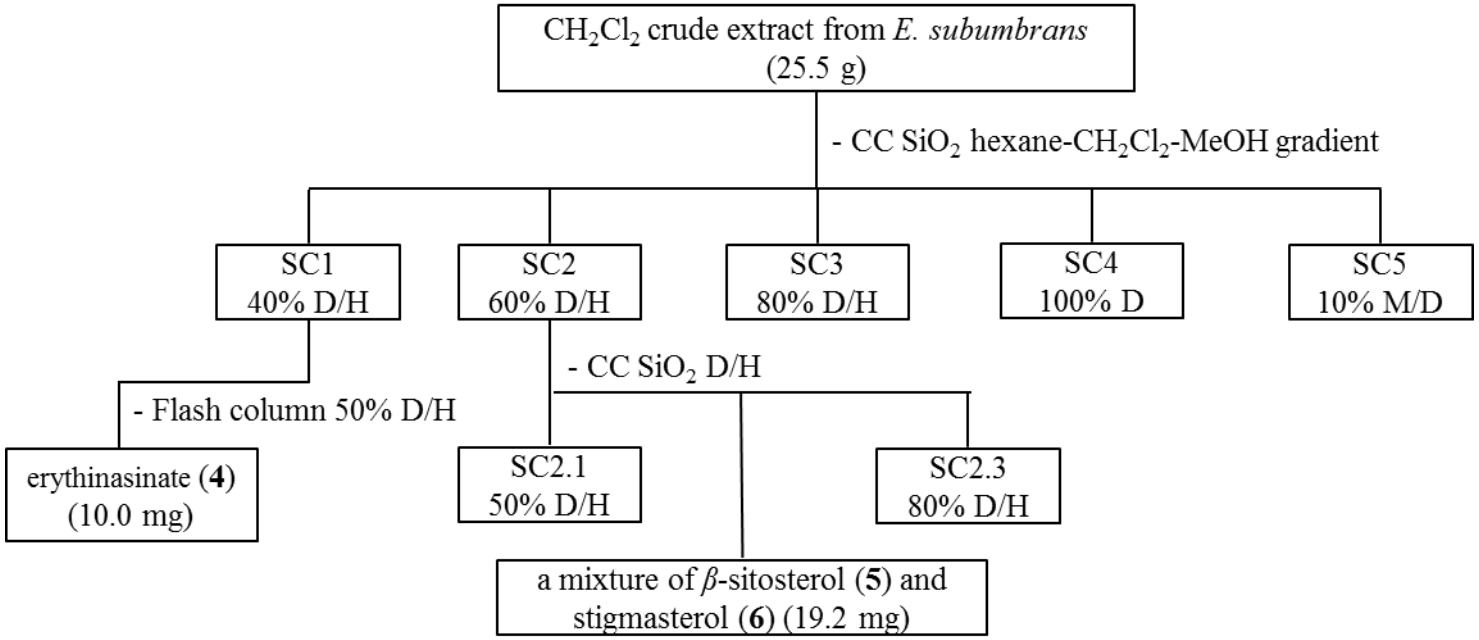
The acetone extract (26.0 g) of *E. subumbrans* stem bark was fractionated by large column chromatography (silica gel; hexane-CH_2Cl_2-MeOH gradient) to obtain nine fractions (SA1-SA9). Fraction SA4 was purified by preparative thin layer chromatography (CH_2Cl_2-MeOH, 95:5) to afford hexacosyl *trans*-ferulate (**7**) (5.3 mg) and lupeol (**8**) (6.9 mg). Fraction SA8 was further fractionated by column chromatography (Hexane-CH_2Cl_2-MeOH gradient) to give three fractions (SA8.1-SA8.3). Fraction SA8.2 was then purified by Chromatotron using hexane and EtOAc as gradient system to furnish *p*-hydroxybenzoic acid (**1**) (4.2 mg).

The isolated compounds were then identified by means of various spectroscopic methods including MS, 1D, and 2D NMR techniques as well as comparison with the previous literature data.

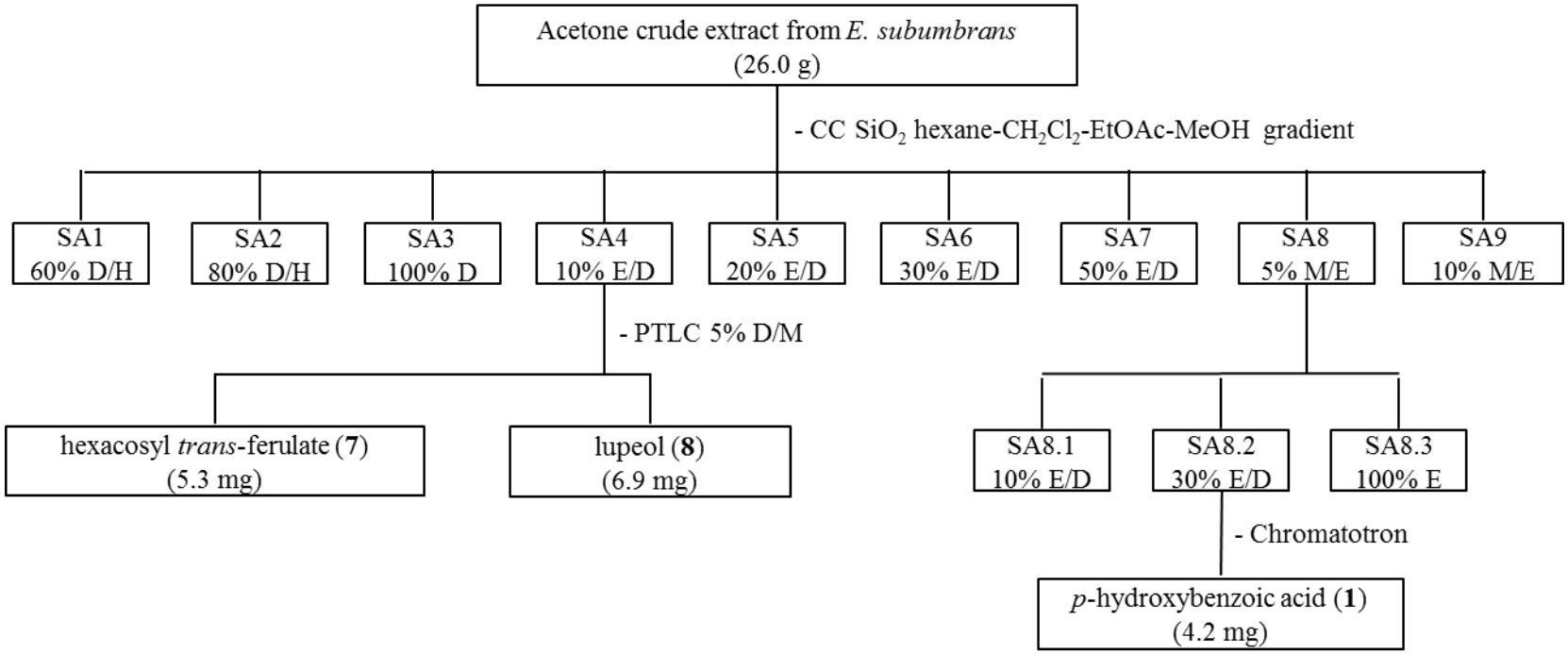
The isolation and purification of all isolated compounds from the stem bark of *E. subumbrans* were briefly summarized in schemes 2.1 to 2.3.



Scheme 2.1 Extraction procedure of *E. subumbrans*



Scheme 2.2 Isolation procedure of the CH_2Cl_2 crude extract from *E. subumbrans*



Scheme 2.3 Isolation procedure of the acetone crude extract from *E. subumbrans*

2.4 Bioassay procedure

2.4.1 The cytotoxicity against KB, HeLa, MCF-7, HepG-2, Colo205, and LLC cell lines by MTT colorimetric assay

All tested compound (1 mg each) were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma), HeLa (human cervix adenocarcinoma), MCF-7 (human breast adenocarcinoma), HepG-2 (liver hepatocellular cells), Colo205 (colorectal adenocarcinoma), and LLC (lung carcinoma) cell lines employing the MTT colorimetric assay. Adriamycin was used as standard according to the method of Kongathip *et al.* [30]. This assay was kindly performed by Natural Products Research Section, Research Division, National Cancer Institute, Thailand.

CHAPTER III

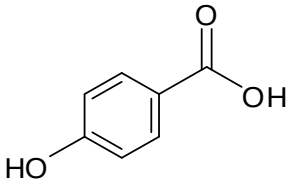
RESULTS AND DISCUSSION

3.1 Properties and structural elucidation of isolated compounds

3.1.1 *p*-Hydroxybenzoic acid (1)

p-Hydroxybenzoic acid was obtained as white crystalline solid that is slightly soluble in water but more soluble in polar organic solvents such as acetone. It is primarily known as the use for the preparation of parabens, which are used as preservatives in cosmetics and some ophthalmic solutions. The ^{1}H NMR spectrum exhibited the following result.

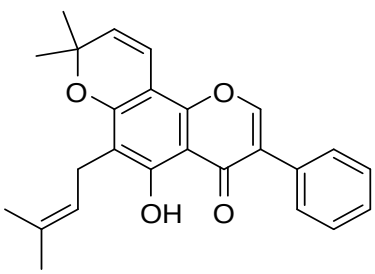
***p*-Hydroxybenzoic acid (1)**: yellow solid, ^{1}H NMR ($CO(CD_3)_2$; δ_H 6.80 (2H, d, *J* = 8.0, H-3,5), 7.80 (2H, d, *J* = 8.0, H-2, 6). This compound was also compared with the authentic sample [31].



3.1.2 Osajin (2)

Osajin was afforded as orange powder that is soluble in water and chloroform. Osajin is flavonoid pigments present in the wood and fruit that usually shown orange color. The ^{1}H NMR spectrum exhibited the following result.

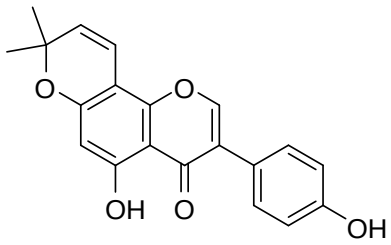
Osajin (2) : orange powder, ^{1}H NMR ($CDCl_3$): δ_H 1.46 (6H, s, CH_3- H-5″, H-6″), 1.68 (3H, s, *Z*-CH_3-4″), 1.81 (3H, s, *E*-CH_3-5″), 3.38 (2H, d, *J* = 6.8 Hz, H-1″), 5.19 (1H, m, H-2″) 6.84 (1H, d, *J* = 10.0 Hz, H-4′), 6.86 (2H, d, *J* = 8.4 Hz, H-3′, H-5′), 7.36 (2H, d, *J* = 8.0 Hz, H-2′, H-6′), 7.89 (1H, s, H-2), 13.05 (1H, s, 5-OH) [32].



3.1.3 Derrone (3)

Derrone was isolated as brown powder that is soluble in water and choloform. Derrone is often found in stem bark. It was first found from Ulex jussiaei since 2002. The ^{1}H NMR and ^{13}C NMR spectrum exhibited the following result.

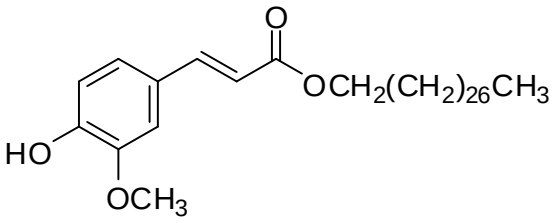
Derrone (3): brown powder, ^{1}H NMR ($CDCl_3$): δ_H 1.47 (6H, s, CH_3-5″,6″), 6.33 (1H, s, H-6), 6.73 (1H, d, *J* = 8.0 Hz, H-4″), 6.88 (2H, d, *J* = 8.0 Hz, H-3′, H-5′), 7.40 (2H, d, *J* = 8.0 Hz, H-2′, H-6′), 7.82 (1H, s, H-2), 13.20 (1H, s, 5-OH) [33]. ^{13}C NMR ($CDCl_3$): δ_C 28.3 (C-5″,6″), 78.1 (C-2″), 94.9 (C-6), 101.2 (C-8), 106.1 (C-10), 114.7 (C-4″), 115.7 (C- 3′,5′), 123.1 (C-1′), 123.7 (C-3), 127.6 (C- 3"), 130.3 (C-2′), 130.3 (C- 6′), 152.7 (C-2,9), 156.8 (C-4′), 159.6 (C-7), 162.5 (C-5), 181.1 (C-4).



3.1.4 Erythinasinate (4)

Erythinasinate was obtained as white powder that is soluble in low polar organic solvents such as hexane. The compound was the major part of the hexane extract and very easy to isolated. The ^{1}H NMR and ^{13}C NMR spectrum exhibited the following result.

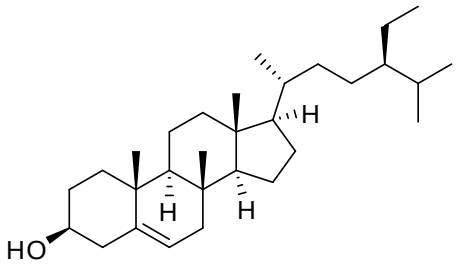
Erythinasinate (4): white powder, ^{1}H NMR ($CDCl_3$) δ_H 0.88 (3H, t, *J* = 6.8 Hz, CH_3-28), 1.25 (46H, m, H4″-H26″1.30 (2H, m, H-27″), 1.39 (3H, m, H-3″), 1.69 (3H, m, H-2″), 3.89 (3H, s, OCH_3-3), 4.19 (2H, t, *J* = 7.5 Hz, H-1″), 6.29 (1H, d, *J* = 16.0 Hz, H-2′), 6.94 (1H, d, *J* = 8.1 Hz, H-5), 6.98 (1H, s, OH-4), 7.07 (1H, dd, *J* = 1.3, 8.1 Hz, H-6), 7.61 (1H, d, *J* = 16.0 Hz, H-1′). ^{13}C NMR ($CDCl_3$) δ_C 0. 14.1 (C-28″), 22.7 (C-27″), 26.0 (C-3″), 28.8 (C-2″), 29.3 -3.8 (C-4″-26″), 60.0 (OCH_3-3), 64.6 (C-1″), 109.4 (C-2′), 114.7 (C-5), 115.8 (C-2), 123.0 (C-6), 127.1 (C-1), 144.6 (C-1′), 146.0 (C-4), 147.9 (C-3), 167.3 (C-3′) [34].



3.1.5 *β*-Sitosterol (5)

β-Sitosterol is obtained as colorless needle together with stigmasterl as a mixture. It is very difficult to separate due to the only different is one double bond change. It has characteristic as waxy powder which little odor. It is one of several phytosterols with chemical structures similar to that of cholesterol. It is hydrophobic and soluble in alcohols. The ^{1}H NMR spectrum exhibited the following result.

***β*-Sitosterol (5)**: colorless needles, ^{1}H NMR ($CDCl_3$) δ_H 0.72 (3H, s, CH_3-18), 0.85 (3H, d, *J* = 6.7 Hz, CH_3-27), 0.87 (3H, d, *J* = 6.7 Hz, CH_3-26), 0.89 (3H, t, *J* = 7.4 Hz, CH_3-29), 0.96 (3H, d, *J* = 6.5 Hz, CH_3-21), 1.05 (3H, s, CH_3-19), 5.39 (1H, m, H-6). This compound was also compared with the authentic sample [35].

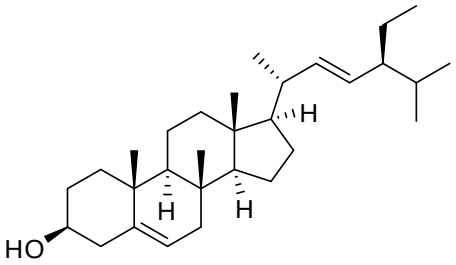


3.1.6 Stigmasterol (6)

Stigmasterol was obtained as colorless needles together with *β*-Sitosterol as a mixture. It is very difficult to separate due to the only different is one double bond change. It is one of several phytosterols with chemical structures similar to that of cholesterol. It is consider an unsaturated plant sterol occurring in the plant fats or oils. The ^{1}H NMR spectrum exhibited the following result.

Stigmasterol (6): colorless needles, ^{1}H NMR ($CDCl_3$) δ_H 0.72 (3H, s, CH_3-18), 0.85 (3H, d, *J* = 6.7 Hz, H-27), 0.87 (3H, d, *J* = 6.7 Hz, H-26), 0.89 (3H, t, *J* = 7.4 Hz, CH_3-29), 0.96 (3H, d, *J* = 6.5 Hz, CH_3-21), 1.05 (3H, s, CH_3-19), 3.56 (1H, m,

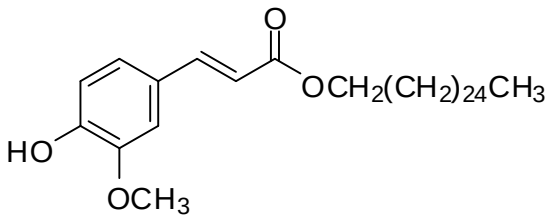
H-3), 5.01 (1H, m, H-22), 5.15 (1H, m, H-23), 5.39 (1H, m, H-6). This compound was also compared with the authentic sample [35].



3.1.7 Hexacosyl *trans*-ferulate (7)

Hexacosyl *trans*-ferulate was afforded as white powder that is soluble in dichloromethane. It has very similar structure to erythinasinate with the different being shorter carbon chain. Mass spectrometer is used in order to determine the structure other than NMR techniques. The ^{1}H NMR and ^{13}C NMR spectrum exhibited the following result.

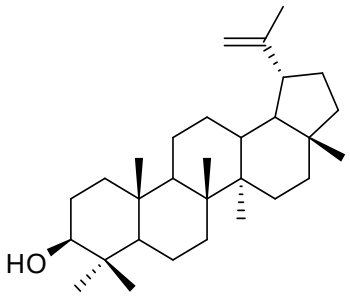
Hexacosyl *trans*-ferulate (7): white powder, ^{1}H NMR ($CDCl_3$) δ_H 0.87 (3H, t, *J* = 8.0 Hz, CH_3-26), 1.18 (42H, m, H-4″-24″), 1.32 (2H, m, H-25″), 1.48 (3H, m, H-3″), 1.64 (3H, m, H-2″), 3.86 (3H, s, OCH_3-3), 4.13 (2H, t, *J* = 7.5 Hz, H-1″), 6.24 (1H, d, *J* = 16.0 Hz, H-2′), 6.97 (1H, d, *J* = 8.0 Hz, H-5), 6.99 (1H, s, OH-4), 7.19 (1H, dd, *J* = 1.3, 8.1 Hz, H-6), 7.56 (1H, d, *J* = 16.0 Hz, H-1). ^{13}C NMR ($CDCl_3$) δ_C 14.1 (C-28″), 22.7 (C-27″), 26.0 (C-3″), 28.8 (C-2″), 29.3-32.8 (C-4″-24″), 55.9 (OCH_3-3), 64.6 (C-1″), 109.3 (C-2′), 114.7 (C-5), 115.8 (C-2), 123.0 (C-6), 127.1 (C-1), 144.6 (C-1'), 146.0 (C-4), 147.9 (C-3), 167.3 (C-3′) [36].



3.1.8 Lupeol (8)

Lupeol was yielded as colorless needles that are soluble in water and polar organic solvents such as acetone. It has several medicinal properties, one being anti-inflammatory. It functions primarily on the interleukin system. The ^{1}H NMR spectrum exhibited the following result.

Lupeol (8): colorless needles; ^{1}H NMR ($CDCl_3$): δ_H 0.66 (1H, d, *J* = 9.1 Hz, H-5), 0.73 (3H, s, H-24), 0.76 (3H, s, H-28), 0.80 (3H, s, H-25), 0.92 (3H, s, H-27), 0.94 (3H, s, H-23), 1.00 (3H, s, H-26), 1.65 (3H, s, H-29), 1.82-1.96 (2H, m, H-21), 2.35 (1H, dt, *J* = 10.9, 5.5 Hz, H-19), 3.16 (1H, dd, *J* = 10.8, 5.1 Hz, H-3), 4.55 (1H, brs, H-30), 4.65 (1H, brs, H-30) [37].



3.2 Bioassay activity of isolated compounds

3.2.1 Cytotoxicity of isolated compounds against cancer cell lines

The cytotoxicity against KB, HeLa, MCF-7, HepG-2, Colo205, and LLC cell lines of all isolated compounds were determined using MTT colorimetric assay and the results were shown in Table 3.1.

Table 3.1. *In vitro* cytotoxicity of compounds **(1-8)** against cancer cell lines.

Compound	IC_{50}(µg/mL)					
	KB	**HeLa**	**MCF-7**	**HepG-2**	**Colo205**	**LLC**
p-Hydroxybenzoic acid (**1**)	58.8	105	>100	>100	>100	>100
Osajin (**2**)	14.5	21.8	16.9	17.4	9.74	16.3
Derrone (**3**)	15.8	19.1	17.3	20.1	15.2	17.1
Erythinasinate (**4**)	46.8	87.2	>100	>100	79.9	>100
A mixture of *β*-sitosterol (**5**) and stigmasterol (**6**)	>100	>100	NT	NT	NT	NT
Hexacosyl *trans*-ferulate (**7**)	52.4	86.9	>100	>100	70.2	>100
Lupeol (**8**)	14.0	13.9	16.7	13.7	7.97	10.6
Adriamycin (standard)	0.115	< 0.1	0.266	0.319	0.078	0.070

KB = human epidermoid carcinoma
HeLa = human cervix adenocarcinoma
MCF-7 = human breast adenocarcinoma
HepG-2 = liver hepatocellular cells
Colo205 = colorectal adenocarcinoma
LLC = lung carcinoma

$IC_{50} \leq 4$ = good activity
$4 < IC_{50} \leq 15$ = moderate activity
$15 < IC_{50} \leq 30$ = weak activity
$IC_{50} > 30$ = inactive

From Table 3.1, isolated compounds from *E. stricta* and *E. subumbrans* were evaluated for their cytotoxicity against on KB, HeLa, MCF-7, HepG-2, Colo205, and

LLC cells. Osajin (**2**) exhibited moderate cytotoxicity against both KB and Colo205 cells with IC_{50} values of 14.5 and 9.74 µg/mL and exhibited weak cytotoxicity against HeLa S-3, MCF-7, HepG-2, and LLC cells with IC_{50} values of 21.8, 16.9, 17.4, and 16.3 µg/mL, respectively. Derrone **(3)** exhibited weak activity against all the tested cells (KB, HeLa, MCF-7, HepG-2, Colo205, and LLC cells) with IC_{50} values of 15.8, 19.1, 17.3, 20.1, 15.2, and 17.1 µg/mL, respectively. Lupeol (**8**) exhibited moderate cytotoxicity against most of the tested cells including KB, HeLa, HepG-2, Colo205, and LLC cells with IC_{50} values of 14.0, 13.9, 13.7, 7.97, and 10.6 µg/mL, respectively, whereas it exhibited weak cytotoxicity against MCF-7 cells with IC_{50} value of 16.7 µg/mL. Nevertheless, the rest of the compounds could be regarded as inactive.

CHAPTER IV

CONCLUSION

4.1 Chemical constituents from the stem bark of *E. stricta*

As a conclusion, the isolated compounds from the hexane and CH_2Cl_2 crudes extracts of the stem bark of *E. stricta* were *p*-hydroxybenzoic acid (**1**), osajin (**2**), derrone (**3**), erythinasinate (**4**), and a mixture of *β*-sitosterol (**5**) and stigmasterol (**6**). The chemical structures of all isolated compounds were characterized by the means of spectral analysis together with comparison with the previous literature data (Figure 4.1).

4.2 Chemical constituents from the stem bark of *E. subumbrans*

In the conclusion, the isolated compounds from the CH_2Cl_2 and acetone crudes, extracted from the stem bark of *E. subumbrans* are *p*-hydroxybenzoic acid **(1)**, erythinasinate (**4**), a mixture of *β*-sitosterol (**5**) and stigmasterol (**6**), hexacosyl *trans*-ferulate (**7**), and lupeol (**8**). The chemical structures of all isolated compounds were characterized by the means of spectral analysis together with comparison with the previous literature data (Figure 4.1).

4.3 Anticancer activity from isolated compounds

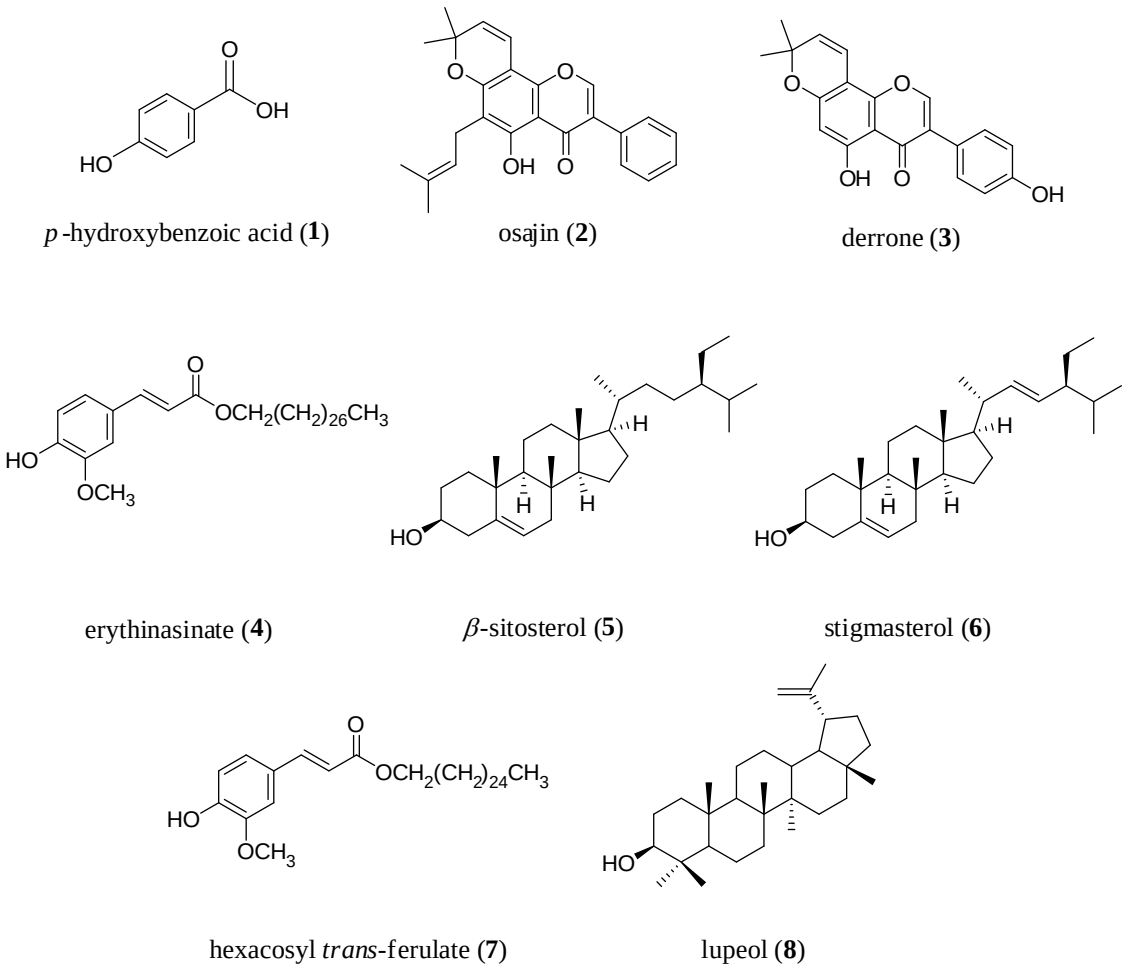
All of the isolated compounds (**1**-**8**) were evaluated for their cytotoxicity against on KB and HeLa cells line. Compounds (**2**) and (**3**) (osajin and derrone**)** exhibited better cytotoxicity against the tested cells lines than the rest of the compounds with the IC_{50} values between moderate activity and weak activity. Lupeol (**8**) exhibited moderate cytotoxicity against most of the tested cell lines (KB, HeLa, HepG-2, Colo205, and LLC cells) except MCF-7 cells with only weak activity. However, the rest of the compounds could be regarded as inactive.

4.3 Limitation of the research

The time of separation and isolation is limited as well as the amount of stem bark from *E. stricta* and *E. subumbrans* we could obtain. The isolated compounds were obtain in very small amount and difficult to perform many kind of activity tests.

4.4 Suggestion for future work

The future work may involve the isolation of more plants to select compounds with high biological activity that could be used in industrial or develop into new drugs. It is possible that new novelty compounds against cancers may be found as well. This will lead to better understanding on the interaction between active compounds and cancer cells, and possible better treatment.



p-hydroxybenzoic acid (**1**) osajin (**2**) derrone (**3**)

erythinasinate (**4**) *β*-sitosterol (**5**) stigmasterol (**6**)

hexacosyl *trans*-ferulate (**7**) lupeol (**8**)

Figure 4.1 All of the isolated compounds (**1-8**) from *E. stricta* and *E. subumbrans*

REFERENCES

[1] Forstl, H., Hentschel, F. and Sattel, H. Age-associated memory impairment and early Alzheimer's disease. **Drug Researh** 45 (1995): 394-397.

[2] Strohl, W. The role of natural products in a modern drug discovery program. **Drug Discovery & Therapeutics** 5 (2000): 39-41.

[3] Fabricant, D.S. and Farnworth, N.R. The value of plants used in traditional medicine for drug discovery. **Environmental Health Perspective** 109 (2001): 69-75.

[4] Russo, M., Spagnuolo, C., Tedesco, I., and Russo G. L. Phytochemistry in cancer prevention and therapy: Truth or dare. **Toxins** 2 (2010): 517-551.

[5] Tapsell, L. C. *et al.* Health benefits of herbs and spices: the pas, the present, the future. **The Medical journal of Australia** 185 (2006): S4-24.

[6] Cragg, G. M. Nature. A vital source of leads for anticancer drug development. **Phytochemistry Review 8**. (2009): 313-331

[7] Waffo, A.F.K. Coombes, P.H. Mulholland, D.A. Nkengfack, A.E. and Fomum, Z. T. Flavones and isoflavones from the west African Fabaceae *Erythrina vogelii*. **Phytochemistry** 67 (2006): 459-463.

[8] Allen, O.N. and Allen, E.K. The Leguminosae, a Source Book of Characteristics, Uses, and Nodulation. **University of Wisconsin Press** (1981): 812.

[9] Ghosal, S., Majumdar, S.K. and Chakrabotti, A. *Erythrina* alkaloids occurrence of (+) *N*-norprotosinomenine and other alkaloids in *Erythrina lithosperma.* **Australian Journal of Chemistry** 24 (1971): 2733-2735.

[10] Barkat, I., Jackson, A.H. and Abdulla, M.I. Further studies of *Erythrina* alkaloids. **Llyodia** 40 (1977): 471-475.

[11] Cordell, G.A. Introduction to alkaloids: A biogenetic approarch. **New York: John Wiley & Son.** (1981).

[12] Telikepalli, H., Gollapudi, S.R. Shokri, A.K. Sandmann, R.A. Isoflavonoids and a cinnamyl phenol from root extracts of *Erythrina variegate*. **Phytochemistry** 29 (1990): 2005-2007.

[13] Chacha, M., Moleta, G.B. and Majinda, R.R.T. Antimicrobial and radical scavenging flavonoids from the stem wood of *Erythrina latissima*. **Phytochemistry** 66 (2005): 99-104.

[14] Yenesew, A., Derese, S., Midiwo, J.O. and Peter, M.G. Antimicrobial flavonoids from the stem bark of *Erythrina burttii.* **Fitoterapia** 76 (2005): 469-472.

[15] Maillard, M., Gupta, M.P. and Hostettmann, K. A new antifungal prenylated flavanones from *Erythrina berteroana.* **Planta Medica** 53 (1987): 563-564.

[16] Tanaka, H., Hirata, M., Etoh, H., Sako, M., Murata, J., Murata, H. and Fukai T. Four new isoflavonoids and a new 2-arylbenzofuran from the roots of *Erythrina variegate.* **Heterocycles** 60 (2003): 2767-2773.

[17] Talla, E., Njamen, D., Mbafor, J.T. and Rios, J.L. Warangalone, the isoflavonoids anti-inflammatory principle of *Erythrina addisoniae* stem bark. **Journal of Natural Products** 66 (2003): 891-893.

[18] Andayi, A.W., Yenesew, A. and Derese, S. Antiplasmodial flavonoids from *Erythrina sacleuxii.* **Planta Medica** 72 (2005): 187-189.

[19] Juma, B.F. and Majinda, R.R.T. Erythrinaline alkaloids from the flowers and pods of *Erythrina lysistemon* and their DPPH radical scavenging properties. **Phytochemistry** 65 (2004): 1397-1404.

[20] Hegde, M.G. Cultivation and uses of *Erythrina* variegate in Western India. **In: *Erythrina* in the New and Old Worlds**. NFTA-CATIE (1993).

[21] Mitscher, L. A., Drake, S., and Okwute, S. K. A modern look at folkloric use of anti-infective agents. **Journal of Natural Products** 50 (1987): 1025-1040.

[22] Harkishan, S., Amrik, S., Vijay, K.K. and Narotam, K. Investigation of *Erythrina* spp. IX. Chemical constituents of *Erythrina stricta* bark. **Journal of Natural Products** 44 (1981): 526-528.

[23] Rukachaisirikul, T., Saekee, A., Tharibun, C. and Suksamrarn, A. Biological activities of the chemical constituents of *Erythrina stricta* and *E. subumbrans.* **Archives of Pharmacal Research** 30 (2007): 1398-1403.

[24] Umamaheswari, M., Asokkumar, K. and Ravi, T.K. *In vitro* xanthine oxidase inhibitory activity of the fractions of *Erythrina stricta* roxb. **Journal of Ethnopharmacology** 124 (2009): 646-648.

[25] Folkers, K. *et al. Erythrina* alkaloids. III. Isolation and characterization of new alkaloids, erythramine. **Journal of the American Chemistry Society** 61 (1939): 1232-1235.

[26] Folkers, K. *et al. Erythrina* alkaloids. X. Isolation and characterization of erysonine and other liberated alkaloids. **Journal of the American Chemistry Society** 63 (1941): 1544-1549.

[27] Prachayasittikul, S., Saraban, P., Cherdtrakulkiat, C., Ruchirawat, S., and Prachayasittikul, V. New bioactive triterpenoids and antimalarial activity of *Diospyros rubra Lec*. **Experimental and Clinical Sciences** 9 (2010) 1-10.

[28] Riss, T.L. and Moravec, R.A. Use of multiple assay endpoints to investigate the effect of incubation time, dose of toxin, and plating density in cell-based cytotoxicity assays. **Assay and drugs development technologies** 2 (2004): 51-62

[29] Fan, F. and Wood, K.V. Bioluminescent assays for high-throughput screening. **Assay and drugs development technologies** 5 (2007): 127-136.

[30] Kongkathip, N., *et al.* Potent antitumor activity of synthetic 1,2-Naphthoquinones and 1,4-Naphthoquinones. **Bioorganic & Medicinal Chemistry** 11(2003): 3179-3191.

[31] Chen, X.,Zhang, J., and Yu, B. Y. J. Biotransformation of p-, m-, and o-hydroxybenzoic acids by Panax ginseng hairy root cultures. **Journal of Molecular Catalysis B** 54, (2008): 72-75.

[32] Delle, G., Scurria,R., Vitali, A., Botta, B., and Cernia, E. Two isoflavones and a flavone from the fruits of Maclura pomifera. **Phytochemistry** 37 (1994) 893-898.

[33] Maximo, P., Lourenco, A., and Roseiro, J.C. A new prenylisoflavone from *Ulex jussiaei*. **Journal of Bioscience** 57 (2002) 609-613.

[34] Nkengfack, A.E., Sanson, D.R. Tempesta, M.S., and Fomun, Z.T. Two new Flavonoids from *Erythrina eriotriocha*. **Journal of Natural Products** 52 (1989): 320-324.

[35] Kamboj, A., and Saluja, A. K. Isolation of Stigmasterol and *β*-sitosterol from petroleum ether extract of aerial parts of *Ageratum conyzoides* (Asteraceae). **Internatinal Journal of Pharmacy and Pharmaceutical Sciences** 3. (2011):94-96.

[36] Zhang, Y., Deng, Z. W., Gao, T. X., Fu, H. Z., and Lin, W. H. Chemical constituents from the mangrove plant **Yaoxue Xuebao** 40 (2005): 935-939.

[37] Prachayasittikul, S., Saraban, P., Cherdtrakulkiat, C., Ruchirawat, S., and Prachayasittikul, V. New bioactive triterpenoids and antimalarial activity of Diospyros rubra Lec. **Experimental and Clinical Sciences** 9 (2010) 1-10.

APPENDIX

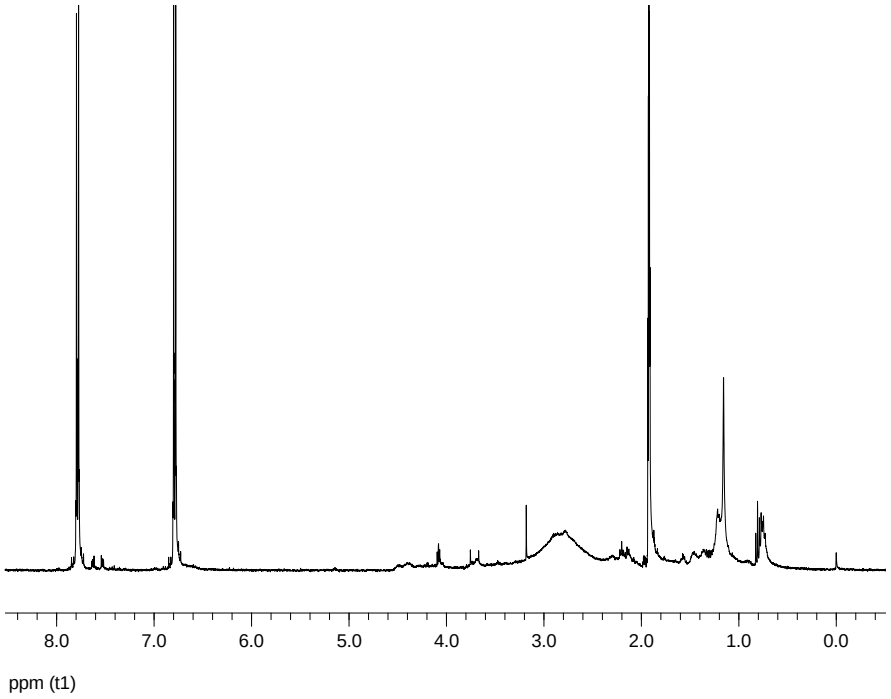


Figure A-1 ^{1}H NMR spectrum $CO(CD_3)_2$ of *p*-hydroxybenzoic acid (**1**).

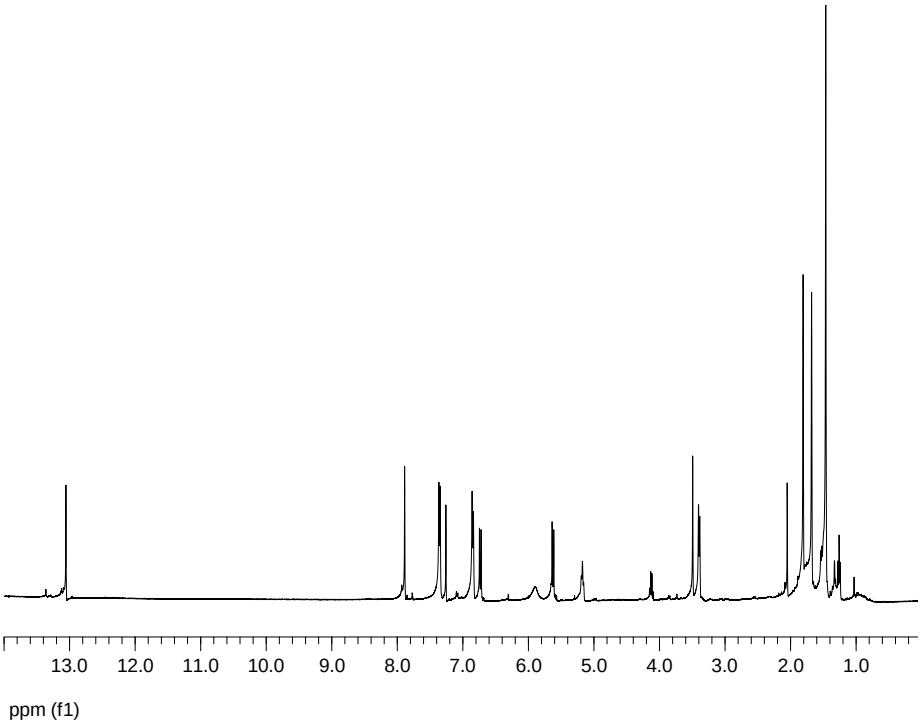


Figure A-2 ^{1}H NMR spectrum ($CDCl_3$) of osajin (**2**).

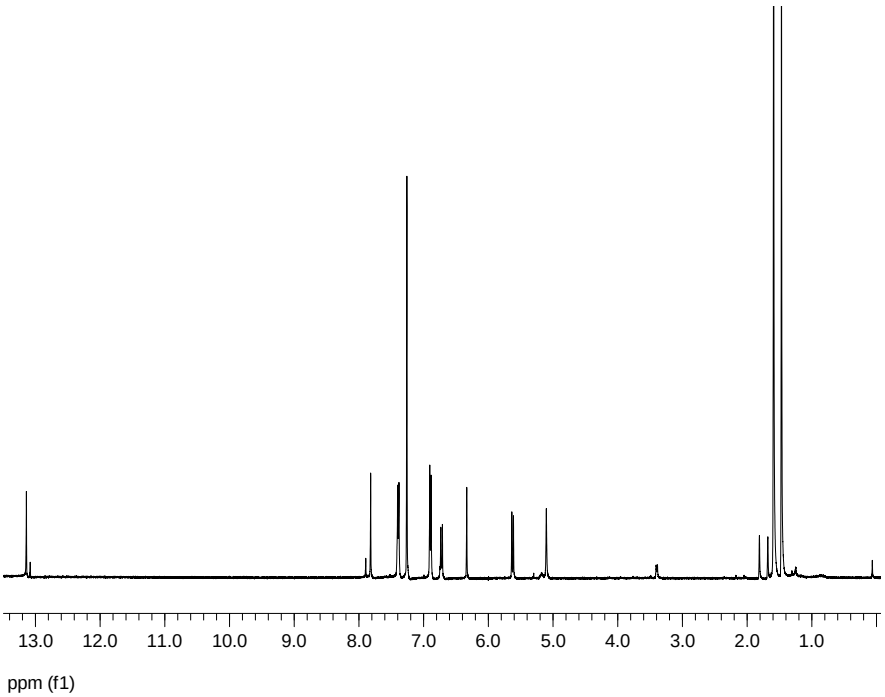


Figure A-3 ^{1}H NMR spectrum ($CDCl_3$) of derone (**3**).

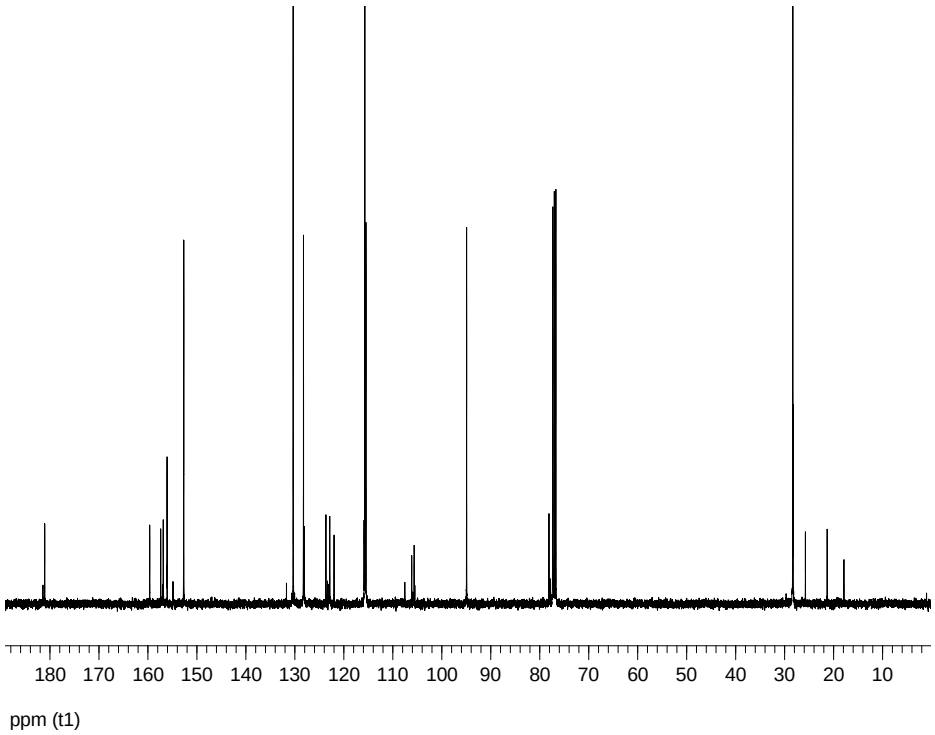


Figure A-4 ^{13}C NMR spectrum ($CDCl_3$) of derone (**3**).

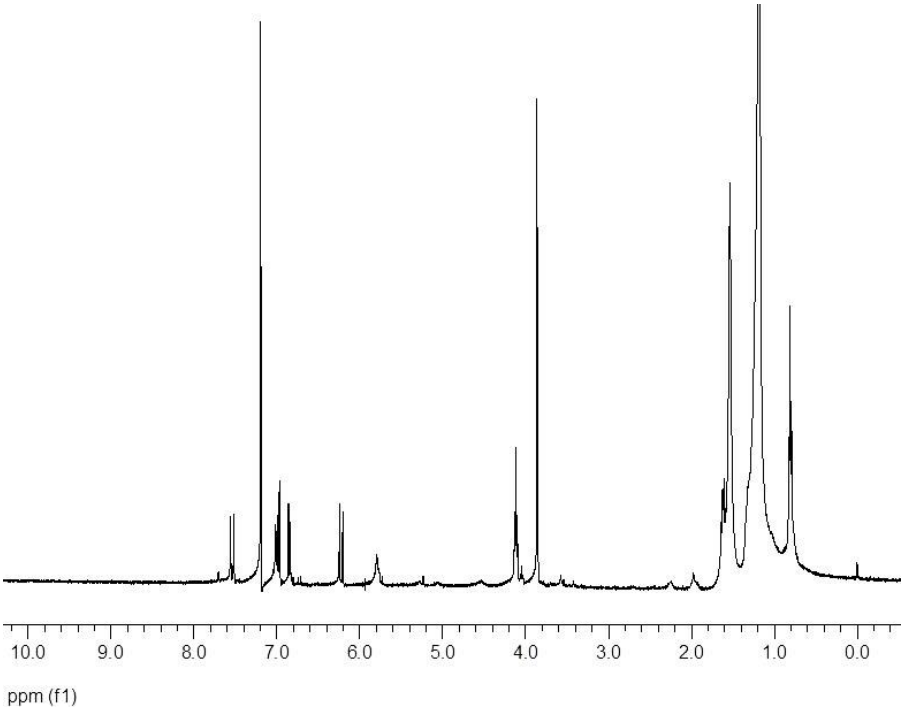


Figure A-5 ^{1}H NMR spectrum ($CDCl_3$) of erythinasinate (**4**).

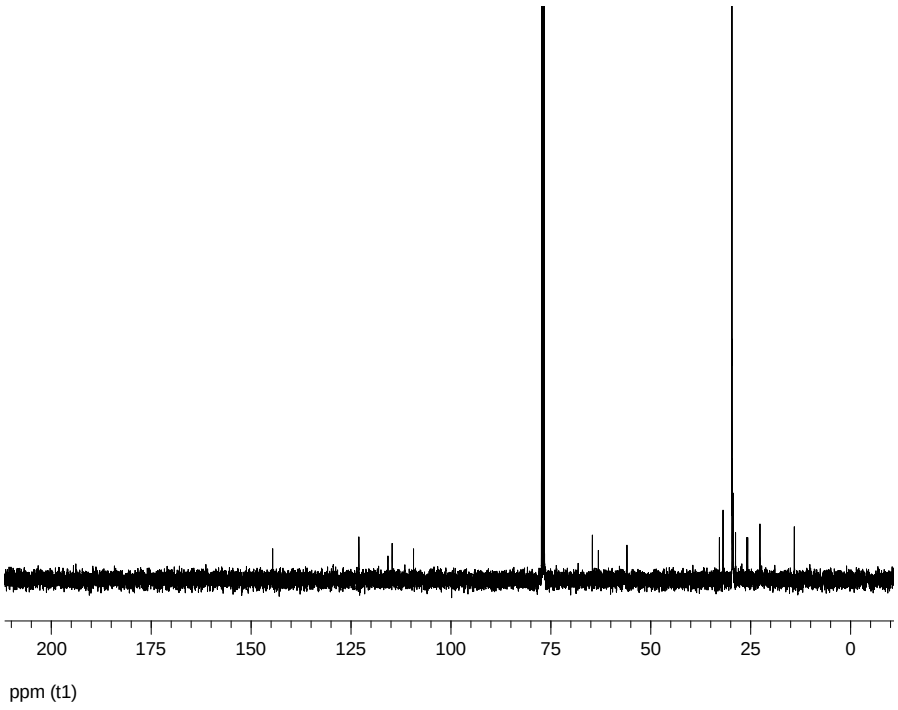


Figure A-6 ^{13}C NMR spectrum ($CDCl_3$) of erythinasinate (**4**).

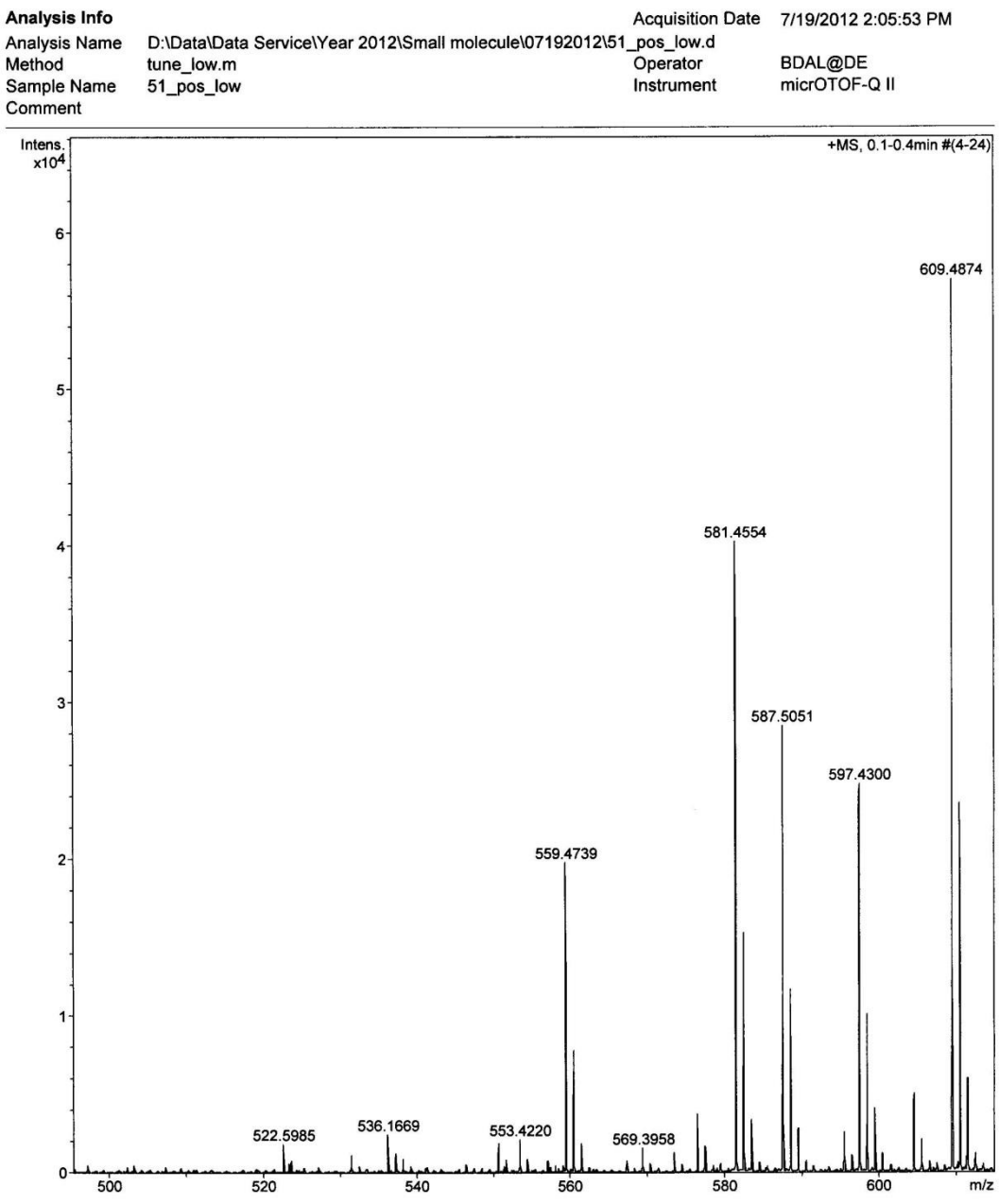


Figure A-7. High resolution mass spectrum of erythinasinate (**4**).

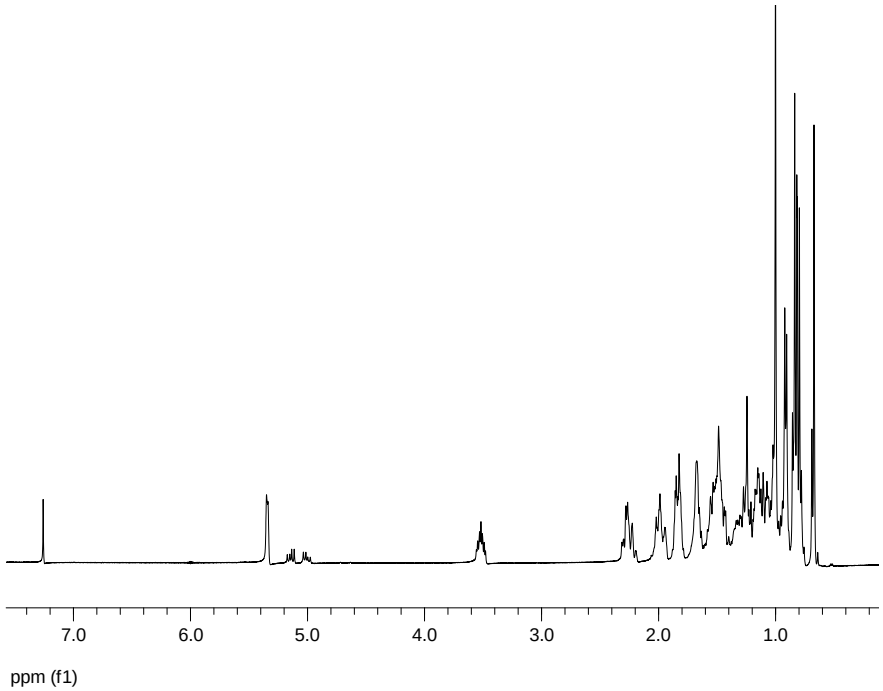


Figure A-8 ^{1}H NMR spectrum ($CDCl_3$) of a mixture of *β*-sitosterol (**5**) and stigmasterol (**6**).

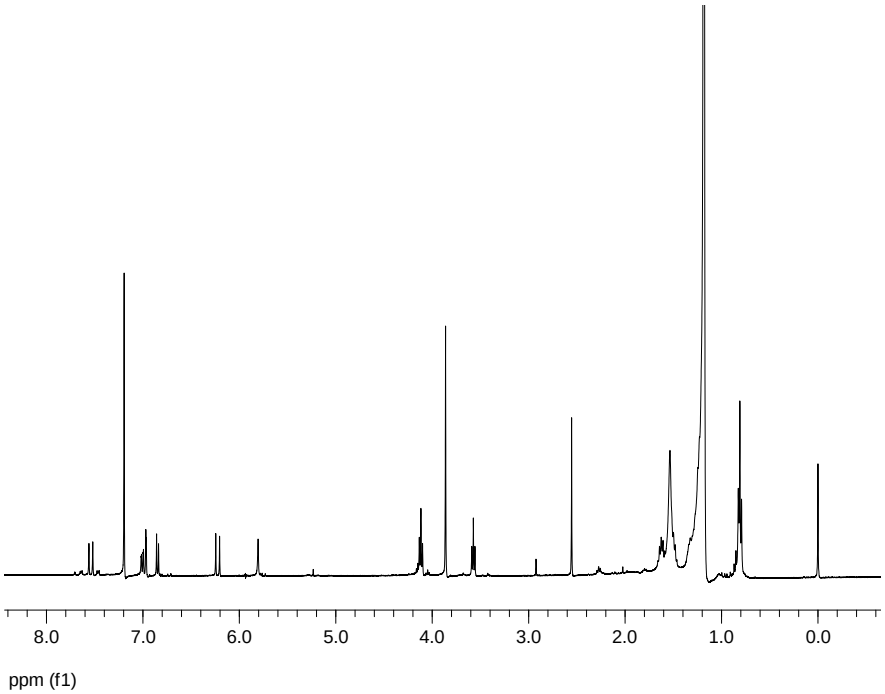


Figure A-9 ^{1}H NMR spectrum ($CDCl_3$) of hexacosyl *trans*-ferulate (**7**).

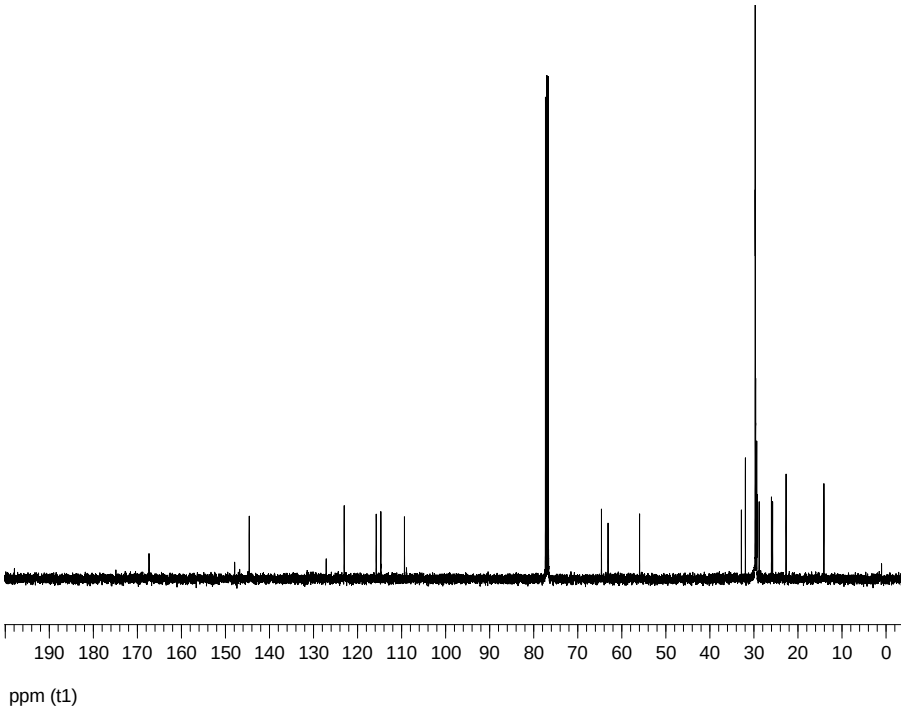


Figure A-10 ^{13}C NMR spectrum ($CDCl_3$) of hexacosyl *trans*-ferulate (**7**).

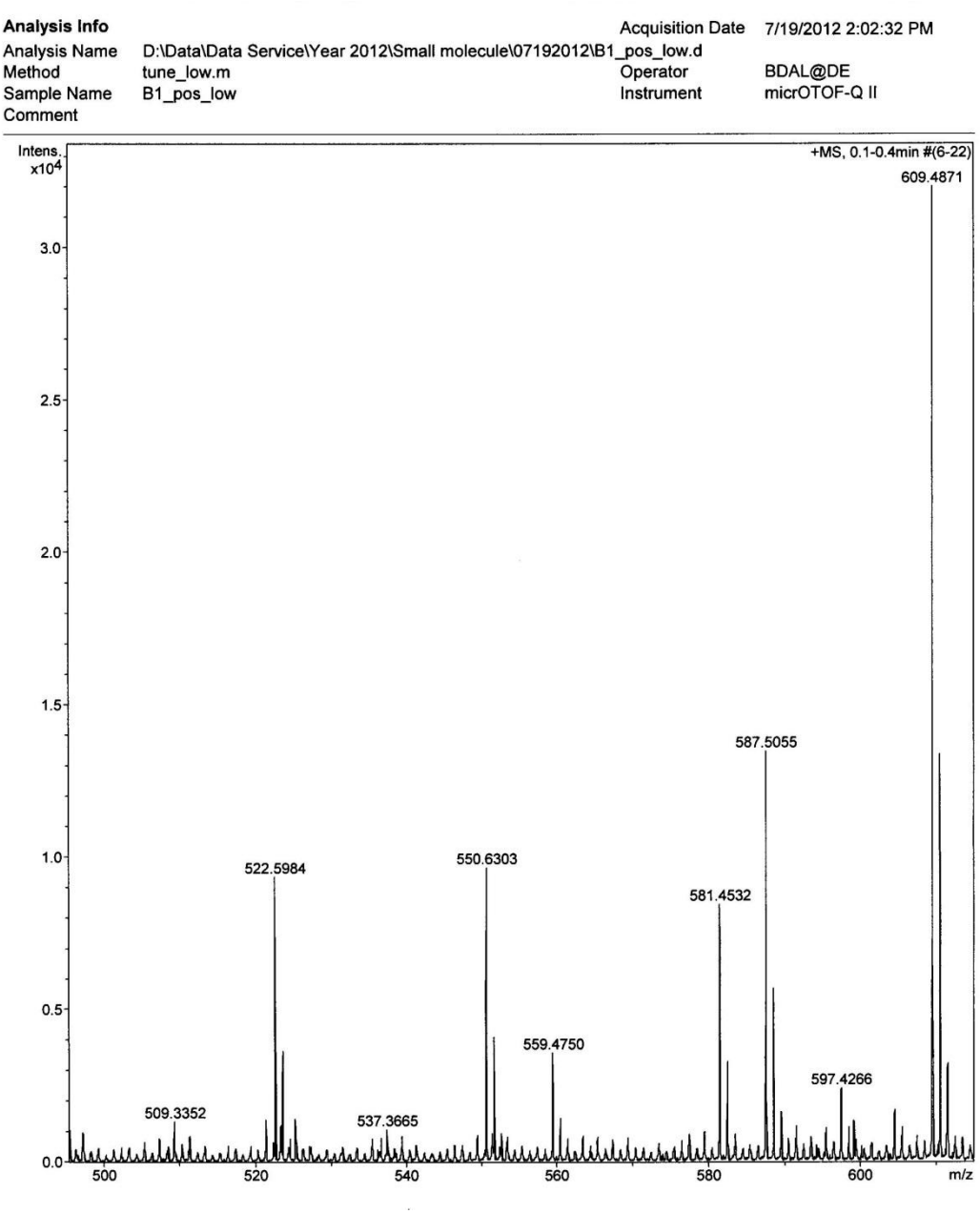


Figure A-11. High resolution mass spectrum of hexacosyl *trans*-ferulate (**7**).

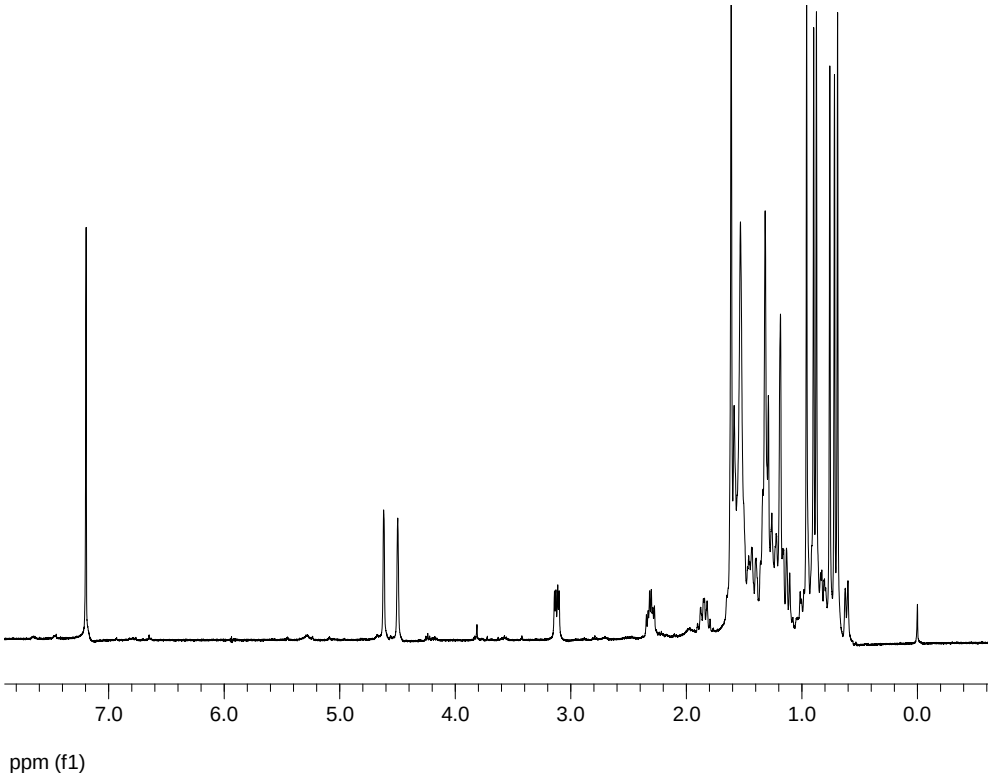


Figure A-12 ^{1}H NMR spectrum ($CDCl_3$) of lupeol (**8**).

VITA

Name:	Mr. Pongsathon Premratanachai
Date of Birth:	28th November, 1985
E-mail:	psttiti@hotmail.com
Address:	99/192 Puttamontonsai 2 road, Bangkaenua, Bangkae, Bangkok, 10160, Thailand.

Education:

2013	M.Sc. (Biotechnology, Chulalongkorn University)
2009	B.Sc (Biotechnology, Melbourne University)