CHEMICAL CONSTITUENTS OF *Knema angustifolia* STEM AND BIOLOGICAL ACTIVITIES

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องค์ประกอบทางเคมีของลำต้นกำลังเลือดม้า *Knema angustifolia* และฤทธิ์ทางชีวภาพ

น.ส.ดุง เล ทิ คิม

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สมุนไพรกำลังเลือดม้า *Knema angustifolia* ถูกใช้เป็นยาตามความเชื่อของแพทย์แผน โบราณสำหรับการบำบัดเชื้อโรคต่างๆ มีรายงานข้อมูลทางเคมีของพืชชนิดนี้น้อย ได้แยกพาราเบน โซควิโนนตัวใหม่ชื่อ angustiquinone (3) และสารที่ทราบสูตรโครงสร้างแล้วสิบสาร ได้แก่ rapanone (1), embellin (2), quercetrin (4), kaempferol (5) bergenin (6), 11-*O*-acetyl bergenin (7), catechin (8), isovanillic acid (9), protocatechuic acid (10) และ gallic acid (11) จากสิ่งสกัดเฮกเซนและเอทิลแอซีเทตของลำต้น *K. angustifolia* ได้ศึกษาโครงสร้างของสาร ที่แยกได้ด้วยการวิเคราะห์ด้วย 1D และ 2D เอ็นเอ็มอาร์, HRMS และการเปรียบเทียบข้อมูลจาก เอกสารอ้างอิง ได้ทดสอบฤทธิ์ยับยั้งเอนไซม์ tyrosinase และ α-glucosidase ของสารประกอบ 1-2 และ 4-11 พบว่า quercetrin (4) และ kaempferol (5) เป็นสารยับยั้ง tyrosinase ที่มี ศักยภาพ ด้วยค่า IC_{50} 42.3±0.26 และ 156.5±0.34 μM สำหรับ rapanone (1) และ embellin (2) แสดงฤทธิ์ยับยั้ง α-glucosidase ที่ดีมาก ด้วยค่า IC_{50} 1.3±0.17 และ 9.25±0.26 μM ตามลำดับ ซึ่งมีฤทธิ์ดีกว่า acarbose ที่เป็นยาที่ใช้ทั่วไป 93.6±0.49 μM

สาขาวิชา	เคมี	ลายมือชื่อนิสิต ....
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Dung Le Thi Kim : CHEMICAL CONSTITUENTS OF *Knema angustifolia* STEM AND BIOLOGICAL ACTIVITIES. Advisor: Asst. Prof. WARINTHORN CHAVASIRI, Ph.D.

The medicinal plant *Knema angustifolia* has been used in Thai folk medicine for the treatment of various diseases, especially related to body tonic or blood tonic agent. Chemical data on this plant are scarce. A novel *para*-benzoquinone, angustiquinone (3), along with ten known compounds, rapanone (1), embelin (2), quercetin (4), kaempferol (5), bergenin (6), 11-*O*-acetyl bergenin (7), catechin (8), isovanillic acid (9), protocatechuic acid (10) and gallic acid (11) were isolated from the *n*-hexane and EtOAc extracts of the stems of *K. angustifolia*. Their structures were unambiguously elucidated using extensive 1D and 2D NMR analyses, high-resolution mass spectrometry along with comparison with literature data. Compounds 1-2 and 4-11 were tested for anti-tyrosinase and α-glucosidase inhibitory activity. Quercetin (4) and kaempferol (5) revealed as potent inhibitors of tyrosinase activity, in which the IC_{50} 42.3±0.26, 156.5±0.34 µM, respectively. Notably, rapanone (1) and embelin (2) exhibited excellent α-glucosidase inhibitory activity with IC_{50} 1.3±0.17 and 9.25±0.26 µM, respectively which much higher than commercial drug acarbose (93.6±0.49 µM).

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

Academic Year: 2019   Advisor's Signature ....

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

1D	One dimensional
2D	Two dimensional
Ac	Acetone
AcOH	Acetic acid
calcd	Calculated
$CDCl_3$	Deuterated chloroform
CC	Column chromatography
CH_2Cl_2	Dichloromethane
d	Doublet
dd	Doublet of doublets
ddd	Doublet of doublet of doublets
DMSO-d_6	Deuterated dimethyl sulfoxide
EtOAc	Ethyl acetate
EtOH	Ethanol
HMBC	Heteronuclear multiple bond correlation
HR-ESI-MS	High resolution electrospray ionization mass Spectroscopy
HSQC	Heteronuclear single quantum correlation
m	Multiplet
MHz	Mega Hertz

MeOH	Methanol
NMR	Nuclear magnetic resonance
ppm	Parts per million (chemical shift value)
s	Singlet
t	Triplet
TLC	Thin-layer chromatography
μL	Microliter (s)
μM	Micromolar

Chapter 1

Introduction

For centuries, natural products and their derivatives have been considered to possess an essential role in medicinal chemistry. Bioactive compounds isolated from a rich diversity of plants, animals and microorganisms have been at the very core of many pharmaceutical drugs. Such a significant example is given by the antibiotics filtrate "penicillin" from *Penicillium notatum*, discovered by Fleming in 1928 thereby creating a massive milestone in the discovery of an efficient method to cure deadly infectious diseases[1]. Notably, pharmaceutical drugs derived from natural products have illustrated more desirable interactions with human cells, albeit with fewer side effects relative to synthetically made pharmaceutical drugs[2]. Moreover, the abundance of a diverse pool of natural chemical skeletons could be useful for scientists in order to improve the drawback of the known products and to pursue novel drug candidates.

Thailand located in Southeast Asia, is known as a tropical country with a variety of plant species, particularly herbs which were utilized in traditional medicine for the prevention or treatment of various diseases[3]. Therefore, these herbal plants could serve as a promising source, and further scientific study should be carried out to aid a medicinal chemistry advance. Specifically, the extracts of *Knema angustifolia* stems collected at Nongkhai province of Thailand, possessed intense cytotoxic activity, coupled with high antioxidant activity – collaborated with the investigation of

Phadungkit and co-workers in 2010[4]. In addition, until the present, there has been no report regarding the chemical constituents. Therefore, it is interesting to establish this plant as a source of bioactive compounds with potential uses in medication. Through the separation and purification process of plant material, the chemical components of *K. angustifolia* will be assessed, as well as their biological activities – for example, enzyme inhibitory activities – will be elucidated.

1.1 Characteristics and traditional usage of plants in *Knema* genus

The *Knema* genus belongs to Myristicaceae family and are widely distributed in tropical habitats situated in Asia, Africa and Australia. In Thailand, at least 12 different species have been identified (**Figure 1.1**)[5].
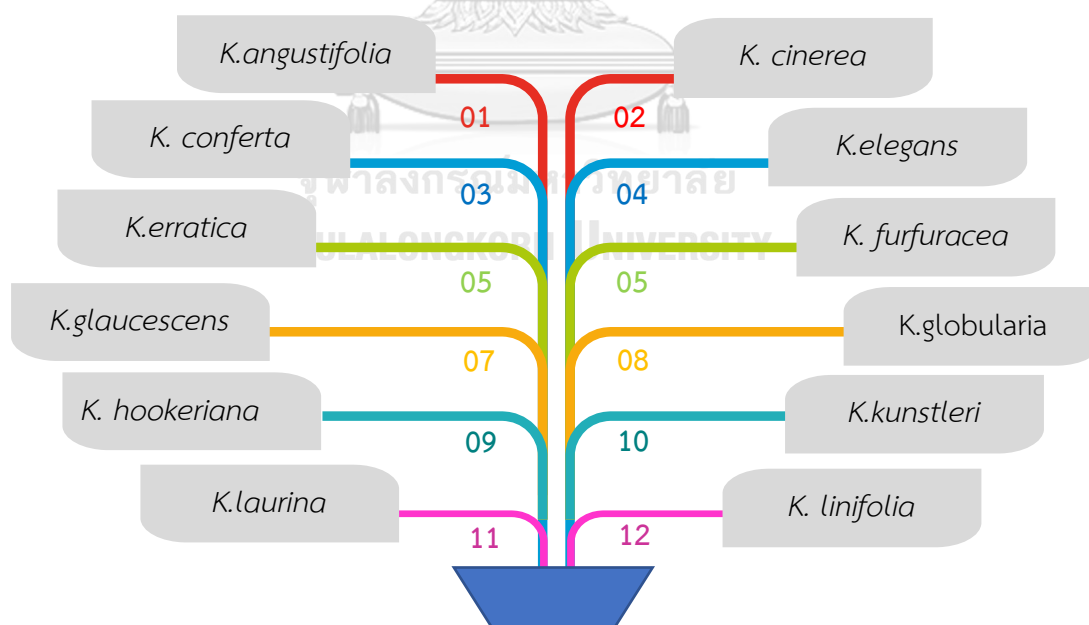
Figure 1.1 Twelve species of *Knema* genus in Thailand

Plants in *Knema* genus have been popularly used as folk medicine in the culture of various countries, especially in the Asian region. For instance, the mixture of *K. globularia* seeds and ointment has been traditionally used for centuries by the indigenous people of Indonesia and China to cure skin diseases, particularly scabies and to apply in the medicinal soap field. In the Philippines, an infusion of the barks of *K. heterophylla* was prepared and considered as a gargle for sore mouth and throat. Other contributions of *Knema* plants in traditional medication were reported in **Table 1.1**.

Table 1.1 Uses of *Knema* genus plants in traditional medicine

Plant	Plant part	Country	Uses or treatment	References
K. angustifolia	Stems	Thailand	Blood tonic or body tonic mediator	6
K. attenuata	-	India	Spleen and breathing disorders.	7
K. furfuraceae	Barks	Thailand	Remedy for sores, pimples, and cancers	8
K. glaucescens	Barks	Indonesia	Abdominal illnesses	9
K. laurina	-	Malaysia	Digestive and inflammatory diseases	10
K. tenuinervia	Barks	Thailand	Therapy for cancer	11

1.2 Chemical constituents of *Knema* genus

The previous investigation on chemical constituents of this genus illustrated the presence of several useful skeletons such as stilbenes, flavonoids, lignans, anacardic acid, alkyl/acyl resorcinol, and other compounds.

Twelve compounds: desoxyrhapontigenin (**1**), 3,4′-dimethoxy-5-hydroxystilbene (**2**), 1-(2-methoxy-4-hydroxyl-phenyl)-3-(3-hydroxy-4-methoxyphenyl)propane (**3**), 1-(2,6-dihydroxyphenyl)-tetradecan-1-one (**4**), malabaricone A (**5**), (*Z*)-1-(2,6-dihydroxy phenyl)-tetradec-(?)-en-1-one (**6**), 1-(2,4,6-trihydroxyphenyl)-tetradecan-1-one (**7**), 1-(2,4,6-trihydroxyphenyl)-9-phenylnonan-1-one (**8**), (*Z*)-1-(2,4,6-trihydroxyphenyl) tetradec-(?)-en-1-one (**9**), (+)-episesamin (**10**), (+)-xanthoxylol (**11**), and (±)-7,4′-di-hydroxy-3′-methoxyflavan (**12**) were isolated from the woods of *K. austrosiamensis* (**Figure 1.2**)[12].

In 1993, from the leaves of *K. furfuraceae*, Zahir and coworkers discovered two new phenylacylphenols: knerachelins A (**13**) and B (**14**) (**Figure 1.2**)[13].

In addition, in 2009, Rangkaew and coworkers isolated a new acyclic diterpene acid: glaucaic acid (**15**), together with seven known compounds: 1-(2,6-dihydroxy- phenyl)tetradecan-1-one (**4**), malabaricone A (**5**), 1-(2,4,6-trihydroxyphenyl)-9-phenyl-nonan-1-one (**8**), dodecanoylphloroglucinol (**16**), sesamin (**17**), asarinin (**18**), myristinin D (**19**) from the fruits of *K. glauca*. Besides, two compounds: (±)-7,4'-dihydroxy-3'-methoxyflavan (**12**) and myristinin A (**20**) were found from the leaves and stems, respectively (**Figure 1.2**)[14].

From the stem barks of *K. glomerata*, Zeng and coworkers reported the presence of three new compounds: kneglomeratanol (**21**), kneglomeratanones A (**22**) and B (**23**), along with ten known compounds: 3-(12'-phenyldodecyl)phenol (**24**), 3-(10'-phenyldecyl)phenol (**25**), 5-pentadecylresorcinol (**26**), 5-(10'-phenyldecyl)-resorcinol (**27**), 5-(12'-phenyldodecyl)resorcinol (**28**), 2,4-dihydroxy-6-(10'-phenyldecyl)- aceto-phenone (**29**), 2-hydroxy-6-(12'-phenyldodecyl)benzoic acid (**30**), formononetin (**31**), biochanin A (**32**), and 8-*O*-methylretusin (**33**) (**Figure 1.2**)[15].

In 2011, Akhtar and coworkers isolated five derivatives of alkenyl phenol and salicylic acid (**Figure 1.1**) from the stem barks of *K. laurina*, including (+)-2-hydroxy-6-(10′-hydroxypentadec-8′(*E*)-enyl)benzoic acid (**34**) and 3-pentadec-10′(*Z*)-enylphenol (**35**), 3-heptadec-10′-(*Z*)-enylphenol (**36**), 2-hydroxy-6-(pentadec-10′-(*Z*)-enyl)benzoic acid (**37**), and 2-hydroxy-6-(10′-(*Z*)-heptadecenyl)benzoic acid (**38**) (**Figure 1.2**)[16].

From *K. globularia*, the investigation of Wenli and coworkers showed the existence of kaempferol-3-*O*-β-D-glucopyranoside (**39**), quercetin-3-*O*-β-D-glucopyra-noside (**40**) in 2000[17] and eight compounds, named taxifolin (**41**), luteolin (**42**), catechin (**43**), 3′,4′,6′ trihydroxyaurone (**44**), 7-megastigmene-3,6,9 triol (**45**), β-sitosterol (**46**), and daucosterol (**47**) in 2002 (**Figure 1.2**)[18].

In 2019, from the EtOAc fraction of *K. pachycarpa* stems, Giap and coworkers discovered two new acetophenone derivatives: knepachycarpanone A (**48**) and knepachycarpanone B (**49**) and a new cardanol derivative knepachycarpanol C (**50**)[19]. In the same year, from the *n*-hexane extract, five new compounds, named

knepachycarpic acid A (**51**), B (**52**), knepachycarpanol A (**53**), B (**54**), and

knepachycarpasinol (**55**)[20] were isolated (**Figure 1.2**).
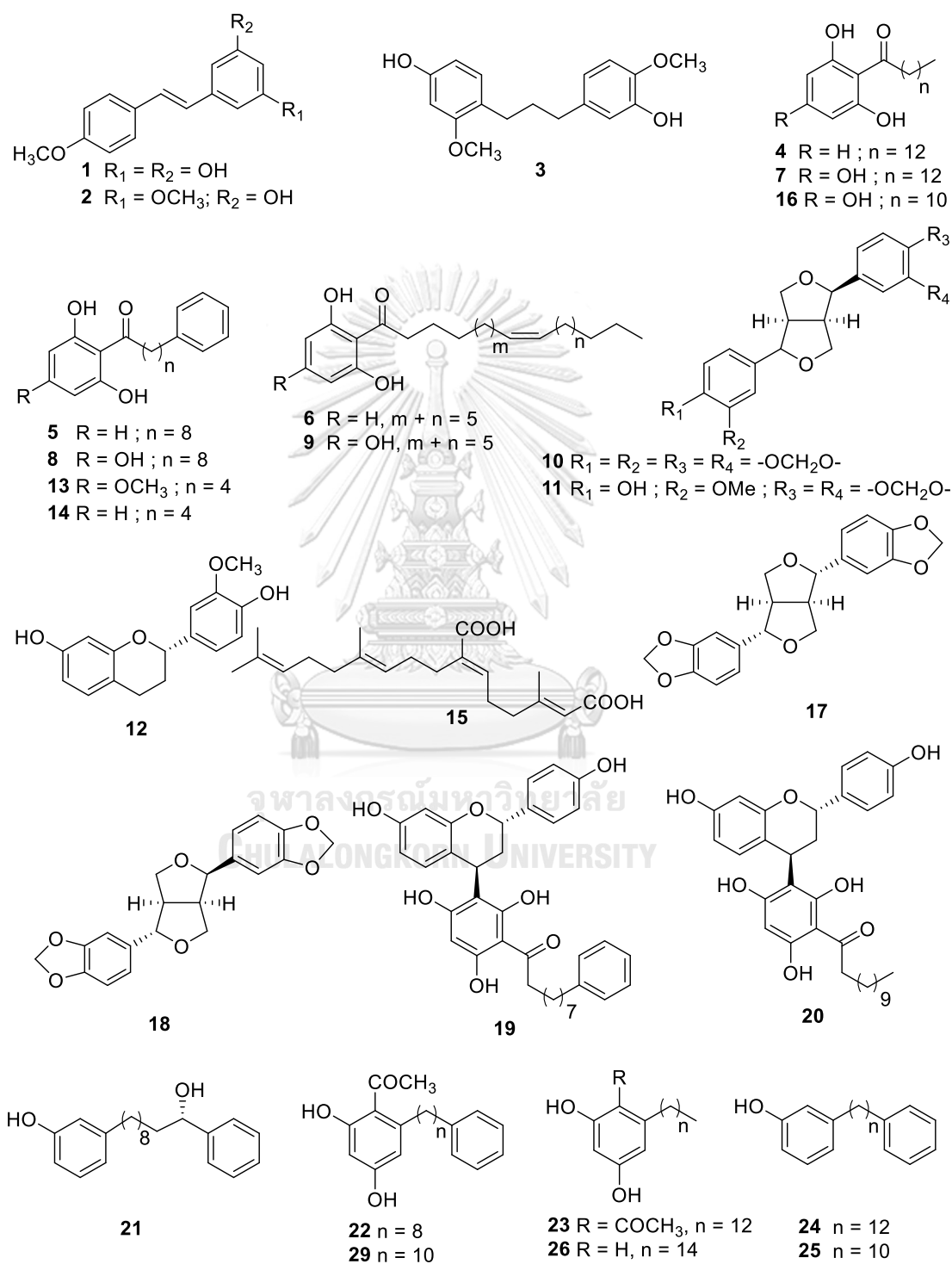
Figure 1.2 Chemical constituents from *Knema* genus

21

22 n = 8
29 n = 10

23 R = COCH₃, n = 12
26 R = H, n = 14

24 n = 12
25 n = 10

27 n = 10
28 n = 12

30

31 R₁ = R₂ = H
32 R₁ = H ; R₂ = OH
33 R₁ = OCH₃ ; R₂ = H

34

35 m = 9 ; n = 3
36 m = 9 ; n = 5

37 m = 9 ; n = 3
38 m = 9 ; n = 5

39 R = H
40 R = OH

41

42

43

44

45

46

47

48

49
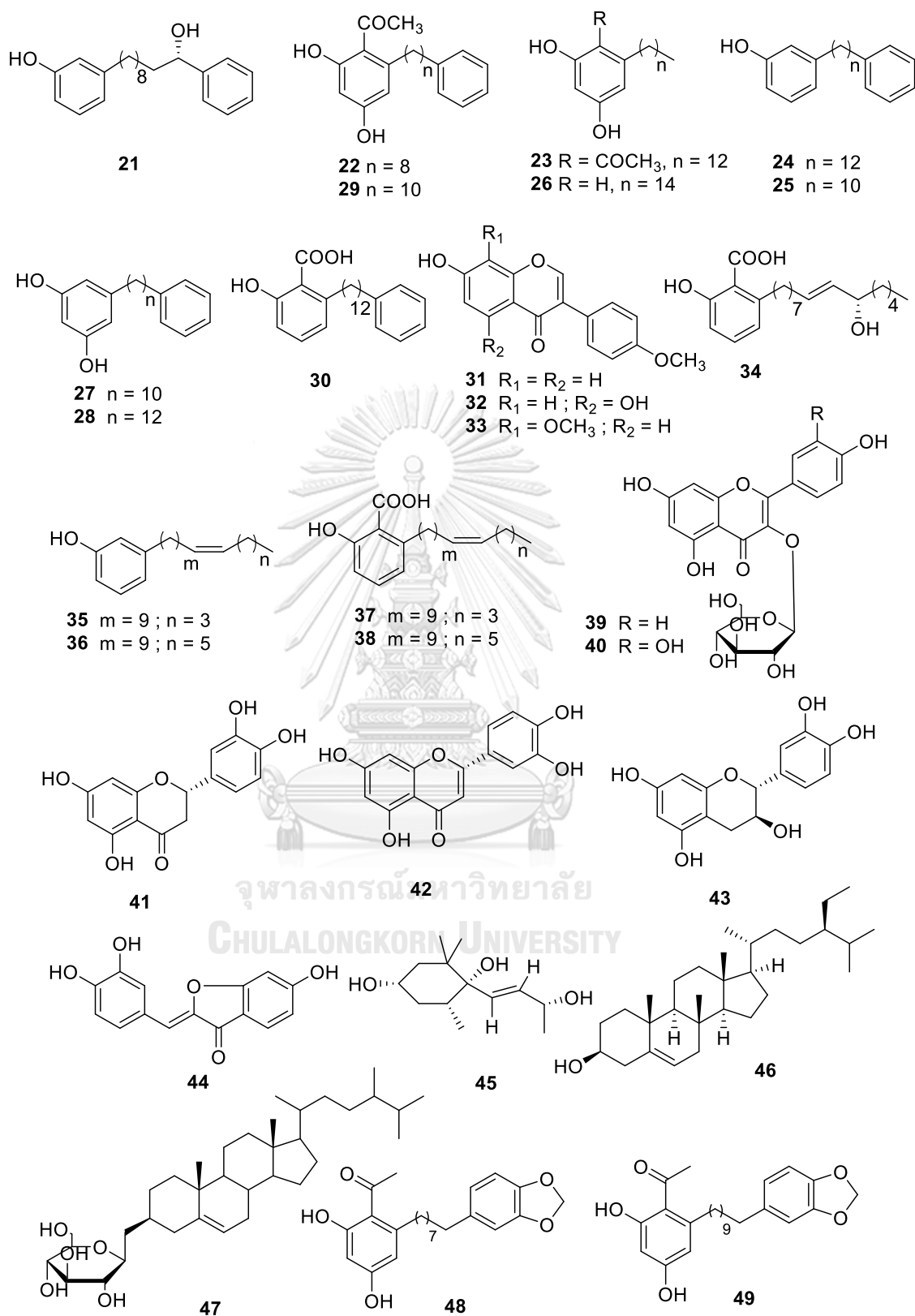
Figure 1.2 Chemical constituents from *Knema* genus (continued)
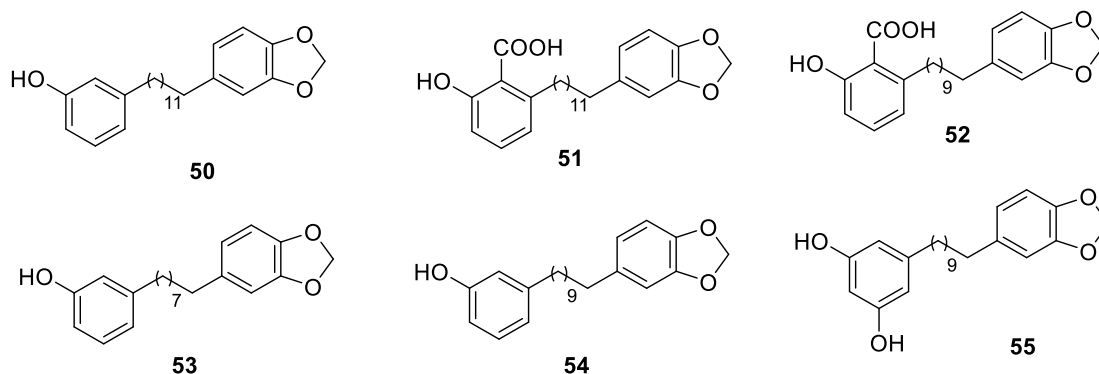
Figure 1.2 Chemical constituents from *Knema* genus (continued)

1.3 Bioactive compounds from *Knema* genus

The isolated compounds from this genus exhibited various pharmacological effects, including antibacterial, antiviral, antimalarial, cytotoxicity and anti-acetylcholinesterase activities. A brief outline of their biological activities were illustrated in **Table 1.2**

Table 1.2 Bioactivities of some *Knema* genus substances

Antibacterial activity		
Compounds	Organism	References
Knerachelins A (**13**) and B (**14**)	- *Staphylococcus aureus* with MIC values 8 and 4 µg/mL for **13** and **14**, respectively - *Streptococcus pneumoniae*, with MIC value 8 µg/mL for both compounds	13

Antiviral activity		
Compounds	Organism	References
Malabaricone A (5)	Malarial parasite *Plasmodium falciparum* (IC$_{50}$ 2.78 µg/mL)	14
Dodecanoylphloroglucinol (16)	Herpes simplex virus type 1 (IC$_{50}$ 3.05 µg/mL)	
Enzyme inhibition activity		
2-hydroxy-6-(10'(*Z*)-heptadecenyl)benzoic acid (38)	Acetylcholinesterase with IC$_{50}$ value of 0.57 µM.	16
Knepachycarpanol A (53) and knepachycarpasinol (55)	Acetylcholinesterase with IC$_{50}$ values of 2.60 and 2.46 mM, respectively	
Cytotoxicity		
Kneglomeratanone B (23)	MCF-7 (IC$_{50}$ 1.12 µg/mL)	15
3-(12'-phenyldodecyl)phenol (24)	A-549 (IC$_{50}$ 1.85 µg/mL) and HT-29 (IC$_{50}$ 2.62 µg/mL)	

1.4 Botanical aspects and traditional usage of *Knema angustifolia*

K. angustifolia (Roxb.) Warb. has been known as "Kamlang Leuat Ma" in Thailand[6]. Besides that, the indigenous people of Thai still called this plant with other names, such as Mamuang Leaut Noi, Phadong Leaut, Phadong Fai or Leaut Kway. It is a medium-sized tree containing the red resin in its bark, usually used by a word meaning "horse blood" in their local names, and the details of botanical characteristics were described below[21]:

Trees: evergreen tree growing 10-20 m tall with twigs slender.

Leaves: simple with the nerves in a fishbone arrangement.

Flowers: the individual plant has either male inflorescences or female inflorescences. While male inflorescences contain 7-8 flowered, female inflorescences have only 1-2 flowered. Its flowers covered with brown woolly hairs outside and cream or yellowish inside.

Fruits: globose, orange with crusty red granules and covered initially with brownish hairs. Fruit stalk 0.5–1.2 cm long and 0.2–0.4 cm broad.

Figure 1.3 Stems (A), leaves (B), male flowers (C) and fruits (D) of *K. angustifolia*

The stems have traditionally been used as whole-body tonic or blood tonic agent. It can be used as an herbal drink for many purposes such as nourish and create blood, cure lymphatic wounds, abscesses, rashes. Moreover, this plant can mix with other herbal plants to make ginseng with various benefits for health.

1.5 Previous study on *K. angustifolia*

Although the plants in *Knema* genus were previously investigated by many scientists; only one paper in 2010 was described the phytochemical screening and biological activities of *K. angustifolia*[4]. In that study, both EtOH and dichloromethane extracts possessed moderate antibacterial activity against *S. aureus* (**Table 1.3**). Moreover, the EtOH extracts showed the modest antioxidant activity compared to ascorbic acid (**Table 1.4**) and high cytotoxicity against lung cancer cell line (NCI-H187) with IC_{50} value of 4.55 µg/mL. The dichloromethane extract showed less potent candidate for both activities.

Table 1.3 Antibacterial activity of *K. angustifolia* extracts

	Zone of inhibition, nm		
	S. aureus ATTC 25923	*E. Coli* ATTC 25922	*P. aeruginosa* ATTC 27853
dichloromethane extract	7.56±0.40		
EtOH extract	10.67±0.57		
amoxycillin 10 µg/disk	10.67±0.57	11.83±0.76	6.67±0.57

Table 1.4 Antioxidant activity of the *K. angustifolia* extracts

	EC$_{50}$, µg/mL
dichloromethane extract	42.25±3.66
EtOH extract	13.90±1.35
ascorbic acid	4.86±0.89

To determine what compounds responsible for their biological activities, based on preliminary testing, the existence of condensed tannins, phenolic compounds, and triterpenes in both extracts were reported. According to this result, *K. angustifolia* was considered not only as a plenty source of bioactive compounds but also as a hopeful plant for in-depth investigation.

1.6 Biological activity

1.6.1 Anti-tyrosinase activity[22]

Tyrosinase is a vital multifunctional enzyme containing copper on the active site that plays a vital role in the biosynthesis of melanin (**Figure 1.4**) by accelerating the hydroxylation reaction. In this process, two types of melanin, including pheomelanin which takes responsibility for the skin's brown/black color and eumelanin which imparts a pink or red color to the skin, were formed. Melanin is considered as the perfect protection against the damage made by the ultraviolet radiation. Less or excess melanin can cause many skin disorders and aesthetic

problems such as dark circles and freckles. Therefore, by controlling the level of melanin, tyrosinase can indirectly avoid some skin's diseases.
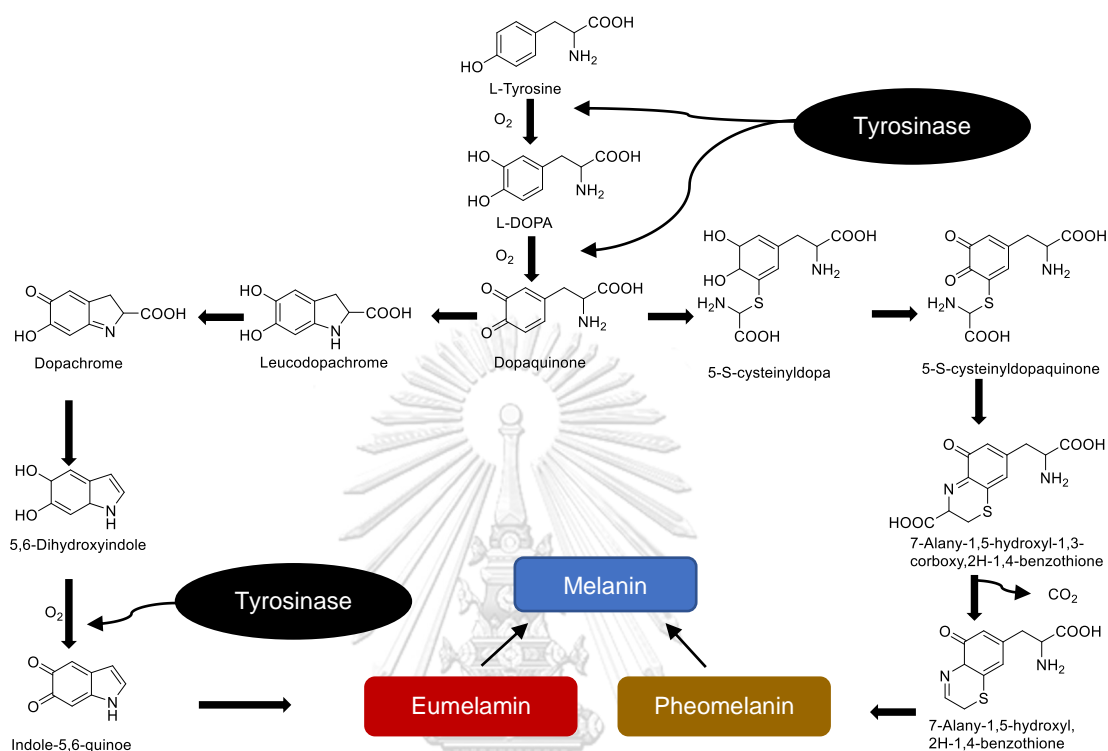
Figure 1.4 Production pathways of melanin biosynthesis

1.6.2 α-glucosidase inhibitory activity

Diabetes has become one of the biggest epidemics of the 21st century. According to the International Diabetes Federation, the number of people affected by diabetes reaches 425 million in 2017, and this figure is expected to rise over the next decades[23].

α-glucosidase inhibitors (AGIs) prevent the fast breakdown of sugars in the blood and convert carbohydrates into monosaccharides. Therefore, it can reduce postprandial blood glucose and also insulin levels[24]. Acarbose, voglibose, and

miglitol are the available oral antidiabetic drugs in the market (**Figure 1.5**). However, they have been associated with some severe side effects related to gastrointestinal[25]. Thus, there is a tremendous interest in finding alternatives antidiabetic agents.
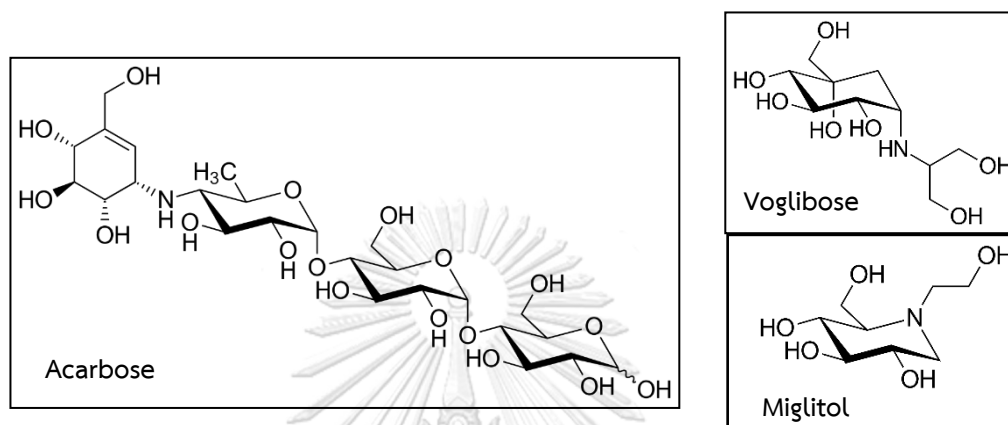
Figure 1.5 Structures of α-glucosidase inhibitor

1.7 The goal of this research

At present, the discovery of new medicine is considered as an essential part of the research. Natural products have played a key role in pharma research, as many medicines are either natural products or derivatives thereof. Thailand has a diverse pool of plant species, particularly herb which can used for the treatment of various diseases. Among them, the stems of *K. angustifolia* have traditionally been used as whole body tonic or blood tonic agent. Moreover, in 2010, the preliminary biological tests demonstrated that the ethanoic extract of this plant possessed strong cytotoxic activity against the lung cancer cell line and had high antioxidant activity. Therefore, the target of this research is summarized that follows:

-       To isolate and elucidate the structures of chemical constituents from *Knema angustifolia* stems.

-       To evaluate the biological activities of isolated compounds, such as anti-tyrosinase and α-glucosidase inhibitory activities.

Chapter 2

Experimental

2.1 Plant material

The stems of *Knema angustifolia* (Roxb.) Warb. were collected from Amnat Charoen province, Thailand in 2017 (**Figure 2.1**). The botanical identification was confirmed and a voucher specimen was deposited in the herbarium of Department of Botany, Faculty of Science, Chulalongkorn University.

Figure 2.1 The stems of *Knema angustifolia*

2.2 Chemical and enzymes

Most solvents [acetone, dichloromethane (CH_2Cl_2), methanol (MeOH), ethanol (EtOH), ethyl acetate (EtOAc) and acetic acid (AcOH)] were purchased from the suppliers and used without further purification, except for *n*-hexane. The chemicals were used as follows: *p*-nitrophenyl-α-D-glucopyranoside (pNPG). Enzyme α-glucosidase from Saccharomyces cerevisiae E.C 3.2.1.20.

2.3 Instruments and equipment

Thin layer chromatography (TLC) analyses were carried out on pre-coated silica gel Merck Kieselgel (60 F_{254}), and spots were analyzed by UV light or visualized by spraying with a solution of 5% vanillin in acidic ethanolic solution followed by heating. The open column was performed using normal phase silica gel (No. 7734, and 9385, Merck) and Sephadex LH-20.

The solvent was evaporated in vacuum using rotatory evaporator Buchi-111. 1D and 2D NMR spectra were acquired on Bruker Advance 500 MHz and 400 MHZ spectrometers. Chemical shifts in ppm were referenced to the corresponding residual solvent signal (CDCl$_3$: δ_H = 7.26, δ_C = 77.2 ppm, acetone-d$_6$: δ_H = 2.05, δ_C = 29.8 ppm, CD$_3$OD: δ_H = 4.87, δ_C = 40.0 ppm, DMSO-d$_6$: δ_H = 2.50, δ_C = 39.5 ppm). The HR-ESI-MS data were recorded on a Bruker microTOF Q-II mass spectrometer.

2.4 Extraction procedure of the *Knema angustifolia* stems

The dried stems of *K. angustifolia* (10 kg) were ground and extracted by maceration with *n*-hexane, CH$_2$Cl$_2$, EtOAc and MeOH, sequentially. Then, the solvent was evaporated in vacuum using rotatory evaporator to obtain the *n*-hexane (**H** 55.9 g, 0.56% w/w); CH$_2$Cl$_2$ (**C** 68.5 g, 0.69% w/w); EtOAc (**Ea** 125.2 g, 1.25% w/w) and MeOH (**M** 89.6 g, 0.90% w/w) extracts (**Scheme 2.1**).
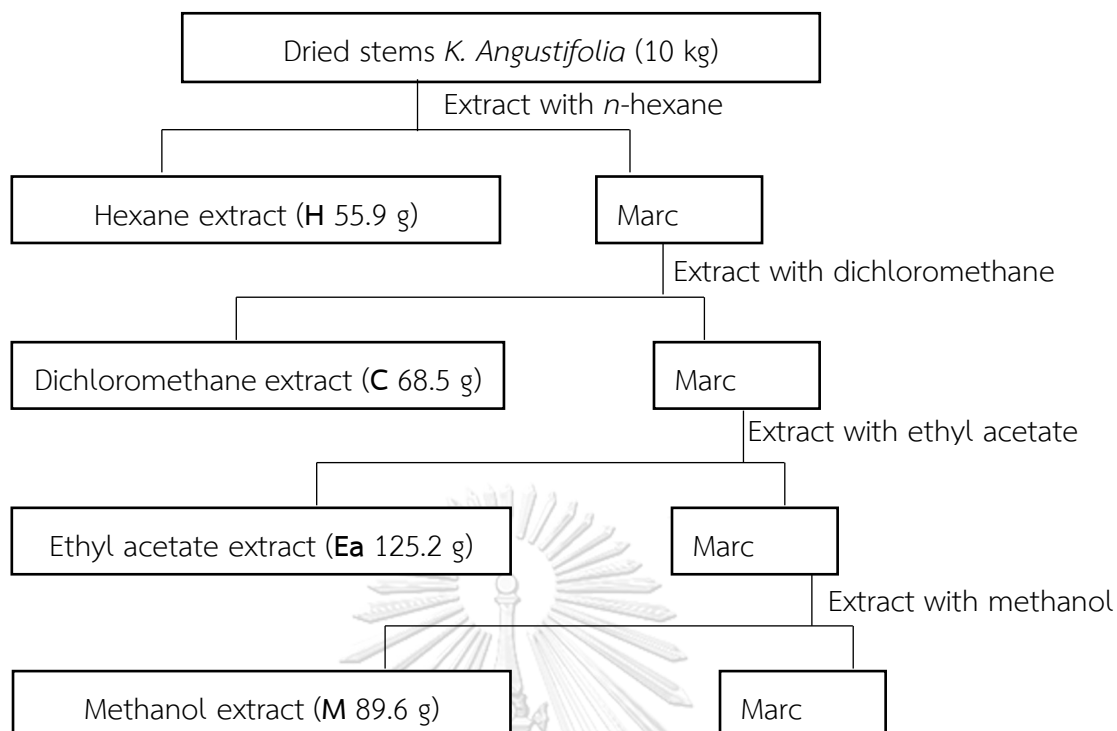
Scheme 2.1 The extraction of *Knema angustifolia* stems

The *n*-hexane (**H**) extract was applied to normal phase silica gel column chromatography (CC), eluted with the gradient of *n*-hexane:EtOAc:acetone (15:1:1 to 5:1:1) to obtain five fractions **H1-5**. Fraction **H5** (8.6 g) was dissolved in *n*-hexane to afford the precipitate **HP** (5.0 g) and the left solution **HS** (3.6 g). The former was crystallized with *n*-hexane to afford **1** (1.1 g). The latter was subjected to Sephadex LH-20, eluted with MeOH to obtain three fractions **HS1-3**. **HS1** (1.2 g) was crystallized with *n*-hexane to afford **2** (210 mg). **HS3** (0.5 g) was rechromatographed by CC, eluted with the solvent system of *n*-hexane:CH_2Cl_2:MeOH:H_2O (10:10:0.4:0.01, v/v/v/v) to give three subfractions **HS3.1-3.3**. **HS3.1** (35 mg) was purified by preparative thin-layer chromatography, eluted with *n*-hexane:CH_2Cl_2:EtOAc:acetone:AcOH

(30:10:4:2.5:0.5, v/v/v/v/v) to afford **3** (1.3 mg). The summary of the separation of *n*-hexane extract can be depicted as shown in **Scheme 2.2**.

The ethyl acetate (**Ea**) extract was subjected to silica gel column chromatograph eluting with CH_2Cl_2:EtOAc:acetone:AcOH (10:4:2.5:1, v/v/v/v), giving six fractions **E1-E6**. **E2** (2.4 g) and **E3** (10.2 g) were selected for rechromatography on Sephadex LH-20, eluted with MeOH:CH_2Cl_2 (1:1), to afford five fractions **E2.1-E2.5** for the former, and five fractions **E3.1-E3.5** for the latter. Further purification of **E2.1** (906.6 mg) using an isocratic mobile phase consisting of a hexane/CH_2Cl_2/EtOH/H_2O (9:10:2:0.05, v/v/v/v) led to the isolation of **4** (7.6 mg) and **5** (4.2 mg). The precipitation occurred in **E3.4** which was subsequently washed many times with acetone to give the pure compound **6** (400.3 mg). Fraction **E3.4** was subjected to silica gel column eluting with hexane/CH_2Cl_2/EtOAc/AcOH (2:1:3:0.1, v/v/v/v) to obtain **7** (35.6 mg). **E3.5** (1.8 g) was subjected to silica gel column, using isocratic hexane/CH_2Cl_2/EtOAc/EtOH/H_2O solvent system (10:15:9:25:0.1, v/v/v/v/v) to grant three subfractions **E3.5.1-E3.5.3**. **E3.5.1** was then purified by column chromatograph with solvent system hexane/acetone/AcOH (4:3:0.06, v/v/v) to obtain **8** (105.4 mg), **9** (7.8 mg), **10** (10.4 mg) and **11** (20.5 mg). The summary of the separation of **Ea** extract is shown in **Scheme 2.3**.

H (55.9 g)

Cc/ S1

H1 (14.3 g) H2 (8.4g) H3 (9.6g) H4 (18.0 g) H5 (8.6 g)

n-hexane

Precipitation Solution
HP (5.0 g) HS (3.6 g)

Crystallized/ *n*-hexane

1 (1.1 g)

Sephadex LH-20/MeOH

HS1 (1.2 g) HS2 (1.9 g) HS3 (0.5 g)

Crystallized/ *n*-hexane

2 (210 mg)

CC/S2

3 (1.3 mg)

Solvents system:

- S1: hexane: EtOAC: acetone (15:1:1 to 5:5:1)
- S2: hexane/CH$_2$Cl$_2$/MeOH/H$_2$O (10:10:0.4:0.01)
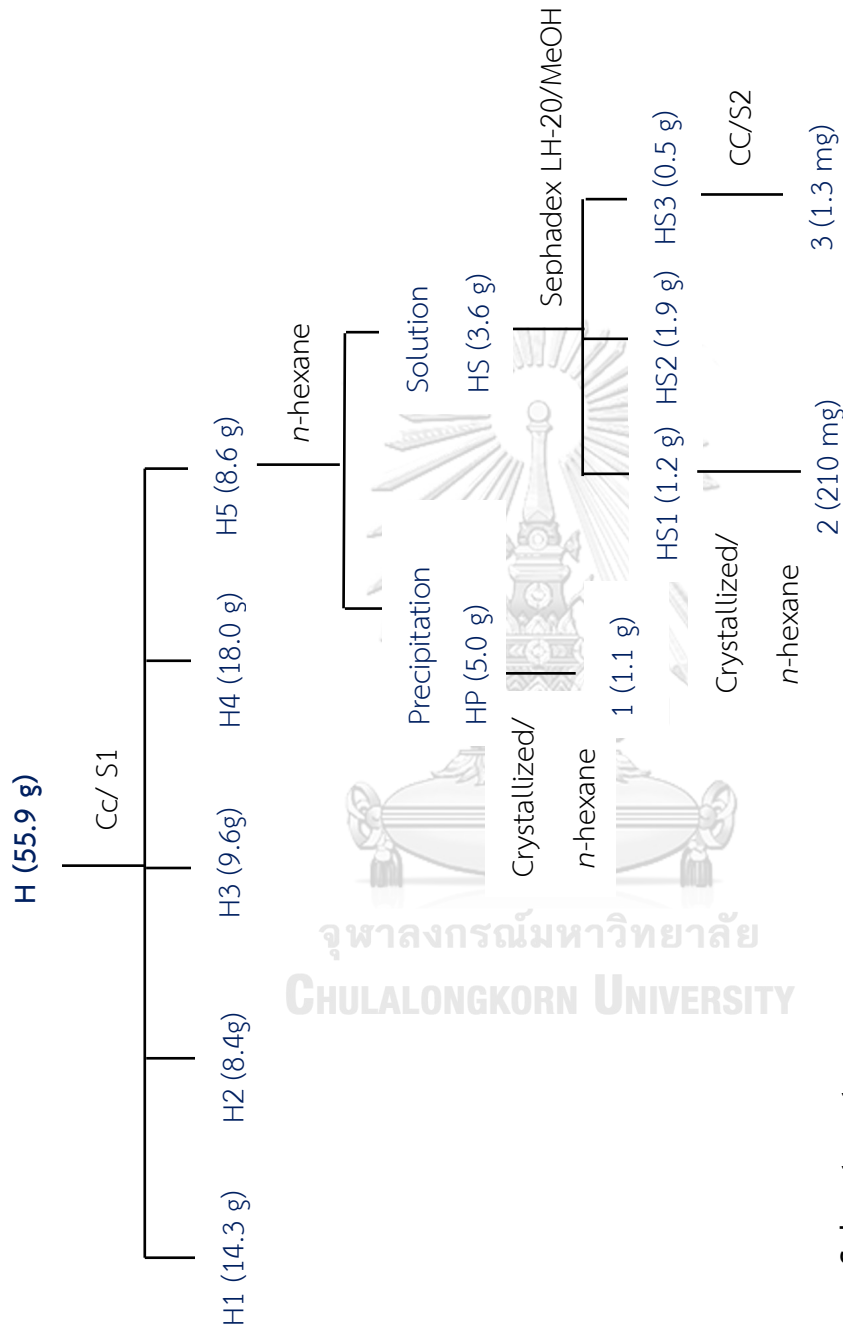
Scheme 2.2 Procedure for the separation of *n*-hexane fraction of *K.angustifolia*

Ea (125.2 g)

Cc/ S3

E1 (5.1 g)

E2 (2.4 g)

E3 (10.2 g)

E4-6 (95.7 g)

Sephadex/S4

E2.1 (906.6 mg)

E2.2 (404.1 mg)

E2.3 (1.3 g)

E3.1-3 (3.5 g)

E3.4 (4.9 g)

E3.5 (1.8 g)

Cc/ S5

4 (7.6 mg)

5 (10.2 mg)

Wash by acetone

Precipitation

6 (400.3 mg)

7 (35.6 mg)

Cc/ S6

Cc/ S7

8 (105.4 mg)

9 (7.8 mg)

10 (10.4 mg)

11 (20.5 mg)
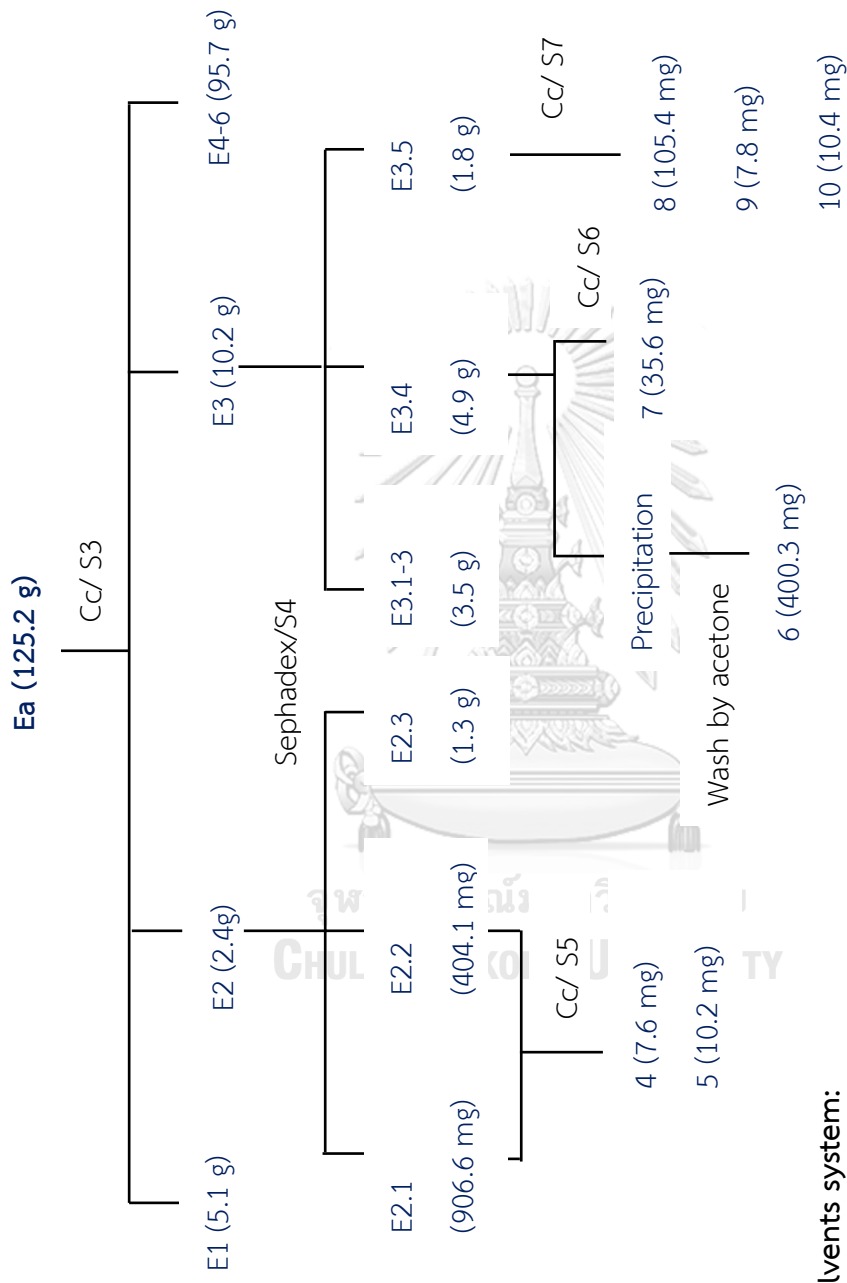
Solvents system:

- S3: CH$_2$Cl$_2$: EtOAc: acetone: HOAc (10:4:2.5:1)

- S4: MeOH:CH$_2$Cl$_2$ (1:1)

- S5: hexane/CH$_2$Cl$_2$/EtOH/H$_2$O (9:10:2:0.05)

- S6: hexane/CH$_2$Cl$_2$/EtOAc/HOAc (2:1:3:0.1)

- S7: hexane/acetone/HOAc (4:3:0.06)

Scheme 2.3 Procedure for the separation of Ea fraction of *K.angustifolia*

2.5 Bioassay Procedures

2.5.1 Anti-tyrosinase activity

The tyrosinase inhibitory activity was performed following the previous method with some modifications[28]. The extracts and isolated compounds were dissolved in the solution compromised of 10% DMSO in buffer, in which two-fold dilution was completed to obtain various concentrations. Briefly, 50 µL of each sample solution in buffer and 50 µL tyrosinase enzyme from a mushroom (250 U/mL) were placed in a 96 well plate. After pre-incubation in 5 minutes, 50 µL of L-tyrosine (5 mM) was later added as a substrate into the mixture and then incubated further for 30 minutes. After that, the absorbance of dopachrome was measured at 490 nm by a micro plate reader. Kojic acid was used as a positive control. The amount of inhibition was expressed as the percentage of concentration necessary to achieve 50% inhibition (IC_{50}). The IC_{50} values were determined by the data analysis.

The percent of tyrosinase inhibition was calculated from the following formula:

$$\% \text{ inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100 \qquad (1)$$

Where $A_{control}$ was the absorbance value at 490 nm of the mixture without the tested sample, and A_{sample} was the absorbance value of mixture contained the tested sample.

2.5.2 α-glucosidase inhibitory activity

The α-glucosidase inhibitory was measured using spectrophotometric method[29]. The amount of product (*p*-nitrophenol) released from the substrate *p*-nitrophenyl-α-D-glucopyranoside (pNPG) determined the hydrolytic activity of α-glucosidase (**Scheme 2.4**).
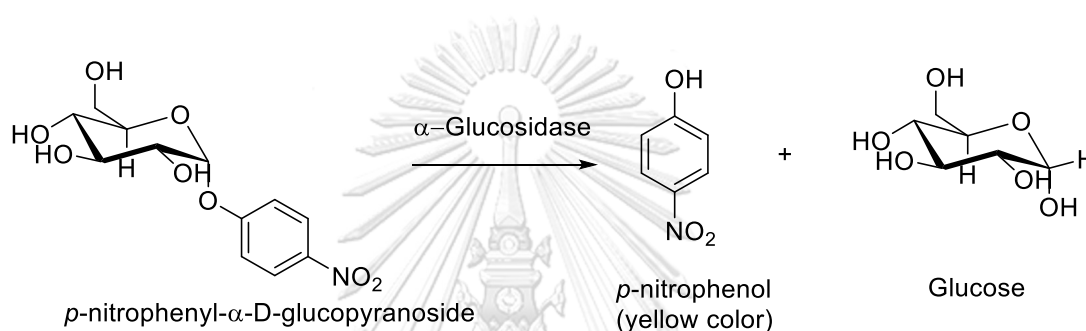
Scheme 2.4 Hydrolysis of p-nitrophenyl-α-D-glucopyranoside by α-glucosidase

Enzyme α-glucosidase from *Saccharomyces cerevisiae* E.C 3.2.1.20 (0.1 U/mL) and pNPG (1mM) as substrate were dissolved in 0.1 mM phosphate buffer (pH 6.9). In the 96-well plates, 10 µL of samples in DMSO or positive control was added with 40 µL of enzyme then incubated at 37 °C for 10 min. Afterward, 50 µL substrate was added into the mixture. The reaction was carried out at 37 °C in 20 minutes, and then 100 µL of 1 M Na_2CO_3 was used to terminate the reaction. Enzymatic activity was quantified by measuring the absorbance at 405 nm (ALLSHENG micro plate reader AMR-100). Acarbose® was used as a standard control and all samples were analyzed in triplicate at different concentrations to obtain the IC_{50} value of compounds. Percentage inhibition was calculated by the equation described below:

$$\% \text{ inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100 \qquad (2)$$

$A_{control}$ is the absorbance of control without tested solution. A_{sample} is the absorbance of control with tested solution

Chapter 3

Results and discussion

The medicinal plant *Knema angustifolia* has been used in Thai folk medicine for the treatment of a wide range of diseases; however chemical data on this plant are scarce. The preliminary screening tests revealed that *K. angustifolia* stems were potential sources for further investigation. Therefore, the main aim of this research was to isolate and elucidate the structures of chemical constituents. The biological activities such as anti-tyrosinase and α-glucosidase were in addition evaluated.

3.1 Preliminary study of crude extracts

According to the procedure shown in **Scheme 2.1**, four extracts including *n*-hexane, CH_2Cl_2, EtOAc, MeOH extracts were gained. They were further applied for preliminary anti-tyrosinase activity. The results are demonstrated in **Table 3.1**.

Table 3.1 Anti-tyrosinase activity of *K. angustifolia* extracts

Extracts	IC_{50} (μg/mL)
n-hexane	50.2±0.7
CH_2Cl_2	118.9±1.8
EtOAc	51.6±1.3
MeOH	115.9±0.8

Based on this result, intense anti-tyrosinase activity belongs to *n*-hexane and EtOAc extracts with IC_{50} value of 50.2 and 51.6 μg/mL, respectively. Thus, these

extracts were chosen as the probable foundation to discover bioactive compounds from this plant.

3.2 Chemical constituents from *K.angustifolia* stems

3.2.1 Separation of *n*-hexane extract

The *n*-hexane extract (55.9 g) as yellow material was applied to normal phase silica gel column, eluted with the gradient of hexane:EtOAc:acetone (15:1:1 to 5:1:1) to obtain five fractions **H1-5**. The results of separation are demonstrated in **Table 3.2**.

Table 3.2 The separation of *n*-hexane extract of *K. angustifolia* stems

Solvent system	Fraction	Weight (g)	Remarks
	H1	5.8	yellow oil
	H2	12.9	brownish-yellow
hexane:EtOAc:acetone	**H3**	15.2	brownish-yellow
(15:1:1 to 5:1:1)	**H4**	10.3	dark brown
	H5	8.6	brownish-yellow

3.2.1.1 Separation of fraction **H5**

H5 (8.6 g) was dissolved in *n*-hexane to afford the precipitate **HP** (5.0 g) and the solution **HS** (3.6 g). The former was crystallized in warming *n*-hexane many times to afford **1** (1.1 g). The detailed separation is shown in **Scheme 3.1**.

H5 (8.6 g)

|
n-hexane

HP (5.0 g)   **HS** (3.6 g)

|
n-hexane

1 (1.1 g)
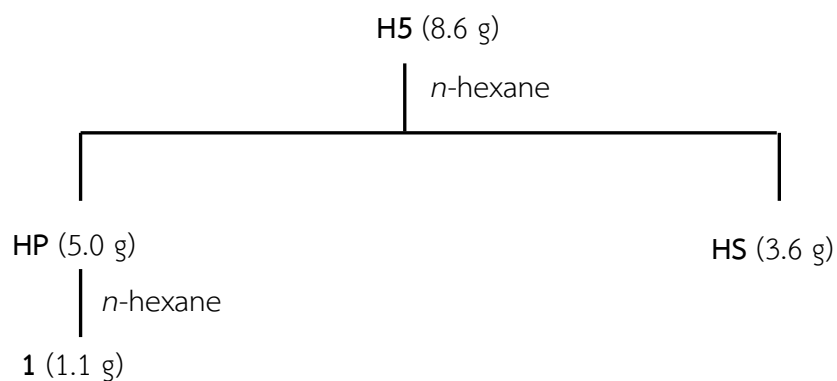
Scheme 3.1 Fractionation of fraction **H5**

HS (3.6 g) was subjected to Sephadex LH-20 column, eluted with MeOH to obtain three fractions **HS1-3**. **HS1** (1.2 g) was recrystallized with *n*-hexane to afford **2** (210 mg). The separation is described in **Scheme 3.2** and **Table 3.3.**

HS (3.6 g)

|
Sephadex LH-20/ MeOH

HS1 (1.2 g)   **HS2** (1.9 g)   **HS3** (0.5 g)

|
n-hexane

2 (210 mg)
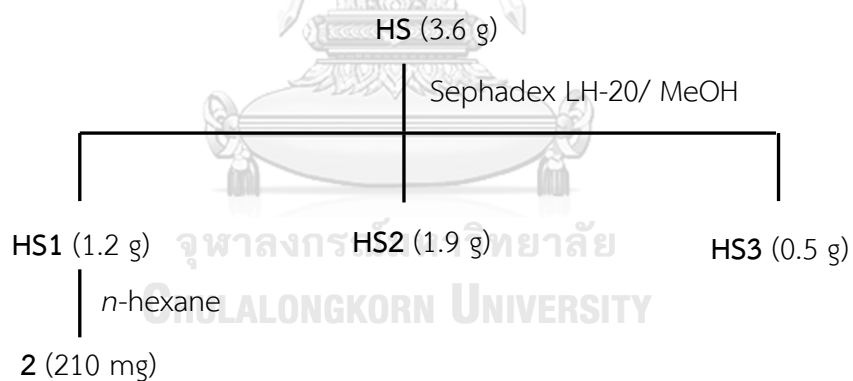
Scheme 3.2 Fractionation and isolation of **HS**

Table 3.3 The separation of **HS**

Solvent system	Fraction	Weight (g)	Remarks
MeOH 100% (Sephadex)	**HS1**	1.2	yellow oil
	HS2	1.9	brownish-yellow
	HS3	0.5	brownish-yellow

   HS3 (0.5 g) was rechromatographed by CC, eluted with the solvent system of

n-hexane:CH_2Cl_2:MeOH:H_2O (10:10:0.4:0.01, v/v/v/v) to give three subfractions **HS3.1-**

3.3. **HS3.1** (35 mg) was purified by preparative thin-layer chromatography, eluted

with n-hexane:CH_2Cl_2:EtOAc:acetone:AcOH (30:10:4:2.5:0.5, v/v/v/v/v) to afford **3** (1.3

mg). The summary of the separation of n-hexane extract can be depicted as shown

in **Scheme 3.3** and **Table 3.4**.

HS3 (0.5 g)

n-hexane: CH_2Cl_2:MeOH:H_2O (10:10:0.4:0.01)

HS3.1 (35 mg)   **HS3.2** (1.9 g)   **HS3.3** (0.5 g)

n-hexane: CH_2Cl_2:EtOAc:Acetone: AcOH

(30:10:4:2.5:0.5)

3 (1.3 mg)

Scheme 3.3 Fractionation and isolation of **HS3**

Table 3.4 The separation of **HS3**

Solvent system	Fraction	Weight (mg)	Remarks
	HS3.1	35	yellow oil
MeOH 100% (Sephadex)	**HS3.2**	167	brownish-yellow
	HS3.3	285	brownish-yellow

3.2.2 Separation of ethyl acetate extract

The Ea extract (125.2 g) as a brownish yellow syrup was applied to normal phase silica gel column and eluted with a solvent system of CH_2Cl_2/EtOAc/acetone/AcOH (10:4:2.5:0.5) to afford six fractions **E1-E6**. The results of separation are shown in **Table 3.5**.

Table 3.5 The separation of Ea extract of *K.angustifolia* stems

Solvent system	Fraction	Weight (g)	Remarks
	E1	5.1	yellow oil
	E2	2.4	yellow syrup
CH_2Cl_2/EtOAc/acetone/AcOH (10:4:2.5:0.5)	**E3**	10.2	brownish-yellow
	E4	27.3	red-brown
	E5	63.7	dark brown
	E6	11.8	dark brown

3.2.2.1 Separation of fraction **E2**

      E2 (2.4 g) was subjected to Sephadex LH-20 and eluted the column with [MeOH: CH$_2$Cl$_2$ (1:1)] to give three fractions **E2.1-E2.3**. After that, fractions **E2.1** and **E.2.2** were applied to silica gel CC using hexane/CH$_2$Cl$_2$/EtOH/H2O solvent system (9:10:2:0.05) led to the isolation of **4** (7.6 mg) and **5** (4.2 mg). The results of separation are shown in **Scheme 3.4** and **Table 3.6**.
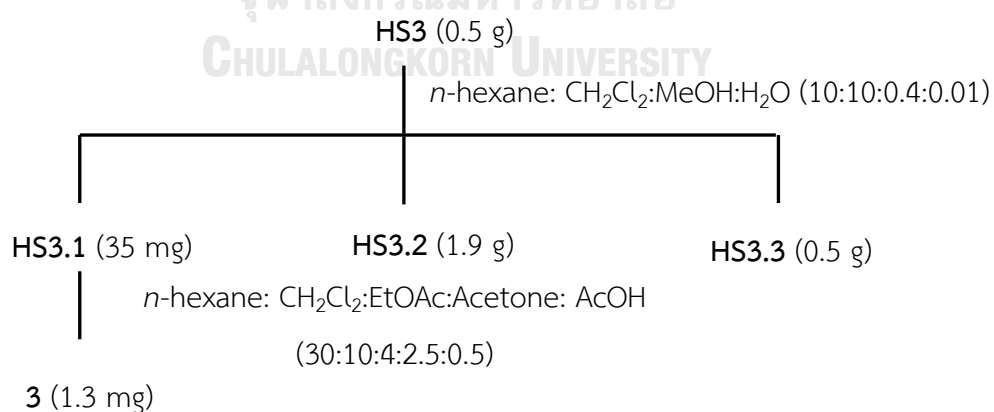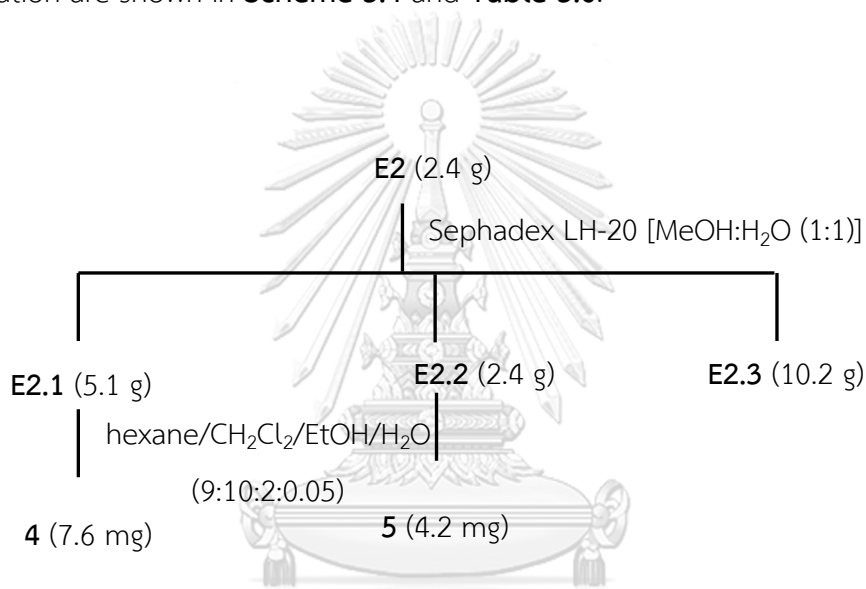

Scheme 3.4 Fractionation and isolation of **E2**

Table 3.6 The separation of **E2**

Solvent system	Fraction	Weight (g)	Remarks
	E2.1	5.1	yellow syrup
MeOH: H$_2$O (1:1)	**E2.2**	2.4	brownish-yellow
(Sephadex)	**E2.3**	10.2	dark brown

3.2.2.2 Separation of fraction **E3**

E3 (10.2 g) was subjected to Sephadex L-20 and eluted the column with [MeOH: CH_2Cl_2 (1: 1)] to give five fractions **E3.1-E3.5** as described in **Table 3.7**.

Table 3.7 The separation of **E3**

Solvent system	Fraction	Weight (g)	Remarks
MeOH: H_2O (1:1) (Sephadex)	**E3.1**	1.1	brown oil
	E3.2	1.5	brownish-yellow
	E3.3	0.9	brownish-yellow
	E3.4	4.9	dark brown
	E3.5	1.8	dark brown

During the evaporating solvent to a small volume, **E3.4** gave some white precipitation. Further washing the precipitation with acetone, the pure compound **6** was obtained. The leftover designated as **E3.4S** was subjected to silica gel column eluting with hexane/CH_2Cl_2/EtOAc/AcOH (2:1:3:0.1) to obtain **7** (35.6 mg). The separation is shown in **Scheme 3.5**.

E3.4 (4.9 g)

Evaporate

Precipitation (1.3 g)

acetone

E3.4S (3.6 g)

hexane/CH$_2$Cl$_2$/EtOAc/HOAc

(2:1:3:0.1)

6 (400.3 mg)

7 (35.6 mg)
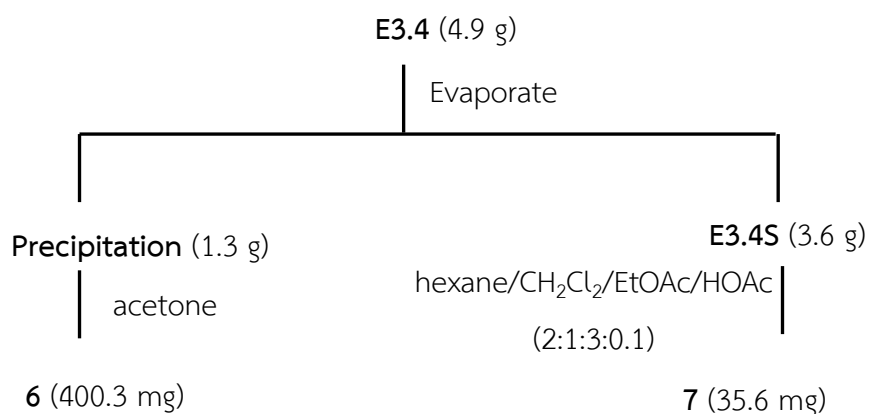
Scheme 3.5 Fractionation and isolation of E3.4

E3.5 (1.8 g) was subjected to silica gel column eluted with isocratic hexane/CH$_2$Cl$_2$/EtOAc/EtOH/H$_2$O solvent system (10:15:9:25:0.1, v/v/v/v/v) to grant three subfractions E3.5.1-E3.5.3 as described in Table 3.8.

Table 3.8 The separation of E3.5

Solvent system	Fraction	Weight (mg)	Remarks
hexane/CH$_2$Cl$_2$/EtOAc/EtOH/H$_2$O (10:15:9:25:0.1)	E3.5.1	874	white solid
	E3.5.2	403	white solid
	E3.5.3	591	brownish-yellow

Fraction E3.5.1 was then purified by column chromatography with hexane/acetone/AcOH (4:3:0.06, v/v/v) to obtain 8 (105.4 mg), 9 (7.8 mg), 10 (10.4 mg) and 11 (20.5 mg).

3.2.3 Structural elucidation of isolated compounds

3.2.3.1 Structural elucidation of compound **1**

 1 (1.1 g, 1.97% yield based on *n*-hexane extract), orange plates, had the molecular formula of $C_{19}H_{30}O_4$ based on an $[M+Na_2-H]$ ion peak at m/z 367.1851 (calcd. for $C_{19}H_{29}Na_2O_4$, 367.1855) (**Figure A.1**) with five double-bond equivalents.

 The ^1H NMR spectrum of **1** (**Figure A.2**) revealed the signal of one singlet olefinic methine (δ_H 5.77), two methylene groups [(δ_H 2.27, t, *J* = 7.5 Hz), (δ_H 1.34, m) and one methyl (δ_H 0.84, t, *J* = 6.0 Hz). Owing to molecular formula requirements and eight protons being evident from ^1H NMR analysis, twenty protons detected at δ_H 1.25-1.30 were considered as ten aliphatic methylene groups. The ^{13}C NMR of **1** (**Figure A.3**) presented two quaternary carbons (δ_C 117.4 and 103.8) and twelve methylene carbons in the linker (δ_C 22.0, 31.3) along with one methyl carbon δ_C 13.9). All reported data of **1** were similar to those of rapanone[30]. Notably, the signal of two carbonyl groups (C-1, C4) and two oxygenated quarternary carbons (C-2 and C-5) not appeared due to the fluxional effect. Thus, **1**, namely rapanone was determined.
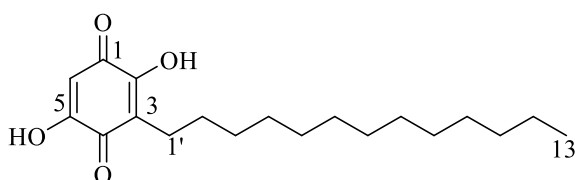
Compound **1**: Rapanone

3.2.3.2 Structural elucidation of compound **2**

2 (210 mg, 0.38% yield based on n-hexane extract), isolated as an orange power, gave a molecular formula of $C_{17}H_{26}O_4$ based on its positive-ion HR-ESI-MS data, which showed [M+Na$_2$-H] peak at m/z 339.1543 (calcd. for $C_{17}H_{25}Na_2O_4$, 339.1543) (**Figure A.4**).

The ^{1}H NMR and ^{13}C NMR data of **2** (**Figures A.5 and A.6**) demonstrated significant similarity with those of **1**, except for the appearance of 16 protons of long-chain methylene groups at δ_H 1.25-1.30 instead of 20, indicating the presence of two fewer methylene groups than rapanone in the side chain. The number of carbon in this moiety further determined based on the mass analysis with the molecular formula $C_{17}H_{26}O_4$. Moreover, all signals in the NMR data of **2** were in agreement with literature reported of embelin by Mahendran (2014)[31]. The structure of embelin **2** was established as shown as follows:
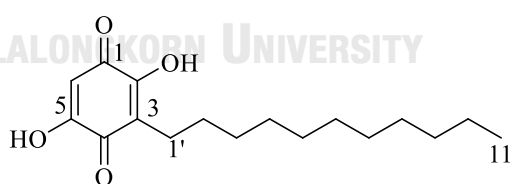
Compound **2**: Embelin

3.2.3.3 Structural elucidation of compound **3**

3 (1.3 mg, 0.002% yield based on *n*-hexane extract) was obtained as yellow amorphous powder. Its HR-ESI-MS peak at m/z 361.2397 ([M–H]-, calcd. for 361.2384) and m/z 723.4864 ([2M–H]-, calcd. for 723.4841) (**Figure A.7)** suggested a molecular formula of $C_{22}H_{34}O_4$ with six indices of hydrogen deficiency.

The ^{1}H NMR spectrum of **3** (**Figure A.8**), in accordance with the HSQC spectrum (**Figure A.10**) indicated the presence of two hydroxy groups (δ_H 8.11 and 7.36), four methyls (δ_H 1.78, 1.33, 1.17 and 0.87), one diastereotopic methylene group [δ_H 2.75 (1H, d, J = 3.0, 15.0 Hz) and 1.84 (1H, d, J = 15.0 Hz, H-2b)] and the signals of a aliphatic long chain connecting of δ_H 2.40, (2H, t, J = 7.5 Hz, H-1'), 1.45 (2H, m), and the consisting of broad signal in the range of 1.20-1.30 ppm]. The ^{13}C NMR spectrum (**Figure A.9**) revealed the existence of two conjugated ketone carbons (δ_C 182.2 and 181.8), six quaternary carbons (δ_C 151.4, 120.3, 117.7, 115.1, 79.8, 29.6), one olefin methine (δ_C 125.2), four methyls (δ_C 28.4, 14.1, 14.1, 14.0), and one methylene (δ_C 47.3) and the methylenes of a long chain in the range of 22 to 30 ppm.

These above chemical features indicated that **3** was a derivative of embelin/rapanone, co-occurred in the same source with the differences at C-3a and C-7a. The location of the aliphatic long-chain was defined at C-6 based on the downfield chemical shift of the methylene δ_H 2.40 (H$_2$-1', t, J = 7.5 Hz) and its HMBC (**Figure A.11**) cross peaks to C-5 (δ_C 151.4), C-6 (δ_C 117.7), and C-7 (δ_C 181.8). The HMBC cross peaks of the 5-OH group (δ_H 7.36) to C-6 and C-4 (δ_C 182.2) indicated its adjacent position toward the aliphatic chain. The spectroscopic data were highly reminiscent of those of embelin/rapanone[30, 32].

The second spin system through C-8-C-3-C-2-C-1-C-9/10 could be deduced based on HMBC correlations. Indeed, all three methyl groups at δ_H 1.78 (H$_3$-8), δ_H

1.33 (H$_3$-9), and δ_H 1.17 (H$_3$-10) gave HMBC correlations to C-2 (δ_C 47.3) while proton H$_2$-2 (2.75 & 1.80) gave the HMBC correlations to C-1, C-3, C-8 and C-9/10, confirming the previous statement. The HMBC correlations of H$_3$-8 (δ_H 1.78) to both carbons C-2 (δ_C 47.3) and C-3 (δ_C 79.8) and the downfield chemical shift of C-3 (δ_C 79.8) defined the location of 3-OH group. Lastly, HMBC correlations of H$_3$-9/10 and H$_2$-2 to C-7a (δ_C 120.3) and of both H$_2$-2 and H$_3$-8 of C-3a (δ_C 115.1) determined the linkages between C-1/C-7a and C-3/C-3a. Altogether, the planar structure of **3**, namely angustiquinone was identified. Unfortunately, the only stereocenter C-3 could not be defined due to the limit amount of **3**.

To the best of our knowledge, angustiquinone represented the first 1,1,3-trimethyl-2,3-dihydro-1H-indene-4,7-dione scaffold bearing a long chain. The key HMBC and chemical shift assignments of **3** were described in **Figure 3.1** and **Table 3.9**.
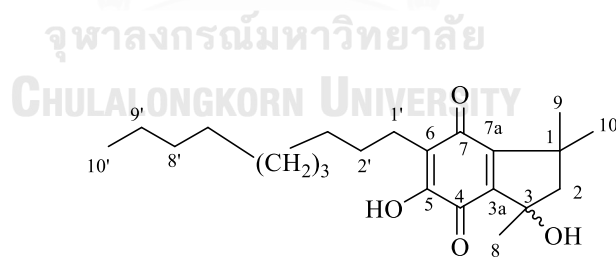
Compound **3**: Angustiquinone
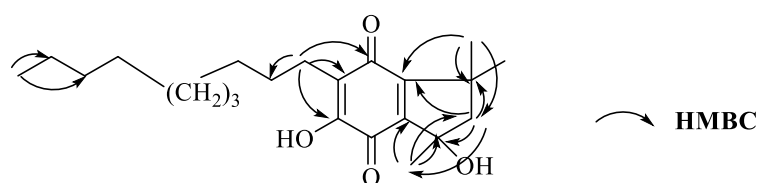
Figure 3.2 Key HMBC correlation of **3**

Table 3.9 The tentative ^{1}H and ^{13}C NMR chemical shift assignments of **1**, **2**, and **3**

Position	**1**[a] (DMSO-d_6)		**2**[a] (DMSO-d_6)		**3**[b] (CDCl$_3$)	
	δ_H, J (Hz)	δ_C	δ_H, J (Hz)	δ_C	δ_H, J (Hz)	δ_C
1		na		na		29.6
2		na		na	2.75, d, 15	47.3
					1.84, d, 15	
3		117.4		117.4		79.8
4		na		na		182.2
5		na		na	6.51, s	151.4
6	5.77, s	103.8	5.77 s	103.8		117.7
7						181.8
8					1.78, s	28.4
9					1.33, s	14.1
10					1.17, s	14.1
3a						115.1
7a						120.3
1′	2.27, t, (7.2)		2.27, t, (7.2)		2.40, t, (7.5)	22.7
2′	1.34, m		1.34, m			
2′- 9′					1.25- 1.33, m	
3′- 10′			1.25 – 1.30, m	22.0 to 31.3		
3′- 12′	1.25 – 1.30, m	22.0 to 31.3				
10′			0.84, t, (6.0)	13.9	0.87, t, (6.5)	14.0
11′						
13′	0.84, t, (6.0)	13.9				

[a]Recorded at 400 MHz, [b]Recorded at 500 MHz.

[na]Carbon peaks not appeared due to fluxional effect

3.2.3.4 Structural elucidation of compound **4**

4 (7.6 mg, 0.006% yield based on EtOAc extract) was obtained as a yellow amorphous powder. The ^{1}H NMR spectrum (**Figure A.12**) revealed one hydrogen-bonded hydroxyl group at $\delta_H$ 12.10 (OH-5), two *meta*-coupled aromatic protons at δ_H 6.21 (1H, d, J = 2.0 Hz, H-6) and 6.47 (1H, d, J = 1.6 Hz, H-8) on ring A, an ABX proton system at δ_H 7.76 (1H, d, J = 2.0 Hz, H-2'), 7.65 (1H, dd, J = 8.4, 2.0 Hz, H-6'), 6.93 (1H, d, J = 8.4 Hz, H-5'), due to a 3', 4'-disubstitution on ring B. The ^{13}C NMR spectrum (**Figure A.13**) showed the occurrence of fifteen carbon signals, including one carbonyl group (δ_C 176.6), five aromatic methine carbons (δ_C 121.5, 116.2, 115.8, 99.2 and 94.5), five aromatic tertiary carbons (all being immediately identified as oxygenated at δ_C 165.1, 162.3, 157.8, 148.4 and 145.9), two aromatic quaternary carbons (δ_C 123.8 and 104.1), and two oxygenated alkenes (δ_C 147.0, 136.8). All NMR data suggested that the structure of **4** could be completed into a flavonol skeleton.

Comparing the ^{1}H and ^{13}C NMR spectra of **4** with those of quercetin[33], **4** was clearly elucidated as quercetin.
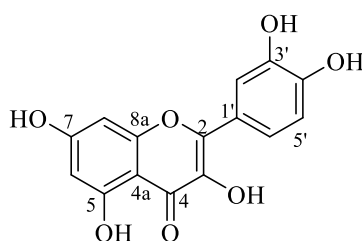
Compound **4**: Quercetin

Table 3.10 The ^{1}H and ^{13}C NMR chemical shift assignments of **4** compared with those of quercetin

Position	δ_H, mult J, (Hz)	δ_c	δ_H, mult J, (Hz)	δ_c
	4[a] (acetone-d_6)		Quercetin[a] (CD$_3$OD)	
2		147.0		148.0
3		136.8		137.2
4		176.6		177.3
5		162.4		162.5
6	6.21, d, (2.0)	99.2	6.20, d, (2.0)	99.3
7		165.1		165.6
8	6.47, d, (1.6)	94.5	6.40, d, (2.0)	94.4
4a		104.2		104.5
8a		157.8		158.3
1'		123.8		124.2
2'	7.76, d, (2.0)	115.8	7.75, d, (2.0)	116.0
3'		145.9		146.2
4'		148.4		148.8
5'	6.93, d, (8.4)	116.3	6.90, d, (8.5)	116.3
6'	7.65, dd, (8.4, 2.0)	121.5	7.65, dd, (8.5, 2.0)	121.7
5-OH	12.12, s			

[a]Recorded at 400 MHz

3.2.3.5 Structural elucidation of compound **5**

5 (10.2 mg, 0.008% yield based on EtOAc extract) was isolated as yellow amorphous powder. The ^{1}H NMR spectral data of **5** (**Figure A.16**) revealed four phenolic groups (δ_H 12.5, 10.81, 20.12, 9.38), an AA'BB' spin system comprising two pairs of two protons doublets at δ_H 8.04 (2H, d, J = 8.8 Hz) and 6.93 (2H, d, J = 8.8 Hz), an AX spin system comprising two doublets protons at δ_H 6.44 (1H, d, J = 2.0 Hz) and 6.20 (1H, d, J = 2.0 Hz). The ^{13}C NMR spectrum (**Figure A.17**) indicated the presence of a carbonyl (δ_C 175.9), six aromatic methine (δ_C 129.5, 115.5, each two carbons and 98.2, 93.5), four tertiary carbons (all oxygenated carbons at δ_C 163.9, 160.7, 159.2 and 156.2), two aromatic quaternary carbons (δ_C 121.7 and 103.1), and two oxygenated alkene (δ_C 146.9 and 135.7).

The ^{1}H and ^{13}C NMR spectral data of **5** was similar to those of **4**, with an addition of one aromatic proton on B-ring. These results when compared to literature data of kaempferol[34], is suggestive that **5** was kaempferol.
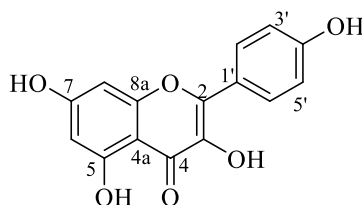
Compound **5**: Kaempferol

Table 3.11 The ^{1}H and ^{13}C NMR chemical shift assignments of **5** compared with those of kaempferol

|	**5**[a] (DMSO-d_6)		Kaempferol[c] (DMSO-d_6)	
Position	δ_H, mult J, (Hz)	δ_c	δ_H, mult J, (Hz)	δ_c
2		146.9		146.8
3		135.7		135.6
4		175.9		175.9
5		156.2		156.2
6	6.20, d, (2.0)	98.2	6.19, d, (1.8)	98.2
7		163.9		163.9
8	6.45, d, (2.0)	93.5	6.44, d, (1.8)	93.5
4a		103.1		103.0
8a		160.7		160.7
1'		121.7		121.7
2'/6'	8.00, d, (8.8)	129.5	8.04, d, (9.0)	129.5
3'/5'	6.93, d, (8.8)	115.5	6.92, d, (9.0)	115.4
4'		159.2		159.2
5-OH	12.48, s			

[a]Recorded at 400 MHz, [c]Recorded at 600 MHz

3.2.3.6 Structural elucidation of compound **6**

　　6 (400.3 mg, 0.32% yield based on EtOAc extract) was isolated as white crystal. The ^{1}H NMR spectrum of **6 (Figure A.18)**, in combination with the HSQC spectrum, exhibited a signal for five hydroxyl groups (δ_H 9.76, 8.44, 5.64, 5.43 and 4.92), one aromatic proton at δ_H 6.99 (1H, s), one methoxy group at δ_H 3.76 (3H, s), five bergenin type oxymethine signals at δ_H 4.96 (1H, d, J = 10.4 Hz), 4.00 (1H, dd, J = 10.0, 9.6 Hz), 3.65 (1H, ddd, J = 9.2, 8.8, 4.4), 3.57 (1H, m), 3.21 (1H, m), and one oxygenated methylene group at δ_H 3.84 (1H, d, J = 11.6 Hz), 3.45 (1H, m).

　　The ^{13}C NMR (**Figure A.19**) revealed the existence of fourteen carbon signals, comprising of one ester carbonyl carbon (δ_C 163.4), five quaternary carbons (δ_C 150.9, 148.1, 140.7, 118.1, 116.0), one methoxyl carbon (δ_C 59.9), five oxymethine carbons (δ_C 81.7, 79.8, 73.7, 72.2, 70.7), one aromatic methine (δ_C 109.5) and one methylene (δ_C 61.1). The position of aromatic proton at δ_H 6.99 at C-7 was confirmed from its HMBC correlation to C-6 (**Figure A.21**).

　　From all above information, both ^{1}H and ^{13}C NMR chemical shift values of **6** were consistent with those reported by De Abreu *et al.* (2008) [35] and Khan *et al.* (2016) [36], therefore **6** was identified as bergenin.
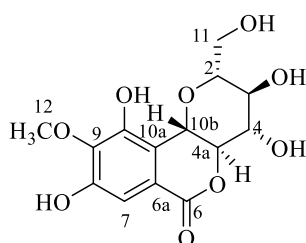
Compound **6**: Bergenin

Table 3.12 The ^{1}H and ^{13}C NMR chemical shift assignments of **6** compared with those of bergenin

Position	4^a (DMSO-d_6)		Bergenin^a(DMSO-d_6)	
	δ_H, mult J, (Hz)	δ_c	δ_H, mult J, (Hz)	δ_c
2	3.57, m	81.7	3.58, ddd, (7.6, 3.2, 1.9)	81.7
3	3.21, m	70.7	3.20, ddd, (8.8, 7.6, 5.0)	70.7
4	3.65, ddd, (9.2, 8.8, 4.4)	73.7	3.65, ddd, (9.5, 8.8, 5.3)	73.7
6		163.4		163.4
7	6.99, s	109.5	6.98, s	109.5
8		150.9		150.9
9		140.7		140.7
10		148.1		148.1
11	3.45, m	61.1	3.44, ddd, (10.9, 8.1, 1.9)	61.1
	3.84, d, (11.6)		3.85, dd, (10.9, 3.2)	
12	3.76, s	59.9	3.78, s	59.8
4a	4.00, dd, (10.0, 9.6)	79.8	4.00, dd, (10.4, 9.5)	79.8
6a		118.1		118.1
10a		116.0		116.0
10b	4.96, d, (10.4)	72.2	4.96, d, (10.4)	72.1
3-OH	5.43, d, (5.6)		5.42, d, (5.0)	
4-OH	5.64, d, (4.4)		5.64, d, (5.3)	
8-OH	9.76, s		9.76, s	
10-OH	8.44, s		8.45, s	
11-OH	4.92, m		4.91, m	

^{a}Recorded at 400 MHz

3.2.3.7 Structural elucidation of compound **7**

7 (35.6 mg, 0.03% yield based on EtOAc extract) was isolated as white solid. The ^{1}H-NMR spectrum (**Figure A.22**) displayed one aromatic methine at δ_H 7.11 (1H, s, H-7), five bergenin type oxymethine signals at δ_H 4.99 (1H, d, J = 10.4 Hz), 4.09 (1H, t, J = 10.0 Hz), 3.87 (1H, m), 3.83 (1H, m), 3.46 (1H, m), one methylene [δ_H 4.66 (1H, dd, J = 12.0, 5.1 Hz) and 4.23 (1H, dd, J = 12.0, 5.1 Hz)], and one methyl at δ_H 2.13 (1H, s). The ^{13}C NMR spectrum (**Figure A.23**) showed the presence of sixteen carbon signals, including two carbonyl groups (δ_C 172.6 and 165.7), six methines (δ_C 111.2, 81.3, 80.4, 75.4, 74.3 and 71.9), one oxygenated methylene (δ_C 64.7), one methyl (δ_C 20.6), one methoxy (δ_C 60.9) and five quaternary carbons, three of which was oxygenated (δ_C 152.4, 149.3 and 142.5).

The comparison in the ^{1}H and ^{13}C NMR spectra between **7** and **6** pointed out the similarity in the chemical structures of two compounds, excepted for the appearance of an acetyl group at δ_H 2.13 (3H, s, H-2') in **7**. This acetyl group could be located at C-11 based on the HMBC correlations observed from the methyl proton at δ_H 2.13 (H3-2') to δ_C 64.7 (C-11) (**Figure A.23**).

The structure was supported by the analysis of the NMR data with those in previous studies[37, 38]. Thus, the structure of 11-*O*-acetyl bergenin (**7**) was established.

Compound **7**: 11-*O*-Acetyl bergenin

3.2.3.8 Structural elucidation of compound **8**

8 (105.4 mg, 0.08% yield based on EtOAc extract) was obtained as pale brown amorphous solid.

The ^{1}H NMR data of **8** (**Figure A.26**) indicated the presence of two protons of a methylene group at δ_H 2.90 (1H, dd, *J* = 5.6, 16.0 Hz, H-4eq) and 2.53 (1H, dd, *J* = 8.4,16.0 Hz, H-4axi), two oxymethine protons at δ_H 4.56 (1H, d, *J* = 8.0 Hz, H-2), 4.00 (1H, m, H-3) in the C-ring, five signals of aromatic protons assigned to an AX type A-ring at δ_H 6.02 (1H, d, *J* = 2.0 Hz, H-6), 5.88 (1H, d, *J* = 2.0 Hz, H-8), and an ABX system in B-ring at δ_H 6.90 (1H, d, *J* = 1.6 Hz, H-2'), 6.79 (1H, d, *J* = 8.4 Hz, H-5'), 6.74 (1H, dd, *J* = 8.0, 2.0 Hz, H6'), suggesting the existence of a flavan-3-ol skeleton. Moreover, the large coupling constant between H-2 and H-3 ($J_{2,3}$ = 8.0 Hz) indicated a 2,3-*trans* stereochemistry in a ring C.

In the ^{13}C NMR (**Figure A.27**), fifteen signals of carbons were observed, in the presence of the aromatic carbon chemical shifts identifiable with the oxygen bearing carbons of the phloroglucinol A-ring at δ_C 157.1, 155.6 and 156.8 for C-5, C-7 and C-

8a, respectively, and the typical catechol B-ring signal at δ_C 145.6(x2) for C-3' and C-4' also confirmed the catechin nature of the core of this molecule.

The spectral data of **8** were compared with the literature[39] and confirmed that the structure of **8** was catechin.

Compound **8**: Catechin
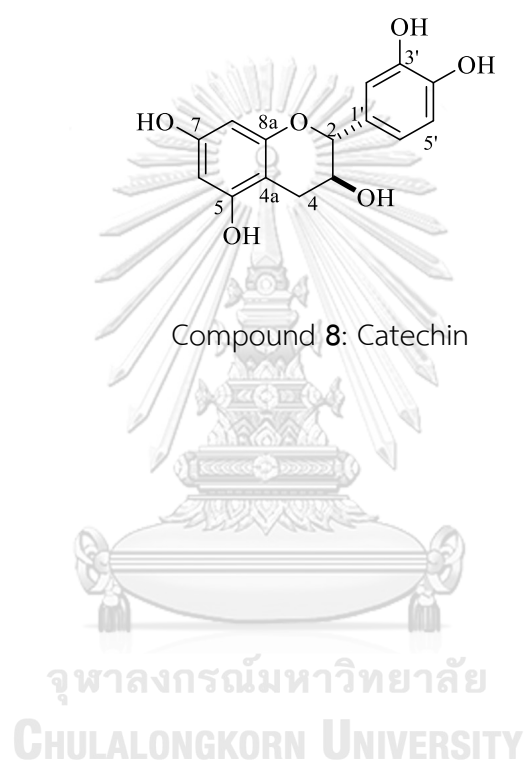
Table 3.13 The ^{1}H and ^{13}C NMR chemical shift assignments of **7** compared with those of 11-*O*-acetyl bergenin

Position	**7**^{a} (CD$_3$OD) δ_H, mult *J*, (Hz)	δ_c	11-*O*-acetyl bergenin^a (CD$_3$OD) δ_H, mult *J*, (Hz)	δ_c
2	3.83, m	80.4	3.81, m	80.3
3	3.46, m	71.9	3.46, m	71.8
4	3.87, m	75.4	3.85, m	75.6
6		165.7		165.8
7	7.11, s	111.2	7.09, s	111.3
8		152.4		152.7
9		142.5		142.4
10		149.3		149.3
11	4.66, dd, (12.0, 5.1)	64.7	4.65, m	64.6
	4.23, dd, (12.0, 5.1)		4.23, m	
12	3.92, s	60.9	3.90, s	60.9
4a	4.09, t, (10.0)	81.3	4.07, dd, (9.6, 10.4)	81.3
6a		119.5		119.4
10a		117.0		117.2
10b	4.99, d, (10.4)	74.3	4.99, d, (10.4)	74.3
1'		172.7		172.6
2'	2.13, s	20.6	2.11	20.6

^{a}Recorded at 400 MHz

Table 3.14 The ^{1}H and ^{13}C NMR chemical shift assignments of **8** compared with those of catechin

| Position | 8[a] (acetone-d_6) | | Catechin[a] (acetone-d_6) | |
	δ_H, mult J, (Hz)	δ_c	δ_H, mult J, (Hz)	δ_c
2	4.56, d, (8.0)	82.5	4.55, d, (7.5)	82.8
3	4.00, m	68.3	3.98, ddd, (8.0, 7.5, 5.5)	68.3
4	2.90, dd, (16.0, 5.6)	28.7	2.91, dd, (16.0, 5.5)	28.8
	2.53, dd, (16.0, 8.4)		2.52, dd, (16.0, 8.0)	
5		157.1		157.2
6	6.02, d, (2.0)	95.4	6.02, d, (2.0)	96.1
7		157.6		157.7
8	5.88, d, (2.0)	96.1	5.87, d, (2.0)	95.3
4a		100.6		100.6
8a		156.8		156.9
1'		132.0		131.8
2'	6.90, d, (1.6)	115.2	6.88, d, (2.0)	115.2
3'		145.6		146.1
4'		145.6		146.0
5'	6.79, d, (8.4)	115.7	6.77, d, (8.0)	115.7
6'	6.74, dd, (8.0, 2.0)	120.0	6.73, dd, (8.1, 2.0)	118.8

[a]Recorded at 400 MHz

3.2.3.9 Structural elucidation of compound **9**

9 (7.8 mg, 0.006% yield based on EtOAc extract) was obtained as white amorphous powder showing the violet spot under the UV light. The ^{1}H NMR spectrum (**Figure A.29**) showed signals of three aromatic protons [δ_H 7.58 (1H, dd, J = 8.4, 2.0 Hz, H-6), 7.55 (1H, d, J = 2.0 Hz, H-2) and 6.90 (1H, d, J = 8.4 Hz, H-5)], indicating the presence of 1,3,4-trisubstitution system in the aromatic ring, and one oxygenated methyl group at δ_H 3.90 (3H, H-8). The ^{13}C NMR spectrum (**Figure A.30**) confirmed the existence of one carbonyl group at δ_C 167.7, C-7, one methoxyl group at δ_C 56.4, C-8, three aromatic methine δ_C 124.9, 115.6 and 113.6), and there quaternary aromatic carbons (δ_C 152.1, 148.1 and 123.0).

These spectroscopic data were compatible with isovanillic acid[40]; therefore **9** was isovanillic acid.
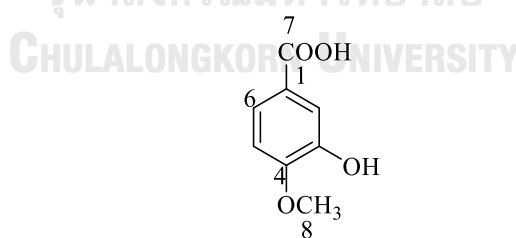
Compound **9**: Isovanillic acid

3.2.3.10 Structural elucidation of compound **10**

10 (10.4 mg, 0.008% yield based on EtOAc extract) was obtained as white amorphous powder showing the violet spot under the UV light. The ^{1}H NMR

spectrum (**Figure A.31**) revealed three aromatic protons in an ABX system at δ_H 7.45

(1H, dd, J = 8.4, 2.0 Hz, H-6), 7.52 (1H, d, J = 2.0 Hz, H-2) and 6.89 (1H, d, J = 8.4 Hz,

H-5). The ^{13}C NMR spectrum (**Figure A.32**) showed the presence of one carbonyl

group at δ_C 167.7, C-7), three aromatic methine (δ_C 123.6, 117.5 and 115.7), and there

quaternary aromatic carbons (δ_C 150.8, 145.6 and 123.6).

After comparing the NMR data of **10** with those of **9**, the difference two

compounds were the loss of methoxyl group at position 4 in case of **10**. Moreover,

this NMR spectrum was similar to that of protocatechuic acid[41], as detailed in **Table
3.17**. **10** was thus elucidated as protocatechuic acid.

Compound **10**: Protocatechuic acid
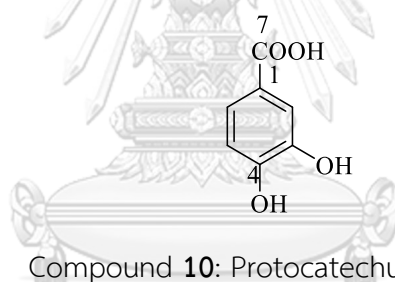
3.2.3.11 Structural elucidation of compound **11**

11 (20.5 mg, 0.016% yield based on EtOAc extract) was isolated as white

amorphous powder. The ^{1}H NMR spectrum (**Figure A.33**) showed the existence of

only two symmetric aromatic protons at δ_H 7.15 (2H, s, H-2, H-6). The ^{13}C NMR

(**Figure A.34**) indicated one carbonyl group at δ_C 167.9, C-7), two aromatic methines

at δ_C 110.2 (x2), C-2, C-6, and four quaternary aromatic carbons (δ_C 146.0 x 2, 138.7

and 122.1).

According to all NMR data, it is suggested that the structure of **11** be gallic acid[42].
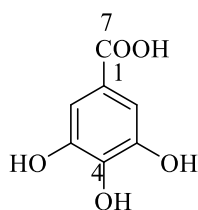
Compound **11**: Gallic acid

Table 3.15 The ^{1}H and ^{13}C NMR chemical shift assignments of **9** compared with those of isovanillic acid

Position	**9**^{a} (acetone-d_6)		Isovanillic acid ^{d}(acetone-d_6)	
	δ_H, mult J, (Hz)	δ_c	δ_H, mult J, (Hz)	δ_c
1		123.0		122.9
2	7.55, d, (2.0)	115.6	7.58, d, (1.6)	115.5
3		148.1		148.1
4		152.1		152.0
5	6.90, d, (8.4)	113.6	6.92, d, (8.4)	113.5
6	7.58, dd, (8.4, 2.0)	124.9	7.61, dd, (8.4, 1.6)	124.8
7		167.7		167.8
8	3.90, s	56.4	3.93, s	56.3

^{a}Recorded at 400 MHz

Table 3.16 The comparative ^{1}H and ^{13}C NMR chemical shift assignments of **10** and protocatechuic acid

Position	**10**[a] (acetone-d_6)		Protocatechuic acid[c] (acetone-d_6)	
	δ_H, mult J, (Hz)	δ_c	δ_H, mult J, (Hz)	δ_c
1		123.2		123.2
2	7.52, d, (2.0)	117.5	7.53, d, (2.0)	117.6
3		145.6		145.5
4		150.8		150.6
5	6.89, d, (8.4)	115.7	6.90, d, (8.3)	115.8
6	7.45, dd, (8.4, 2.0)	123.6	7.48, dd, (8.3, 2.0)	123.7
7		167.7		167.7

[a]Recorded at 400 MHz, [c]Recorded at 600 MHz

Table 3.17 The comparative ^{1}H and ^{13}C NMR chemical shift assignments of **11** and gallic acid

Position	**11**[a] (acetone-d_6)		Gallic acid[a] (CDCl$_3$)	
	δ_H, mult J, (Hz)	δ_c	δ_H, mult J, (Hz)	δ_c
1		122.1		120.5
2/6	7.15, s	110.2	6.9, s	108.8
3/5		146.0		145.5
4		138.7		138.1
7		167.9		167.6

[a]Recorded at 400 MHz

3.3 Biological activities of isolated compounds from the stem of *K.angustifolia*

The separation of *K. angustifolia* stems led to the isolation of eleven compounds. These compounds were further evaluated for the biological activities including anti-tyrosinase and α-glucosidase inhibition activity.

3.3.1 Anti-tyrosinase

The tyrosinase assay was performed by the method of Larik *et al.* (2017) with slight adjustments. Kojic acid was used as a standard positive control.

The results of *K. angustifolia* extracts for anti-tyrosinase activity were mentioned in the first part of the preliminary biological activity test, both *n*-hexane and EtOAc extracts exhibited as the potent tyrosinase inhibitory sources. Thus, the isolated compounds were chosen to evaluate for that of activity as presented in **Table 3.18**.

Due to a shortage of material, angustiquinone (**3**) could not be assayed for the activity of mushroom tyrosinase. Rapanone (**1**) and embelin (**2**), contained the long-chain saturated carbons in their molecules, possessed the value of IC_{50} more than 200 μM. This might be implied that the steric effect of the long chain reduced the activity.

Table 3.18 Anti-tyrosinase activity of isolated compounds from *K. angustifolia*

Compounds	IC_{50} (µM)
Rapanone (**1**)	> 200
Embelin (**2**)	> 200
Angustiquinone (**3**)	NT
Quercetin (**4**)	43.3±0.26
Kaempferol (**5**)	156.5±0.34
Bergenin (**6**)	NA
11-*O*-acetyl bergenin (**7**)	NA
Catechin (**8**)	> 200
Isovanilllic acid (**9**)	> 200
Protocatechuic acid (**10**)	> 200
Gallic acid (**11**)	> 200
Kojic acid	36.1±1.07

NT: not tested, NA: not active

From the literature review for flavonoids, it has been demonstrated that 3-hydroxyl-4-ketone moiety, which makes the chelate with copper, was the key to determine the inhibitory activity, and the loss of one factor will complete abolishing the activity. This hypothesis was confirmed in our research. While quercetin (**4**) and kaempferol (**5**) exhibited moderate activity with IC_{50} value 43.3±0.26 and 156.5±0.34 µM, respectively, catechin (**8**) displayed IC_{50} more than 200 µM. In addition, **4** was

found to be 3.6-fold more active than **5**. The only difference between these two compounds is that **4** has an extra hydroxyl group at C-3′, which is consist of with the literature findings[43].

Bergenin (**6**) and its derivative 11-*O*-acetyl bergenin (**7**) were inactive toward mushroom tyrosinase. It could be indicated that the bulky glycosides moiety in the structures of **6** and **7** effected on the active site of the enzyme by preventing the inhibitor.

For the monophenolic compounds, isovanillic acid (**9**), protocatechuic acid (**10**), and gallic acid (**11**) revealed weak inhibitors against mushrooms tyrosinase with IC_{50} value more than 200 µM.

3.3.2 α-Glucosidase inhibitory activity

α-Glucosidase inhibitory assay of extracts and isolated compounds from *K. angustifolia* stems was evaluated using Ramadhan's method with slight modification[29]. Acarbose, an oral anti-diabetes drug, was used as a positive control. The lower IC_{50} values demonstrated the stronger enzymatic inhibition. The results are demonstrated in **Tables 3.19** and **3.20**.

Table 3.19 α-glucosidase inhibitory activity of *K. angustifolia* extracts

Extracts	IC$_{50}$ (µg/mL)
n-hexane	20.91±0.40
CH$_2$Cl$_2$	10.10±0.32
EtOAc	< 2
MeOH	< 2

In general, all herbal extracts showed potential activity. Notably, the higher α-glucosidase inhibitory activity was detected from both EtOAc and MeOH extracts. Previous studies have illustrated that the strong α-glucosidase inhibitors were the essential sources for the treatment of diabetes mellitus type 2. Therefore, the discovery of the bioactive compounds from *K. angustifolia* may warrant further investigation for its ability to promote good health.

Table 3.20 α-glucosidase inhibitory activity of isolated compounds from *K. angustifolia*

Compounds	IC$_{50}$ (μM)
Rapanone (**1**)	9.25±0.26
Embelin (**2**)	1.30±0.17
Angustiquinone (**3**)	NT
Quercetin (**4**)	71.20±1.04
Kaempferol (**5**)	23.15±1.25
Bergenin (**6**)	NA
11-*O*-acetyl bergenin (**7**)	87.70±1.01
Catechin (**8**)	151.60±1.88
Isovanilllic acid (**9**)	> 200
Protocatechuic acid (**10**)	> 200
Gallic acid (**11**)	> 200
Acarbose	93.63±0.49

NT: not tested, NA: not active

According to the results in **Table 3.20,** rapanone (**1**) and embelin (**2**), *p*-benzoquinone derivatives from *K. angustifolia*, showed tremendous activity compared to other isolated compounds with IC$_{50}$ 9.3±0.26 and 1.3±0.17 μM, respectively. The result also displayed that **1** and **2** gave much higher inhibitory activity than commercial α-glucosidase inhibitor acarbose with IC$_{50}$ 93.6±0.49 μM. **2**

with better inhibitory effect was reported to possess antidiabetic effect towards induced diabetic rats, which probably explained the excellent activity towards α-glucosidase[44]. The reduction of the alkane chain in **2** led to better inhibitory activity compared to **1**.

The isolated flavonoids including quercetin (**4**), kaempferol (**5**), catechin (**8**) also showed moderate α-glucosidase inhibitor with IC_{50} 71.2±1.04, 23.2±1.25 and 151.6±1.88 µM. Previous reports illustrated that the activity of some flavonoids showed better activities than commercial diabetic drug acarbose. Various kinetic mechanisms of flavonoids were also established[25, 45, 46]. The structure-activity relationships (SARs) of these compounds established by Zeng *et al.* suggested that the hydroxyl groups on rings A, B, and C were essential for α-glucosidase inhibition. In detail, 4′-OH substitution on ring B and 3-OH substitution on ring C, which could interact with the positively charged groups on the enzyme, was considered as a crucial role for the activity. Moreover, another study reported that the existence of a hydroxyl group at the C-3 ′position of ring B decreased the inhibitory effect on the enzyme[47]. The fact that quercetin (**4**) had a better effect than kaempferol (**5**) was consistent with these SARs.

11-*O*-acetyl bergenin (**7**) good activity with IC_{50} 87.7±1.01 µM compared to bergenin which showed no activity towards α-glucosidase. The acetyl group in 11-*O*-acetyl bergenin played a vital role in increasing α-glucosidase activity compared to

the hydroxyl group at bergenin itself. It was also reported that the derivatives of bergenin such as 11-*O*-benzoylbergenin, 11-*O*-(3′,4′-dimethoxybenzoyl)-bergenin, 11-*O*-vanilloylbergenin and 11-*O*-protocatechuoylbergenin revealed better activity towards α-glucosidase[48].

Similarly to anti-tyrosinase activity, isovanillic acid (**9**), protocatechuic acid (**10**) and gallic acid (**11**) displayed IC_{50} more than 200 µM. Unfortunately, the new compound angustiquinone (**3**) was unable to determine for α-glucosidase activity due to the minimum amount of compound. To the best of our knowledge, this is the first report of rapanone (**1**) embelin (**2**), and 11-*O*-acetyl bergenin (**7**) as α-glucosidase inhibitor.

Base on the results, the detected compounds could be responsible for the biological activities of *n*-hexane and EtOAc extracts.

จุฬาลงกรณ์มหาวิทยาลัย

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Chapter 4

Conclusions

4.1 Chemical constituent of *K. angustifolia* stems

This is the first report on the chemical constituents of *K. angustifolia* plant. Further repeated column chromatograph of *n*-hexane and EtOAc extracts led to the isolation of one novel quinone: angustiquinone (**3**) and ten known compounds: rapanone (**1**), embelin (**2**), quercetin (**4**), kaempferol (**5**), bergenin (**6**), 11-*O*-acetyl bergenin (**7**), catechin (**8**), isovanillic acid (**9**), protocatechuic acid (**10**), gallic acid (**11**). The structures of isolated compounds are shown in **Figure 4.1**. Each structure was unambiguously elucidated using 1D and 2D NMR analyses, in addition to the use of literature for the comparison of the experimental data acquired.

- **Para-benzoquinone**

(1)   (2)   (3)

Flavonoids

(4)   (5)   (8)
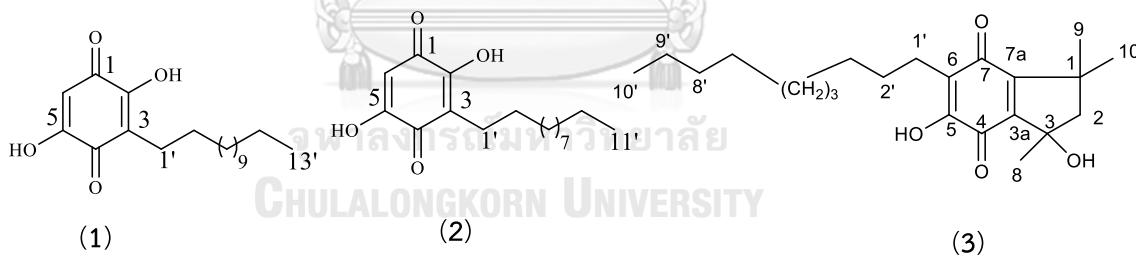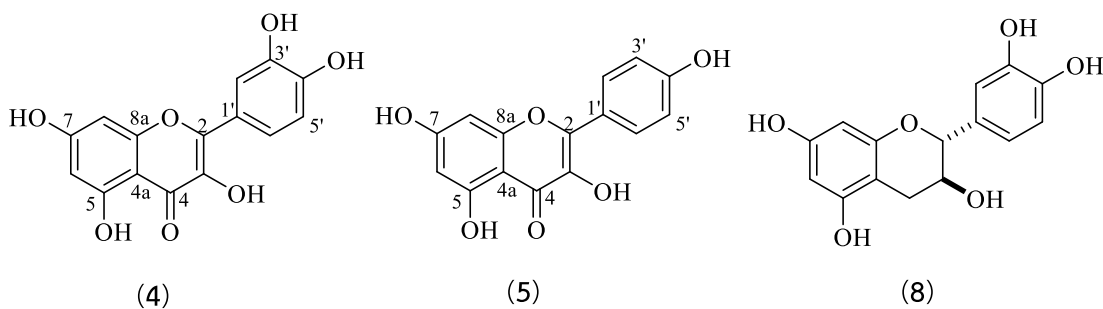
- **Monoaromatic ring**
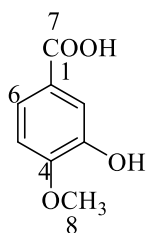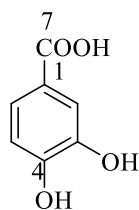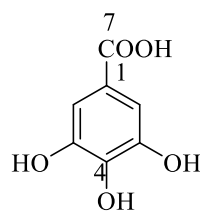
(9)   (10)   (11)

- **Other skeletons**
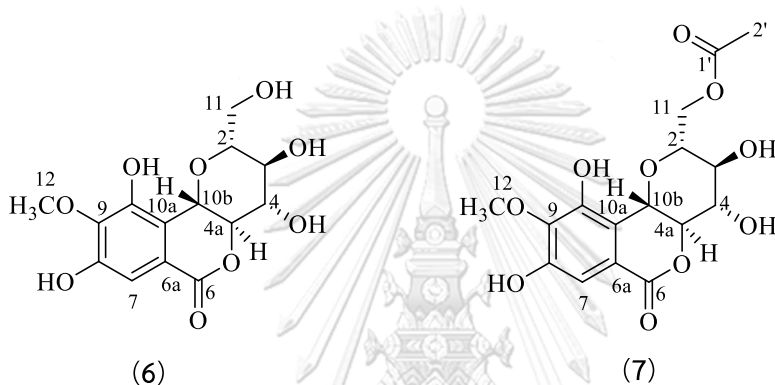
(6)   (7)

4.2 Biological activities

The isolated substances were evaluated for the biological activities including anti-tyrosinase, α-glucosidase inhibitory activity.

According to the results of anti-tyrosinase activity, quercetin (**4**) showed the highest inhibitory effect with IC_{50} 42.3±0.25 µM, followed by kaempferol (**5**) with IC_{50} 156.5±0.34. Interestingly, the activity of both compounds were lower than that of a positive control, kojic acid (IC_{50} 36.1±1.07).

The bright point of this research is the discovery of promising α-glucosidase inhibitors. Most compounds including **1, 2, 4, 5** and **8** showed excellent activity

better than the commercial drug acarbose. Especially, rapanone (**1**) and embelin (**2**) exhibited as the best candidates, with 10 and 72-folds, respectively. Therefore, it could serve as auspicious substances for designing new potent α-glucosidase inhibitors.

4.3 Suggestions for future work

This investigation has disclosed that *K. angustifolia* had the potential to provide medicinally beneficial compounds. Therefore, it is necessary to continue to isolate more compounds and evaluate their biological activities for applying on medicinal fields. Notably, rapanone **(1)** and embelin (**2**) revealed the potent candidates for α-glucosidase inhibitory activity, thus the further studies such as kinetic mechanism could be investigated. Moreover, synthesis of their derivatives is one of the excellent directions to develop the benefits of natural products.

APPENDIX

Generic Display Report
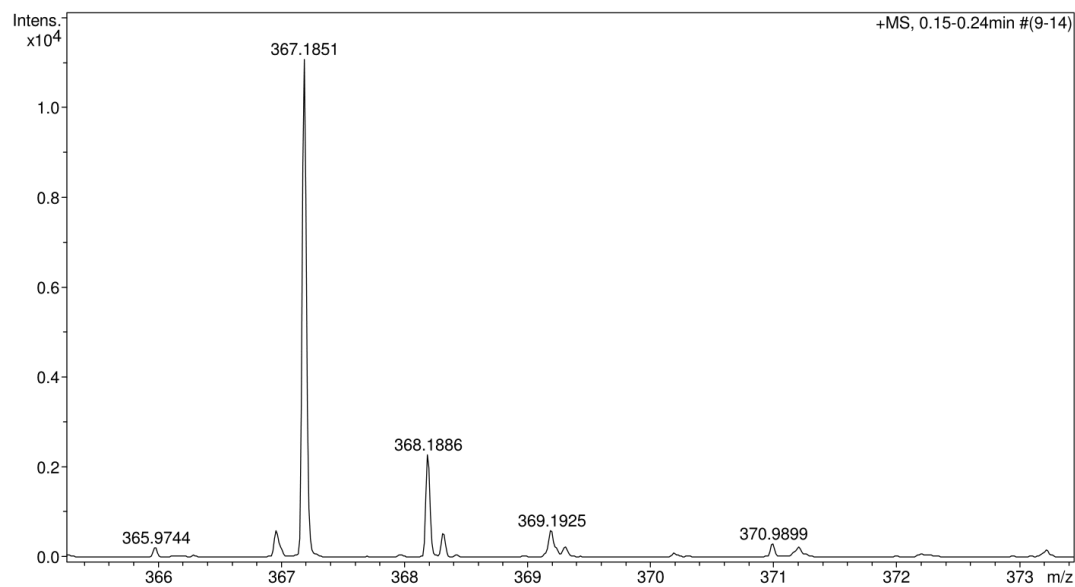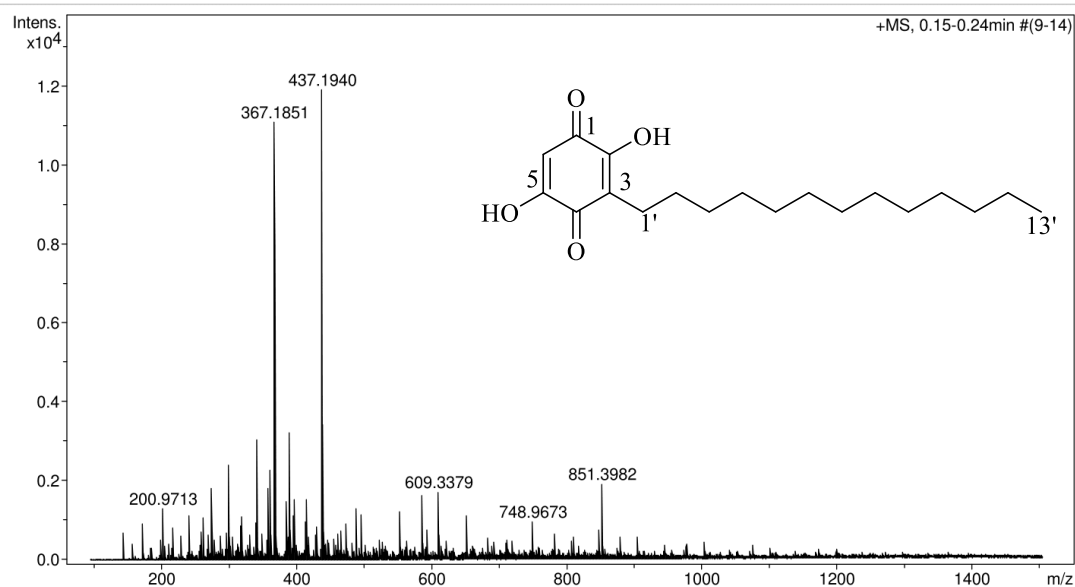
Figure A.1 HR-ESI-MS spectrum of **1** (pos)
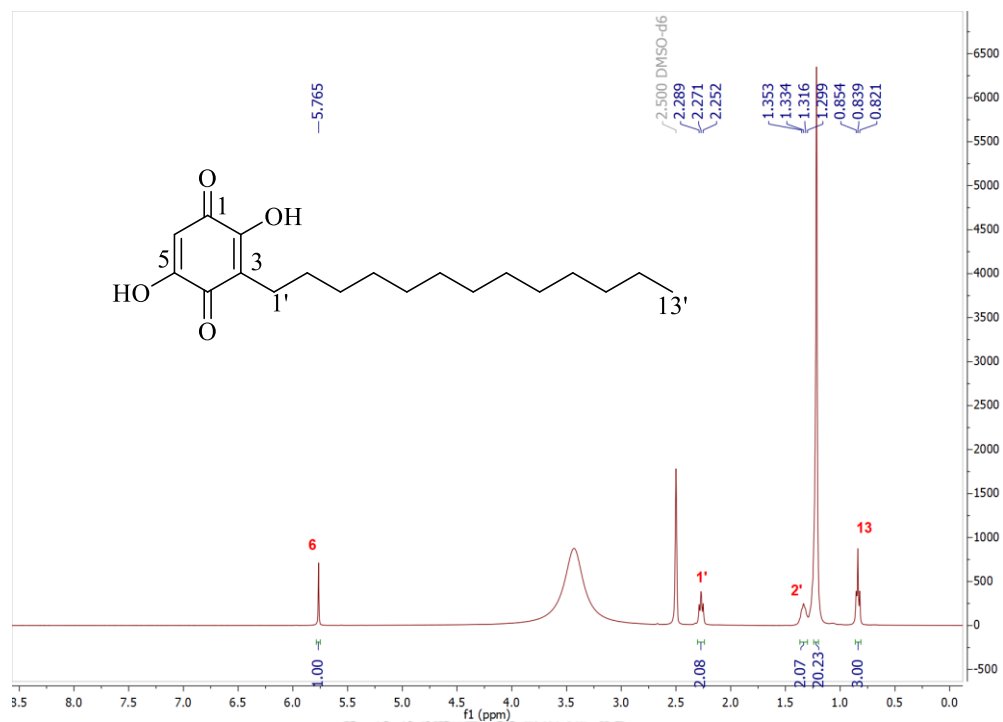
Figure A.2 The ^{1}H NMR (400 MHz) spectrum of **1** (DMSO-d_6)
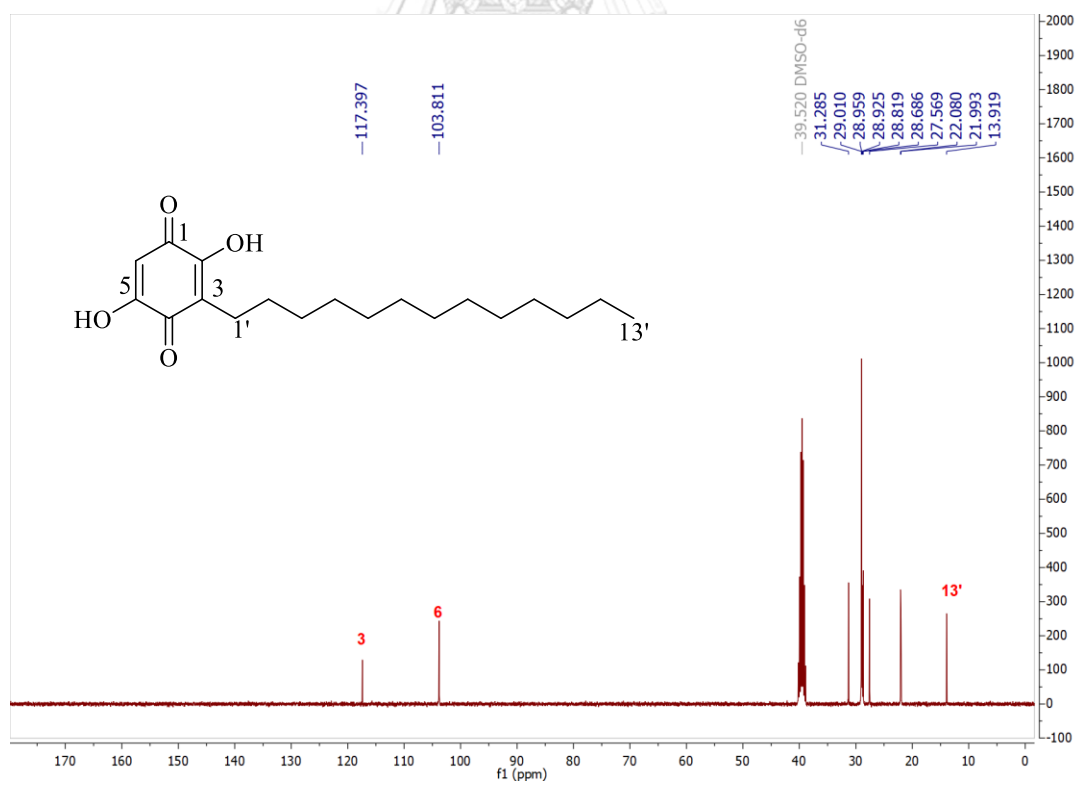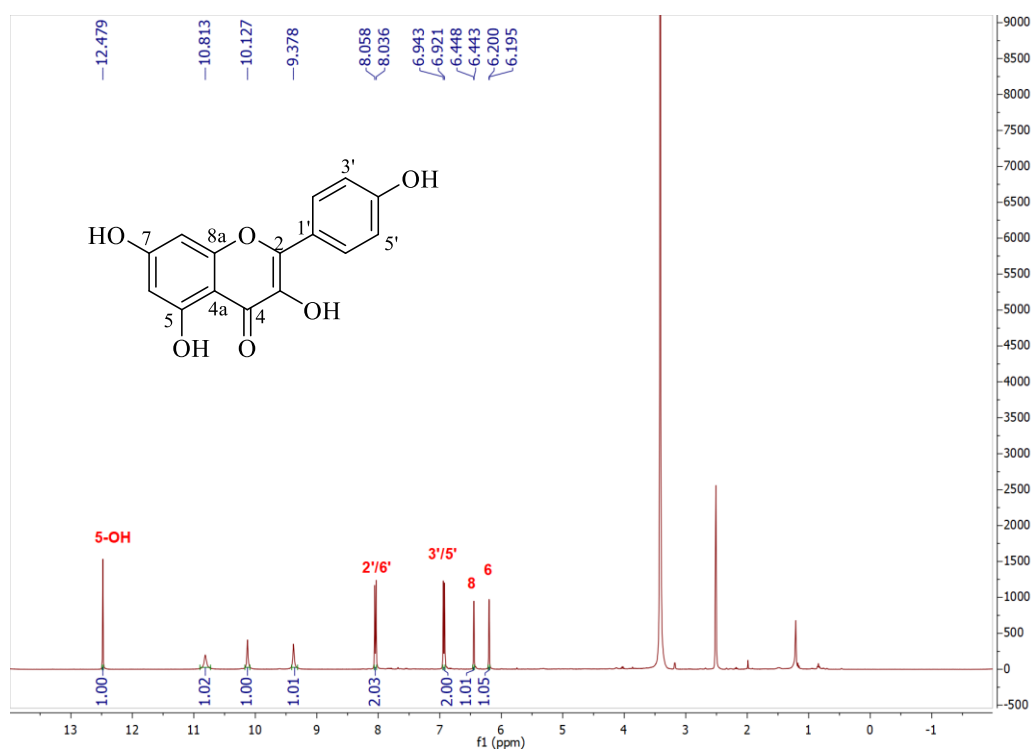

Figure A.3 The ^{13}C NMR (100 MHz) spectrum of **1** (DMSO-d_6)

Generic Display Report
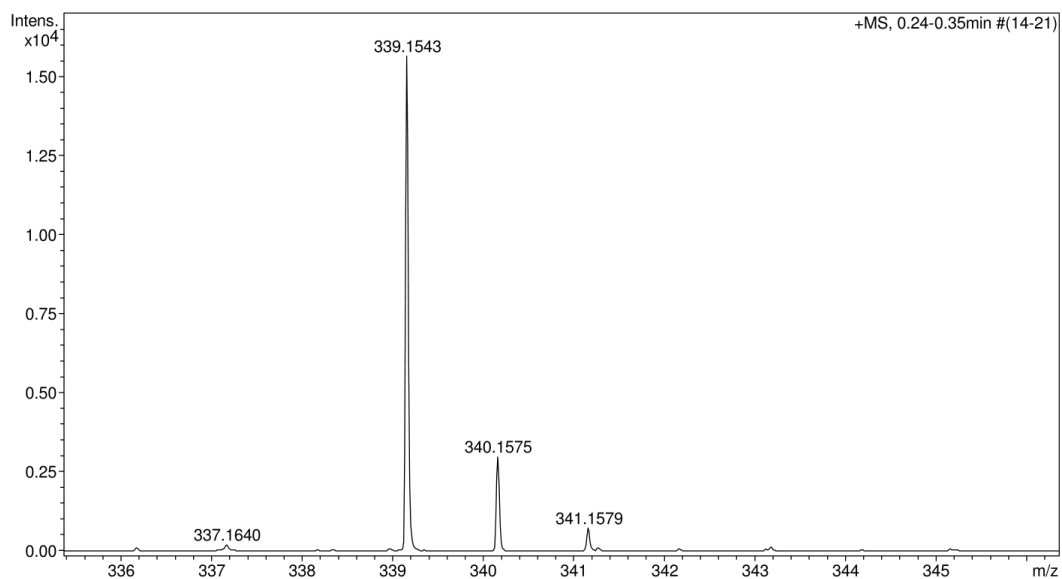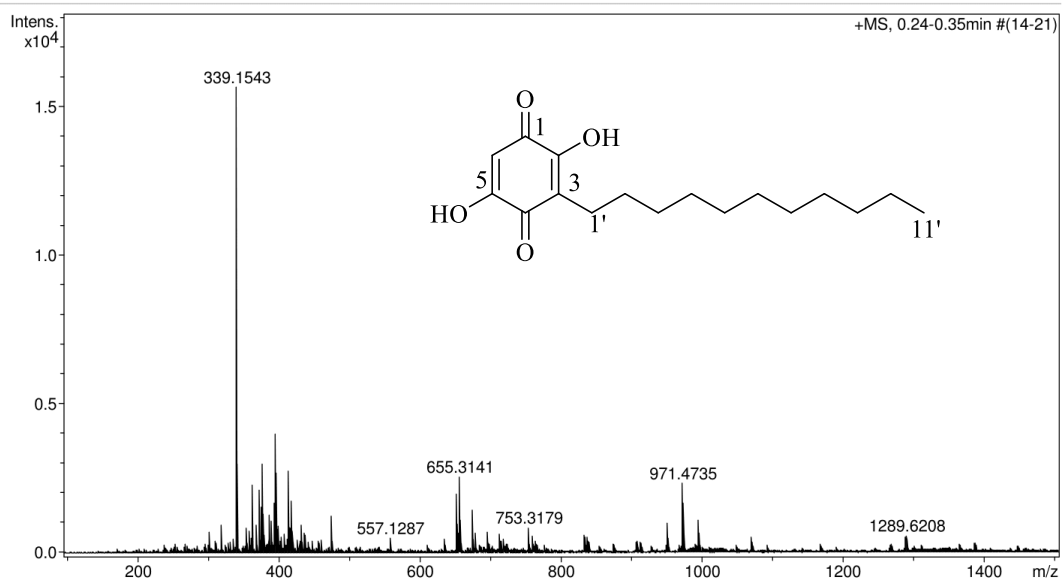
Figure A.4 HR-ESI-MS spectrum of **2** (pos)
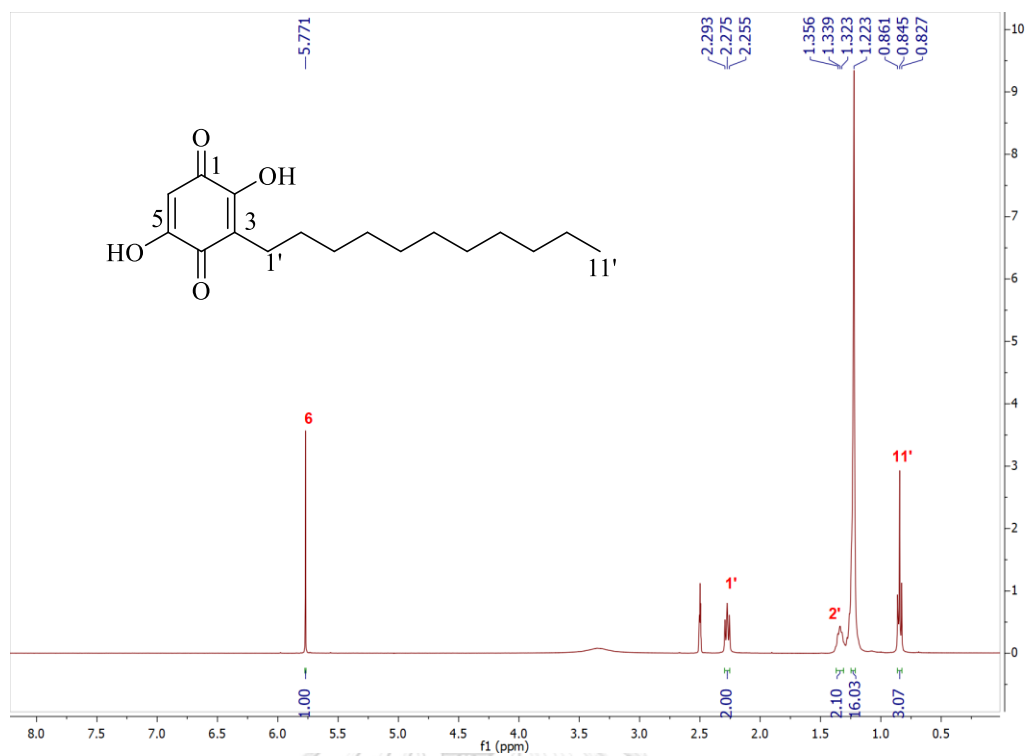
Figure A.5 The ^{1}H NMR (400 MHz) spectrum of **2** (DMSO-d_6)
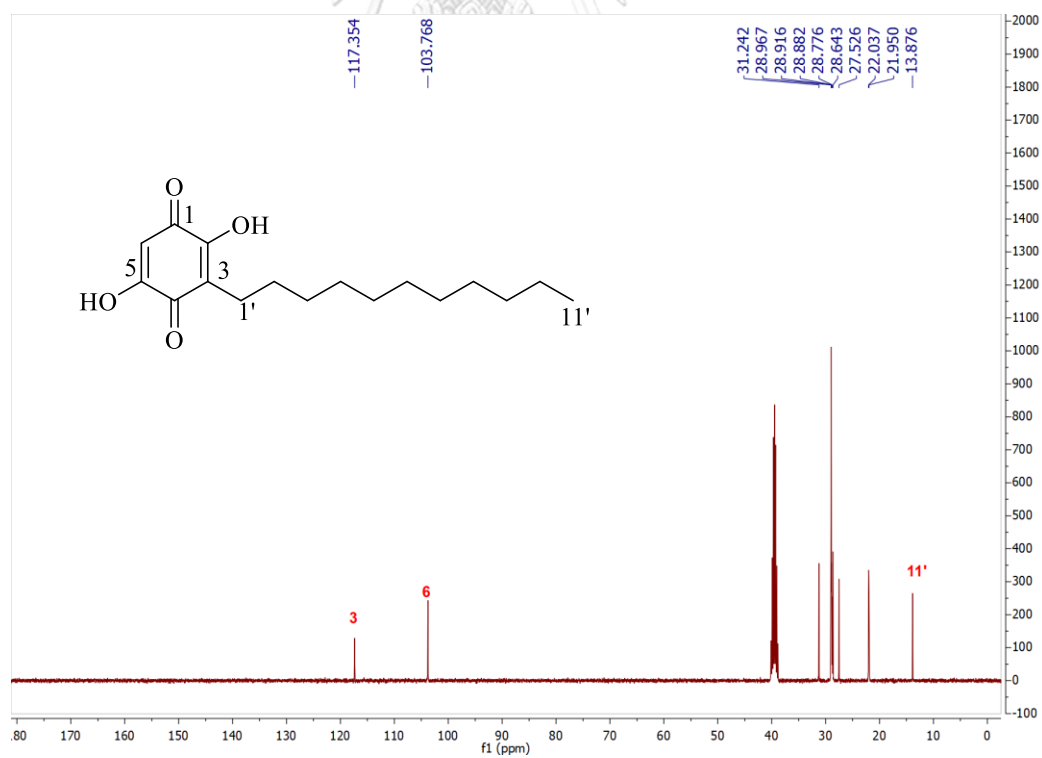
Figure A.6 The ^{1}H NMR (400 MHz) spectrum of **2** (DMSO-d_6)
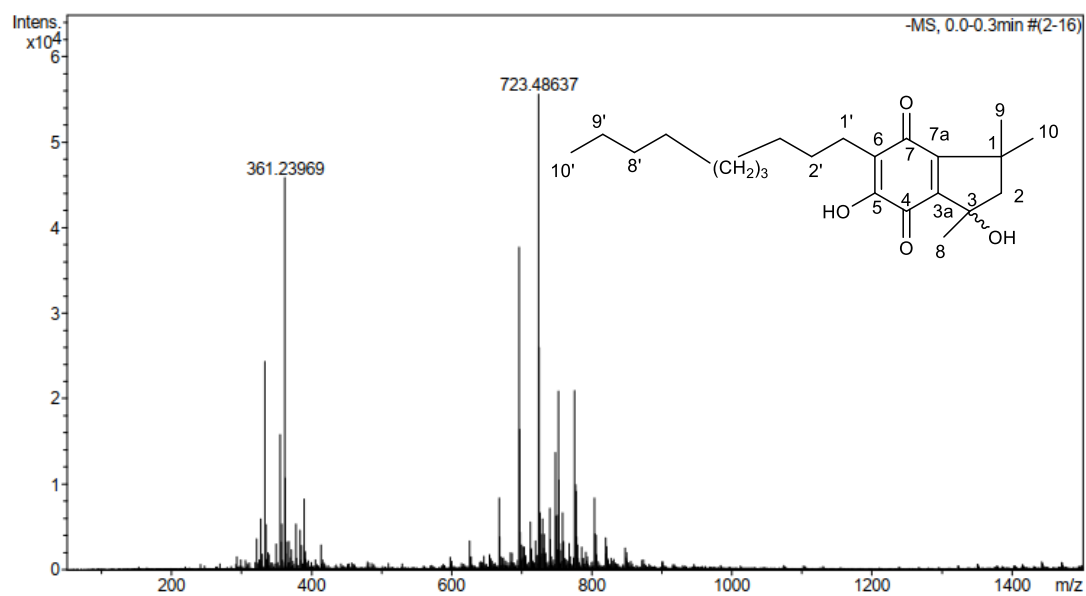
Figure A.7 HR-ESI-MS spectrum of **3** (neg)
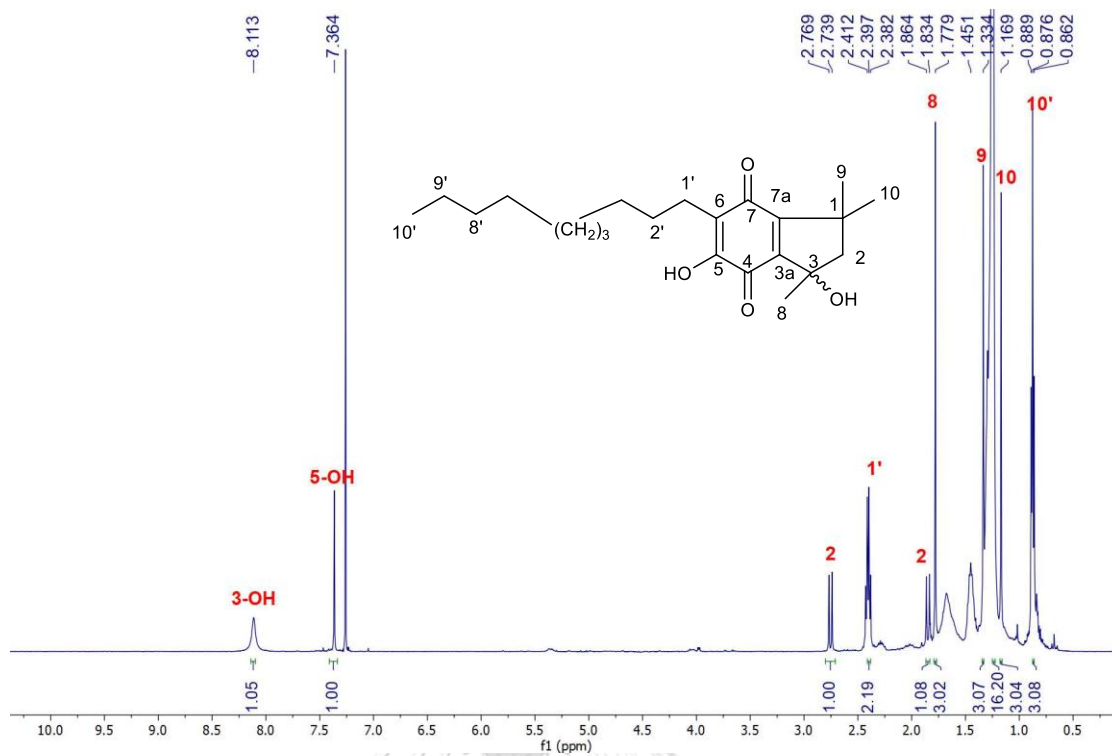
Figure A.8 The ^{1}H NMR (500 MHz) spectrum of **3** (CDCl$_3$)
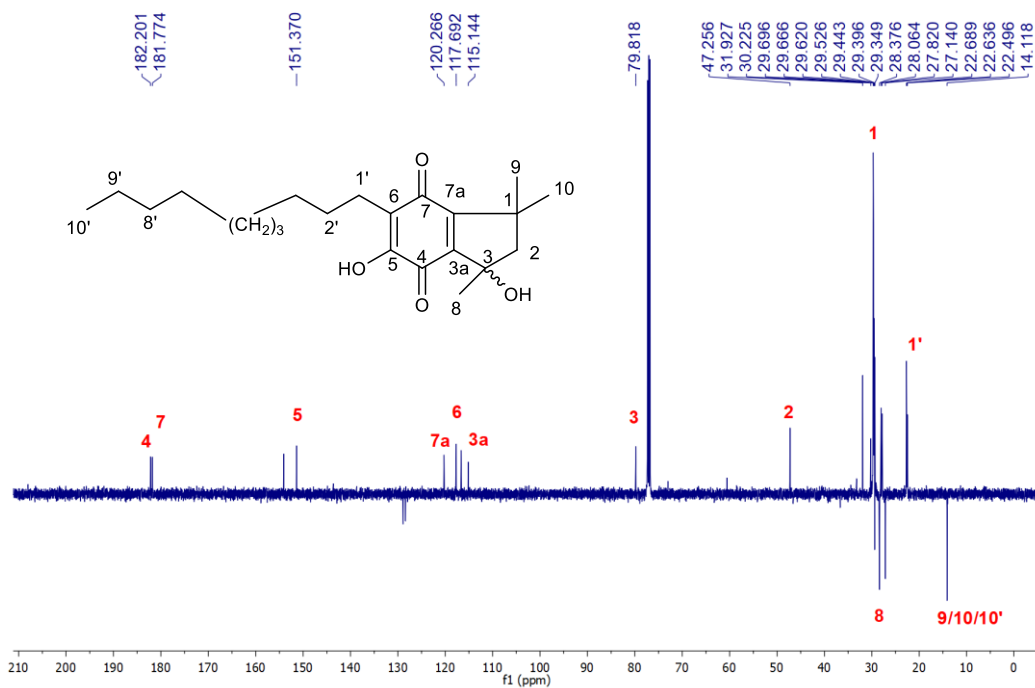
Figure A.9 The ^{13}C NMR (JMOD, 125 MHz) spectrum of **3** (CDCl$_3$)
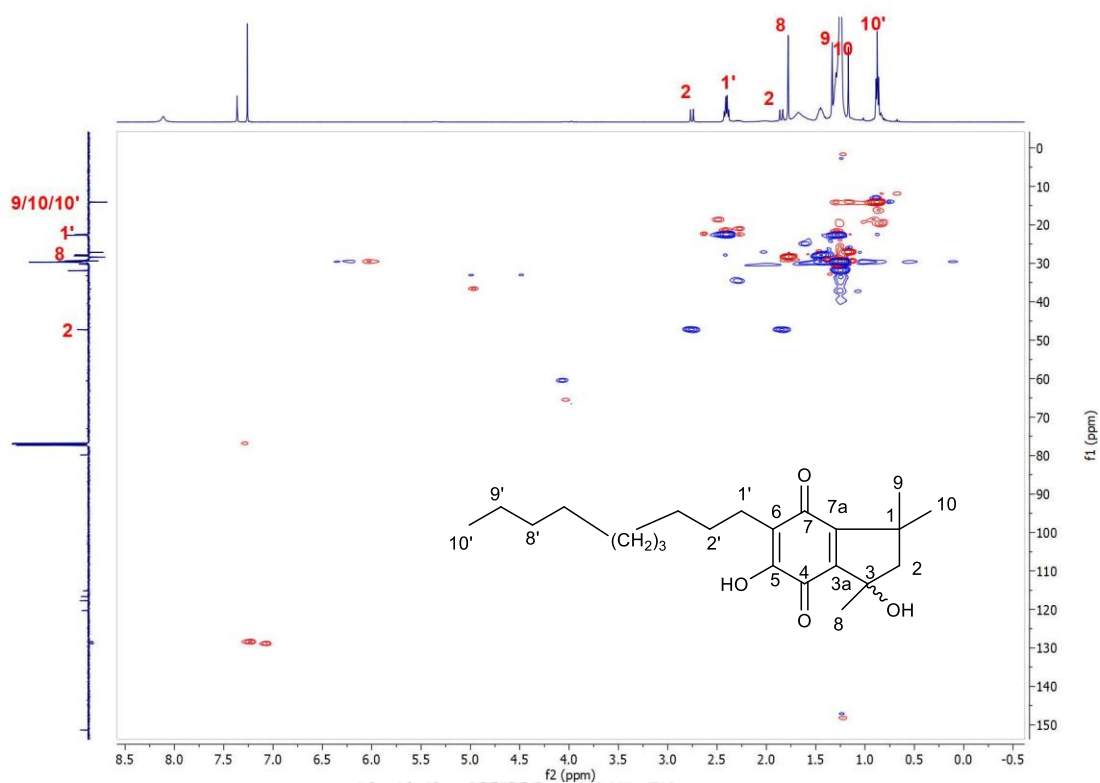
Figure A.10 The HSQC spectrum of **3** (CDCl$_3$)
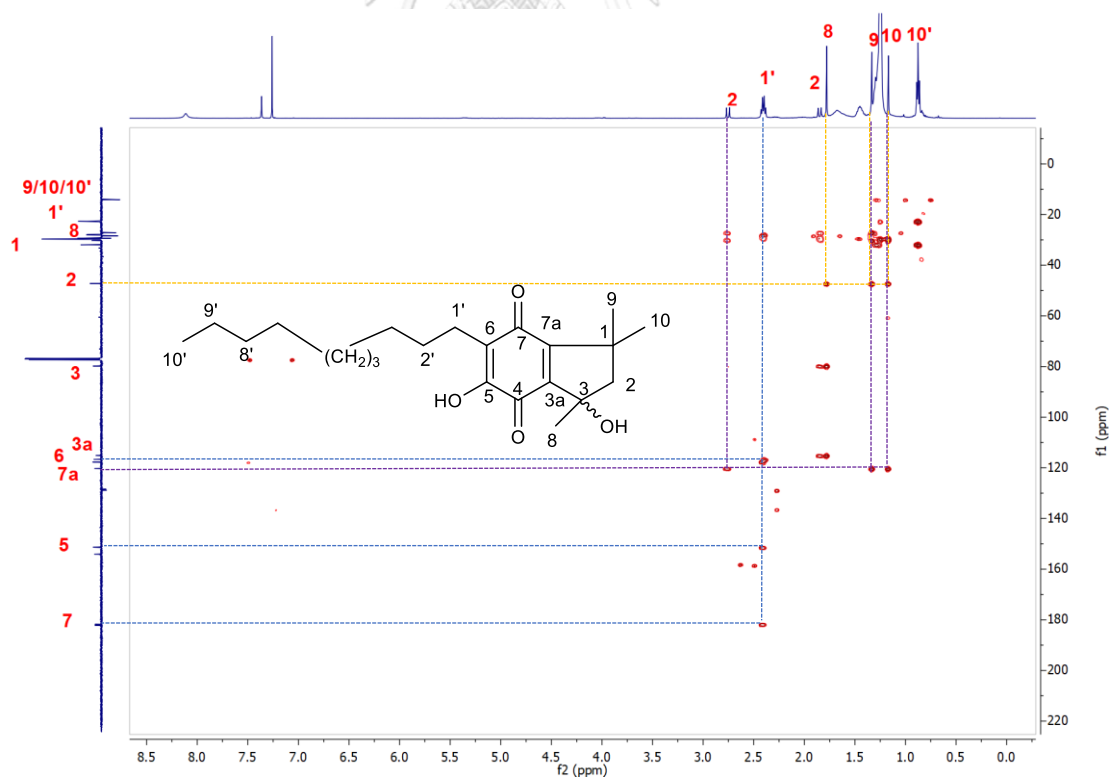
Figure A.11 The HMBC spectrum of **3** (CDCl$_3$)
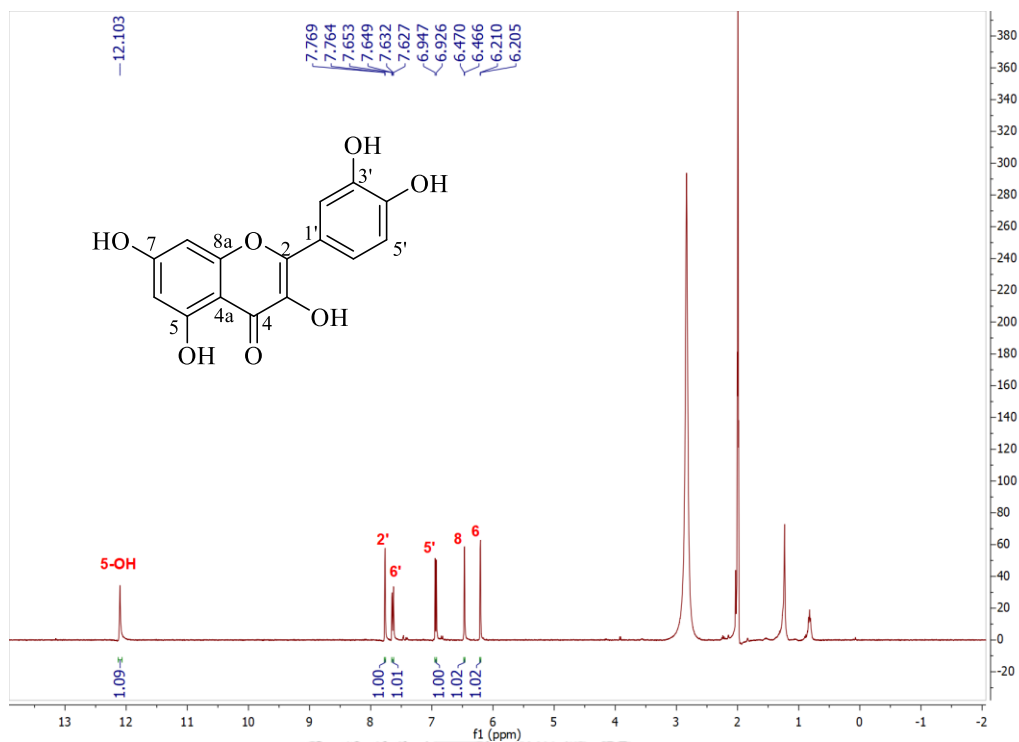
Figure A.12 The ^{1}H NMR (400 MHz) spectrum of **4** (acetone-d_6)
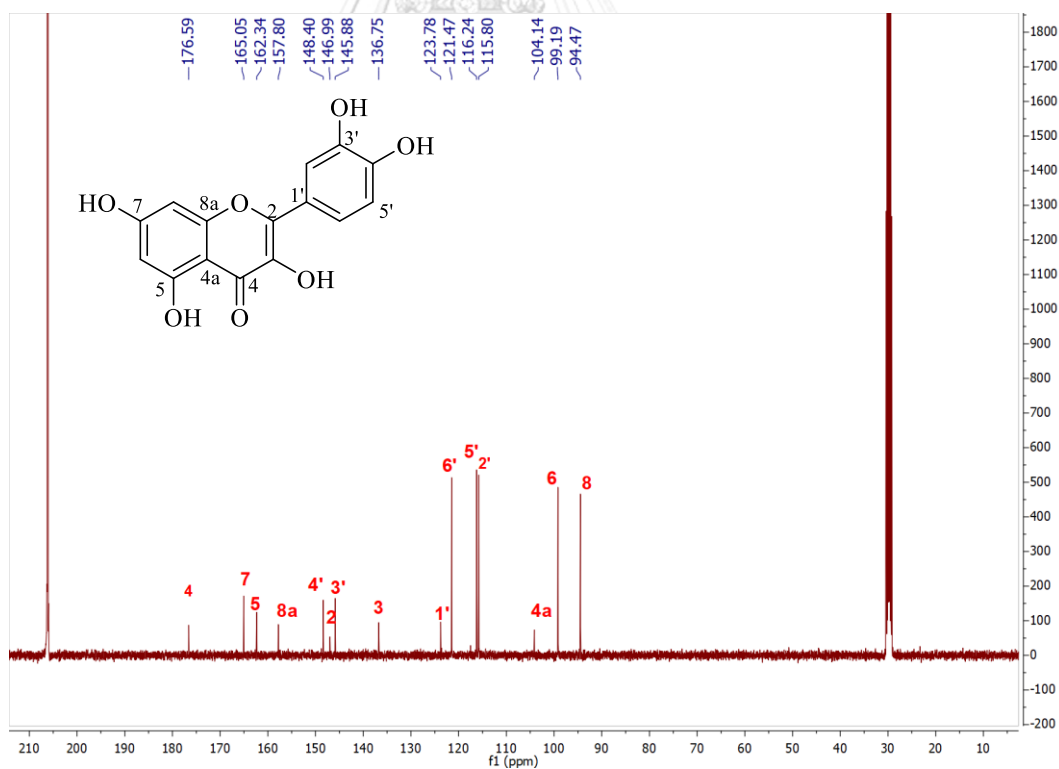
Figure A.13 The ^{13}C NMR (100 MHz) spectrum of **4** (acetone-d_6)

Figure A.14 The HSQC spectrum of **4** (acetone-d_6)

Figure A.15 The HMBC spectrum of **4** (acetone-d_6)
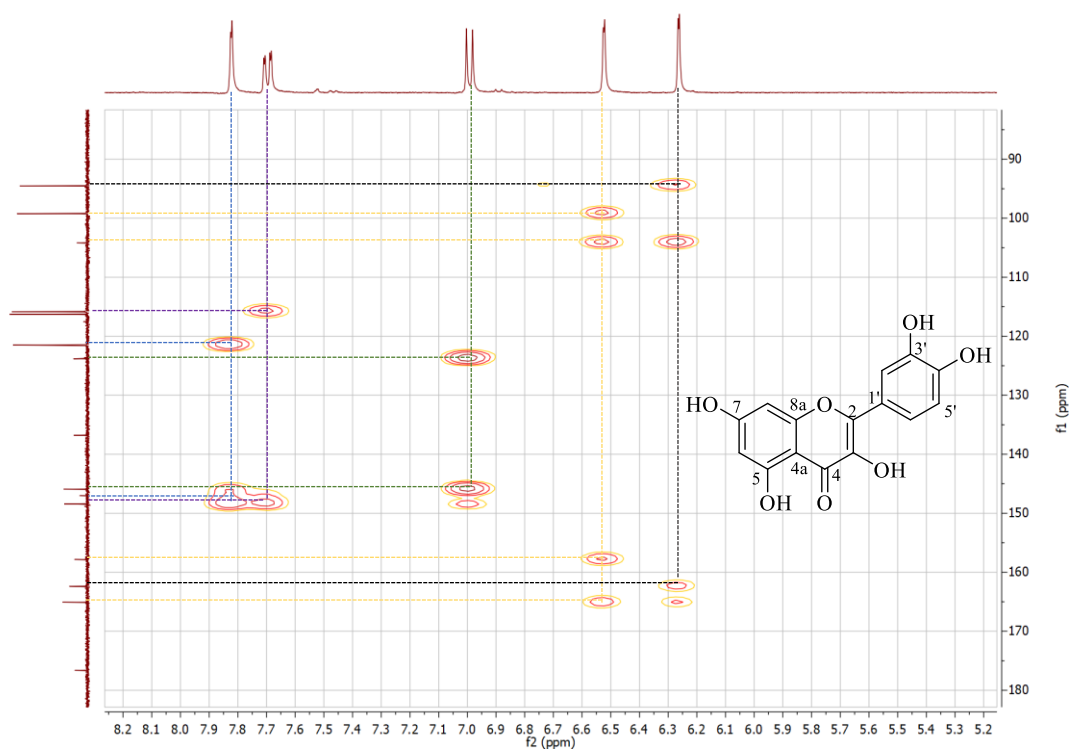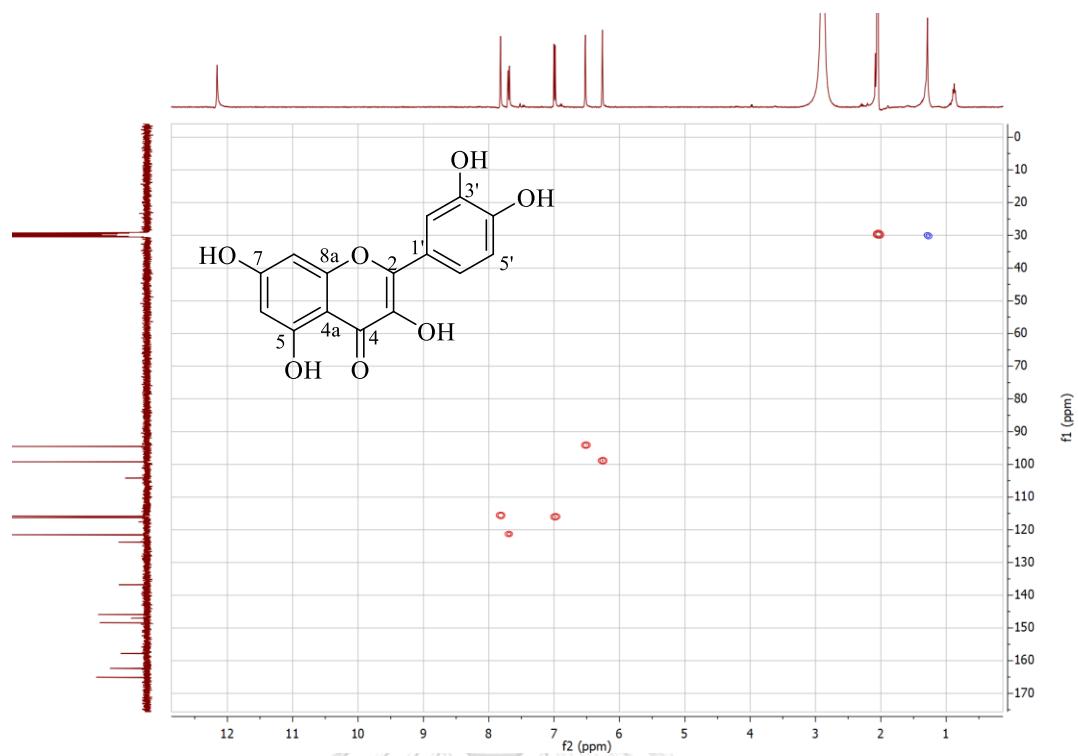
Figure A.16 The ^{1}H NMR (400 MHz) spectrum of **5** (DMSO-d_6)
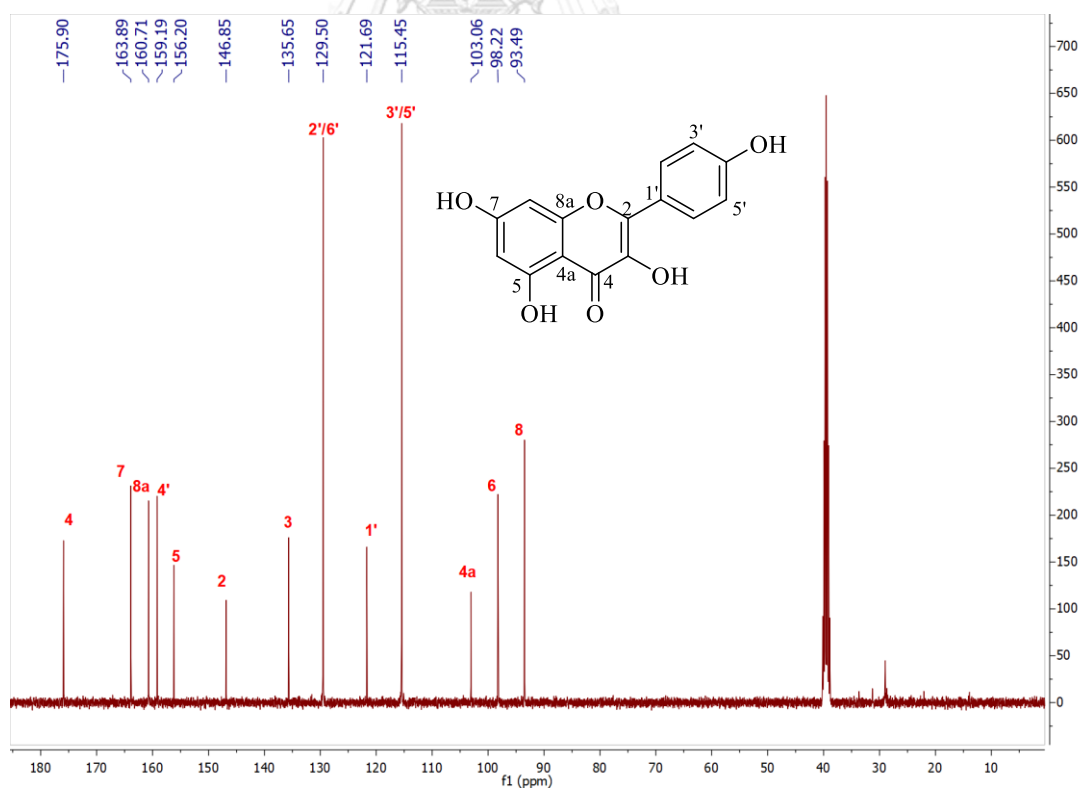
Figure A.17 The ^{13}C NMR (100 MHz) spectrum of **5** (DMSO-d_6)
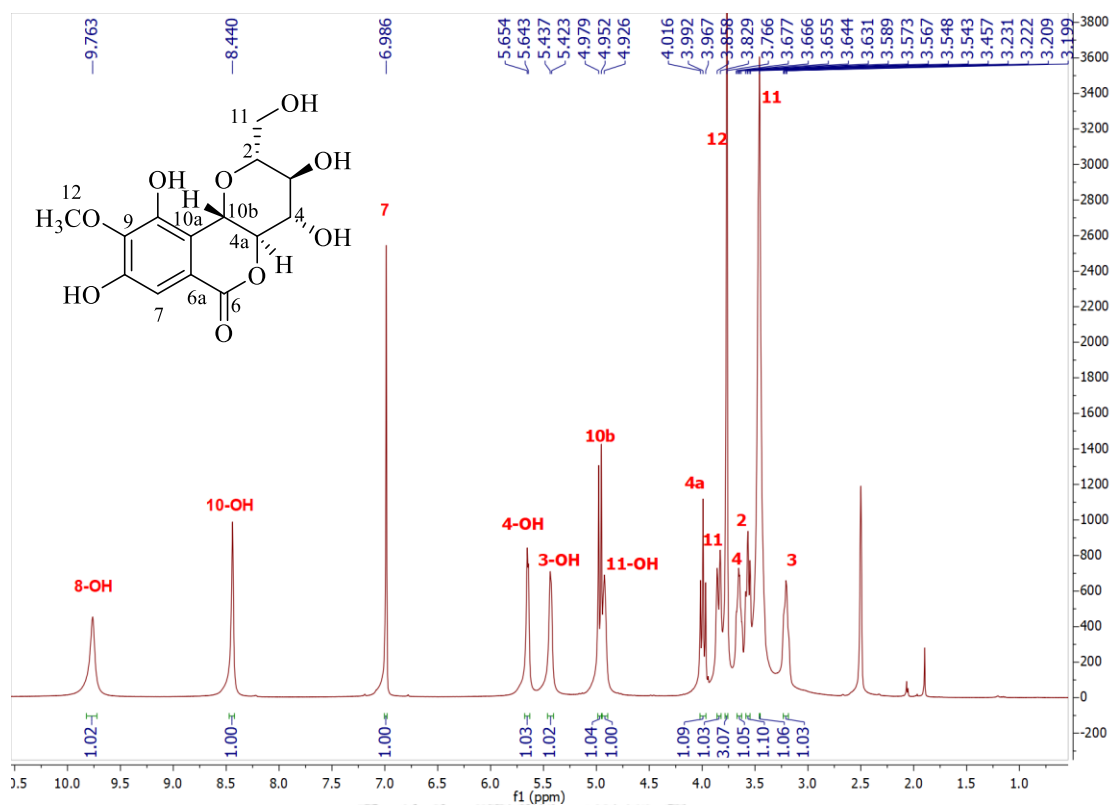
Figure A.18 The ^{1}H NMR (400 MHz) spectrum of **6** (DMSO-d_6)
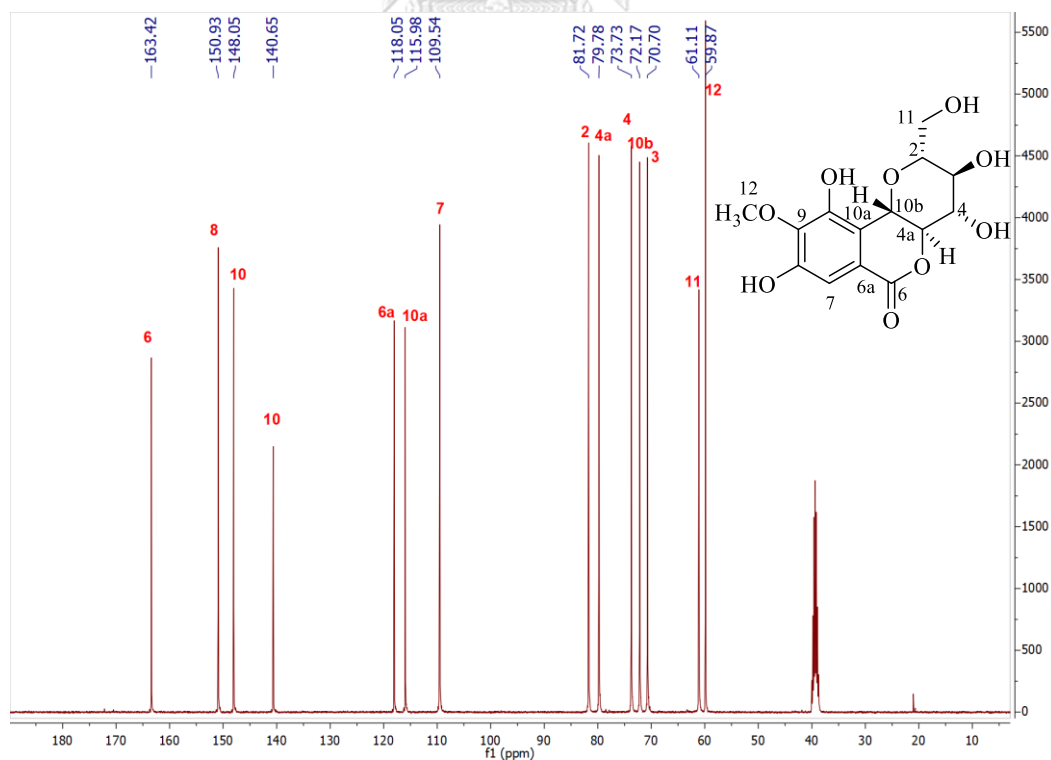
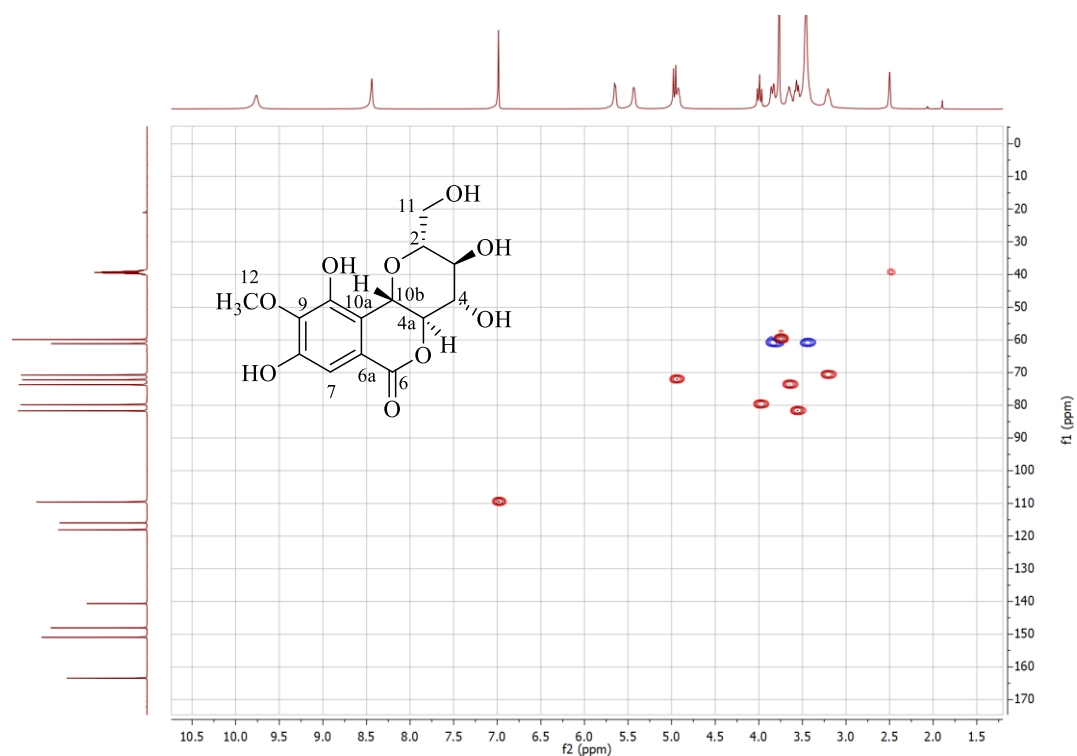**Figure A.19** The ^{13}C NMR (100 MHz) spectrum of **6** (DMSO-d_6)
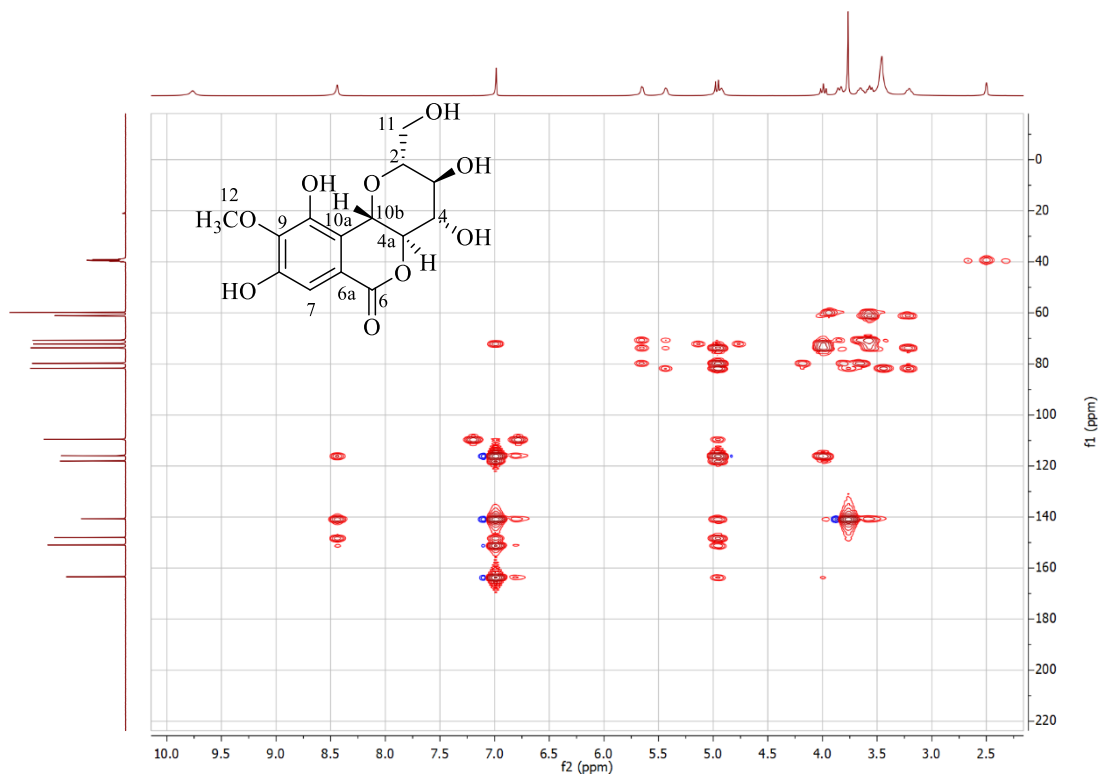
Figure A.20 The HSQC spectrum of **6** (DMSO-*d₆*)
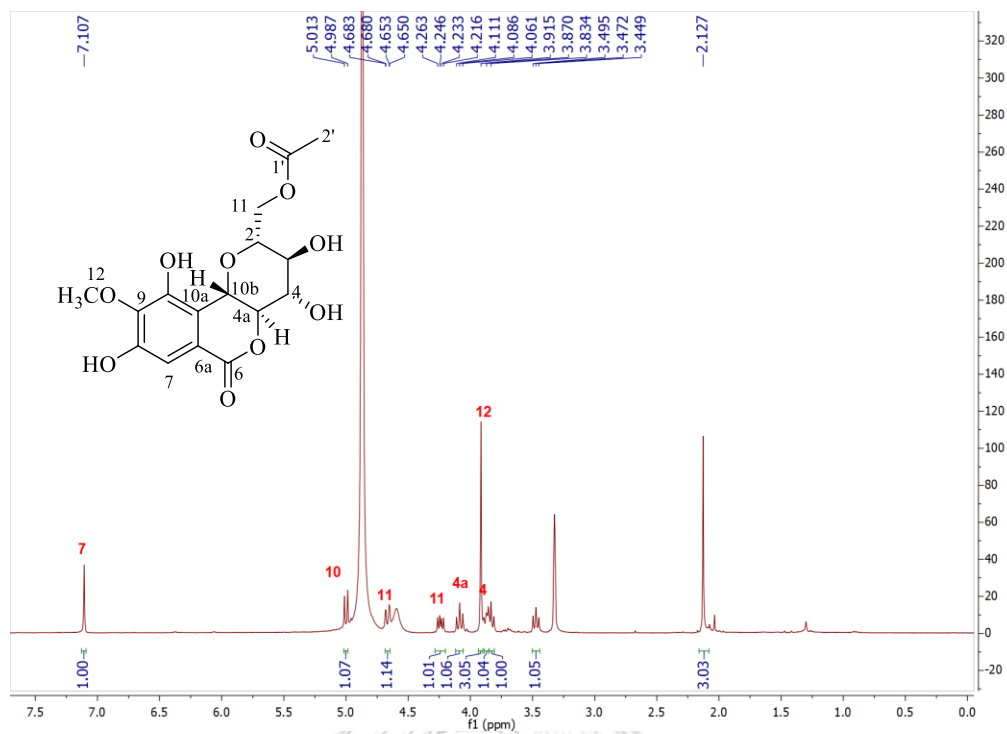

Figure A.21 The HMBC spectrum of **6** (DMSO-*d₆*)
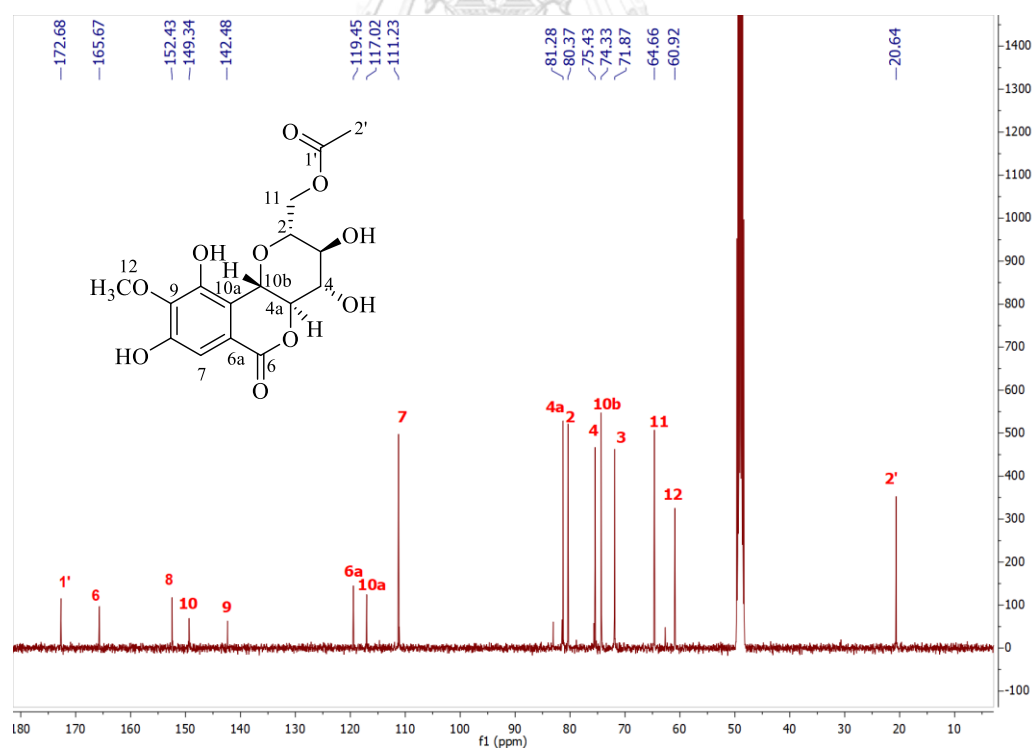
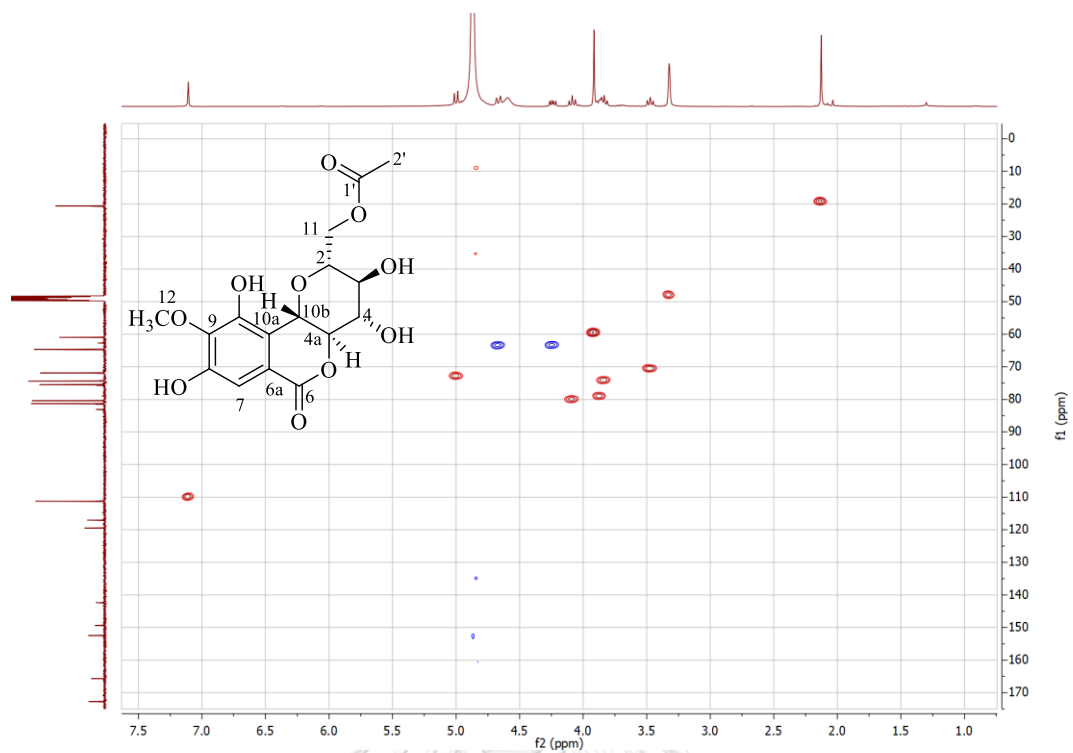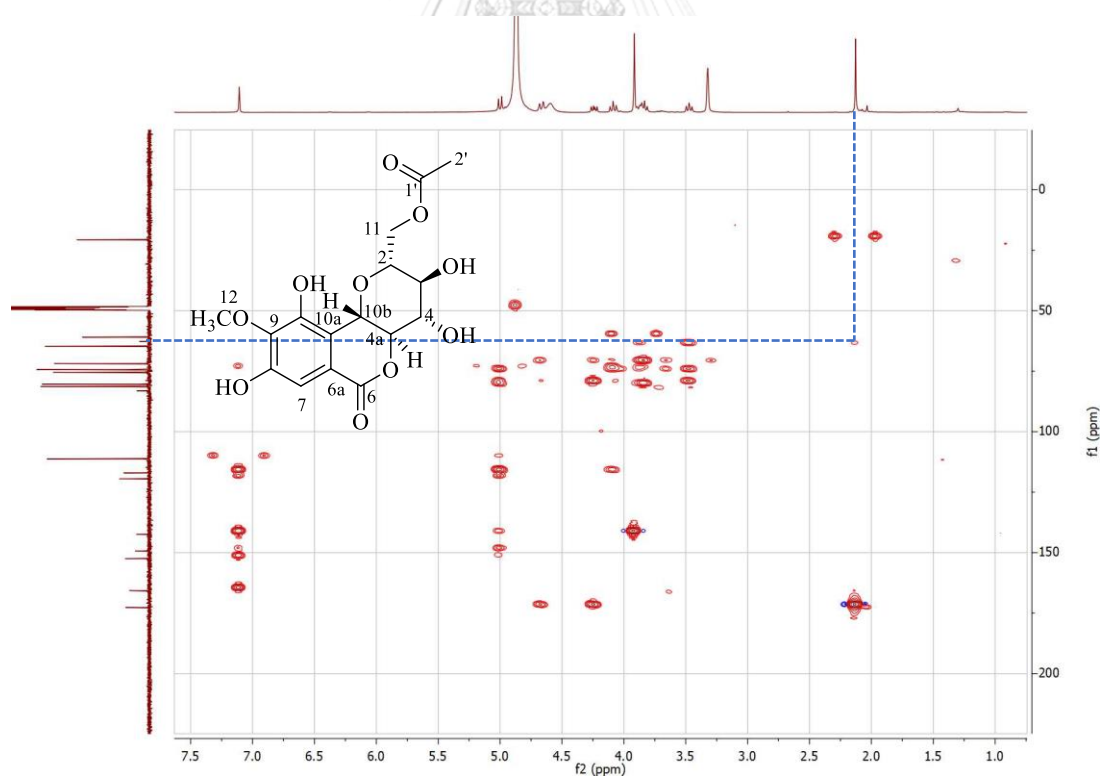**Figure A.22** The ^{1}H NMR (400 MHz) spectrum **7** (CD$_3$OD)

Figure A.23 The ^{13}C NMR (100 MHz) spectrum of **7** (CD$_3$OD)

Figure A.24 The HSQC spectrum of **7** (CD₃OD)

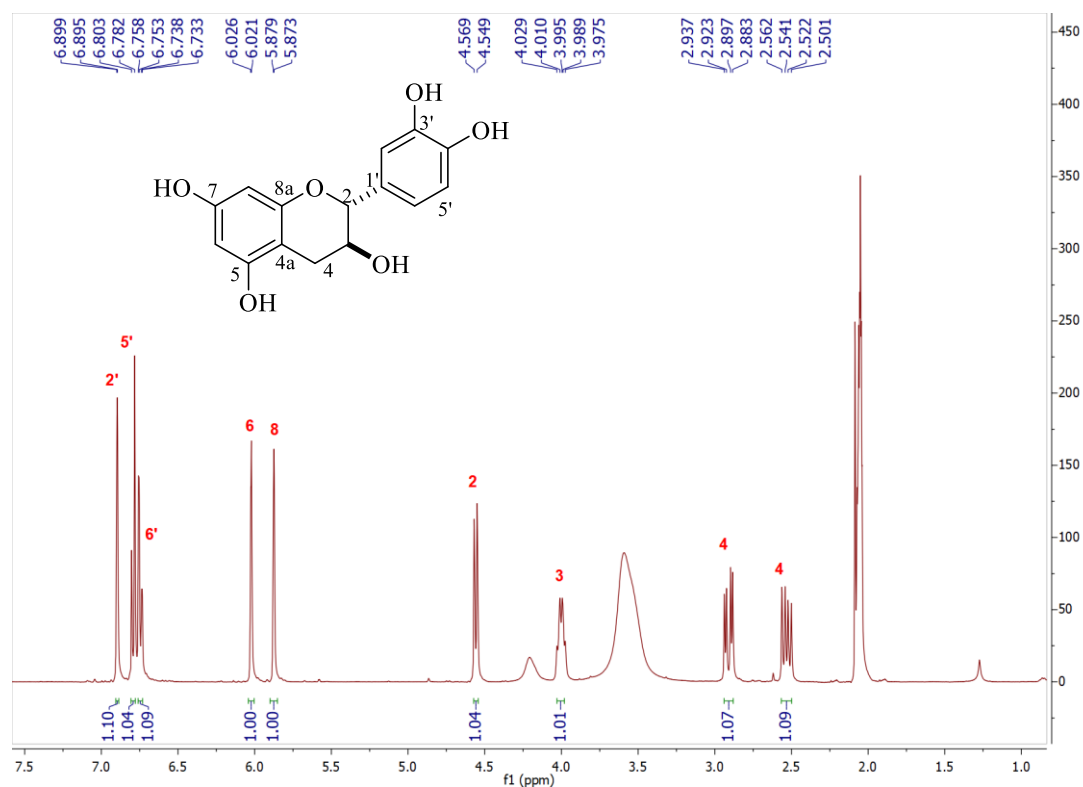**Figure A.25** The HMBC spectrum of **7** (CD₃OD)

Figure A.26 The ^{1}H NMR (400 MHz) spectrum of **8** (acetone-d_6)
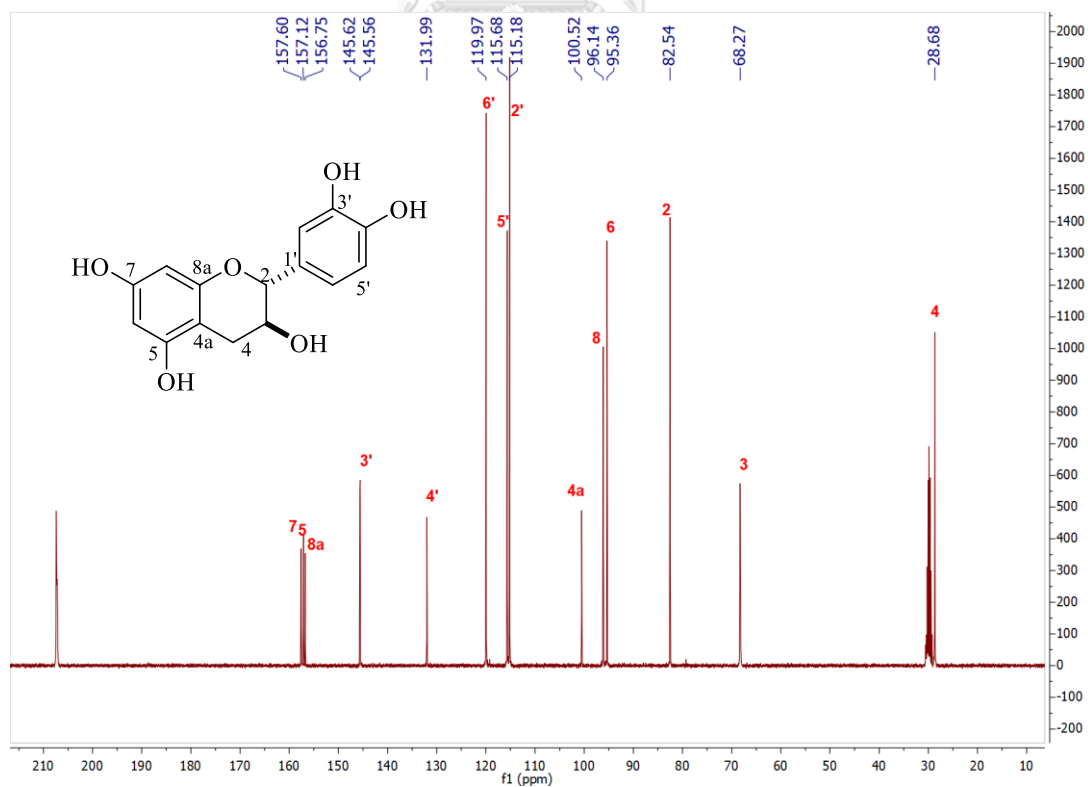
Figure A.27 The ^{13}C NMR (100 MHz) spectrum of **8** (acetone-d_6)
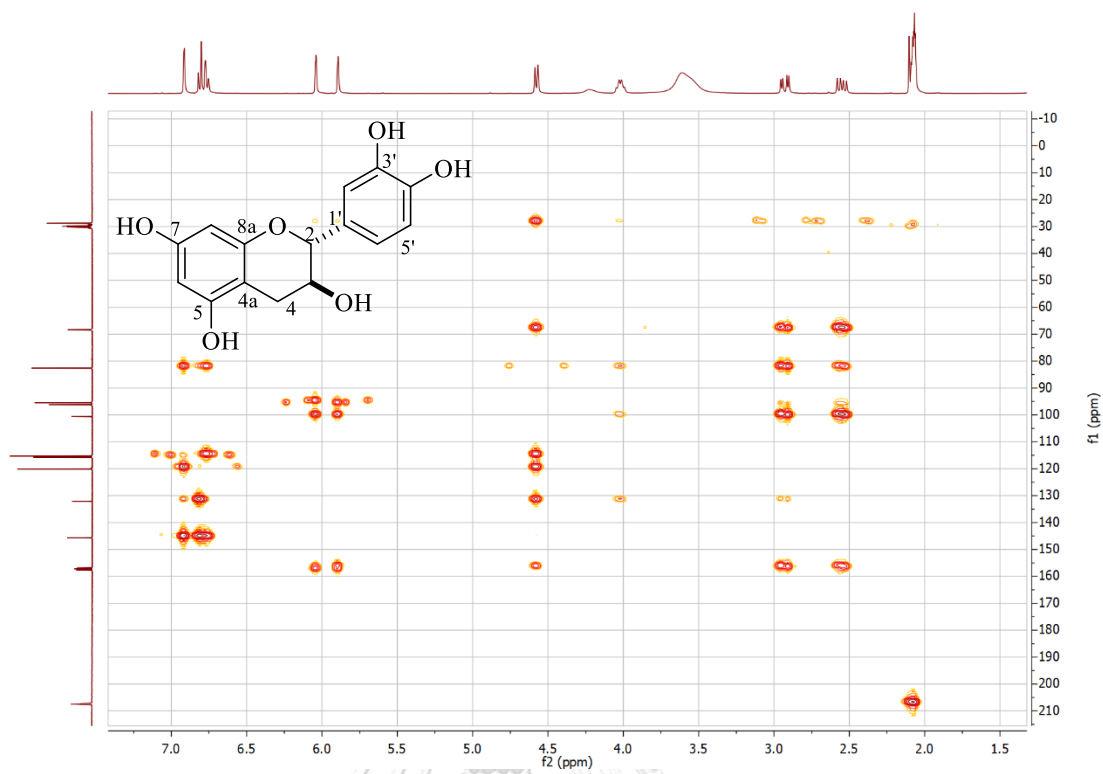
Figure A.28 The HMBC spectrum of **8** (acetone-*d₆*)
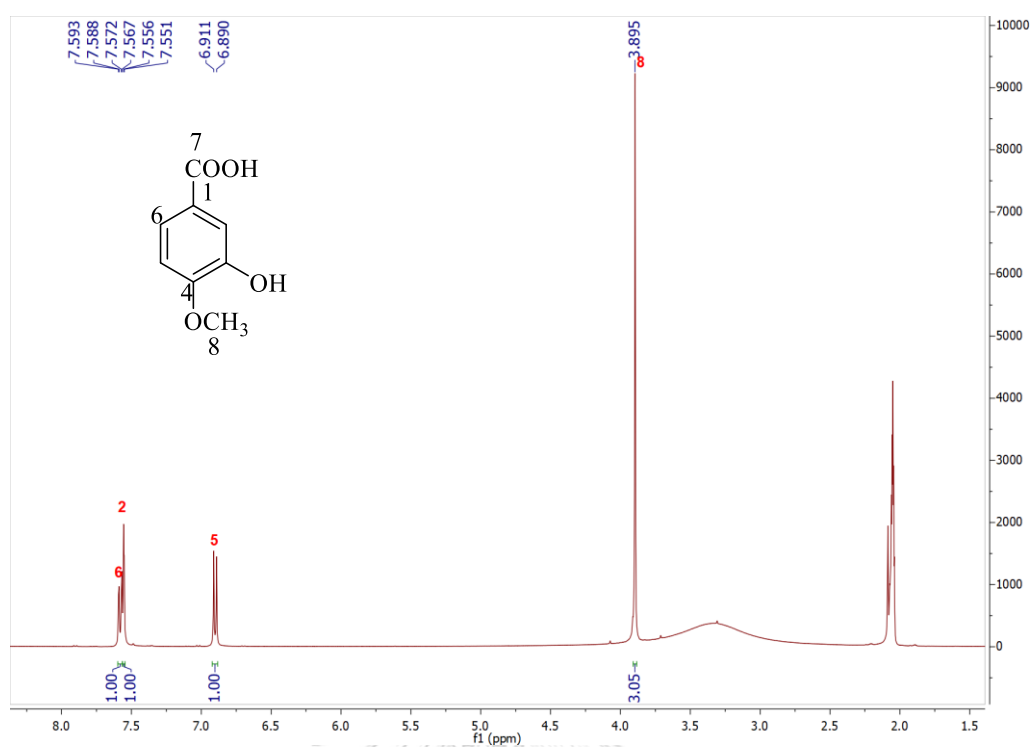
Figure A.29 The ^{1}H NMR (400 MHz) spectrum of **9** (acetone-d_6)
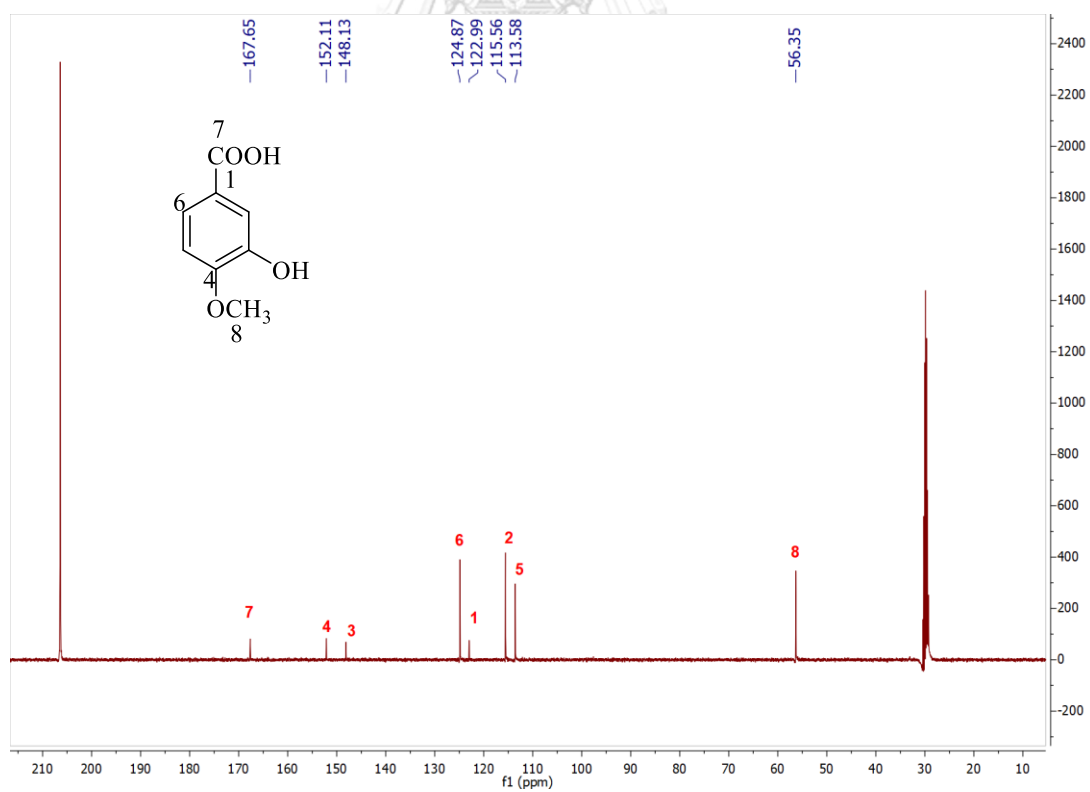
Figure A.30 The ^{13}C NMR (100 MHz) spectrum of **9** (acetone-d_6)

Figure A.31 The ^{1}H NMR (400 MHz) spectrum of **10** (acetone-d_6)

Figure A.32 The ^{13}C NMR (100 MHz) spectrum of **10** (acetone-d_6)

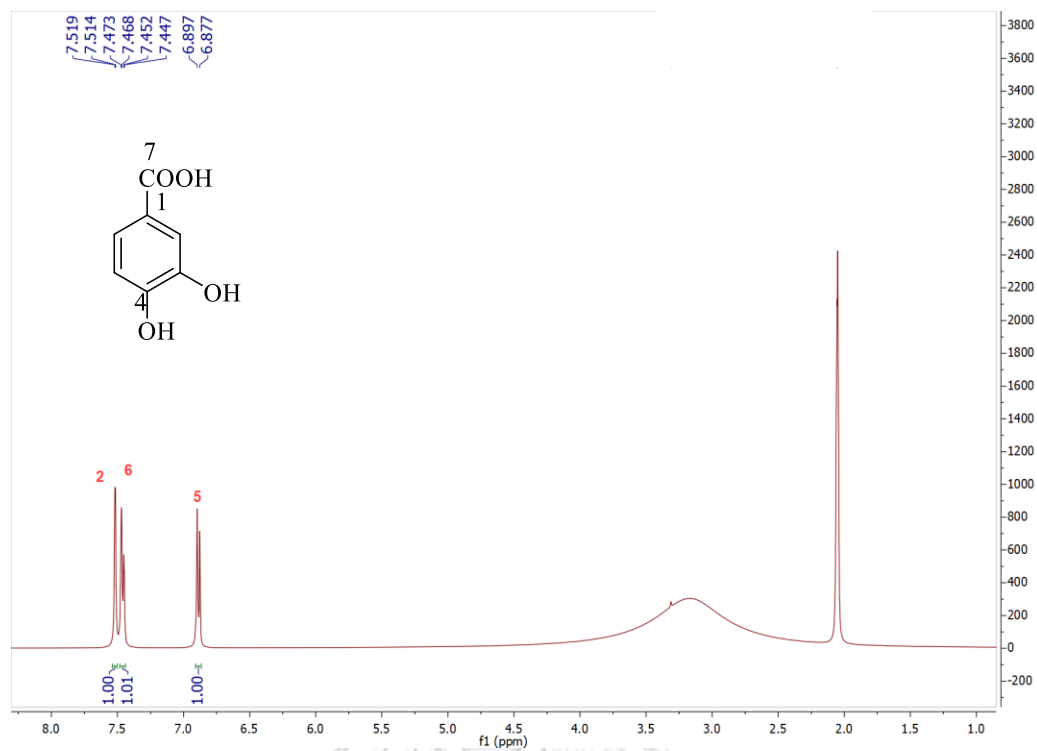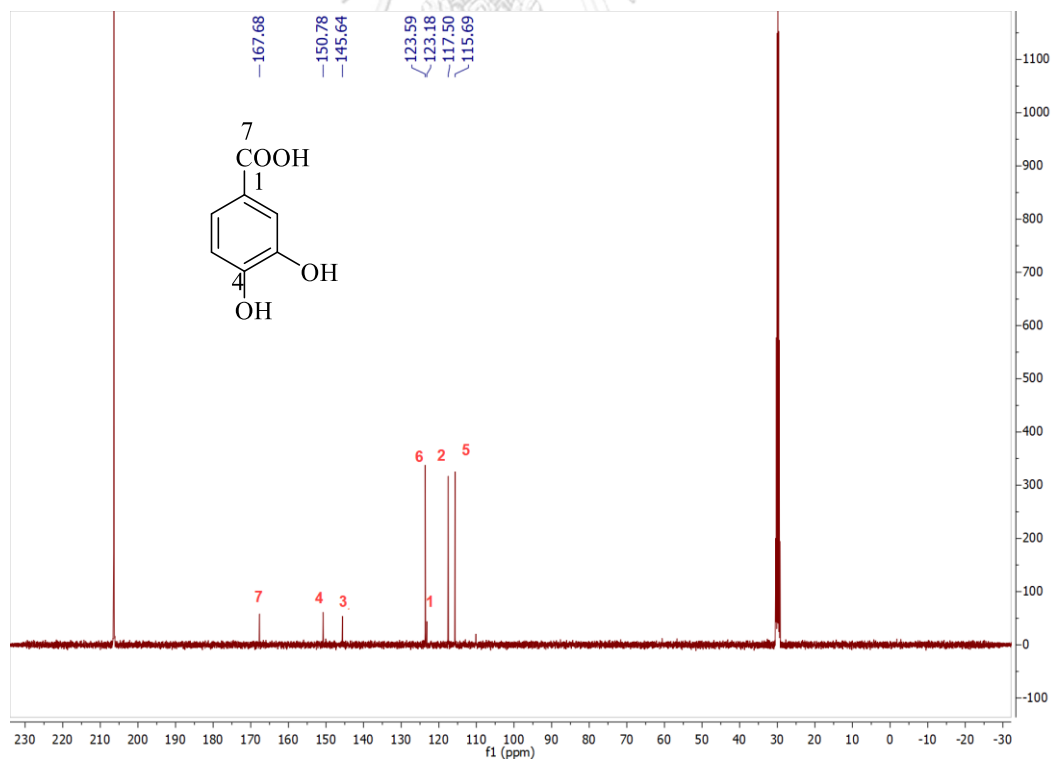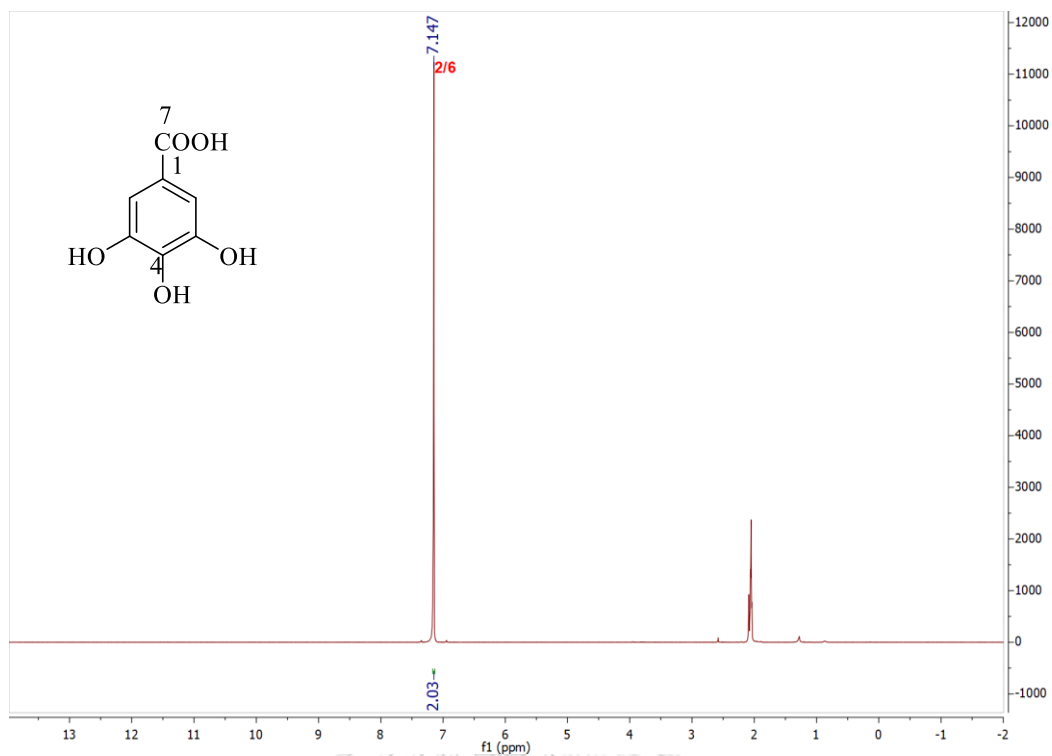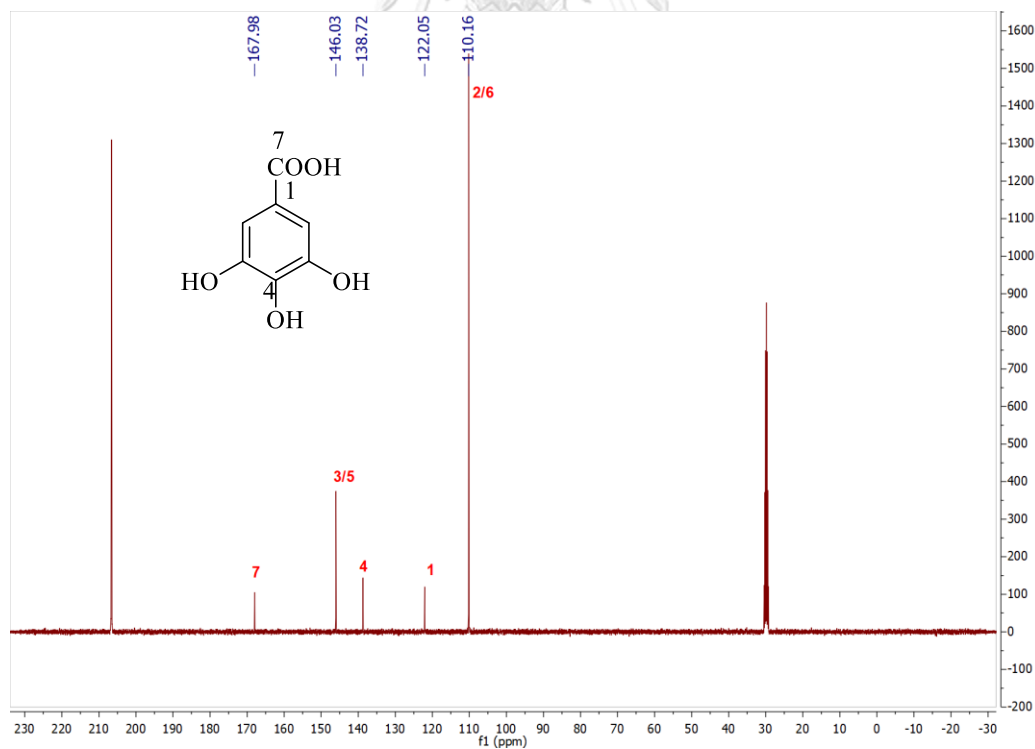**Figure A.33** The ^{1}H NMR (400 MHz) spectrum of **11** (acetone-d_6)

Figure A.34 The ^{13}C NMR (100 MHz) spectrum of **11** (acetone-d_6)

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