การสังเคราะห์และฤทธิ์ทางชีวภาพของอนุพันธ์กรดแอนาคาร์ดิกและคาร์ดานอลจาก
ของเหลวที่ได้จากเปลือกผลมะม่วงหิมพานต์

นางสาวกุลวดี แต้มสำเภาเลิศ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF ANACARDIC ACID AND CARDANOL DERIVATIVES FROM CASHEW NUT SHELL LIQUID

Miss Kulwadee Tamsampaoloet

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
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Thesis Title	SYNTHESIS AND BIOLOGICAL ACTIVITIES OF ANACARDIC ACID AND CARDANOL DERIVATIVES FROM CASHEW NUT SHELL LIQUID
By	Miss Kulwadee Tamsampaoloet
Field of Study	Chemistry
Thesis Advisor	Assistant Professor Warinthorn Chavasiri, Ph.D.

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กุลวดี แต้มสำเภาเลิศ : การสังเคราะห์และฤทธิ์ทางชีวภาพของอนุพันธ์กรดแอนาคาร์ดิกและคาร์ดานอลจากของเหลวที่ได้จากเปลือกผลมะม่วงหิมพานต์ (SYNTHESIS AND BIOLOGICAL ACTIVITIES OF ANACARDIC ACID AND CARDANOL DERIVATIVES FROM CASHEW NUT SHELL LIQUID) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.วรินทร ชวศิริ, 113 หน้า.

ของเหลวที่ได้จากเปลือกผลมะม่วงหิมพานต์ (ซีเอ็นเอสแอล) ประกอบด้วยกรดแอนาคาร์ดิก 1 (สารหลัก) คาร์ดานอล 2 และคาร์ดอล 3 การแยกของผสมเหล่านี้ด้วยเซมิเพร์พเอชพีแอลซี ได้สารเก้าตัวซึ่งได้นำไปทดสอบฤทธิ์ต้านการอักเสบและฤทธิ์ต้านอนุมูลอิสระ ทั้งกรดแอนาคาร์ดิก 1 และคาร์ดานอล 2 ไม่เป็นพิษต่อ RAW 264.7 cells ในขณะที่คาร์ดอลเป็นพิษที่ความเข้มข้น 50 ไมโครโมลาร์ กรดแอนาคาร์ดิก $C_{15:3}$ 7 ที่ความเข้มข้น 100 ไมโครโมลาร์ สามารถลดการสร้างไนตริกออกไซด์ได้สูงสุดถึง 71% สำหรับฤทธิ์ต้านการเกิดอนุมูลอิสระพบว่ากรดแอนาคาร์ดิก 5-7 และคาร์ดอล 14-15 และสารประกอบอิ่มตัว 4 และ 12 สามารถจับซุปเปอร์ออกไซด์แอนไอออนจาก PMS-NADH พบว่าฤทธิ์ต้านอนุมูลอิสระของกรดแอนาคาร์ดิกขึ้นอยู่กับจำนวนพันธะคู่บนสายโซ่ กรดแอนาคาร์ดิก 1 และคาร์ดอล 3 เป็นสารที่มีฤทธิ์ฆ่าแบคทีเรีย *Propionibacterium acnes* KCCM 41747 และเป็นสารที่มีผลยับยั้งแบคทีเรีย *Streptococcus mutans* ATCC 25175, *Streptococcus sobrinus* KCCM 11898, *Staphylococcus aureus* ATCC 25923 และ *Salmonella typhi* ATCC 422 ได้สังเคราะห์อนุพันธ์ของกรดแอนาคาร์ดิก 20 ตัวและของคาร์ดานอล 12 ตัว สารที่สังเคราะห์ได้พบว่าเป็นสารใหม่ 25 ตัว อนุพันธ์เอสเทอร์และอีเทอร์ของกรดแอนาคาร์ดิก 35-42 ก็สามารถจับซุปเปอร์ออกไซด์แอนไอออนที่สร้างจาก PMS-NADH โดยเฉพาะอย่างยิ่งเมื่อหมู่แทนที่ของกรดแอนาคาร์ดิกที่มีคาร์บอนอะตอมแปดอะตอม เอสเทอร์ 37 และอีเทอร์ 42 มีฤทธิ์ที่ดี นอกจากนี้พบว่า อนุพันธ์กรดแอนาคาร์ดิก 32, 35, 39-40 มีฤทธิ์ต้านแบคทีเรียที่ดีถึงดีเยี่ยม และอนุพันธ์ไทโอเซมิคาร์บาไซด์ของคาร์ดานอล 53 แสดงฤทธิ์ต้านแบคทีเรีย *S. sobrinus* ได้ดีมาก

ภาควิชา เคมี ลายมือชื่อนิสิต .....

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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KULWADEE TAMSAMPAOLOET: SYNTHESIS AND BIOLOGICAL ACTIVITIES OF ANACARDIC ACID AND CARDANOL DERIVATIVES FROM CASHEW NUT SHELL LIQUID. ADVISOR: ASST. PROF. WARINTHORN CHAVASIRI, Ph.D., 113 pp.

Cashew nut shell liquid (CNSL) contains three mixtures of anacardic acid 1 (major), and cardanol 2 and cardol 3. The separation of these mixtures by semi-prep HPLC furnished nine compounds which were subsequently subjected to anti-inflammatory and antioxidant activity tests. Both anacardic acid 1 and cardanol 2 displayed no cytotoxicity, while cardol 3 revealed cytotoxicity at 50 μM on Raw 264.7 cells. Anacardic acid $C_{15:3}$ 7 exhibited the highest reducing NO production by 71% at 100 μM. For antioxidant assay, anacardic acid 5-7 and cardol 14-15, and their hydrogenated compounds, 4 and 12 could scavenge superoxide anion radicals by PMS-NADH. The antioxidant activity of anacardic acid was found to depend on the number of double bond on side chain. Anacardic acid 1 and cardol 3 were bactericidal agents for *Propionibacterium acnes* KCCM 41747 and bacteriostatic agents for *Streptococcus mutans* ATCC 25175, *Streptococcus sobrinus* KCCM 11898, *Staphylococcus aureus* ATCC 25923 and *Salmonella typhi* ATCC 422. Twenty anacardic acid and twelve cardanol derivatives were synthesized. Among them, twenty-five compounds have not been reported. The ester and ether derivatives of anacardic acid 35-42 could also display scavenging superoxide anion radicals by PMS-NADH, particularly anacardic acids ester 37 and ether 42 with eight carbon atoms. Additionally, anacardic acid 32, 35, 39-40 displayed good to excellent antibacterial activity against tested bacteria. Cardanol thiosemicarbazide 53 showed very good activity against *S. sobrinus*.

Department: Chemistry Student's Signature .....
Field of Study: Chemistry Advisor's Signature .....
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LIST OF ABBREVIATIONS

CH_3CN	acetonitrile
$Ca(OH)_2$	calcium hydroxide
conc.	concentrated
CH_2Cl_2	dichloromethane
$CHCl_3$	chloroform
CCl_3CN	trichloroacetonitrile
d	doublet (NMR)
dd	doublet of doublets (NMR)
DMEM	Dulbecco's Modified Eagle Medium
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
dt	doublet of triplets (NMR)
equiv.	equivalent (s)
EDTA	ethylenediaminetetraacetic acid
Et	ethyl
Et_3N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol

FBS	Fetal Bovine Serum
g	gram (s)
h	hour (s)
HCl	hydrochloric acid
H_2SO_4	sulfuric acid
IC_{50}	inhibition concentration 50 %
IZ	inhibition zone
J	coupling constant (NMR)
K_2CO_3	potassium carbonate
KI	potassium iodide
LPS	lipopolysaccharide
m	multiplet (NMR)
MBC	minimum bactericidal concentration
MeOH	methanol
MIC	minimal inhibitory concentration
µg	microgram (s)
M	molar (s)
mg	milligram (s)
min	minute (s)
mL	milliliter (s)
mmol	millimole (s)

MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NADH	β-Nicotinamide adenine dinucleotide
Na_2SO_4	sodium sulfate
nm	nanometer (s)
NMR	nuclear magnetic resonance
°C	degree of Celsius
Pd/C	palladium on activated charcoal
Ph	phenyl
PMS	phenazine methosulfate
PPh_3	triphenylphosphine
ppm	part per million
rt	room temperature
s	singlet (NMR)
$SnCl_4$	Tin(IV) chloride
t	triplet (NMR)
t_R	retention time
TLC	thin layer chromatograph
UV	ultraviolet
%	percent
α	alpha
δ	chemical shift

CHAPTER I

INTRODUCTION

Anacardium occidentale Linn (cashew tree, Anacardiaceae family) a tropical evergreen tree is an originally native tree of Brazil. Cashew tree has been imported into Thailand since 1901 and much cultivated in the southern and the northeastern parts. At present, this plant has been economically important. Usually, the kernel is an edible part so the shell becomes waste which however could be added the value by extraction or pressing to get cashew nut shell liquid (CNSL). CNSL contains three main components: anacardic acid, cardanol and cardol being studied in medicinal and industrial applications [1].

1.1 Botanical characteristics of *Anacardium occidentale* Linn.

The cashew tree produces cashew seed and cashew apple. Pseudo fruit or cashew apple is a pear shape and ripen into red or yellow. It can be eaten fresh or used for the preparation of jams, juices or sweet desserts. True fruit or cashew seed is shaped like kidney. It grows at the end of the cashew apple. The true fruit contains a single seed or kernel, known as a nut and liquid, a potent skin irritant chemically related to the better-known allergenic oil. The cashew tree and fruit are shown in Figure 1.1.

Figure 1.1 a) cashew tree b) cashew fruit

1.2 Cashew nut shell liquid (CNSL)

CNSL is a byproduct from cashew industry and obtains from the spongy layer between the inner and the outer shell of cashew nut by extraction or press. It is a dark brown liquid with slightly smell (Figure 1.2). It contains three main alkenyl phenolic components and certain decomposition and polymerization products. The phenolic components are a salicylic acid derivative (anacardic acid **1**), a monophenol derivative (cardanol **2**), and a resorcinol derivative (cardol **3**) (Figure 1.3). CNSL itself is useful as larvicides and possesses antioxidant activity. Moreover, it can be utilized as a starting material for organic syntheses and an alternative source of phenolic compounds. The most alternative consideration for CNSL use can be its low cost and chemically reactive in nature [2-4].

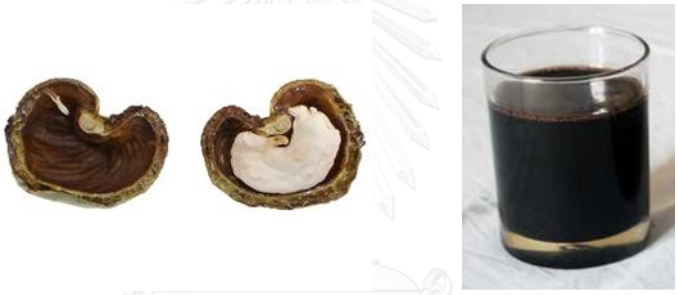


Figure 1.2 a) cashew nut shell b) CNSL

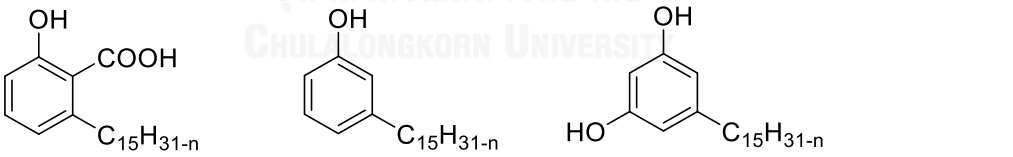


Figure 1.3 Three major substances of CNSL 1-3

1.3 Antioxidant

In biological systems, free radicals are implied as the causes of pathological physiologies. There are many types of free radicals: for oxygen derived species is known as reactive oxygen species (ROS), for nitrogen derived species is called as reactive nitrogen species (RNS). The active oxygen radicals such as superoxide radicals, hydroxyl

radicals, and peroxyl radicals, play an important role in oxidative stress related to the pathogenesis of different diseases.

Antioxidants are compounds that inhibit or delay the oxidation of other molecules. They are also known as free radical scavengers or oxidation inhibitor. Antioxidants are capable to neutralize free radicals by accepting or donating electron(s) to eliminate the unpaired condition of the radical. Antioxidants can be categorized based on their activity as enzymatic and non-enzymatic systems. An enzymatic antioxidant is for example superoxide dismutase (SOD), glutathione peroxidase (GSHPx), catalase *etc.* and a non-enzymatic antioxidant includes glutathione, vitamin C, vitamin E, plant extract *etc.*

Antioxidant activity cannot be measured directly but rather by the effects of the antioxidant in controlling the extent of oxidation. The most common methods used in determination of antioxidant capacity such as 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) radical ($ABTS^{\bullet+}$) scavenging, 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) radical scavenging, Fe^{3+}–Fe^{2+} transformation assay, ferric reducing antioxidant power (FRAP) assay, cupric ions (Cu^{2+}) reducing power assay (Cuprac), peroxyl radical scavenging, superoxide anion radical ($O_2^{\bullet-}$) scavenging, hydrogen peroxide (H_2O_2) scavenging, and hydroxyl radical ($OH^{\bullet}$) scavenging [5, 6].

1.4 Antibacterial activity

Most of human, animal and plant diseases are caused by pathogenic microbes (fungi, bacteria, and algae). Infections due to fungi and bacteria have been a major cause of death in higher organisms. In the past, many antibiotics were isolated from natural sources such as plants, bacteria, or fungi and later they were synthesized and introduced in clinical practices. The discovery and development of new antimicrobial agent is therefore a going on process. There are many bioassays to evaluate antimicrobial activity. One of the major methods for antimicrobial activity is agar diffusion method.

The agar diffusion method is the process of measuring inhibition zones which were generated after incubation the sample on seeded agar. The diameter of the clear zone around the well is compared between samples and standard antibiotics [7].

1.5 Pathogenic bacteria

A pathogenic organism is an organism capable of causing disease and illness in its host. The term is most often used for agent which disrupts the normal physiology of a multi cellular animal, human, or plant.

A pathogenic bacteria is a bacteria that causes infectious diseases. Although the vast majority of the bacteria are harmless or beneficial but a few bacteria are pathogenic. This research involves with animal pathogenic bacteria including *Streptococcus mutans* and *Streptococcus sobrinus* causing caries decay of teeth, *Staphylococcus aureus* and *Propionibacterium acnes* causing skin infections, and *Salmonella typhi* being the causative agent of typhoid fever [8] (Figure 1.4).

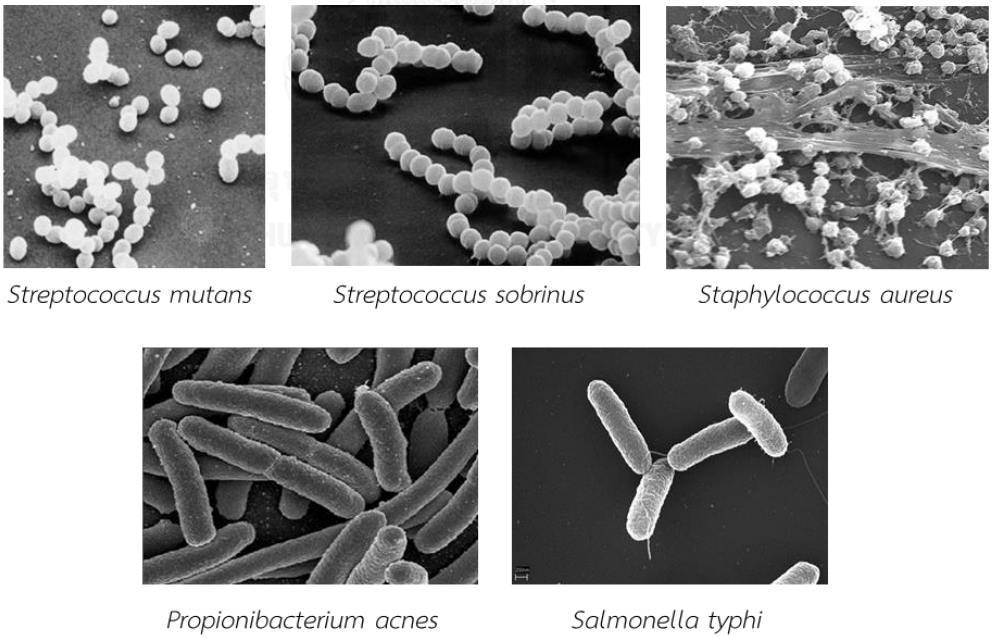


Figure 1.4 Selected pathogenic bacteria used in this study

1.5.1 *Streptococcus mutans*

S. mutans is gram-positive cocci shaped and facultative anaerobe bacteria, normally found in the human oral cavity and is a major contributor of tooth decay. *S. mutans* is mesophilic and grow at temperatures between 18-40 °C. It is cariogenic microorganism that plays metabolizing sucrose to lactic acid using the enzyme glucansucrase and produces an acidic environment, which demineralizes the superficial structure of the tooth. So it is the main causative agent of dental cavities.

1.5.2 *Streptococcus sobrinus*

S. sobrinus is a gram-positive, non-motile, non-spore forming bacteria and a spherical shaped. It is an anaerobic bacteria which grows in pairs or chains. *S. sobrinus* in particular lives in the tooth cavities of humans. It is found in high quantities in the form of biofilm or plaque in patients with dental caries. Usually, *S. sobrinus* has an optimal growth at 37 ℃ and thrives in a slightly acidic environment. It closely connects with species *S. mutans* within humans and enhances the formation of caries within teeth.

1.5.3 *Staphylococcus aureus*

S. aureus is a gram-positive cocci in clusters, non-motile, and non-spore forming. It is an opportunistic pathogen and a facultative anaerobes. Commonly, *S. aureus* occurs as a commensal on human skin and lives in the nose or on the skin of human. *S. aureus* can cause many diseases from minor skin infections. Its incidence is from skin, soft tissue, bone, joint, respiratory, and endovascular to wound infections.

1.5.4 *Propionibacterium acnes*

P. acnes is a gram-positive, non-spore forming, and rod shaped bacteria. It typically grows as an aerotolerant anaerobic. *P. acnes* is the causative agent of acne pimples. The combination of digestive products (fatty acids) and bacterial antigens stimulates an intense local inflammation that bursts the hair follicle. Then, a lesion forms on the surface of the skin in the form of a pustule (Whitehead). Moreover, *P.*

acnes is the causative of other infections such as corneal ulcers, heart valves and prosthetic devices, and central nervous system shunts.

1.5.5 *Salmonella typhi*

S. typhi is a gram-negative, non-spore forming and rod shaped bacteria. It has a complex regulatory system, which mediates its response to the changes in its external environment. It is living in intestinal organ and can grow under both an aerobic and anaerobic conditions. *S. typhi* is the causative agent of typhoid fever which causes a serious, often fatal disease. The symptoms of typhoid fever include nausea, vomiting, fever and death. It can only infect humans, and no other host has been identified.

1.6 Anti-inflammatory

Inflammatory is a biological response of body tissues to injury. It is a protective response that involves immune cells, blood vessels, and molecular mediators. The objective of inflammation is to eliminate the initial cause of cell injury, initiate tissue repair and clear out necrotic cells and tissues damaged from the original insult and the inflammatory process. A few of inflammation could lead to progressive tissue destruction by the harmful stimulus (*e.g.* bacteria) and compromise the survival of the organism. In contrast, acute inflammation may lead to a host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (*e.g.* gallbladder carcinoma). Inflammation is therefore normally closely regulated by the body.

1.6.1 RAW 264.7 cell line

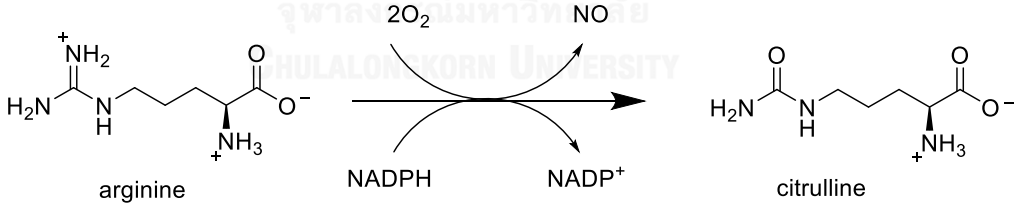
Macrophage is important in immune regulation and possible role in immunity has created a need for homogenous cell populations and for large number of macrophages. It was developed and used to study problems in macrophage biology. Macrophage cell lines were derived from many ways as tumors, or tumor-like cells, and bone marrow cells. In this research, RAW 264.7 cell line from mouse monocyte macrophage (murine macrophage) is a secondary cell culture, established from an

ascites of a tumour induced in a male mouse. Cells will pinocytose neutral red and phagocytose zymosan [9, 10].

1.6.2 Lipopolysaccharide (LPS)-treated RAW 246.7 cell lines

Murine macrophage cells 264.7 express adenosine receptors and play critical roles in the immune system and inflammatory response and, when activated, these cells secrete nitrogen intermediates and pro-inflammatory cytokines such as TNF-α, IL-1, IL-6 and enzyme (COX-2 and iNOS) by activation of transcription factors. RAW 264.7 cells can be useful as a model for study of inflammatory process and to test adenosine receptor ligands and evaluate their effects as pro- or anti-inflammatory drugs.

LPS is endotoxin from gram-negative bacteria using for simulating RAW 264.7 cells. LPS usually uses for stimulating macrophage to generate inducible nitric oxide synthase (iNOS) which produces nitric oxide from L-arginine. Nitric oxide exerts its role in host defence due to its antibacterial and virustatic properties. However, if NO production gets out of control, damage of host cells occurs due to the cytotoxic potential of NO. Therefore, NO is discussed as an important regulator in states of inflammatory diseases, including hepatic inflammatory conditions [9, 11].



1.7 Literature review

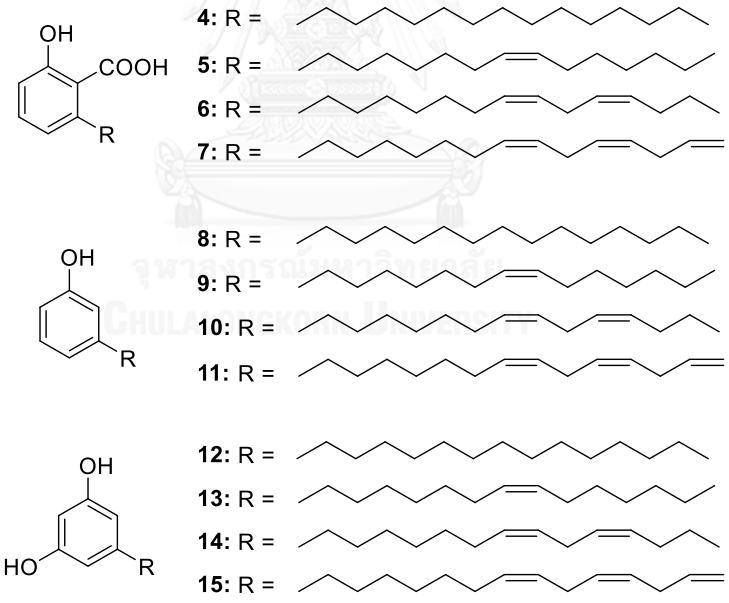
1.7.1 Constituents of CNSL

In 1986, Kubo and co-workers reported that 12 components were separated from CNSL. There were differences in side chains of anacardic acid **1**, cardanol **2**, cardol **3** and 2-methylcardols**.** All compounds were tested against *Biomphalaria glabratus*, snail vectors of schistosomiasis. The molluscicidal activity of anacardic acid **1** is

increased in direct proportion to the degree of unsaturation in the alkenyl side chain. In case of cardol **3** and 2-methylcardols, the activity decreased in inverse proportion to the degree of unsaturation in the side chain. Moreover, carboxyl group on the ring resulted in a large increase in activity [12].

In 1991, Himejima and Kubo examined the antimicrobial activity of 16 phenolic components from CNSL on four typical microorganism: a Gram-positive bacteria, a gram-negative bacteria, a yeast, and a mold. Most of them exhibited potent antibacterial activity against only Gram-positive bacteria, among which were *S. mutans* and *P. acnes* [13].

In 1993, Toyomizu and co-workers investigated 16 components from CNSL. Among the constituents isolated, anacardic acid $C_{15:0}$ **4** was good potent enzyme inhibitors of **α**-glucosidase and anacardic acid $C_{15:3}$ **7** was the most active inhibitor against aldose reductase, while it showed moderate inhibitor for invertase [14].



In the same year, Kubo and co-workers reported a series of anacardic acid **1** against *Bacillus subtilis*, *Brevibacterium ammoniagenes*, *S. aureus*, *S. mutans* and *P. acnes*. Moreover, anacardic acid **1** with different side chain lengths were synthesized, and their antimicrobial activity was tested. For *S. aureus*, the anacardic acid with C-10 alkyl side chain was the most active; for *P. acnes, S. mutans*, and *B. ammoniagenes*, the anacardic acid with C-12 alkyl side chain was the most effective [15].

In 1993, Muroi and Kubo reported that anacardic acid **1** from cashew apple and 6-alkyl salicylic acid possessed bactericidal activity against *S. mutans* by time-kill curve method. The carbon length comprised 12 carbon atoms in the side chain showed maximum activity against this cariogenic bacterium. In addition, anacardic acid $C_{15:3}$ **7** was used to combine with other natural substances to enhance antibacterial activity. Synergistic bactericidal activity was found in the combination of anacardic acid $C_{15:3}$ **7** and anethole or linalool [16].

In 1994, Shobha and co-workers addressed that anacardic acid-$C_{15:1}$ **5** exhibited the highest potency (IC_{50} 50 μM), followed by cardol $C_{15:3}$ **15** (IC_{50} 60 μM) against soybean lipoxygenase-1. The other unsaturated phenolic compounds were of intermediate potency [17].

In 1994, Kubo and co-workers explored 16 phenolic compounds isolated from CNSL and examined for their tyrosinase inhibitory activity. Two active compounds: anacardic acid $C_{15:3}$ **7** and cardol $C_{15:3}$ **15** exhibited characteristic competitive inhibition for the oxidation of 3,4-dihydroxyphenyldanine (L-DOPA) by mushroom tyrosinase [18].

In 1994, Kubo and co-workers reported that the activity of anacardic acid $C_{15:3}$ **7** was enhanced when combined with anethole, farnesol and β-caryophyllene. However, owing to the strong odor of anethole, this combination may not be completely agreeable. On the other hand, combination between anacardic acid $C_{15:3}$ **7** and β-caryophyllene or farnesol showed that they were synergism [19].

In 2003, Kubo and co-workers reported that unsaturated alkyl side chain of anacardic acid was associated with increasing the inhibition of Methicillin Resistant *Staphylococcus aureus* (MRSA). Moreover, the activity was significantly enhanced in combination between anacardic acid $C_{15:3}$ **7** and anacardic acid $C_{12:0}$. [20].

In 2004, Olajide and co-workers reported the evaluation of the methanol extract of *Anacardium occidentale* stem bark against the LPS-induced septic shock, and LPS-induced microvascular permeability in mice. The highest dose of the extract (200 mg/kg) produced a 100% protection against death from sepsis while pentoxifylline (100 mg/kg), a class IV phosphodiesterase inhibitor and *N*(omega)-nitro-L-arginine

methyl ester (L-NAME) (5 mg/kg), a nitric oxide inhibitor offered 100% protection against LPS-induced septic shock [21].

In 2004, Masuoka and Kubo reported that anacardic acid $C_{15:3}$ **7** inhibited both uric acid formation and the generation of superoxide radicals by xanthine oxidase *via* binding at a salicylic acid binding site in Mo-pterin domain to cause the cooperative inhibitions and binding at the hydrophobic site of xanthine to enhance the inhibition ability [22].

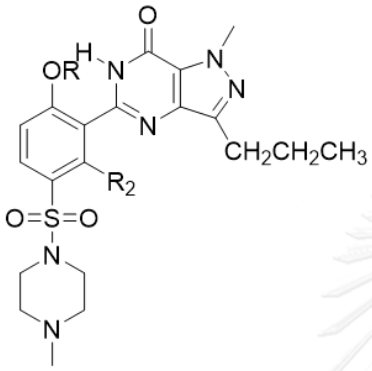
In 2006, Trevisan and co-workers evaluated the antioxidant activity of alkyl phenol in cashew products. Anacardic acid **1** (10.0 mg/mL) displayed the highest antioxidant capacity (IC_{50} 0.60 mM) compared to cardanol **2** and cardol **3** (IC_{50} > 4.0 mM). The antioxidant capacity of anacardic acid $C_{15:3}$ **7** was more related to the inhibition of superoxide generation (IC_{50} 0.04 mM) and xanthine oxidase (IC_{50} 0.30 mM) than the scavenging of hydroxyl radicals [23].

In 2006, Kubo and co-workers reported that anacardic acid **1** prevented the generation of superoxide radicals by inhibiting xanthine oxidase without radical scavenging activity. Anacardic acid $C_{15:1}$ **5** inhibited the soybean lipoxygenase-1 catalyzed oxidation of linoleic acid with IC_{50} 6.8 μM which was a slow, reversible reaction without residual enzyme activity. The inhibition kinetics indicated that anacardic acid $C_{15:1}$ **5** was a competitive inhibitor. Moreover, anacardic acids acted as antioxidants in various ways as inhibition of the reactive oxygen species production and chelating divalent metal ions such as Fe^{2+} or Cu^{2+} [24].

In 2012, Teerasripreecha and co-workers reported that cardanol **2** and cardol **3** could be isolated from Thai *Apis mellifera* propolis extracts and tested with in vitro anti-proliferation across five cancer cell lines and the control Hs27 cell line. Cardanol **2** showed ranging of IC_{50} from 10.8 to 29.3 μg/mL and cardol **3** revealed less than 3.13 to 5.97 μg/mL. Moreover, both substances induced cytotoxicity and cell death without DNA fragmentation in cancer cells, but only an antiproliferation response in the control Hs27 cells [25].

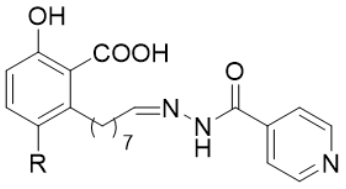
1.7.2 Derivatives of anacardic acid and cardanol

In 2002, Paramashivappa and co-workers reported the modification of anacardic acid to sildenafil analogues for phosphodiesterase-5 (PDE_5) inhibition activity (sildenafil is a potent PDE_5 inhibitor and an orally active drug for the treatment of erectile dysfunction). Pentadecyl side chain of anacardic acid **16-17** decreased PDE_5 inhibition comparing with sildenafil [26].



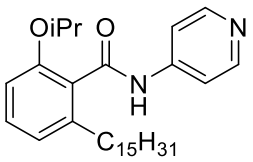
	R	R_2	IC_{50}
16	CH_2CH_3	$C_{15}H_{31}$	145
17	CH_3	$C_{15}H_{31}$	125
sildenafil	CH_2CH_3	H	38

In 2007, Swamy and co-workers synthesized isonicotinoylhydrazone derivatives of anacardic acid at C_8-aldehyde and tested against *Mycobacterium smegmatis* mc^2155 and their synergistic effects in combination with isoniazid (tuberculosis drug). Two derivatives **18-19** showed lesser inhibitory activity compared to isoniazid, but showed significant higher activity when treated synergistically with isoniazid using pathogenic strain *M. tuberculosis* $H_{37}Rv$ [27].



	R	MIC (μg/mL) against M. smegmatis mc^2155
18	H	4
19	NO_2	5
isoniazid		1.5

In 2009, Chandregowda and co-workers synthesized benzamide derivatives of anacardic acid $C_{15:0}$ **4** and tested cytotoxic activity on HeLa cell. Three derivatives **20-22** were more potent with the respective IC_{50} 11.02, 13.55, and 15.29 mM comparing with garcinol which is a cell permeable histone acetyltransferase (HAT) inhibitor [28].



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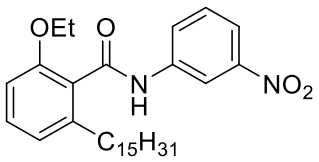

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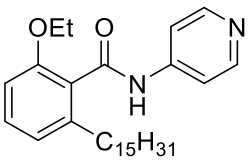
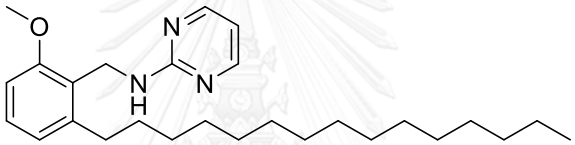


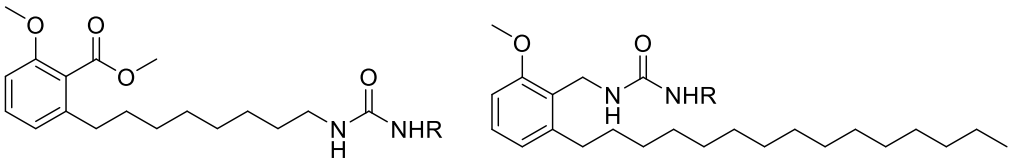
22

In 2011, Vempati and co-workers prepared *N*-substituted benzylamine analogues of anacardic acid $C_{15:0}$ **4** and tested on antibacterial activity with *S. aureus*, *E. coli*, *Pseudomonas aeruginosa* and *S. Pyogens*. Compound **23** showed excellent activity compared to ampicillin and chloramphenicol and comparable activity with ciprofloxacin. Most synthesized compounds displayed good or moderate activities compared to ampicillin and chloramphenicol but inferior activities compared to ciprofloxacin and norfloxacin [29].

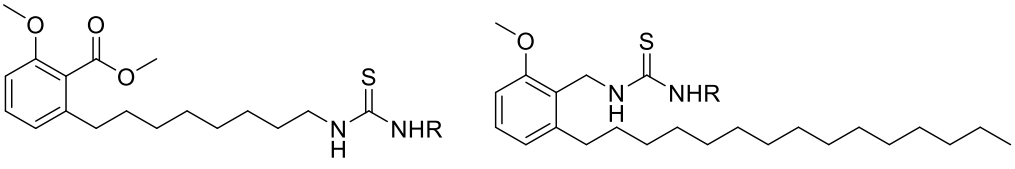


23

During 2011 and 2012, Reddy and co-workers synthesized urea and thiourea derivatives at C-8 side chain and C-2 on aromatic of anacardic acid. All compounds were evaluated on antibacterial activity for gram-positive and negative bacteria. Most of the compounds showed good activity comparing with ampicillin [30, 31].

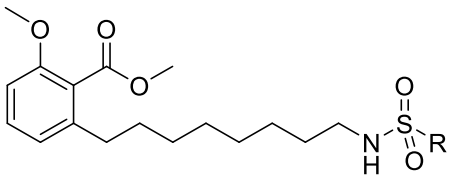


urea derivatives



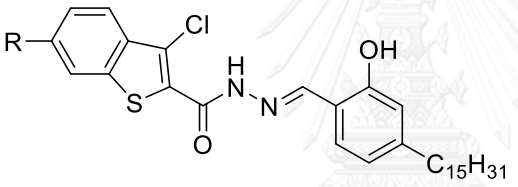
thiourea derivatives

In 2012, Reddy and co-workers synthesized sulfonamide derivatives at C-8 alkyl chain of anacardic acid and were tested for antibacterial activity. Most of the compounds showed higher antibacterial activity compared with ampicillin [32].

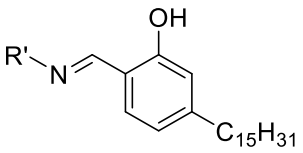


sulfonamide derivatives

In 2014, Naganagowda and co-workers synthesized benzothiophene and Schiff base derivatives from cardanol **2**. The results showed that synthesized compounds exhibited low to moderate antibacterial activity against *S. aureus* and *B. subtilis* and significant antifungal activity against *Candida albicans* and *Chrysosporium pannical* [33].



benzothiophene derivatives



Schiff base derivatives

From the literature review, constituents of CNSL and their derivatives were examined for various biological activities such as antimicrobial activity, tyrosinase inhibition, and antioxidant activity [13, 18, 23]. In this research, three major activities including the scavenging activity of superoxide anion generated by PMS-NADH, NO production as anti-inflammatory activity and antibacterial activity of natural compounds from CNSL and their derivatives were examined. Thus, the objectives of this research could be summarized as:

- To isolate constituents of CNSL (anacardic acid **1**, cardanol **2** and cardol **3**)

- To synthesize derivatives of anacardic acid **1** and cardanol **2**.

- To test biological activities including antioxidant (PMS-NADH and DPPH), antibacterial, and anti-inflammatory (NO production) activities of isolated and synthesized compounds.

CHAPTER II

EXPERIMENTAL

2.1 Raw material

Cashew nut shell liquid (CNSL) was kindly received as gift from Udomkij Company (Chonburi) in March 2013.

2.2 Chemicals

All solvents used in this research were purified prior to use by standard methodologies. The reagents used for synthesis were purchased from Sigma-Aldrich chemical company or otherwise stated and were used without further purification.

2.3 Instruments and equipment

Thin layer chromatography (TLC) was performed on aluminum sheets pre-coated with silica gel (Merck's, Kieselgel 60 PF_{254}). Column chromatography was performed on silica gel (Merck's silica gel 60 G Art 7734 (70-230 mesh)). The ^{1}H and ^{13}C NMR spectra were performed in deuterated chloroform ($CDCl_3$) and deuterated dimethylsulfoxide (DMSO-d_6) on Varian nuclear magnetic resonance spectrometer, model Mercury plus 400 NMR spectrometer and Bruker advanced 400 NMR spectrometer. Semi-preparative HPLC was carried out on Waters 600 controller with Waters 2996 photodiode array detector. Antioxidant assay was evaluated on Beckman spectrophotometer, model DU® 7500.

2.4 Separation of CNSL by extraction method

CNSL (50 g) was dissolved in 5% aqueous MeOH (300 mL) and $Ca(OH)_2$ (50 g) was added in portions under stirring. After that the reaction mixture was stirred at 50 °C for 5 h. The reaction was monitored by TLC for the absence of CNSL. After

completion of the reaction, the precipitated calcium anacardate was filtered and washed with MeOH and the precipitate was dried under vacuum evaporator. Calcium anacardate was suspended in water, acidified with conc. HCl and stirred for 1 h. The resultant solution was extracted with EtOAc. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to yield anacardic acid **1** [1] (42.1 g, 84 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.35 (t, *J* = 7.9 Hz, 1H), 6.86 (d, *J* = 8.3 Hz, 1H), 6.77 (d, *J* =7.5 Hz, 1H), 5.85-4.96 (-C**H**=CH_2 and -CH=C**H$_2$** on alkyl chain) and 2.99-0.85 (**H** on alkyl chain).

The filtrate left from the filtration of calcium anacardate was evaporated under reduced pressure and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain crude product which was purified by silica gel column eluting with hexane/EtOAc to afford cardanol **2** (1.1 g, 2 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.14 (t, *J* = 7.6 Hz, 1H), 6.76 (d, *J* = 7.6 Hz, 1H), 6.65 (d, *J* = 7.9 Hz, 2H), 5.81-5.01 (-C**H**=CH_2 and -CH=C**H$_2$** on alkyl chain), and 2.95-0.89 (**H** on alkyl chain) and cardol **3** (319.1 mg, 0.1 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 6.24 (d, *J* = 2.3 Hz, 2H), 6.18 (t, *J* = 2.3 Hz, 1H), 5.85-4.97 (-C**H**=CH_2 and -CH=C**H$_2$** on alkyl chain) and 2.82-0.92 (**H** on alkyl chain).

2.5 Separation of CNSL by decarboxylation method

$Ca(OH)_2$ (4 g) was added into CNSL (200 g). The mixture was stirred at 120 °C for 3 h. The reaction was cooled to room temperature and hexane (200 mL) was added and then stirred for 30 min. After that the liquid was separated and evaporated under vacuum to obtain brown liquid which was distilled under reduced pressure to afford cardanol **2** [34] (84.7 g, 42 %yield).

2.6 Separation of the constituents of CNSL by semi-preparative HPLC

50 mg of anacardic acid **1** in 1 mL CH_3CN was prepared and subjected to semi-prep HPLC using the following conditions: mobile phase consisted of CH_3CN–water–acetic acid (80:20:1) run with isocratic mode at a flow rate of 3.0 mL/min. The alkyl

phenols were detected with a photodiode array detector set at 278 nm [23]. The peaks at t_R 35, 42, 49, and 67 min were collected and dried to yield compounds **4**-**7** [35, 36].

6-Pentadecylsalicylic acid (**4**) or saturated anacardic acid [37]: white solid (9.0 mg, 18 %yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 11.01 (s, 1H), 7.36 (t, *J* = 7.9 Hz, 1H), 6.87 (dd, *J* = 8.3, 1.2 Hz, 1H), 6.78 (dd, *J* = 7.5, 1.2 Hz, 1H), 2.98 (t, *J* = 8.0 Hz, 2H), 1.59 (m, 2H), 1.26 (m, 19H) and 0.88 (3H, t, *J* = 6.8 Hz).

6-[8(*Z*)-Pentadecenyl]salicylic acid (**5**) [35]: yellow liquid (15.7 mg, 31 %yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 11.04 (s, 1H), 7.35 (t, *J* = 7.9 Hz, 1H), 6.86 (d, *J* = 8.3 Hz, 1H), 6.77 (d, *J* = 7.5 Hz, 1H), 5.35 (t, *J* = 4.8 Hz, 2H), 2.97 (t, *J* = 7.9 Hz, 2H), 2.01 (m, 4H), 1.60 (m, 2H), 1.29 (m, 16H) and 0.88 (t, *J* = 6.5 Hz, 3H).

6-[8(*Z*),11(*Z*)-Pentadecadienyl]salicylic acid (**6**) [35]: yellow liquid (12.8 mg, 26 %yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 11.07 (s, 1H), 7.35 (t, *J* = 7.9 Hz, 1H), 6.86 (d, *J* = 8.3 Hz, 1H), 6.77 (d, *J* = 7.5 Hz, 1H), 5.37 (m, 4H), 2.97 (dd, *J* = 9.1, 6.6 Hz, 2H), 2.77 (t, *J* = 6.3 Hz, 2H), 2.01 (m, 4H), 1.33 (m, 12H) and 0.90 (t, *J* = 7.4 Hz, 3H).

6-[8(*Z*),11(*Z*),14-Pentadecatrienyl]salicylic acid (**7**) [35]: yellow liquid (12.3 mg, 25 %yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 11.09 (s, 1H), 7.35 (t, *J* = 7.9 Hz, 1H), 6.86 (d, *J* = 8.3 Hz, 1H), 6.76 (d, *J* = 7.5 Hz, 1H), 5.81 (m, 1H), 5.39 (m, 4H), 5.01 (m, 2H), 2.97 (t, *J* = 7.9 Hz, 3H), 2.79 (dd, *J* = 13.7, 7.5 Hz, 4H), 2.05 (m, 2H), 1.59 (m, 2H) and 1.34 (m, 8H).

By the same fashion, the constituents of cardanol **2** and cardol **3** were separated. For cardanol **2**, compounds **9-11** were accomplishedly collected at t_R 38, 57 and 87 min, whereas for cardol **3**, the peaks at t_R 16 and 23 min were isolated to yield compounds **11-12**.

3-[8(*Z*)-Pentadecenyl]phenol (**9**) [38]: yellow liquid (15.4 mg, 31 %yield) ^{1}H NMR ($CDCl_3$) δ_H ppm): 7.14 (t, *J* = 7.7 Hz, 1H), 6.75 (d, *J* = 7.6 Hz, 1H), 6.65 (d, *J* = 8.4 Hz, 2H), 5.35 (m, 2H), 2.55 (t, *J* = 7.8 Hz, 2H), 2.01 (m, 4H), 1.59 (m, 2H), 1.29 (m, 16H), and 0.89 (t, *J* = 6.5 Hz, 3H).

3-[8(*Z*),11(*Z*)-Pentadecadienyl]phenol (**10**) [38]: yellow liquid (22.8 mg, 46 %yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.14 (t, *J* = 7.7 Hz, 1H), 6.75 (d, *J* = 7.5 Hz, 1H), 6.65

(d, *J* = 8.1 Hz, 2H), 5.36 (m, 4H), 2.78 (t, *J* = 6.3 Hz, 2H), 2.55 (t, *J* = 7.8 Hz, 2H), 2.04 (m, 4H), 1.58 (m, 12H), and 0.91 (t, *J* = 7.4 Hz, 3H).

3-[8(*Z*),11(*Z*),14-Pentadecatrienyl]phenol (**11**) [38]: yellow liquid (11.4 mg, 23 %yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.14 (t, *J* = 7.6 Hz, 1H), 6.76 (d, *J* = 7.6 Hz, 1H), 6.65 (d, *J* = 7.9 Hz, 2H), 5.83 (m, 1H), 5.40 (m, 4H), 5.03 (m, 2H), 2.82 (dt, *J* = 16.1, 6.1 Hz, 4H), 2.56 (t, *J* = 7.7 Hz, 2H), 2.04 (m, 2H), 1.60 (m, 2H), and 1.29 (m, 8H).

5-[8(*Z*),11(*Z*)-Pentadecadienyl]resorcinol (**14**) [39]: brown liquid (36.5 mg, 73 %yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 6.24 (d, *J* = 2.3 Hz, 2H), 6.17 (t, *J* = 2.3 Hz, 1H), 5.36 (dd, *J* = 8.1, 4.8 Hz, 4H), 2.78 (t, *J* = 6.3 Hz, 2H), 2.48 (t, *J* = 7.8 Hz, 2H), 2.04 (m, 4H), 1.56 (m, 2H), 1.33 (m, 8H), and 0.91 (t, *J* = 7.4 Hz, 3H).

5-[8(*Z*),11(*Z*),14-Pentadecatrienyl]resorcinol (**15**) [39]: brown liquid (13.4 mg, 27 %yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 6.25 (d, *J* = 2.3 Hz, 2H), 6.17 (t, *J* = 2.3 Hz, 1H), 5.82 (m, 1H), 5.39 (m, 4H), 5.02 (m, 2H), 2.80 (dt, *J* = 14.1, 7.9 Hz, 4H), 2.46 (t, *J* = 7.7 Hz, 2H), 2.04 (m, 2H), 1.55 (m, 2H), and 1.28 (m, 8H).

2.7 Hydrogenation of anacardic acid 1, cardanol 2 and cardol 3

Anacardic acid **1** (20 g) in MeOH (50 mL) and 10% Pd/C (0.5 g) were mixed under H_2 atmosphere in a Parr apparatus and the completeness of the reaction was monitored by TLC. The completed reaction was filtered through a celite bed to remove a catalyst. The filtrate was evaporated under vacuum. The product was purified by crystallization to afford saturated anacardic acid **4** [29] (16.7 g, 84 %yield).

3-Pentadecylphenol (**8**) or saturated cardanol [40] (14.9 g, 75 %yield) was obtained from the same procedure mentioned above using cardanol **2** (20 g). ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.15 (t, *J* = 7.7 Hz, 1H), 6.77 (d, *J* = 7.6 Hz, 1H), 6.68 (d, *J* = 8.4 Hz, 2H), 5.91 (s, 1H), 2.56 (t, *J* = 7.9 Hz, 2H), 1.60 (t, *J* = 7.6 Hz, 2H), 1.30 (m, 24H) and 0.91 (t, *J* = 6.6 Hz, 3H).

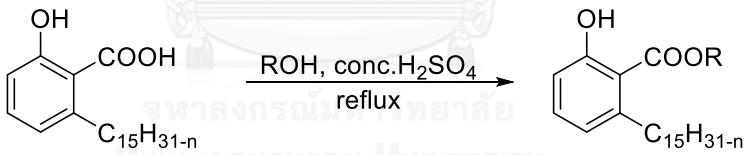
Cardol **3** (19.4 mg) in EtOAc (8 mL) and 10%Pd/C (0.5 mg) were added and stirred under H_2 balloon for 24 h. The complete reaction was filtered through a celite

bed to remove a catalyst. The filtrate was evaporated under vacuum and further purified by silica gel column eluting with hexane/EtOAc to afford 5-pentadecylresorcinol (**12**) or saturated cardol [41] (16.1 mg, 83 %yield): 6.24 (s, 2H), 6.18 (s, 1H), 2.47 (t, *J* = 7.8 Hz, 2H), 1.55 (m, 2H), 1.25 (m, 24H) and 0.87 (t, *J* = 6.6 Hz, 3H).

2.8 Synthesis of anacardic acid derivatives

2.8.1 Esters of anacardic acids

Anacardic acid **1** (10.0 g) was dissolved in MeOH (or EtOH) (140 mL) and conc. H_2SO_4 (15 mL) was added slowly at 0 °C. The mixture was refluxed for 20 h. The reaction was cooled down to room temperature and poured into iced water (300 mL). The mixture was extracted with EtOAc for 3 times. The combined organic layers were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel column eluting with hexane/EtOAc to achieve the desired esters **24** and **25** [29, 42].



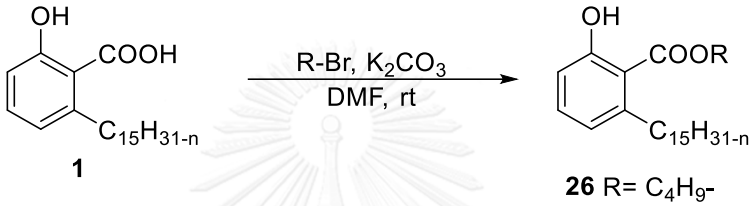
1

24 R= CH_3-
25 R= C_2H_5-

Methyl 2-hydroxy-6-pentadecyl(alkenyl) benzoate (**24**): orange liquid (4.5 g, 42 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 11.13 (s, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 6.85 (d, *J* = 8.3 Hz, 1H), 6.74 (d, *J* = 7.4 Hz, 1H), 4.98-5.84 (-C**H**=CH_2 and -CH=C**H$_2$** on alkyl chain), 3.98 (s, 3H) and 0.88-2.99 (**H** on alkyl chain).

Ethyl 2-hydroxy-6-pentadecyl(alkenyl) benzoate (**25**): yellow liquid (2.8 g, 26 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 11.26 (s, 1H), 7.30 (t, *J* = 7.8 Hz, 1H), 6.84 (d, *J* = 8.3 Hz, 1H), 6.72 (d, *J* = 7.4 Hz, 1H), 4.98-5.83 (-C**H**=CH_2 and -CH=C**H$_2$** on alkyl chain), 4.46 (q, *J* = 7.2 Hz, 2H) and 0.89-2.99 (**H** on alkyl chain).

Anacardic acid **1** (1 eq) was dissolved in DMF (20 mL) and K_2CO_3 (1.2 eq) and alkyl bromide or benzyl bromide (1.2 eq) were added in portions. The mixture was stirred at room temperature and was followed by TLC. When the reaction completed, the mixture was diluted with water and extracted with EtOAc (3 times). The organic layer was washed with water and brine respectively, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The mixture was purified by silica gel column eluting with hexane/EtOAc to achieve the desired ester products **26**-**30** [43].



1

26 R= C_4H_9-
27 R= C_6H_{13}-
28 R= C_8H_{17}-
29 R= $C_{12}H_{25}$-
30 R= CH_2-Ph-

n-Butyl 2-hydroxy-6-pentadecyl(alkenyl) benzoate (**26**): using **1** and 1-bromobutane as starting materials, this mixture was obtained as yellow liquid (406.8 mg, 20 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 11.30 (s, 1H), 7.28 (t, *J* = 7.9 Hz, 1H), 6.84 (d, *J* = 8.3 Hz, 1H), 6.71 (d, *J* = 7.5 Hz, 1H), 5.82–4.97 (-C**H**=CH_2 and -CH=C**H$_2$** on alkyl chain), 4.38 (t, *J* = 6.7 Hz, 2H), 2.91 (dd, *J* = 9.1, 6.7 Hz, 2H), 2.81 (dd, *J* = 13.0, 6.3 Hz, 2H) and 2.04–0.89 (**H** on alkyl chain).

n-Hexyl 2-hydroxy-6-pentadecyl(alkenyl) benzoate (**27**): using **1** and 1-bromohexane as starting materials, this mixture was obtained as yellow liquid (528.8 mg, 24 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 11.29 (s, 1H), 7.28 (t, *J* = 8.0 Hz, 1H), 6.84 (d, *J* = 8.7 Hz, 1H), 6.71 (d, *J* = 7.5 Hz, 1H), 5.82–4.95 (-C**H**=CH_2 and -CH=C**H$_2$** on alkyl chain), 4.37 (t, *J* = 6.8 Hz, 2H) and 3.40–0.91 (**H** on alkyl chain).

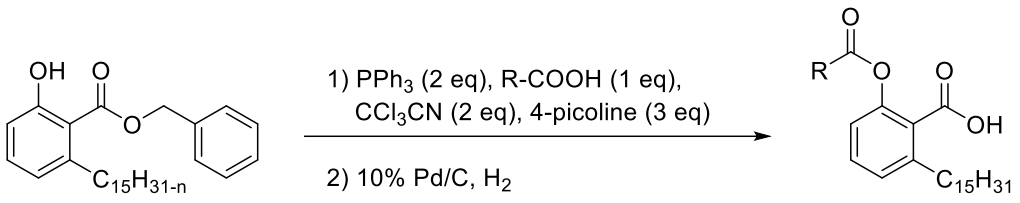
n-Octyl 2-hydroxy-6-pentadecyl(alkenyl) benzoate (**28**): using **1** and 1-bromooctane as starting materials, this mixture was obtained as yellow liquid (1.1 g, 49 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 11.28 (s, 1H), 7.28 (t, *J* = 8.0 Hz, 1H), 6.83 (d, *J* =

8.3 Hz, 1H), 6.71 (d, *J* = 7.5 Hz, 1H), 5.81-5.01(-C**H**=CH_2 and -CH=C$\mathbf{H_2}$ on alkyl chain), 4.36 (t, *J* = 6.7 Hz, 2H), 3.40 (t, *J* = 6.9 Hz, 6H) and 2.90-0.88 (**H** on alkyl chain).

n-Dodecyl 2-hydroxy-6-pentadecyl(alkenyl) benzoate (**29**): using **1** and 1-bromododecane as starting materials, this mixture was obtained as yellow liquid (1.6 g, 61 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 11.30 (s, 1H), 7.26 (t, *J* = 8.0 Hz, 1H), 6.84 (d, *J* = 8.3 Hz, 1H), 6.71 (d, *J* = 7.4 Hz, 1H), 5.82-5.03 (-C**H**=CH_2 and -CH=C$\mathbf{H_2}$ on alkyl chain), 4.37 (t, *J* = 6.7 Hz, 2H), 3.40 (t, *J* = 6.9 Hz, 7H), 2.91 (m, 2H), 2.82 (m, 2H) and 2.06-0.89 (**H** on alkyl chain).

Benzyl 2-hydroxy-6-pentadecyl(alkenyl) benzoate (**30**): using **1** and benzyl bromide as starting materials, this mixture was obtained as yellow liquid (1.1 g, 49 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 11.26 (s, 1H), 7.50–7.24 (m, 6H), 6.87 (dd, *J* = 8.3, 1.3 Hz, 1H), 6.71 (dd, *J* = 7.5, 1.3 Hz, 1H), 5.40 (s, 2H), 5.85-5.05 (-C**H**=CH_2 and -CH=C$\mathbf{H_2}$ on alkyl chain) and 2.84-0.94 (**H** on alkyl chain).

PPh_3 (2 eq) in CH_2Cl_2 (2 mL) was added to a mixture of selected acid (1 eq) and CCl_3CN (2 eq) in CH_2Cl_2 (4 mL). The mixture was stirred at room temperature for approximately 1 h. The mixture of benzyl 2-hydroxy-6-pentadecyl(alkenyl) benzoate **30** (1 eq) and 4-picoline (3 eq) was added to the above mixture. The reaction was continued stirring at 37-40 °C and followed by TLC. When the reaction completed, the mixture was extracted with 10% HCl and saturated $NaHCO_3$, respectively. The organic layer was dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by silica gel eluting with hexane/EtOAc to achieve the ester product. The EtOAc (8 mL) containing obtained ester (1 eq) was hydrogenolyzed using H_2 balloon in the presence of 10% Pd/C (0.3 eq) and followed by TLC. The completed reaction was filtered through a celite bed to remove a catalyst. The filtrate was evaporated under vacuum to dryness and was further purified by silica gel column eluting with hexane/EtOAc to afford the target compounds **31-38** [44].



30 (1 eq)

31 R= Ph-
32 R= 2-Cl-Ph-
33 R= 2-Br-Ph-
34 R= 2-OH-Ph-
35 R= CH_3-
36 R= C_3H_7-
37 R= C_7H_{15}-
38 R= $C_{11}H_{23}$-

2-(Benzoyloxy)-6-pentadecylbenzoic acid (**31**): using **30** and benzoic acid as starting materials, this compound was obtained as white solid (147 mg, 47 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 8.14 (m, 2H), 7.60 (dd, *J* = 7.4, 1.6 Hz, 1H), 7.50–7.39 (m, 3H), 7.15 (dd, *J* = 17.8, 7.9 Hz, 2H), 2.74 (t, *J* = 7.9 Hz, 2H), 1.60 (m, 2H), 1.13–1.41 (m, 24H) and 0.87 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm): 170.2, 165.0, 148.6, 143.4, 133.7, 131.1, 130.3 (2C), 129.2, 128.6 (2C), 127.4, 125.1, 120.5, 33.8, 31.9, 31.4, 29.7 (4C), 29.7 (2C), 29.6, 29.5, 29.4, 29.4, 22.7 and 14.1.

2-((2-Chlorobenzoyl)oxy)-6-pentadecylbenzoic acid (**32**): using **30** and 2-chlorobenzoic acid as starting materials, this compound was obtained as white solid (140 mg, 42 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 8.15 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.99 (d, *J* = 7.8 Hz, 1H), 7.60 (t, *J* = 7.5 Hz, 1H), 7.45 (m, 2H), 7.16 (m, 2H), 2.75 (t, *J* = 8.0 Hz, 2H), 1.61 (m, 2H), 1.25 (m, 24H) and 0.87 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm): 170.3, 165.0, 148.6, 143.4, 133.7, 133.1, 131.9, 131.2, 131.1, 130.2, 128.6, 127.4, 126.7, 120.5, 33.8, 31.9, 31.4, 29.7 (4C), 29.7 (2C), 29.6, 29.5, 29.4, 29.4, 22.7 and 14.1.

2-((2-Bromobenzoyl)oxy)-6-pentadecylbenzoic acid (**33**): using **30** and 2-bromobenzoic acid as starting materials, this compound was obtained as white solid (179 mg, 49 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 8.16 (d, *J* = 7.6 Hz, 1H), 7.98 (m, 1H), 7.52–7.33 (m, 3H), 7.17 (m, 2H), 2.77 (t, *J* = 8.0 Hz, 2H), 1.63 (m, 2H), 1.25 (m, 24H) and 0.88 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm): 170.7, 164.2, 148.5, 143.7, 133.7, 133.1, 131.3, 131.1, 130.2, 128.6, 127.8, 127.4, 125.0, 120.5, 33.9, 31.9, 31.4, 29.7 (4C), 29.7 (2C), 29.6, 29.5, 29.4, 29.4, 22.7 and 14.1.

2-((2-Hydroxybenzoyl)oxy)-6-pentadecylbenzoic acid (**34**): using **30** and 2-benzyloxybenzoic acid as starting materials, this compound was obtained as white solid (171 mg, 53 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 10.25 (s, 1H), 8.00 (d, *J* = 7.9 Hz, 1H), 7.51 (t, *J* = 7.9 Hz, 1H), 7.46 (t, *J* = 7.9 Hz, 1H), 7.22 (d, *J* = 7.7 Hz, 1H), 7.13 (d, *J* = 8.1 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 6.91 (t, *J* = 7.6 Hz, 1H), 2.70 (t, *J* = 8.0 Hz, 2H), 1.55 (t, *J* = 7.7 Hz, 2H), 1.24 (m, 24H) and 0.81 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm): 170.5, 168.5, 162.2, 147.9, 143.9, 136.5, 131.3, 130.4, 127.9, 124.9, 120.5, 119.5, 117.8, 111.6, 33.8, 31.9, 31.4, 29.7 (4C), 29.7 (2C), 29.6, 29.5, 29.4, 29.4, 22.7 and 14.1.

2-Acetoxy-6-pentadecylbenzoic acid (**35**): using **30** and acetic acid as starting materials, this compound was obtained as white solid (86 mg, 32 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.40 (t, *J* = 7.9 Hz, 1H), 7.16 (d, *J* = 7.7 Hz, 1H), 6.99 (d, *J* = 8.1 Hz, 1H), 2.77 (m, 2H), 2.29 (s, 3H), 1.62 (p, *J* = 7.4 Hz, 2H), 1.25 (m, 24H) and 0.87 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm): 171.5, 169.4, 148.6, 143.6, 131.3, 127.5, 120.6, 115.8, 33.9, 31.9, 31.5, 29.7 (4C), 29.7 (2C), 29.6, 29.6, 29.4, 29.4, 22.7, 20.9 and 14.1.

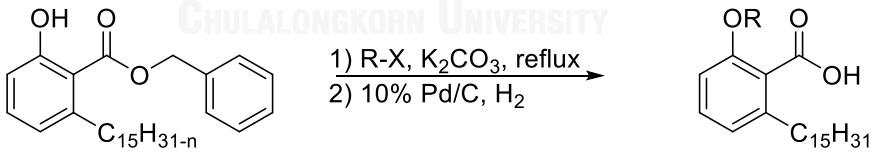
2-Butanoyloxy-6-pentadecylbenzoic acid (**36**): using **30** and butanoic acid as starting materials, this compound was obtained as white solid (72 mg, 25 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.38 (t, *J* = 7.9 Hz, 1H), 7.14 (d, *J* = 7.7 Hz, 1H), 6.98 (d, *J* = 8.1 Hz, 1H), 2.76 (t, *J* = 7.9 Hz, 2H), 2.53 (t, *J* = 7.4 Hz, 2H), 1.77 (m, 2H), 1.60 (m, 2H), 1.25 (m, 24H), 1.02 (t, *J* = 7.4 Hz, 3H) and 0.87 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm): 175.7, 171.9, 147.7, 143.4, 135.2, 122.7, 120.5, 115.8, 36.4, 36.1, 32.0, 31.4, 29.7 (4C), 29.7 (2C), 29.6, 29.5, 29.4, 29.4, 22.7, 18.3, 14.1 and 13.6.

2-Octanoyloxy-6-pentadecylbenzoic acid (**37**): using **30** and octanoic acid as starting materials, this compound was obtained as white solid (148 mg, 46 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.39 (t, *J* = 7.9 Hz, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 6.99 (d, *J* = 8.1 Hz, 1H), 2.79 (t, *J* = 7.9 Hz, 2H), 2.56 (t, *J* = 7.5 Hz, 2H), 1.75 (m, 2H), 1.63 (m, 2H), 1.27 (m, 32H) and 0.88 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm): 172.1, 171.7, 148.5, 143.4, 135.2, 122.7, 120.5, 115.8, 36.4, 34.2, 33.8, 31.9, 31.6, 31.4, 29.7 (4C), 29.7 (2C), 29.6, 29.5, 29.4, 29.4, 24.8, 24.7, 22.7, 22.6, 14.1 and 14.0.

2-Dodecanoyloxy-6-pentadecylbenzoic acid (**38**): using **30** and lauric acid as starting materials, this compound was obtained as white solid (203 mg, 56 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.38 (t, *J* = 7.9 Hz, 1H), 7.14 (d, *J* = 7.7 Hz, 1H), 6.97 (d, *J* = 8.1 Hz, 1H), 2.76 (t, *J* = 7.9 Hz, 2H), 2.54 (t, *J* = 7.5 Hz, 2H), 1.72 (m, 2H), 1.60 (m, 2H), 1.38 (m, 40H) and 0.87 (t, *J* = 6.7 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm): 176.1, 172.1, 148.6, 143.4, 135.2, 122.7, 120.5, 115.8, 36.4, 34.2, 34.1, 33.8, 32.0, 31.9, 31.4, 29.7 (4C), 29.7 (2C), 29.6, 29.5, 29.4, 29.4, 29.3, 29.2, 29.1, 29.1, 24.7, 22.7 (2C) and 14.1 (2C).

2.8.2 Ethers of anacardic acid

The mixture of benzyl 2-hydroxy-6-pentadecyl(alkenyl) benzoate **30** (1 eq), anhydrous K_2CO_3 (5 eq) and alkyl halide (24 eq) in acetone was refluxed overnight. The completed reaction was extracted with EtOAc and water. The organic layer was dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by silica gel column eluting with hexane/EtOAc to achieve ether product. The obtained ether (1 eq) in EtOAc (5 mL) was stirred under H_2 balloon with 10% Pd/C (0.3 eq) for 24 h. The catalyst was removed by filtering through a celite bed. The filtrate was evaporated under vacuum to obtain product which was purified by silica gel column eluting with hexane/EtOAc to afford the target products **39-42** [29].



30

*X= Br or I

39 R= CH_3-
40 R= C_2H_5-
41 R= C_4H_9-
42 R= C_8H_{17}-

2-Methoxy-6-pentadecylbenzoic acid (**39**) [26]: using **30** and iodomethane as starting materials, this compound was obtained as white solid (72 mg, 29 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.30 (t, *J* = 8.0 Hz, 1H), 6.88 (d, *J* = 7.7 Hz, 1H), 6.80 (d, *J* = 8.3 Hz, 1H), 3.80 (s, 3H), 2.78 (t, *J* = 7.9 Hz, 2H), 1.60 (m, 2H), 1.41–1.13 (m, 24H) and 0.88 (t, *J* = 6.6 Hz, 3H).

2-Ethoxy-6-pentadecylbenzoic acid (**40**) [28]: using **30** and ethyl bromide as starting materials, this compound was obtained as white solid (101 mg, 39 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.30 (t, *J* = 8.0 Hz, 1H), 6.88 (d, *J* = 7.7 Hz, 1H), 6.80 (d, *J* = 8.3 Hz, 1H), 4.14 (q, *J* = 7.0 Hz, 2H), 2.78 (t, *J* = 7.9 Hz, 2H), 1.60 (m, 2H), 1.45 (t, *J* = 7.0 Hz, 3H), 1.41–1.13 (m, 24H) and 0.88 (t, *J* = 6.6 Hz, 3H).

2-Butyloxy-6-pentadecylbenzoic acid (**41**): using **30** and 1-bromobutane as starting materials, this compound was obtained as white solid (49 mg, 17 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.30 (t, *J* = 8.0 Hz, 1H), 6.88 (d, *J* = 7.7 Hz, 1H), 6.81 (d, *J* = 8.3 Hz, 1H), 4.08 (t, *J* = 6.4 Hz, 2H), 2.79 (t, *J* = 7.9 Hz, 2H), 1.80 (m, 2H), 1.60 (p, *J* = 7.5 Hz, 2H), 1.49 (m, 2H), 1.41–1.13 (m, 24H), 0.97 (t, *J* = 7.4 Hz, 3H) and 0.88 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm): 171.1, 156.4, 143.4, 131.0, 122.4, 121.7, 109.8, 69.0, 34.0, 31.9, 31.4, 31.1, 29.7 (6C), 29.6 (2C), 29.5, 29.4, 22.7, 19.2, 14.1 and 13.8.

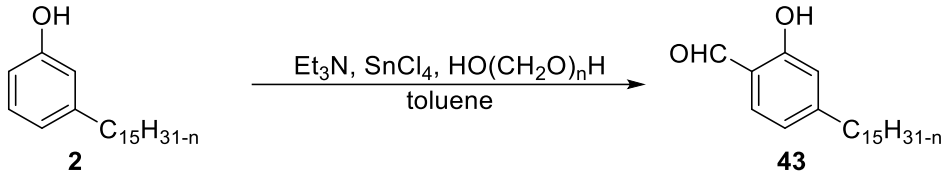
2-(Octyloxy)-6-pentadecylbenzoic acid (**42**): using **30** and 1-bromooctane as starting materials, this compound was obtained as white solid (96 mg, 30 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.29 (t, *J* = 8.0 Hz, 1H), 6.86 (d, *J* = 7.7 Hz, 1H), 6.80 (d, *J* = 8.3 Hz, 1H), 4.05 (t, *J* = 6.6 Hz, 2H), 2.77 (t, *J* = 8.0 Hz, 2H), 1.81 (m, 2H), 1.59 (q, *J* = 7.6 Hz, 2H), 1.44 (m, 2H), 1.41–1.13 (m, 32H), and 0.88 (dt, *J* = 7.3, 3.7 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm): 171.2, 156.4, 143.4, 131.0, 122.4, 121.7, 109.8, 69.3, 34.0, 31.9, 31.8, 31.4, 29.7 (6C), 29.6 (2C), 29.5, 29.4, 29.3, 29.2, 29.1, 25.9, 22.7, 22.6, 14.1 and 14.0.

2.9 Synthesis of cardanol derivatives

2.9.1 Synthesis of formylated cardanol

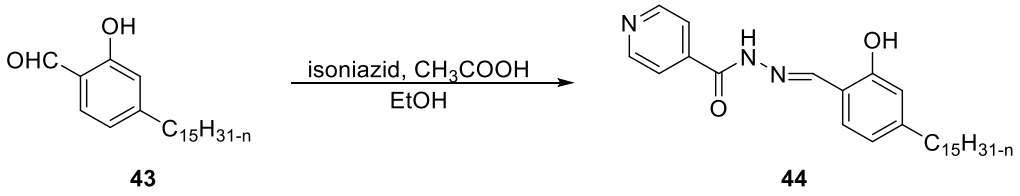
Et_3N (0.75 eq) and $SnCl_4$ (0.1 eq) were added to a solution of cardanol **2** (1 eq) in toluene (40 mL). The mixture was stirred under N_2 atmosphere at room temperature for 1 h then paraformaldehyde (2.75 eq) was added. The reaction was stirred at 100 °C for 8 h. The completed reaction was cooled down to room temperature, poured into water, and acidified to pH 2 with 10% HCl. The mixture was extracted with EtOAc and washed with water and brine, respectively. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain crude product which was

purified by silica gel column to afford a pale yellow liquid **43** [45] (4.69 g, 72 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 11.06 (s, 1H), 9.83 (s, 1H), 7.45 (d, *J* = 7.8 Hz, 1H), 6.84 (d, *J*= 8.0 Hz, 1H), 6.81 (s, 1H), 5.81-5.01 (-C**H**=CH_2 and -CH=C**H$_2$** on alkyl chain) and 2.95-0.89 (**H** on alkyl chain).



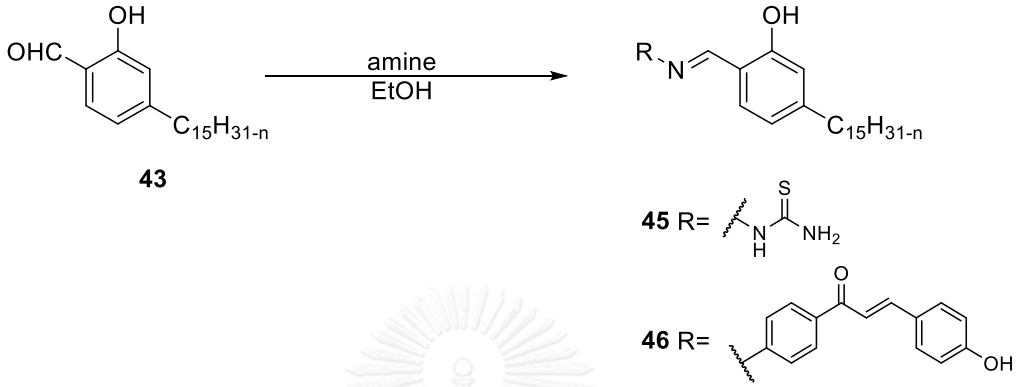
2.9.2 Imine derivatives

(E)-N'-(2-Hydroxy-4-pentadecyl(alkenyl)benzylidene)isonicotinohydrazide (**44**): formylated cardanol **43** (1 eq) and isoniazid (2 eq) were dissolved with EtOH (10 mL) and a few drops of glacial acetic acid was added. The mixture was refluxed for 18 h and cooled down to room temperature. The reaction was poured into water and extracted with EtOAc (3 times). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain crude product which was purified by column chromatography to give an orange solid [27] (75 mg, 27 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 12.26 (s, 1H), 11.06 (s, 1H), 8.77 (d, *J* = 5.0 Hz, 2H), 8.59 (s, 1H), 7.81 (m, 2H), 7.45 (d, *J* = 8.2 Hz, 1H), 6.73 (m, 2H), 5.01-5.81 (-C**H**=CH_2 and -CH=C**H$_2$** on alkyl chain) and 0.81-2.48 (**H** on alkyl chain).



(E)-2-(2-hydroxy-4-pentadecyl(alkenyl)benzylidene)hydrazine-1-carbothioamide (**45**): formylated cardanol **43** (1 eq) and thiosemicarbazide (2 eq) were dissolved with EtOH (20 mL). The mixture was refluxed for 24 h and cooled down to room temperature. Then the reaction was poured into water and extracted with EtOAc (3 times). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain crude product which was purified by

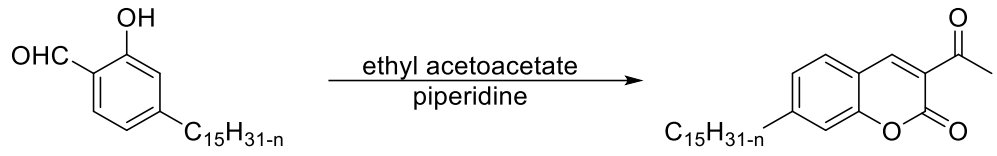
silica gel column to obtain an orange solid (220 mg, 88 %yield); ^{1}H NMR (DMSO-d_6) δ_H (ppm): 11.31 (s, 1H), 9.74 (s, 1H), 8.29 (s, 1H), 8.07 (s, 1H), 7.85 (s, 1H), 7.77 (d, *J* = 7.9 Hz, 1H), 6.63 (m, 2H), 5.01-5.81 (-C**H**=CH_2 and -CH=C**H$_2$** on alkyl chain) and 0.81-2.48 (**H** on alkyl chain).



(*E*)-1-(4-(((*E*)-2-hydroxy-4-pentadecyl(alkenyl)benzylidene)amino) phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**46**) was synthesized by the same method above, using **43** and (*E*)-1-(4-aminophenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one as starting materials, obtaining a yellow solid (176 mg, 52% yield); ^{1}H NMR (DMSO-d_6) δ_H (ppm): 12.81 (s, 1H), 10.12 (s, 1H), 8.97 (d, *J* = 1.6 Hz, 1H), 8.21 (dd, *J* = 8.7, 1.7 Hz, 2H), 7.73 (m, 4H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.51 (d, *J* = 7.6 Hz, 2H), 6.2 (m, 4H), 5.01-5.81 (-C**H**=CH_2 and -CH=C**H$_2$** on alkyl chain) and 0.81-2.48 (**H** on alkyl chain).

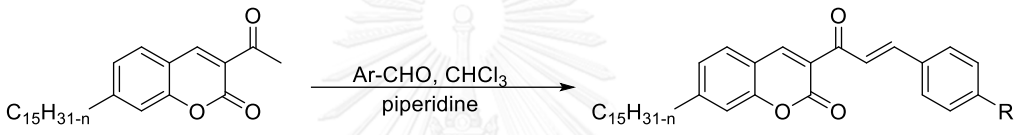
2.9.3 Synthesis of 3-acetylcoumarin-cardanol and its derivatives

Formylated cardanol **43** (1 eq), ethyl acetoacetate (1.2 eq), and a few drops of piperidine were stirred at room temperature for 30 min. The reaction was neutralized with 1M HCl, extracted with EtOAc (3 times) and washed with water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain crude product which was purified by silica gel column to obtain a yellow solid **47** [46] (1.92 g, 64 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 8.49 (s, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.14 (d, *J* = 7.8 Hz, 2H), 4.96-5.81 (-C**H**=CH_2 and -CH=C**H$_2$** on alkyl chain), 2.70 (s, 3H) and 0.89-2.95 (**H** on alkyl chain).



43 **47**

3-Acetylcoumarin-cardanol **47** (1 eq) and selected aromatic aldehyde (1 eq) were dissolved in $CHCl_3$ (1-1.5 mL) and a catalytic amount of piperidine (50 µL) was added. The reaction mixture was refluxed for 24 h. After completion of reaction, the solvent was evaporated under reduced pressure and the crude product was purified by silica gel column eluting with hexane/EtOAc to afford the desired compounds **48-50**.



47

48 R= OH-
49 R= OMe-
50 R= $C(CH_3)_3$-

(E)-3-(3-(4-Hydroxyphenyl)acryloyl)-7-pentadecyl(alkenyl)-2H-chromen-2-one (**48**): the compound was prepared as described above, using **47** and 4-hydroxybenzaldehyde, obtaining as an orange solid (100 mg, 52 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 8.61 (d, *J* = 1.6 Hz, 1H), 7.86 (t, *J* = 1.7 Hz, 1H), 7.59 (td, *J* = 6.7, 3.4 Hz, 3H), 7.14–7.25 (m, 3H), 6.92 (dd, *J* = 8.7, 1.8 Hz, 2H), 5.00-5.70 (-C**H**=CH_2 and -CH=C**H$_2$** on alkyl chain) and 0.83-2.71 (**H** on alkyl chain).

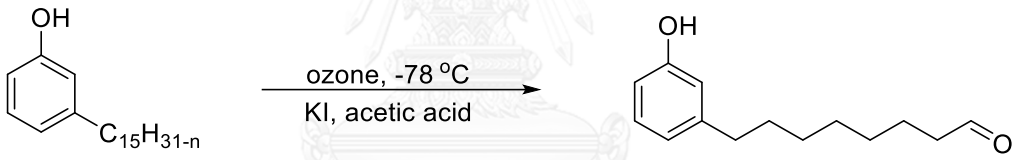
(E)-3-(3-(4-Methoxyphenyl)acryloyl)-7-pentadecyl(alkenyl)-2H-chromen-2-one (**49**): the product was synthesized by the same method above, using **47** and 4-methoxybenzaldehyde, giving as a yellow solid (243 mg, 47 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 8.57 (s, 1H), 7.86 (s, 2H), 7.64 (d, *J* = 8.3 Hz, 2H), 7.56 (d, *J* = 7.9 Hz, 1H), 7.14-7.22 (m, 2H), 6.8-6.96 (m, 2H), 3.85 (d, *J* = 5.0 Hz, 3H), 5.00-5.80 (-C**H**=CH_2 and -CH=C**H$_2$** on alkyl chain) and 0.92-2.74 (**H** on alkyl chain).

(*E*)-3-(3-(4-(*tert*-Butyl)phenyl)acryloyl)-7-pentadecyl(alkenyl)-2H-chromen-2-one (**50**): the product was prepared as described above, starting **47** and 4-*tert*-butylbenzaldehyde, obtaining as a yellow solid (281 mg, 41 %yield); ^{1}H NMR ($CDCl_3$)

δ_H (ppm): 8.57 (s, 1H), 7.92 (m, 2H), 7.62 (m, 2H), 7.56 (d, *J* = 7.9 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.18 (m, 2H), 4.9-5.80 (-C**H**=CH_2 and -CH=C$\mathbf{H_2}$ on alkyl chain) and 0.91-2.70 (**H** on alkyl chain and three methyl group of 4-*tert*-butyl benzaldehyde).

2.9.4 Synthesis of 8-(3-hydroxyphenyl)octanal and its derivatives

Cardanol **2** was dissolved in MeOH: CH_2Cl_2 (1:4), and stirred under ozone at -78 °C for 2 h. After that KI and acetic acid were added into the reaction and continued stirring at room temperature for 1 h. The reaction was extracted with 10 % thiosulfate solution and water. The organic solution was evaporated under reduced pressure. The crude product was purified by silica gel column to afford a product **51** [47] as a yellow liquid [27, 47] (257.6 mg, 34 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 9.72 (d, *J* = 2.1 Hz, 1H), 7.09 (t, *J* = 7.7 Hz, 1H), 6.68 (m, 3H), 2.51 (t, *J* = 7.8 Hz, 2H), 2.40 (td, *J* = 7.3, 2.0 Hz, 2H), 1.58 (m, 4H) and 1.27 (m, 6H).



2 **51**

(*E*)-*N*'-(8-(3-Hydroxyphenyl)octylidene)isonicotinohydrazide (**52**): 8-(3-hydroxyphenyl)octanal **51** (1 eq) and isoniazid (2 eq) were dissolved with EtOH (10 mL) and a few drops of glacial acetic acid was added. The mixture was refluxed for 24 h and cooled down to room temperature. The reaction was poured into water and extracted with EtOAc (3 times) [27]. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain crude product which was purified by silica gel column to give an orange solid (87.6 mg, 55 %yield); ^{1}H NMR ($CDCl_3$) δ_H (ppm) 11.63 (s, 1H), 9.24 (s, 1H), 8.72 (m, 2H), 7.72 (m, 3H), 7.01 (t, *J* = 7.7 Hz, 1H), 6.54 (s, 3H), 2.43 (t, *J* = 7.9 Hz, 2H), 2.25 (m, 2H), 1.48 (m, 4H) and 1.24 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm): 161.3, 157.1, 154.1, 150.1, 143.7, 140.6, 129.1, 121.4, 119.0, 115.1, 112.5, 35.1, 31.9, 30.7, 28.5, 28.5, 28.5 and 25.8.

(*E*)-2-(8-(3-hydroxyphenyl)octylidene)hydrazine-1-carbothioamide (**53**) was synthesized by the same method above but glacial acetic acid was not used, using **51** and thiosemicarbazide, obtaining a yellow solid (82.1 mg, 41 %yield); ^{1}H NMR (DMSO-d_6) δ_H (ppm): 11.00 (s, 1H), 9.24 (s, 1H), 8.61 (s, 1H), 7.99–7.85 (m, 1H), 7.31-7.47 (m, 2H), 7.01 (t, *J* = 7.7 Hz, 1H), 6.54 (q, *J* = 8.6, 8.2 Hz, 2H), 2.43 (t, *J* = 7.7 Hz, 4H), 2.14 (d, *J* = 7.5 Hz, 2H) and 1.27 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm): 177.6, 157.2, 147.6, 143.6, 129.0, 118.9, 115.1, 112.5, 35.1, 31.6, 30.8, 29.0, 28.5, 25.8 and 24.3.

2.10 Biological activity

2.10.1 Antibacterial activity

Test strains: *Propionibacterium acnes*, *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus sobrinus*, and *Salmonella typhi*

Medium: nutrient agar (NA) medium was prepared by mixing agar (15 g) and nutrient (8 g) in water (1,000 mL). The mixture was heated and stirred to homogenize, then sterilized in autoclave at 121 °C for 1 h and cooled down to room temperature.

Nutrient broth (NB) medium was prepared by dissolving nutrient (8 g) with water (1,000 mL) and sterilized in autoclave at 121 °C for 1 h and cooled down to room temperature.

Agar diffusion assay: nutrient agar medium was heated and poured into a sterile Petri dish. The agar is allowed to set and harden at room temperature, then holes are cut using a sterile cork borer in the center of area. Agar plugs were removed and the bacterial culture was swapped on agar. The testing sample 30 µL (dissolved in acetone) was pipetted into holes. The plates were incubated at 37 °C for 24 h. After incubation, the diameter of the zones of inhibition was measured [7].

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): To all wells were added 50 µL of nutrient broth (NB) and the serial dilutions, six concentrations, were performed using a multichannel pipette. Tips were discarded after using such that each well had 50 µL of the

compounds in serially descending concentrations. After that, each well was added 30 µL NB and bacterial suspension 10 µL (1 to $2x10^8$ CFU/mL obtained from the 0.5 McFarland standards) was added by pipette to each well. Each plate had a set of controls: a column with a broad-spectrum antibiotic as positive control (chloramphenicol in serial dilution), a column with all solutions with the exception of the test compound (EtOH), and a column with all solutions with the exception of the bacterial solution adding 10 µL of nutrient broth instead. The plates were prepared in triplicate and incubated at 37 °C for 18-24 h. The colorimetric assay, 30 µL of 0.01% resazurin as oxidation-reduction indicator was added into each well to give blue color, then left for 10 min. the MIC was the lowest concentration of well which still had blue color. All blue color solution 10 µL were transferred onto the new plate contain agar medium and then swapped. The plates were incubated at 37 °C for 18-24 h. The MBC was the lowest concentration that bacteria did not grow [7].

2.10.2 Antioxidant assay

2.10.2.1 Scavenging activity on DPPH radicals

First, 1 mL of 100 mM acetate buffer (pH 5.5), 1.87 mL of EtOH and 0.1 mL of 3 mM DPPH solution in EtOH were put into a test tube and measured the absorbance at 517 nm for starting point. Then 0.03 mL of the sample solution in DMSO (using DMSO as a control) were added to the tube, the mixture was incubated for 20 min at 25 °C and measured the absorbance at 517 nm again. The decrease of the absorbance was calculated and expressed as % scavenged DPPH molecules [22].

2.10.2.2 Scavenging activity for the O_2^- generated by the PMS-NADH system

The mixture consisted of 2.82 mL of 40 mM Na_2CO_3 buffer (containing 0.1 mM EDTA pH 10.0), 0.03 mL of 0.5% bovine serum albumin, 0.03 mL of 2.5 mM nitro blue tetrazolium, 0.06 mL of sample solution (10 mM sample in DMSO) and 0.03 mL of 7.8 mM NADH. The reaction starts after adding of 0.03 mL of 155 µM phenazine methosulfate and was measured the absorbance at 560 nm for 60 sec. The control was used 0.06 mL DMSO instead of sample. The reaction rate was calculated from the

proportional increase of the absorbance and scavenging activity of sample was expressed as the inhibition percentage [48].

2.10.2.3 Assay of hydrogen peroxide generated by the PMS-NADH system

The reaction mixture consisted of 5.7 mL of 40 mM Na_2CO_3 buffer containing 0.1 mM EDTA (pH 10.0), 0.12 mL of 10 mM sample (used DMSO as control), 0.06 mL of 0.5% bovine albumin serum and 0.06 mL of 7.8 mM NADH. The reaction started by the addition of 0.06 mL of 155 µM phenazine methosulfate. After 0 and 120 s, 1.5 mL of the reaction mixture was put into 1.5 mL of reagent solution (64.5 µM *meso*-tetra*kis*(4-methyl-pyridyl)-porphinatoiron(III) pentachloride, 13.3 mM *N,N*-dimethylaniline, 2.76 mM 3-methyl-2-benzothiazolinone hydrazone, and 1.0 mM EDTA in 0.13 M HCl) for stopping the reaction and determination of hydrogen peroxide. The mixture was incubated at 25 °C for 1 h and measured the absorbance at 590 nm [48].

2.10.3 Anti-inflammatory assay

2.10.3.1 Cell culture

The RAW 264.7 mouse macrophage cells were cultured in DMEM supplemented with 10% FBS at 37 °C under a humidified atmosphere of 5% CO_2.

2.10.3.2 MTT assay for testing cell viability

RAW 264.7 cells were mechanically scraped, plated at a density of 1.5×10^4 cells/mL onto 96-well plates containing 100 µL of DMEM medium, and incubated in a 37 °C, 5% CO_2 incubator overnight. After overnight incubation, the cells were treated with samples in the presence or absence of LPS (2 µg/mL) according to the experimental design. After 24 h, the medium was removed and replaced with 180 µL of fresh medium. Then, 20 µL of 5 mg/mL MTT was added to each well and the cells were further incubated for an additional 3 h. MTT was removed and cells were lysed with 100 µL/well DMSO. The optical density was measured at 540 nm on a microplate reader [49].

2.10.3.3 Measurement of nitric oxide production

To investigate the effect of samples on nitric oxide from LPS-treated cells, RAW 264.7 cells (1.5×10^4 cells/mL) seeded into 96-well plates were treated with samples and 2 µg/mL LPS for 24 h at 37 °C in 5% CO_2 incubator. Cell-free supernatants 100 µL were collected and reacted with 100 µL Griess solution (0.1 g sulfanilamide + 0.01 g *N*-1-napthylethylenediamine dihydrochloride 625 µL phosphoric acid in 20 mL water). The mixture was incubated at 37 °C for 10 min and measured the absorbance at 540 nm on microplate reader. The amount of nitrite in the samples was calculated from a sodium nitrite standard curve.

CHAPTER III

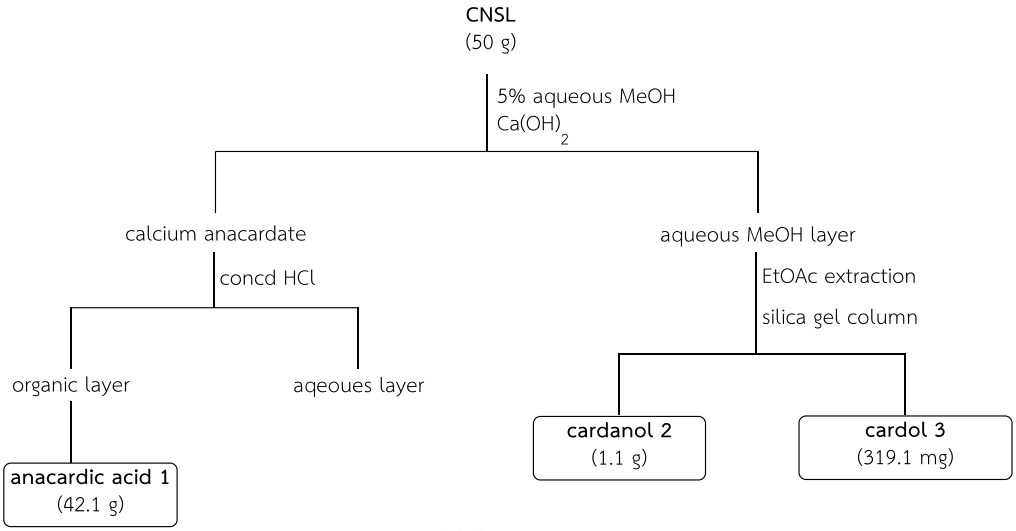
RESULTS AND DISCUSSION

The separation of cashew nut shell liquid (CNSL) was carried out by three methods: extraction, decarboxylation, and semi-prep HPLC. The separated components from semi-prep HPLC were tested on anti-inflammatory and antioxidant activities. The hydroxyl and carboxyl groups of anacardic acid **1** were modified to observe the effect of chain length using alkyl halide or alcohol. Benzoyl derivatives on hydroxyl were synthesized using benzoic acid derivatives. For cardanol **2**, the structures were transformed into imine and coumarin derivatives *via* formylated cardanol **43** with selected amines and ethyl acetoacetate, respectively. All synthesized derivatives were well-characterized and tested on antioxidant and bactericidal activities.

3.1 Separation of cashew nut shell liquid (CNSL)

3.1.1 By extraction method

Anacardic acid **1** was obtained from acidification of calcium anacardate with conc. HCl. Cardanol **2** and cardol **3** were isolated by silica gel column from the filtrate left from the filtration of calcium anacardate (Scheme 3.1). For this method, anacardic acid **1** was obtained as a major product, while cardanol **2** and cardol **3** were minor ones. All substances were characterized by ^{1}H NMR. The spectra showed the same pattern with the signals of side chain around 1-6 ppm and aromatic signals in the region of 6-8 ppm. The ^{1}H NMR signals on aromatic of three substances showed slightly different appearance. The signals of anacardic acid **1** displayed triplet at 7.35 ppm of one proton and two doublet of two protons at 6.86 and 6.77 ppm, respectively. This pattern was usually observed for aromatic protons of anacardic acid **1** in Figure 3.1. The proton signals of cardanol **2** appeared three signals in the pattern of triplet, doublet and doublet at 7.14, 6.76 and 6.65 ppm, respectively (Figure 3.2). Whereas cardol **3** showed two proton signals of aromatic at 6.24 ppm as two proton-doublet and 6.18 ppm of one proton-triplet in Figure 3.3.



Scheme 3.1 The separation of anacardic acid **1**, cardanol **2**, and cardol **3** from CNSL

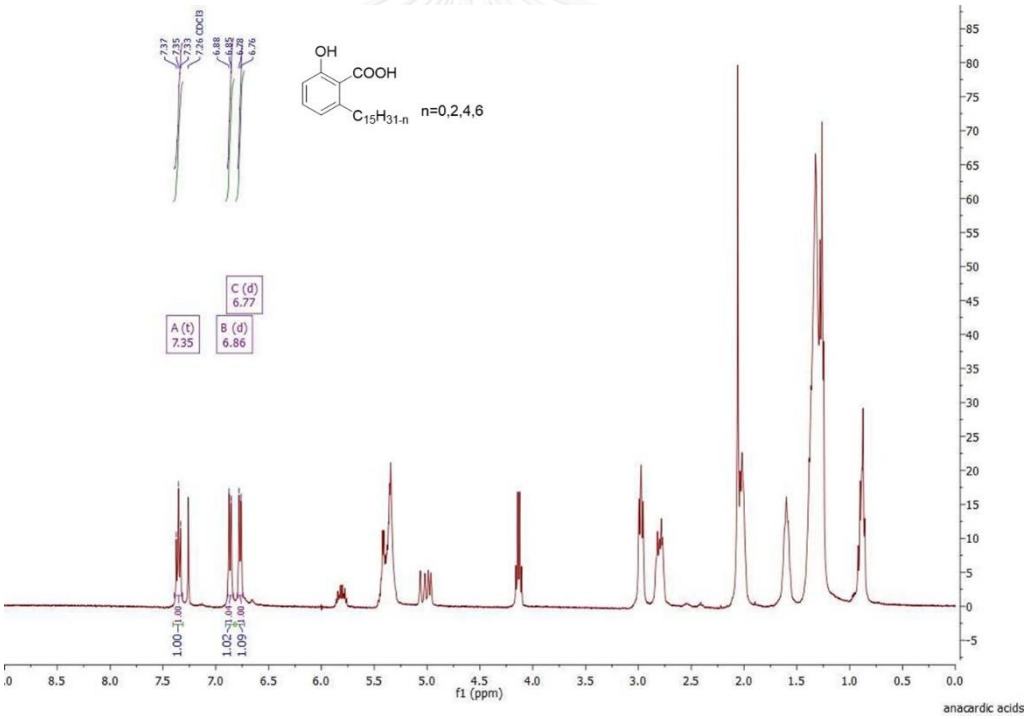


Figure 3.1 The ^{1}H NMR spectrum ($CDCl_3$) of anacardic acid **1**

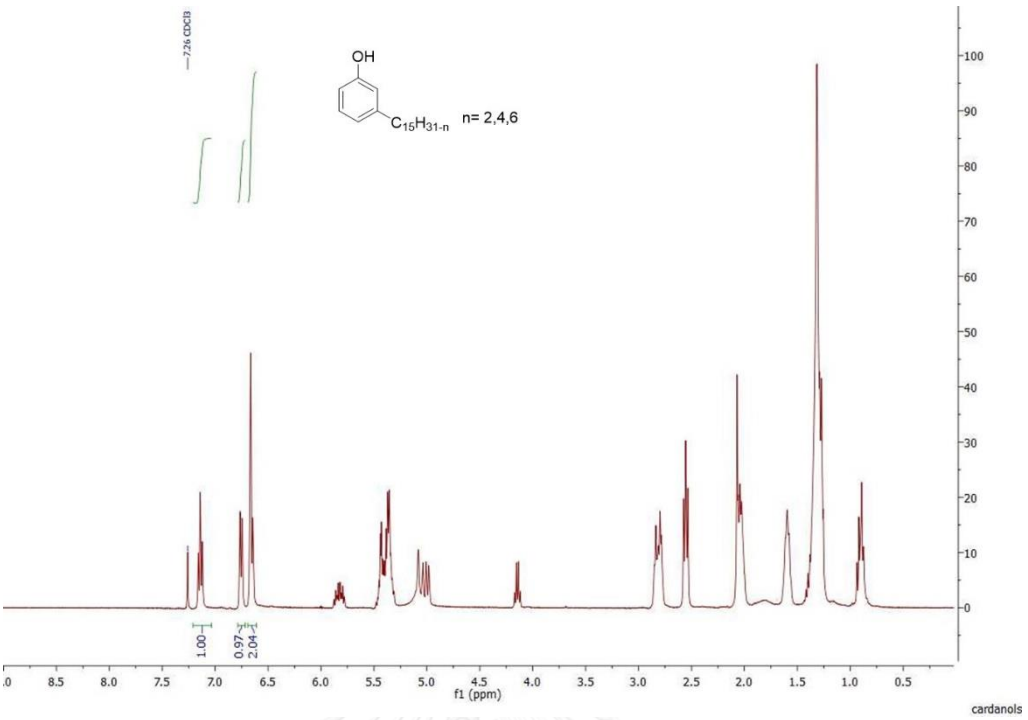


Figure 3.2 The ^{1}H NMR spectrum ($CDCl_3$) of cardanol **2**

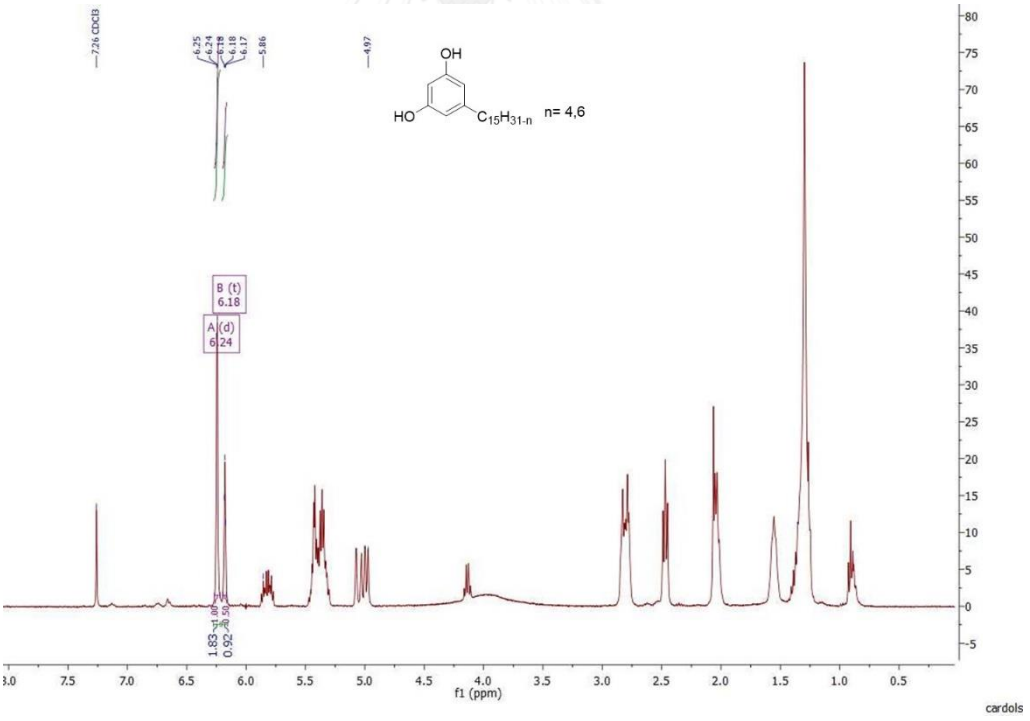
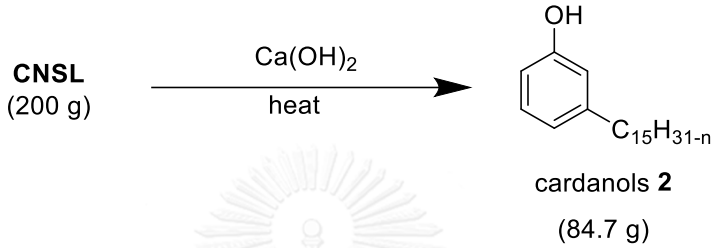


Figure 3.3 The ^{1}H NMR spectrum ($CDCl_3$) of cardol **3**

3.1.2 By decarboxylation method

Since CNSL contains anacardic acid **1** as a major product, the simplest way to prepare cardanol **2** can be achieved by decarboxylation of anacardic acid **1** [34]. CNSL was thus distillated with $Ca(OH)_2$ under reduced pressure to yield cardanol **2**. For this method, cardanol **2** is only the product obtained (84.7 g). The product was characterized by ^1H NMR (Figure 3.2).



3.1.3 By semi-preparative HPLC

The HPLC chromatograms of anacardic acid **1**, cardanol **2** and cardol **3** are presented in Figure 3.4.

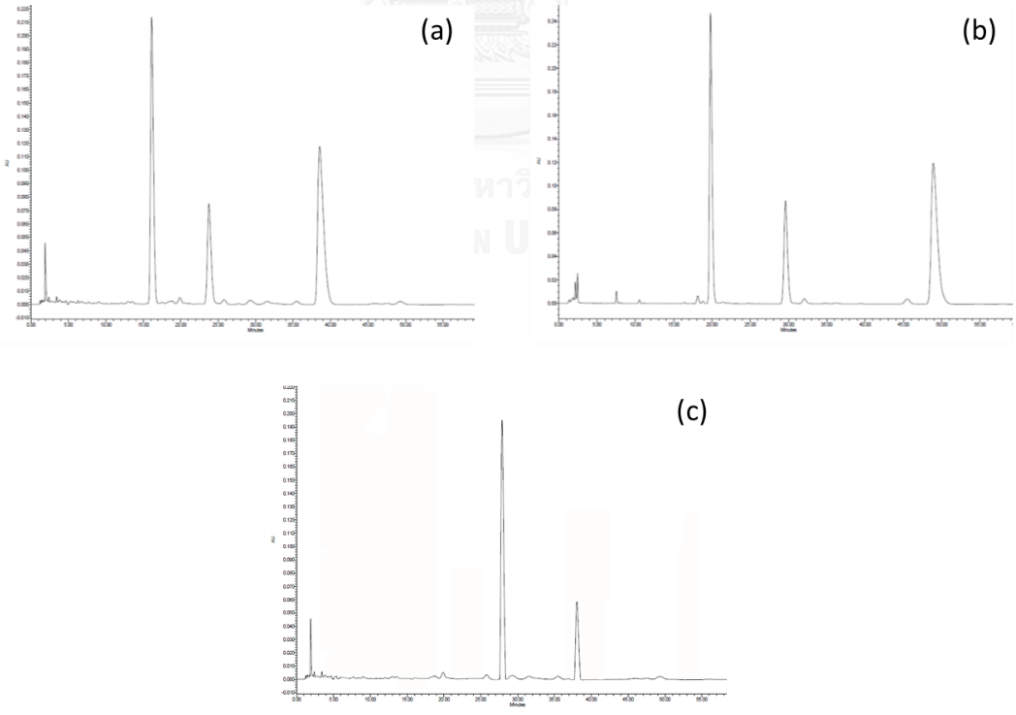


Figure 3.4 The HPLC chromatograms of a) anacardic acid **1**, b) cardanol **2**, c) cardol **3**

Using semi-prep HPLC with mobile phase consisting of CH_3CN–water–acetic acid (80:20:1) in isocratic mode with a photodiode array detector set at 278 nm, 4 components **4-7** could be separated from anacardic acid **1**. By the same method, the separation of cardanol **2** and cardol **3** furnished three compounds **9-11** and two compounds **14-15,** respectively. All isolated compounds were characterized by ^{1}H NMR [35, 36] and their structures are presented in Figure 3.5.

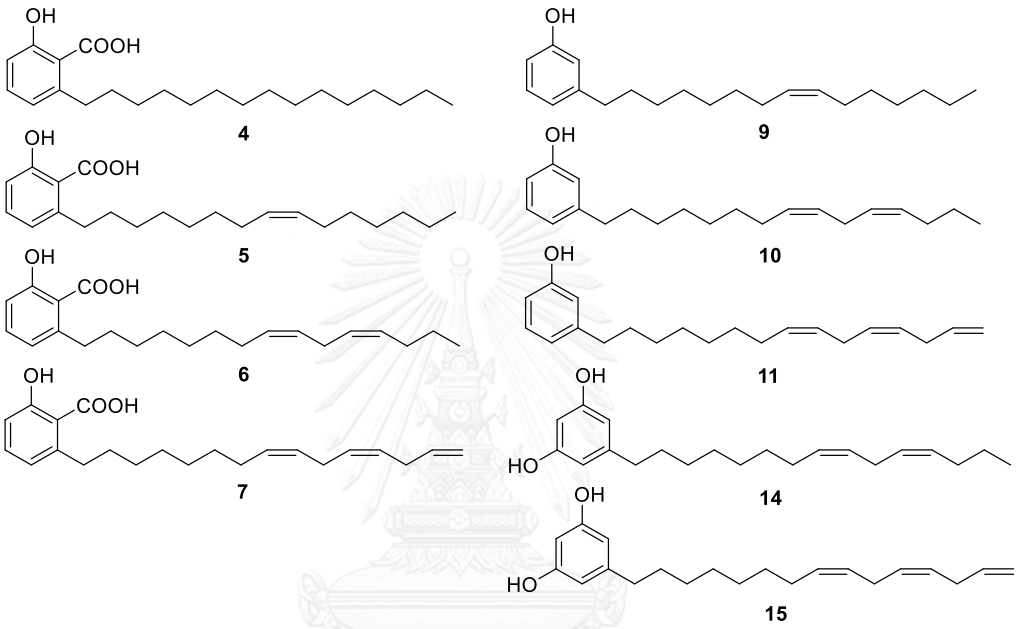


Figure 3.5 The structures of isolated components from anacardic acid **1**, cardanol **2** and cardol **3** by semi-prep HPLC

3.1.4 Hydrogenation of anacardic acid 1, cardanol 2 and cardol 3

Three saturated compounds: saturated anacardic acid **4**, saturated cardanol **8**, and saturated cardol **12** (Figure 3.6) were prepared *via* hydrogenation of corresponding starting materials using H_2 in the presence of 10% Pd/C [29]. All compounds were characterized by ^{1}H NMR.

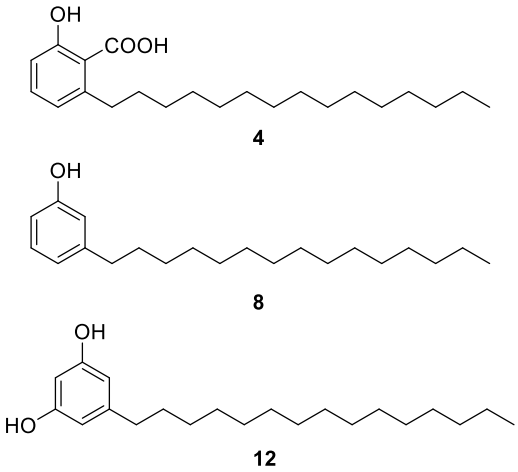


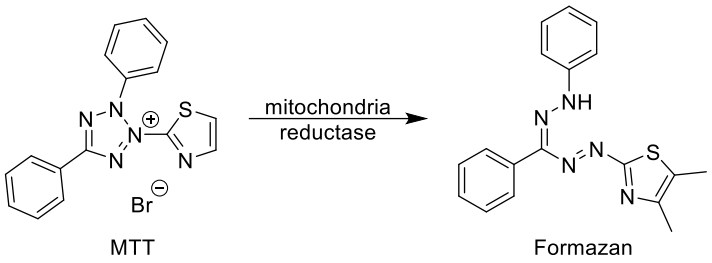
Figure 3.6 The structures of saturated anacardic acid **4**, cardanol **8** and cardol **12**

3.2 Biological activity studies of isolated compounds from CNSL and saturated constituents

As aforementioned, eight isolated constituents of CNSL **5-7, 9-11, 14-15** and three saturated compounds **4, 8** and **12** were evaluated for two selected biological activities: anti-inflammatory and antioxidant.

3.2.1 Anti-inflammatory activity

There are many ways to evaluate anti-inflammatory activity [50, 51]. The simple method is to measure NO production. The inflammatory of cells induces nitric oxide synthase (iNOS) generation, and then iNOS oxidizes L-arginine to produce L-citrulline and NO. Nowadays, LPS is the best recognized inducer for activating macrophage to produce NO that was assessed the inflammatory activity. In addition, MTT assay was used for investigating the viability of cells *via* reducing MTT to formazan by mitochondria reductase of active cells. The acceptance value for living cells should be more than 70% [52]. In this research, major constituents of CNSL were screened for cell viability by MTT assay and selected compounds were further tested on NO assay.



3.2.1.1 Preliminary study on MTT assay of anacardic acid 1, cardanol 2 and cardol 3

After incubation with anacardic acid **1**, cardanol **2** and cardol **3** in amount ranging from 12.5 to 50 µM (using DMSO as control), existing RAW 264.7 cells were determined by measuring the purple formazan at 560 nm. The %cell viability was calculated as shown in Figure 3.7.

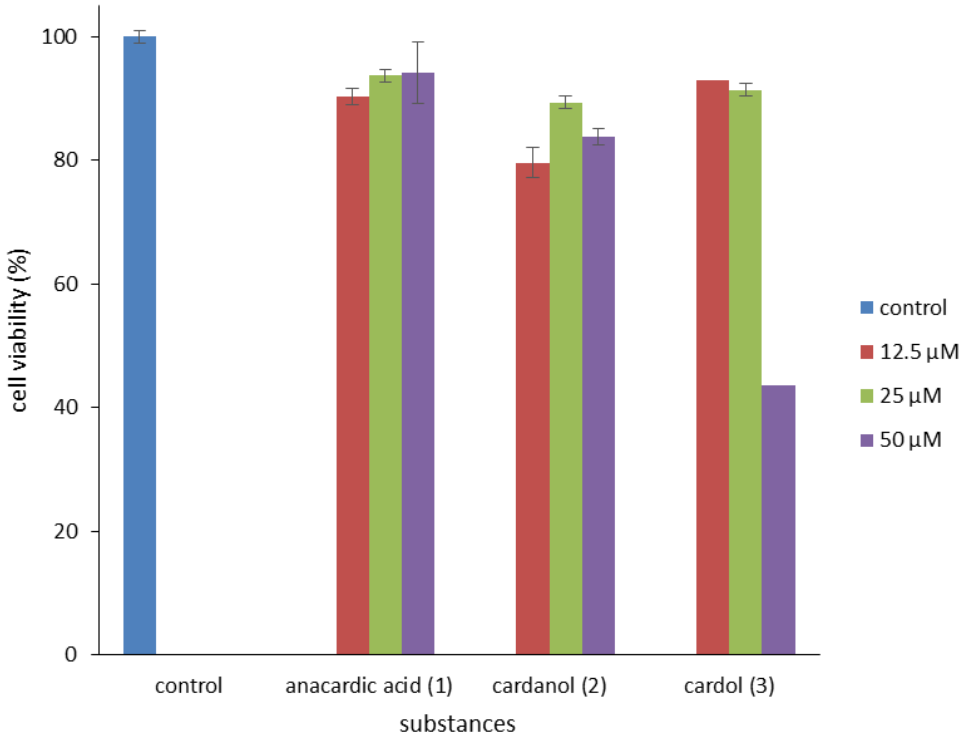


Figure 3.7 Effects of anacardic acid **1**, cardanol **2** and cardol **3** on the viability of RAW 264.7 cells by MTT assay. Data are presented as means ± SEM.

Figure 3.7 indicates that %cell viability of the systems incubated with anacardic acid **1** and cardanol **2** was 90-94 and 80-89, respectively. With the range of 12.5-50 μM, these two compounds revealed no cytotoxic on RAW 264.7 cells. For cardol **3**, at low concentration (< 50 μM) no cytotoxic was observed (91 and 92% cell viability for 12.5 and 25 μM). On the other hand, at concentration of 50 μM only 43% living cells were detected. This result stated that cardol **3** at 50 μM was toxic and not appropriate for further study. On the independent parallel study on MTT assay of LPS-stimulated cells (data not shown), no cytotoxicity was observed for anacardic acid **1** and cardanol **2**. So, these two substances **1** and **2** were selected for next study on the inhibition of NO production.

3.2.1.2 Inhibitory activity on NO production of anacardic acid 1 and cardanol 2

The inhibitory activity of anacardic acid **1** and cardanol **2** against NO production was evaluated. LPS-stimulated cells induced over-expression of iNOS resulting in inflammation creation. The generated NO complexed with Griess reagent was analyzed by UV-Vis at 540 nm as shown in Figure 3.8.

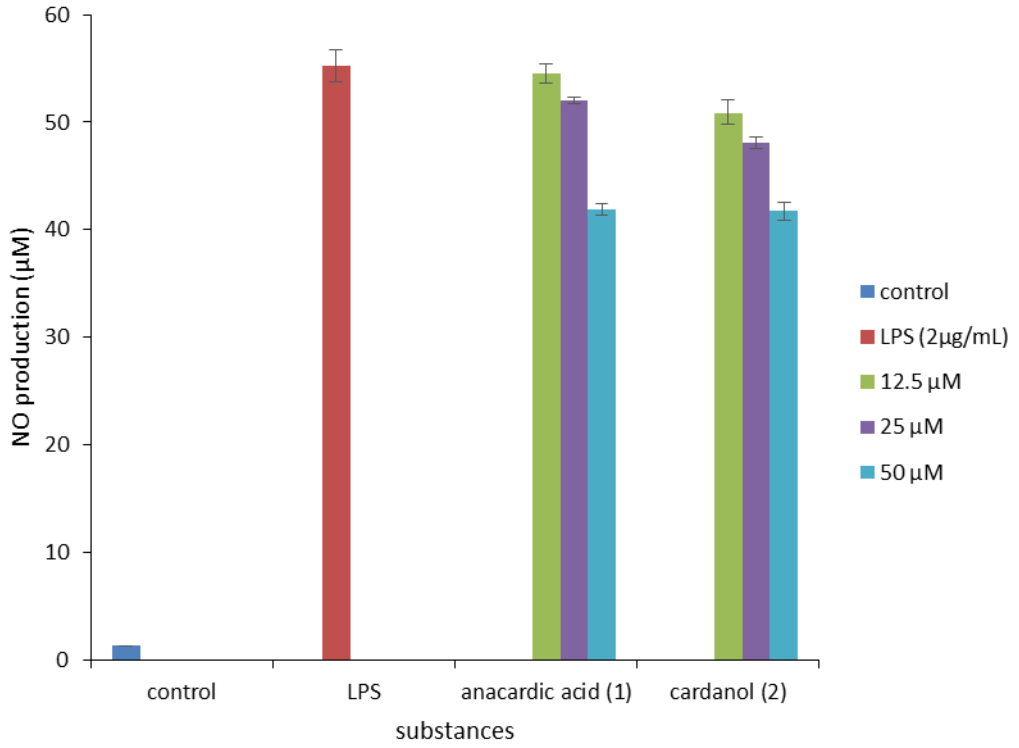
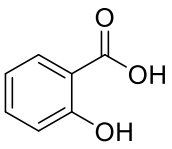
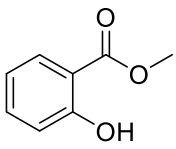


Figure 3.8 The inhibitory activity of NO production on LPS-stimulated cells treated with anacardic acid **1** and cardanol **2**

Both anacardic acid **1** and cardanol **2** were promising substances for further study since they displayed the positive trend for NO production reduction compared to LPS-stimulated cells (the highest NO production condition). According to previous literatures, salicylic acid and methyl salicylate showed anti-inflammatory activity. While the former inhibited the expression of iNOS stimulated by LPS and interferon gamma (IFN-**γ**) in alveolar macrophages of rats and in hepatocytes at the level of translation, Kim and co-workers reported that the latter also inhibited the NF**κ**B, a transcription factor regulating genes in inflammation [53-55]. Being possessed the same core structure as salicylic acid and methyl salicylate, anacardic acid **1** was further examined by separation into its constituents. Each constituent **4-7** was evaluated for cell viability and NO production.



salicylic acid



methyl salicylate

3.2.1.3 Determination on MTT assay and NO production of anacardic acid constituents 4-7

Each anacardic acid constituent **4-7** was examined on MTT assay and NO production and compared with salicylic acid and methyl salicylate at the concentration ranging of 12.5-100 μM. The results are presented in Figures 3.9 and 3.10.

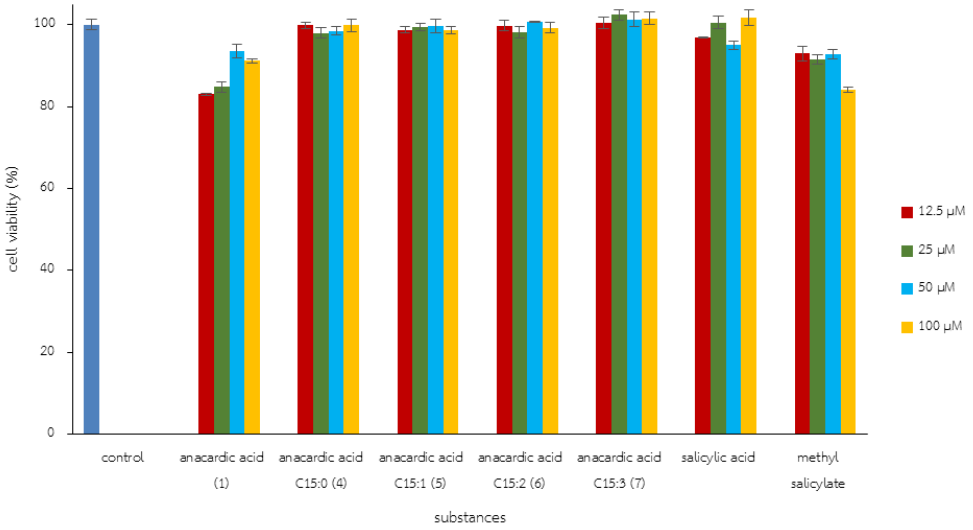


Figure 3.9 Effects of anacardic acid constituents **4-7** on the viability of RAW 264.7 cells by the MTT assay. Data are presented as means ± SEM

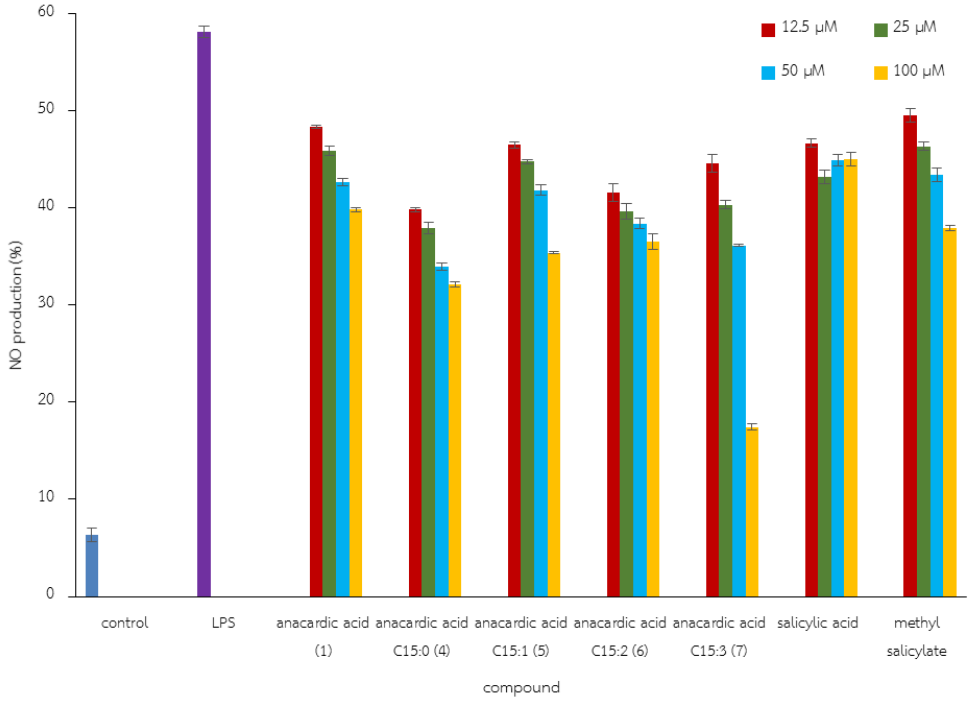


Figure 3.10 The inhibitory activity on NO production of LPS-stimulated cells when treated with anacardic acid constituents **4-7**, salicylic acid and methylsalicylate. Data are presented as means ± SEM

Four constituents of anacardic acids **4-7**, salicylic acid and methyl salicylate did not exhibit cytotoxicity with RAW 264.7 cells as shown in Figure 3.9. These results showed the same trend with MTT assay on LPS-stimulated cells. Then, all compounds were further examined on NO production inhibition. Figure 3.10 indicates that LPS-stimulated cells increased NO production by 9.7-fold (58 μM), as compared to control (6 μM). Significant NO production inhibition could be observed in the system containing four constituents of anacardic acid **4-7**, salicylic acid or methyl salicylate. It should be mentioned that with the range of 12.5–100 μM, salicylic acid and methyl salicylate could suppress the accumulation of NO production by 22-29% and 15-34%, respectively. Four constituents: anacardic acid $C_{15:0}$ **4**, $C_{15:1}$ **5** and $C_{15:2}$ **6** revealed this same trend by 20–45%, even better for anacardic acid $C_{15:3}$ **7** by 22-71%. To our best knowledge, this is the new findings for anacardic acid on NO production of RAW 264.7 cell macrophage. These results suggested that the side chain of anacardic acid

revealed a profound effect on NO production inhibition compared with salicylic acid and methyl salicylate at the same concentration. The number of double bond in side chain also displayed the influence on the activity. To illustrate this, three double bonds of anacardic acid **7** revealed better results than the others **4-6**. Interestingly, anacardic acid $C_{15:3}$ **7** at 100 μM showed better activity than salicylic acid and methyl salicylate. It should also be mentioned that the highest concentration of anacardic acid $C_{15:3}$ **7** in this experiment (100 μM) displayed excellent NO production inhibition with still no toxicity to cells.

3.2.1.4 Conclusion and suggestion for future work

All anacardic acid **1**, cardanol **2** and cardol **3** could decrease the NO production and did not affect on the viability of cells. In the future work, the constituents of anacardic acid will be explored on mechanism of anti-inflammatory in cytokine as tumor necrosis factor (TNF-α), interleukin 6 (IL-6) and interleukin 1 (IL-1β). For cardanol **2** and cardol **3**, the constituents of both compounds should be tested on anti-inflammatory.

3.2.2 Antioxidant activity

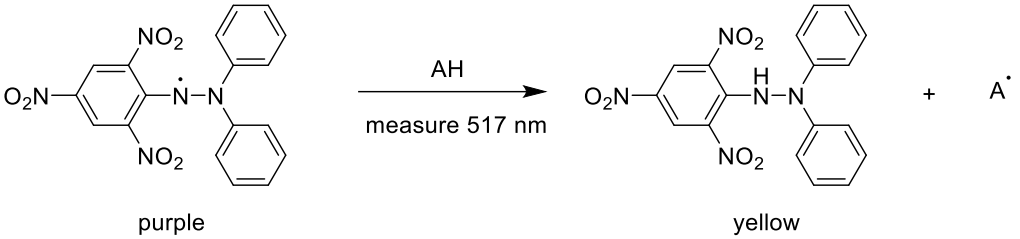
Antioxidant activity could be assessed using many protocols. Previously, antioxidant activities such as DPPH, hypoxanthine/xanthine oxidase, lipoxygenase, superoxide anion and uric acid generated by xanthine oxidase [23, 24] of CNSL constituents have been addressed. Two antioxidant assays for CNSL: DPPH, and superoxide anion generated by PMS-NADH system were chosen in this study. The latter nevertheless has not been reported.

DPPH radicals react with proton donated substances leading to the change of purple of DPPH radicals to yellow. The change of the strong absorption band of DPPH detected at 517 nm would directly relate to antioxidant activity. The percentage of DPPH inhibition is defined by equation as shown below [56].

$$\% \text{ scavenging of DPPH radicals} = \frac{(A_0 - A_1)}{A_0} \times 100$$

A_0 is the absorbance of control (DMSO)

A_1 is the absorbance of sample

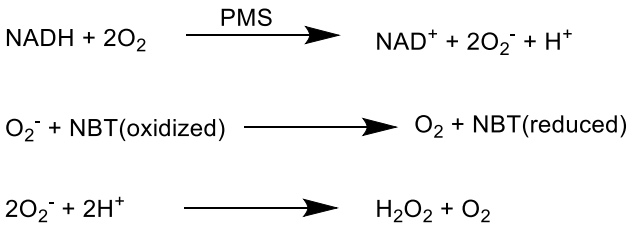


In PMS-NADH system, the superoxide anion is generated from O_2 using NADH as a reductant in the presence of an indicator, nitro blue tetrazolium (NBT) which turns blue when reduced by superoxide [57]. The color change can be monitored spectrophotometrically in the visible range at 560 nm. In addition, O_2^- dismutases into O_2, and H_2O_2 which is detected by *meso-tetra*kis(1-methyl-4-pyridyl)-porphinatoiron(III)pentachloride (FeTMPyP) and measured at 590 nm [48]. The percentage of superoxide anion scavenging activity can be obtained from the equation mentioned below. In this study, the isolated constituents of CNSL **5-7, 9-11, 14-15** and saturated compounds **4, 8, 12** were examined.

$$\% \text{ scavenging of superoxide anion} = \frac{(A_0 - A_1)}{A_0} \times 100$$

A_0 is the absorbance of control (DMSO)

A_1 is the absorbance of sample



3.2.2.1 DPPH radical-scavenging activity of separated components 5-7, 9-11, 14-15 and saturated compounds 4, 8, 12

DPPH, a stable non-biological radical, is widely used for screening the antioxidant activity of hydrogen donating of antioxidant substances. The percentage of DPPH radicals scavenging activity of separated components **5-7, 9-11, 14-15** and saturated compounds **4, 8, 12** (100 μM) is presented in Figure 3.12 together with two positive polyphenols: resveratrol (100 μM) and gallic acid (10 μM) [58, 59].

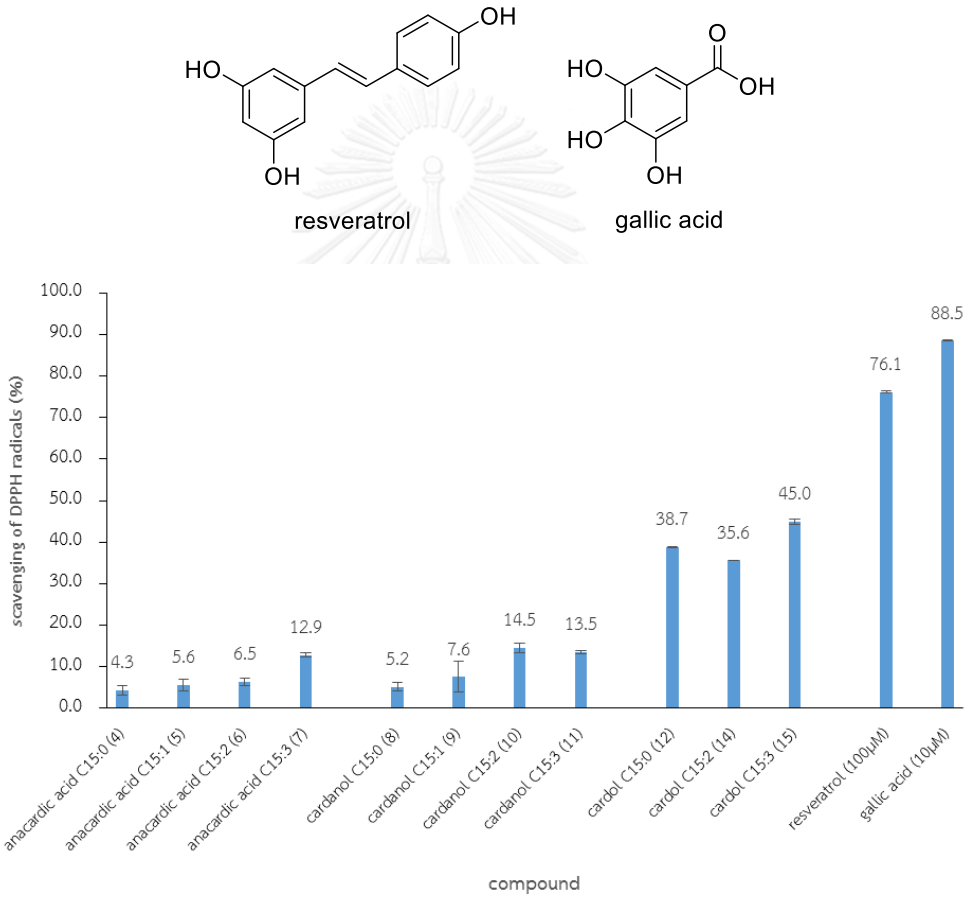


Figure 3.11 % Scavenging of DPPH radicals of separated components **5-7, 9-11, 14-15** and saturated compounds **4, 8, 12**. Data are presented as means ± SEM

% Scavenging DPPH radicals of compounds **4-12** and **14-15** (100 μM) were determined and compared with resveratrol (100 μM), and gallic acid (10 μM). All

separated components displayed low % inhibition of DPPH radicals. Cardol **12, 14, 15** (35-45%) exhibited low antioxidant activity; however higher than cardanol **8-11** (5-14%) and anacardic acid **4-7** (4-13%). These results were agreeable with the previous research [24].

3.2.2.2 Scavenging superoxide anion radicals generated and H_2O_2 formation by PMS-NADH system of constituents 5-7, 9-11, 14-15 and saturated compounds 4, 8, 12

The superoxide anion is a free radical that plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide and the hydroxyl radical. The scavenging superoxide radicals of thirteen compounds: **4-12** and **14-15**, resveratrol and salicylic acid (0-200 μM) were determined by detecting the color change of NBT. Those compounds, resveratrol and salicylic acid were measured for 60 s at 560 nm and calculated into % relative scavenging activity as shown in Figures 3.12 and 3.14.

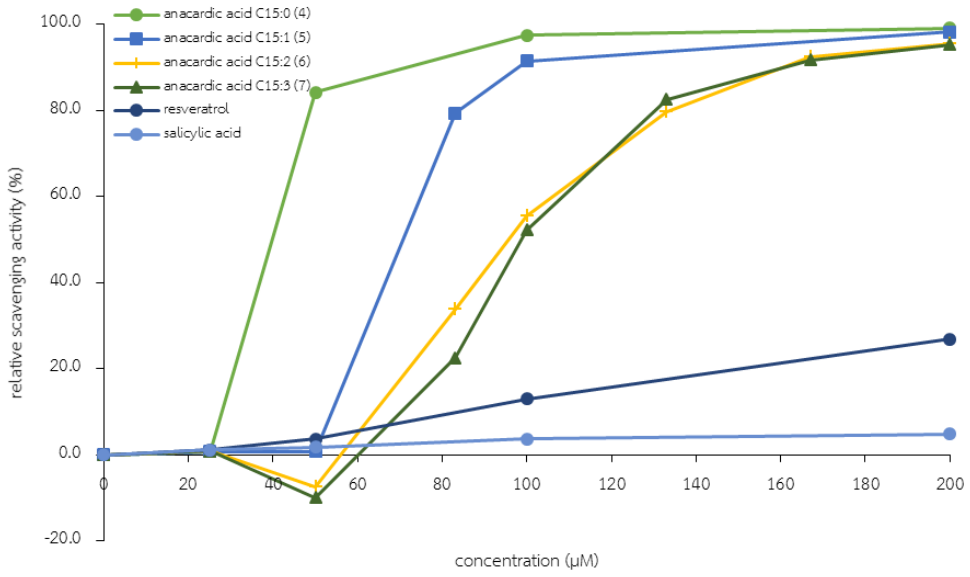


Figure 3.12 % Superoxide anion radicals scavenging activity generated by PMS-NADH of four anacardic acid **4-7**, resveratrol and salicylic acid

From Figure 3.12, anacardic acid **4-7** exhibited higher scavenging activity against superoxide anion radicals than resveratrol, while salicylic acid at the same concentration did not show activity. According to the result, resveratrol showed weak activity in PMS-NADH system. In 2010, it was reported that this compound could scavenge well in superoxide from photochemically-reduced riboflavin [60]. In this research, the reaction was immediately measured after mixing. This assumed that resveratrol was less active to scavenge superoxide anion radical than anacardic acid. Furthermore, superoxide anion radical scavenging activity of anacardic acid **4-7** was examined to demonstrate the hypothesis that the number of double bond in side chain and hydrophilic head affected on the scavenging activity. Interestingly, the scavenging activities of four anacardic acid **4-7** showed significantly different as presented in Figure 3.13.

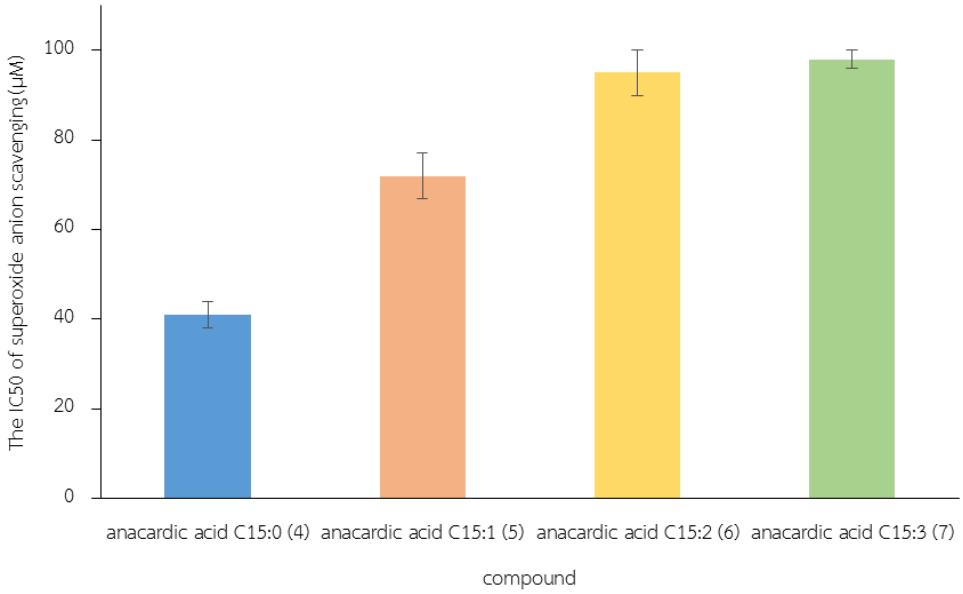


Figure 3.13 The IC_{50} value of superoxide anion radicals scavenging activity generated by PMS-NADH of four anacardic acid **4-7**. Data are presented as means ± SEM

Anacardic acid $C_{15:0}$ **4**, a saturated anacardic acid represented the best superoxide anion scavenger (41±3 μM), followed by anacardic acid $C_{15:1}$ **5**, $C_{15:2}$ **6** and $C_{15:3}$ **7** with IC_{50}'s of 72±5, 95±3 and 98±2 μM, respectively (Figure 3.13). These results

indicated that the more double bond present, the less antioxidant activity was observed. It was believed that the saturated side chain may form micelle better than the unsaturated compounds [61]. In this experimental, the results were different from previous research that the more double bond conferred greater activity in xanthine oxidase system [23]. Anacardic acid $C_{15:3}$ **7** suppressed superoxide generation and inhibited xanthine oxidase rather than scavenging superoxide anion. This meant that anacardic acid may reduce superoxide anion in different pathway. In addition, compared the scavenging activity of anacardic acid (salicylic acid derivative) to its parent compound, salicylic acid, it was found that the presence of pentadecyl(alkenyl) side chain on C-6 of salicylic acid (anacardic acid) displayed more potent activity. The IC_{50} of salicylic acid was more than 200 μM while those of anacardic acid **4-7** were less than 100 μM (Figure 3.12). Anacardic acid was amphiphilic molecules, so that their hydrophobic properties dominated the properties of the molecule. These results were in good agreement with previous reports that salicylic acid acted only as a scavenger of reactive oxygen species, whereas anacardic acid potently inhibit the generation of superoxide anion by xanthine oxidase [22].

In addition, the scavenging activity against superoxide anion radicals of the constituents of cardanol **8-11** and those of cardol **12, 14-15** were determined as presented in Figure 3.14.

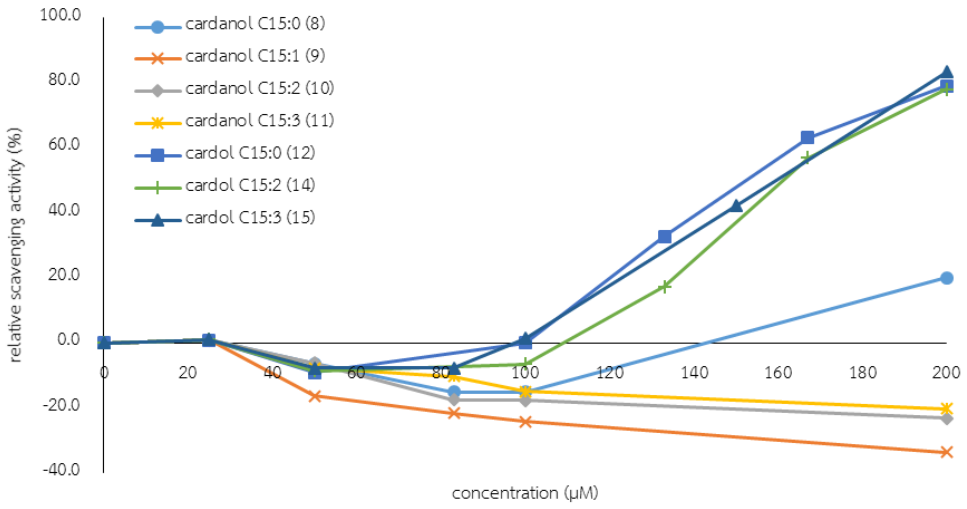
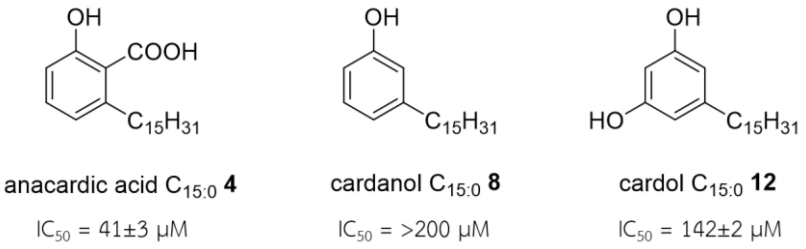


Figure 3.14 Superoxide anion radicals scavenging activity generated by PMS-NADH of four cardanol **8-11** and three cardol **12, 14-15**

Figure 3.14 shows that cardol **12, 14-15** scavenged superoxide anion while those of cardanol **8-11** did not show this activity. The IC_{50}'s of cardol $C_{15:0}$ **12**, $C_{15:2}$ **14** and $C_{15:3}$ **15** were 142±2, 155±2 and 151±3 μM, respectively. In this particular case, the number of the double bonds in pentadecyl(alkenyl) side chain did not have an essential effect on the activity. This showed that the core structure was crucial for displaying this activity.

The functionality of those isolated compounds was also found to be important for the activity. To illustrate this, saturated anacardic acid **4** exhibited more scavenging activity against superoxide anion than saturated cardol **12** and saturated cardanol **8** as displayed in Figure 3.15.



anacardic acid $C_{15:0}$ **4** — IC_{50} = 41±3 μM

cardanol $C_{15:0}$ **8** — IC_{50} = >200 μM

cardol $C_{15:0}$ **12** — IC_{50} = 142±2 μM

Figure 3.15 The effect of hydrophilic head on scavenging superoxide anion

The hydrophilic head of all saturated anacardic acid **4**, cardanol **8**, and cardol **12** are different as shown in Figure 3.15. Salicylic acid head group of anacardic acid **4** displayed the lowest IC_{50} (41±3 μM), while cardol **12** (resorcinol head group) showed 142±2 μM which was better activity than cardanol **8** (IC_{50} >200 μM). These results assumed that the more polarity of core structure, the more activity was observed. Anacardic acid contained one carboxyl and one hydroxyl; cardol contained two hydroxyls and cardanol just contained only one hydroxyl. Carboxylic group may assist the molecule to dissolve in the system containing polar solution better than hydroxyl group. When the polarity increased, the interaction between these groups and superoxide anion induced superoxide anion scavenging activity. In addition, anacardic acid could generate more stable radical than cardol and cardanol, the greater scavenging activity were detected [62].

According to the superoxide anion radical scavenging activity, anacardic acid **4-7** revealed the best activity. The generated H_2O_2 in this system was mostly converted from superoxide anion which was not scavenged by compounds. Thus H_2O_2 generation from PMS-NADH system of anacardic acid **4-7** was determined to verify this phenomenon. The results are collected in Table 3.1.

Table 3.1 H_2O_2 generation by PMS-NADH system of anacardic acids **4-7**

Compound	H_2O_2 formation (μM)
anacardic acid $C_{15:0}$ **4**	0.18 ± 0.00
anacardic acid $C_{15:1}$ **5**	0.48 ± 0.01
anacardic acid $C_{15:2}$ **6**	0.83 ± 0.02
anacardic acid $C_{15:3}$ **7**	0.89 ± 0.01
salicylic acid	1.07 ± 0.01
gallic acid	3.19 ± 0.14

From Table 3.1, the H_2O_2 generated from the system containing saturated anacardic acid **4** was 0.18±0.00 μM, followed by anacardic acid $C_{15:1}$ **5** (0.48±0.01 μM), anacardic acid $C_{15:2}$ **6** (0.83±0.02 μM) and anacardic acid $C_{15:3}$ **7** (0.89±0.01 μM). These results supported that the increment of the number of double bond, the decreasing

superoxide anion scavenging, and the increasing H_2O_2 formation were monitored. This assumed that those compounds could scavenge superoxide anion so the low remaining superoxide anions converted to H_2O_2 were detected. While salicylic acid (1.07±0.01 μM) and gallic acid (3.19±0.14 μM) displayed high value of H_2O_2 formation. These results clearly evidenced that anacardic acid **4-7** could scavenge superoxide anion generated by PMS-NADH.

3.2.2.3 Conclusion

Eight isolated constituents **5-7, 9-11, 14-15** and three saturated compounds **4**, **8**, **12** were tested on antioxidant in DPPH and PMS-NADH system. For DPPH assay, all eleven compounds displayed low scavenging DPPH radicals. In PMS-NADH system anacardic acid **4-7** and cardol **12**, **14-15** could scavenge superoxide anion. The contrast of the results was possibly due to the fact that they could reduce radical with different pathways. In addition, low H_2O_2 formation was detected for anacardic acid **4-7**. So anacardic acid **4-7** was assigned as unique antioxidants.

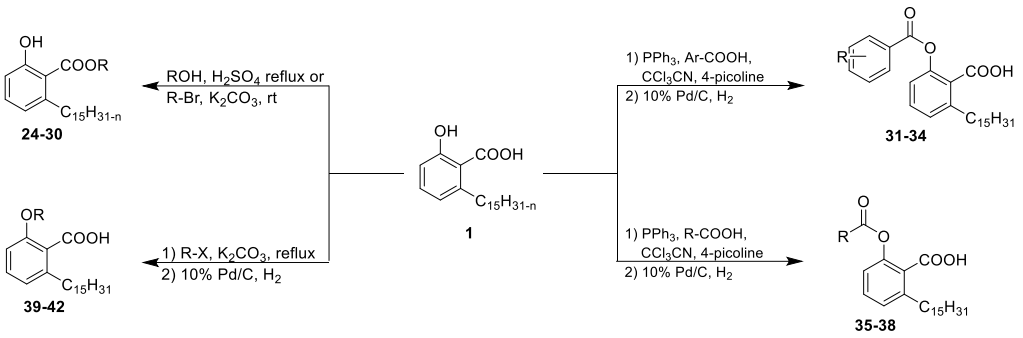
3.3 Synthesis and biological activities of anacardic acid, cardanol and their derivatives

According to previous literatures, anacardic acid **1** possessed a variety of biological activities. These facts provided anacardic acid **1** as an interesting substance leading to its structural modification and biologically active evaluation. In the case of cardanol **2**, a few biological activities have been reported. This study aims to functionalize cardanol **2** by introducing imine group, and to synthesize coumarin *via* formylating at C-2, and to evaluate antibacterial activity.

3.3.1 Synthesis and characterization of anacardic acid derivatives

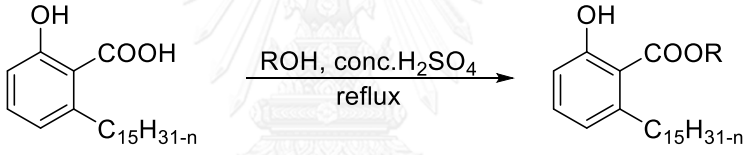
Various derivatives of anacardic acid **1** such as isonicotinoylhydrazone, benzylamine and benzamide were reported to possess several biological activities [27-

29]. Considering the functional groups of anacardic acid **1**, a carboxyl group at C-1 and a hydroxyl group at C-2 could be modified to furnish its analogues. In this research, two types as ester and ether derivatives of anacardic acid were prepared.



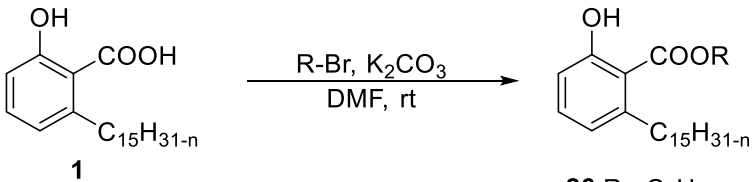
3.3.1.1 Ester derivatives

Fifteen esters of anacardic acid **24-38** were synthesized by two major methods: esterification and nucleophilic substitution *via* acid chloride.



24 R= CH_3-
25 R= C_2H_5-

Esters of anacardic acids **24-25** were synthesized *via* esterification of anacardic acid **1** with MeOH or EtOH in the presence of conc. H_2SO_4 under reflux to obtain orange and yellow products in 42 and 26 %yield, respectively (Table 3.2). Both compounds were characterized by ^{1}H NMR. The spectra showed a significant methylene proton signal of ester at δ_H 3.98 and 4.46.



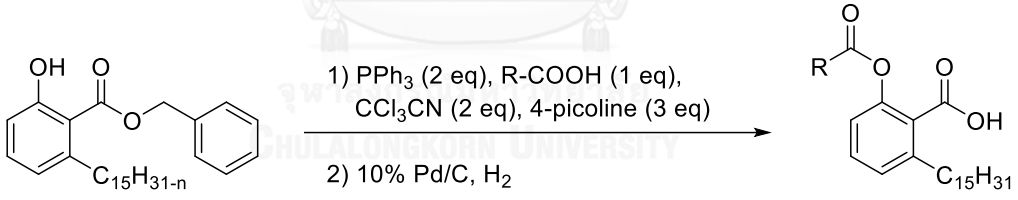
26 R= C_4H_9-
27 R= C_6H_{13}-
28 R= C_8H_{17}-
29 R= $C_{12}H_{25}$-
30 R= Ph-CH_2-

Table 3.2 Synthesis of ester analogues on carboxyl group **24-30**

Entry	Alcohol / alkyl halide	Compound	% Isolated yield
1	methanol	**24**	42
2	ethanol	**25**	26
3	1-bromobutane	**26**	20
4	1-bromohexane	**27**	24
5	1-bromooctane	**28**	49
6	1-bromododecane	**29**	61
7	benzyl bromide	**30**	49

In addition, compounds **26-30** were prepared from the reaction between anacardic acid **1** and alkyl bromide under basic condition using K_2CO_3 to yield yellow liquid (Table 3.2). All compounds were characterized by ^{1}H NMR which presented the identity signal of two methylene protons of ester approximately at δ_H 4.35-4.50.

Eight new esters of anacardic acid **31-38** were synthesized using carboxylic acid as a starting material *via* generated acid chloride, and hydrogenolysis by Pd/C under H_2 atm. The results are demonstrated in Table 3.3.



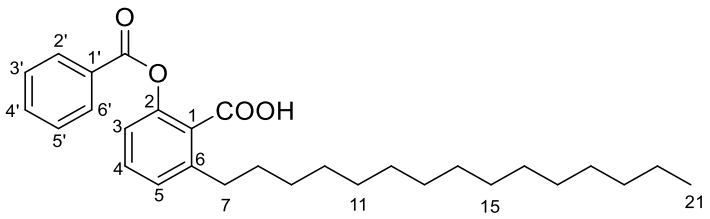
30 (1 eq)

31 R= Ph-
32 R= 2-Cl-Ph-
33 R= 2-Br-Ph-
34 R= 2-OH-Ph-
35 R= CH_3-
36 R= C_3H_7-
37 R= C_7H_{15}-
38 R= $C_{11}H_{23}$-

Table 3.3 Synthesis of ester analogues via acid chloride **31-38**

Entry	Acid	Compound	% Isolated yield
1	benzoic acid	**31**	47
2	2-chlorobenzoic acid	**32**	42
3	2-bromobenzoic acid	**33**	49
4	2-hydroxybenzoic acid	**34**	53
5	acetic acid	**35**	32
6	butyric acid	**36**	25
7	octanoic acid	**37**	46
8	dodecanoic acid	**38**	56

From Table 3.3, benzyl 2-hydroxy-6-pentadecyl(alkenyl) benzoate **30** (1 eq) reacted with selected acid chloride which was generated from carboxylic acid (1 eq), PPh_3 (2 eq) and CCl_3CN (2 eq) [44]. Then the reactions were hydrogenolyzed using H_2 and 10% Pd/C for removing benzyl to carboxyl group, and at the same time for hydrogenating unsaturation on the side chain. Benzoic acid, 2-chloro, 2-bromo, and 2-hydroxybenzoic acids were used to react with compound **30** to give the corresponding 2-(benzoyloxy)-6-pentadecylbenzoic acid **31**, 2-((2-chlorobenzoyl)oxy)-6-pentadecylbenzoic acid **32**, 2-((2-bromobenzoyl)oxy)-6-pentadecylbenzoic acid **33**, and 2-((2-hydroxybenzoyl)oxy)-6-pentadecylbenzoic acid **34** in 47, 42, 49, and 53 %yield, respectively in entries 1-4. By the same method, acetic acid, butyric acid, octanoic acid and dodecanoic acid were used to give 2-acetoxy-6-pentadecylbenzoic acid **35**, 2-butanoyloxy-6-pentadecylbenzoic acid **36**, 2-octanoyloxy-6-pentadecylbenzoic acid **37**, and 2-dodecanoyloxy-6-pentadecylbenzoic acid **38** in 32, 25, 46, and 56 %yield, respectively (entries 5-8). Comparison the structure of carboxylic acids employed with their % isolated yield suggested that % yield of compounds **35-38** increased when using carboxylic acids with more carbon atoms in alkyl chain. The structures of modified compounds **31-38** were characterized by ^{1}H and ^{13}C NMR.



The ^{1}H NMR spectrum of 2-(benzoyloxy)-6-pentadecylbenzoic acid **31** in Figure 3.17 reveals the signals for two aromatic protons at δ_H 8.14 (H-2′, H-6′), an aromatic proton at δ_H 7.60 (H-3), three aromatic protons at δ_H 7.50-7.39 (H-3′, H-4′ and H-5′), two aromatic protons at δ_H 7.15 (H-4, H-5), two protons at δ_H 2.74 (H-7), two protons at δ_H 1.60 (H-8), twenty four protons at δ_H 1.13–1.41 (H-9 to H-20), and three methyl protons at δ_H 0.87 (H-21). Comparison this spectrum with that of **4** found that the singlet hydroxyl proton H-6 was not appeared. Five aromatic protons of benzoyl group were inferred from the presence of 8.14 (m, 2H), and 7.39–7.50 (m, 3H). The ^{13}C NMR spectrum (Figure 3.17) presents the characteristic carbonyl carbon signals of carboxyl and ester at δ_C 170.2 and 164.9, respectively. Two aromatic signals at δ_C 148.6 (C-2) and 143.4 (C-6) were observed. The peaks at δ_C 133.7, 131.1, 130.2 (2C), 129.2, 128.6 (2C), 127.4, 125.1 and 120.5 indicated the presence of ten aromatic carbons. The chemical shifts at δ_C 22.7-33.8 were observed from the presence of fourteen carbons of side chain (C-7 to C-20). The signal of a methyl carbon at δ_C 14.1 (C-21) was detected. For compounds **32-34**, their spectra presented the same pattern as shown in Tables 3.4 and 3.5.

Table 3.4 The ^{1}H NMR chemical shifts of anacardic acid derivatives **31-34**

position	Compound/ chemical shift (ppm)			
	31	32	33	34
1				
2				
3	7.60	7.60	7.33-7.52	7.22
4	7.15	7.16	7.17	6.91
5	7.15	7.16	7.17	7.13
6				
7	2.74	2.71	2.77	2.70
8	1.6	1.61	1.63	1.55
9-20	1.13-1.41	1.25	1.25	1.24
21	0.87	0.87	0.88	0.81
1′				
2′	8.14			
3′	7.39-7.50	7.99	7.98	7.02
4′	7.39-7.50	7.45	7.33-7.52	7.51
5′	7.39-7.50	7.45	7.33-7.52	7.46
6′	8.14	8.15	8.16	8.00
-O**H**				10.25

Table 3.5 The ^{13}C NMR chemical shifts of anacardic acid derivatives **31-34**

Compound	Chemical shift of ^{13}C NMR (ppm)
31	170.2 (**C**OOH-), 165.0 (**C**OOR-), 148.6 (C-2), 143.4 (C-6), 133.7, 131.1, 130.2 (2C), 129.2, 128.6 (2C), 127.4, 125.1 and 120.5 (aromatic carbon), 33.8-22.7 (C-7 to C-20) and 14.1 (C-21).
32	170.3 (**C**OOH-), 165.0 (**C**OOR-), 148.6 (C-2), 143.4 (C-6), 133.7, 133.1, 131.9, 131.2, 131.1, 130.2, 128.6, 127.4, 126.7, 120.5 (aromatic carbon), 33.8-22.7 (C-7 to C-20) and 14.1 (C-21).
33	170.7 (**C**OOH-), 164.1 (**C**OOR-), 148.5 (C-2), 143.7 (C-6), 133.7, 133.1, 131.3, 131.1, 130.3, 128.6, 127.8, 127.4, 125.0, 120.5 (aromatic carbon), 33.9-22.7 (C-7 to C-20) and 14.1 (C-21).
34	170.5 (**C**OOH-), 168.5 (**C**OOR-), 162.2, 147.9 (C-2), 143.9 (C-6), 136.5, 131.3, 130.4, 127.9, 124.9, 120.5, 119.5, 117.8, 111.6 (aromatic carbon), 33.8-22.7 (C-7 to C-20) and 14.1 (C-21).

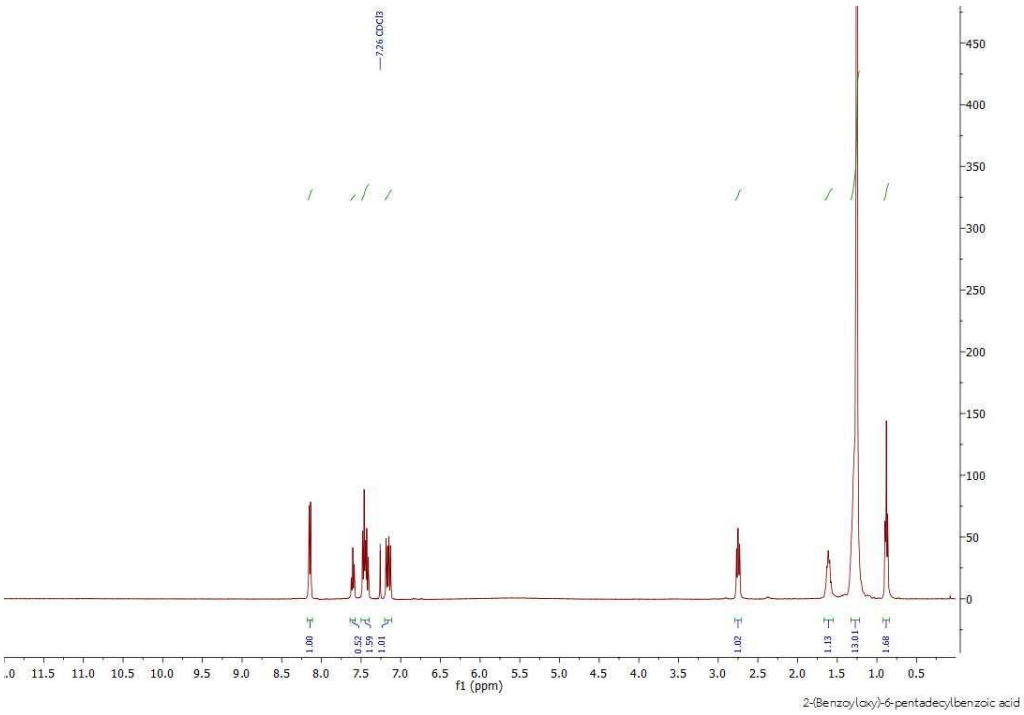


Figure 3.16 The ^{1}H NMR spectrum ($CDCl_3$) of 2-(benzoyloxy)-6-pentadecylbenzoic acid **31**

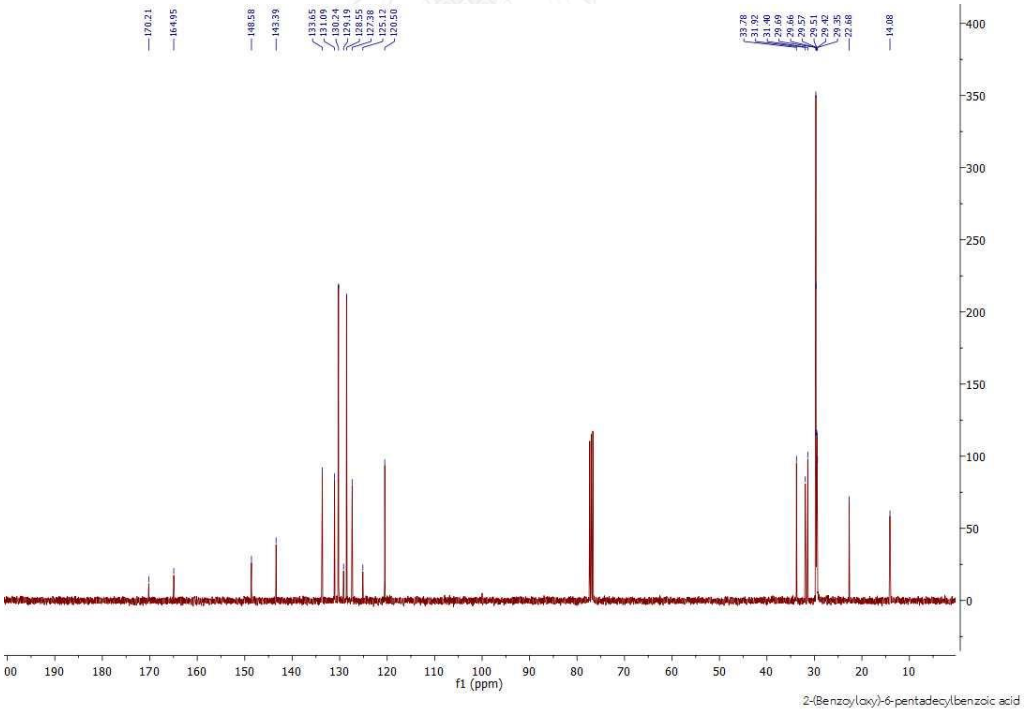


Figure 3.17 The ^{13}C NMR spectrum ($CDCl_3$) of 2-(benzoyloxy)-6-pentadecylbenzoic acid **31**

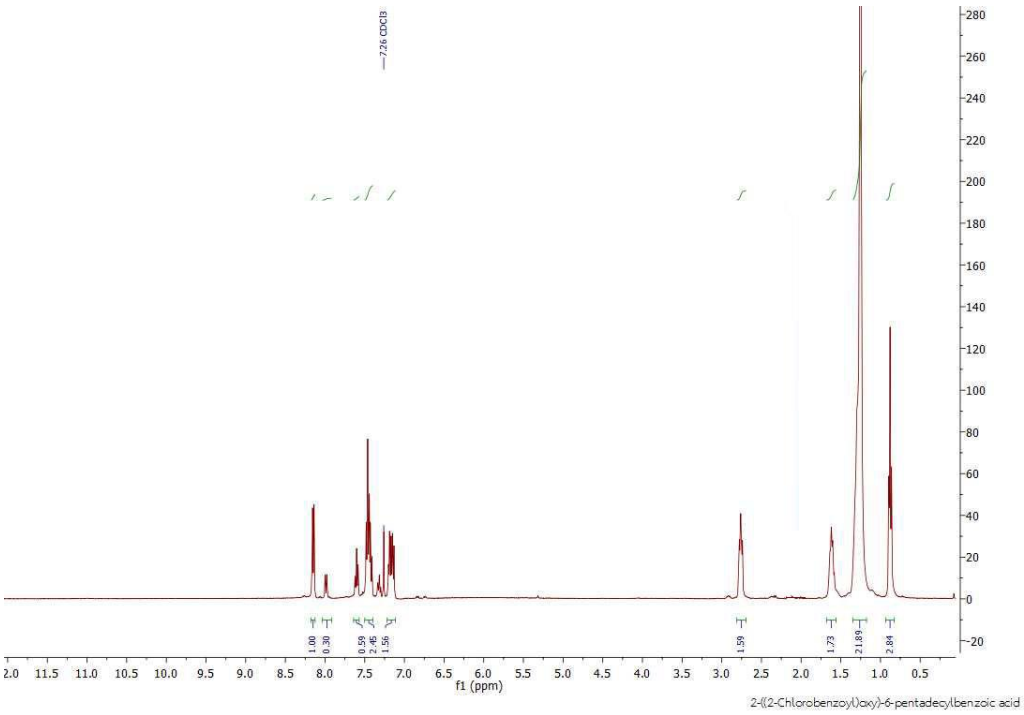


Figure 3.18 The ^{1}H NMR spectrum ($CDCl_3$) of 2-((2-chlorobenzoyl)oxy)-6-pentadecylbenzoic acid **32**

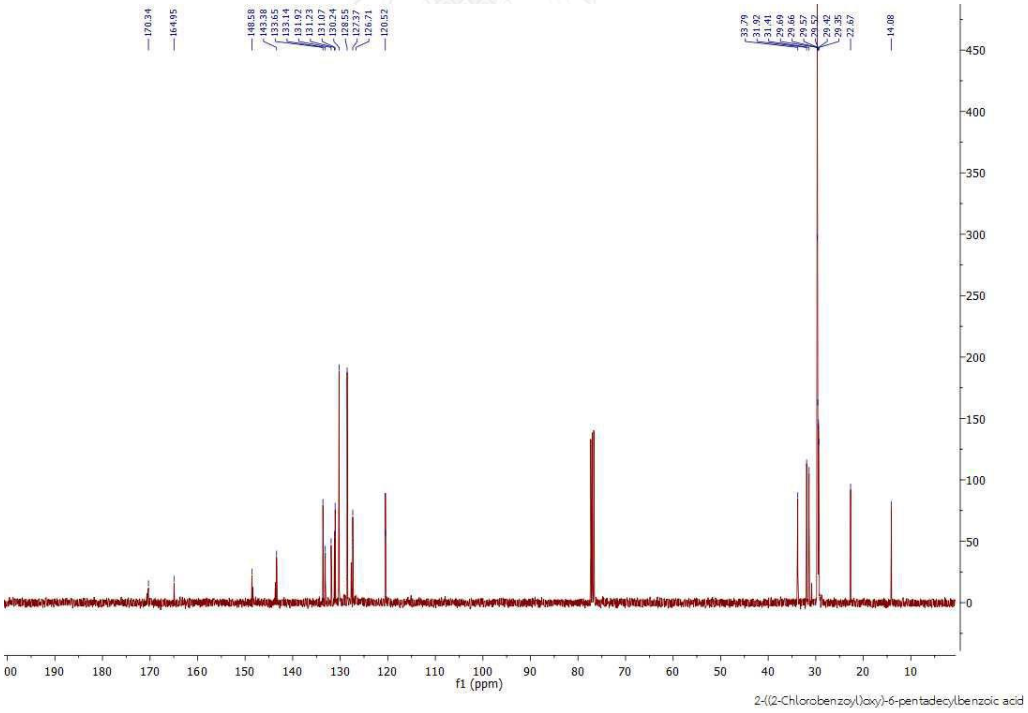


Figure 3.19 The ^{13}C NMR spectrum ($CDCl_3$) of 2-((2-chlorobenzoyl)oxy)-6-pentadecylbenzoic acid **32**

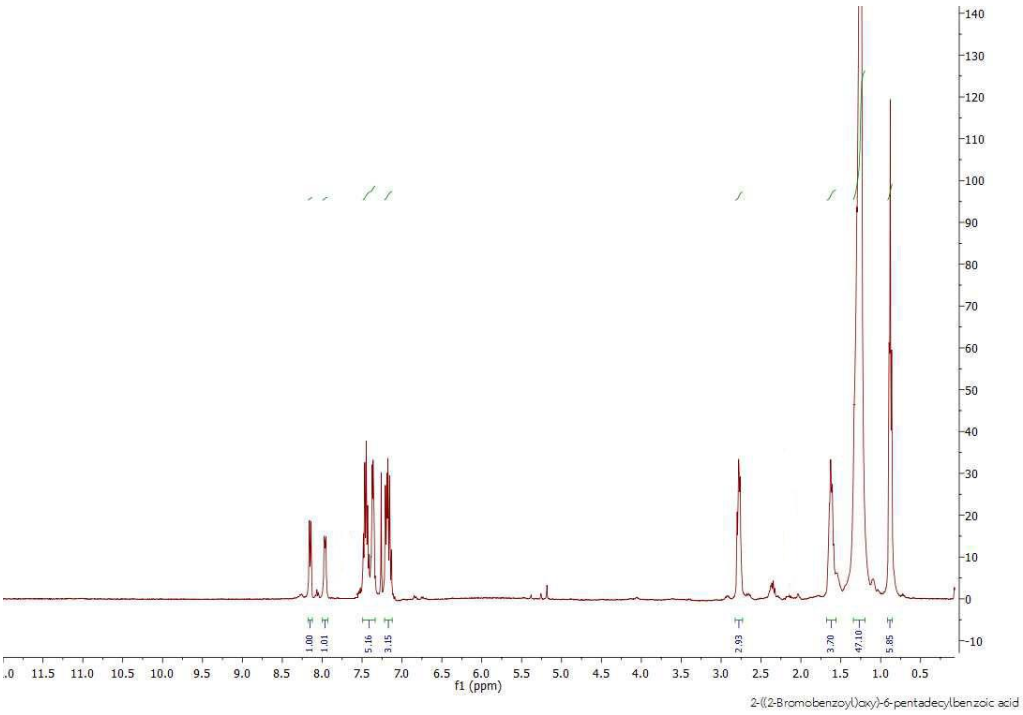


Figure 3.20 The ^{1}H NMR spectrum ($CDCl_3$) of 2-((2-bromobenzoyl)oxy)-6-pentadecylbenzoic acid **33**

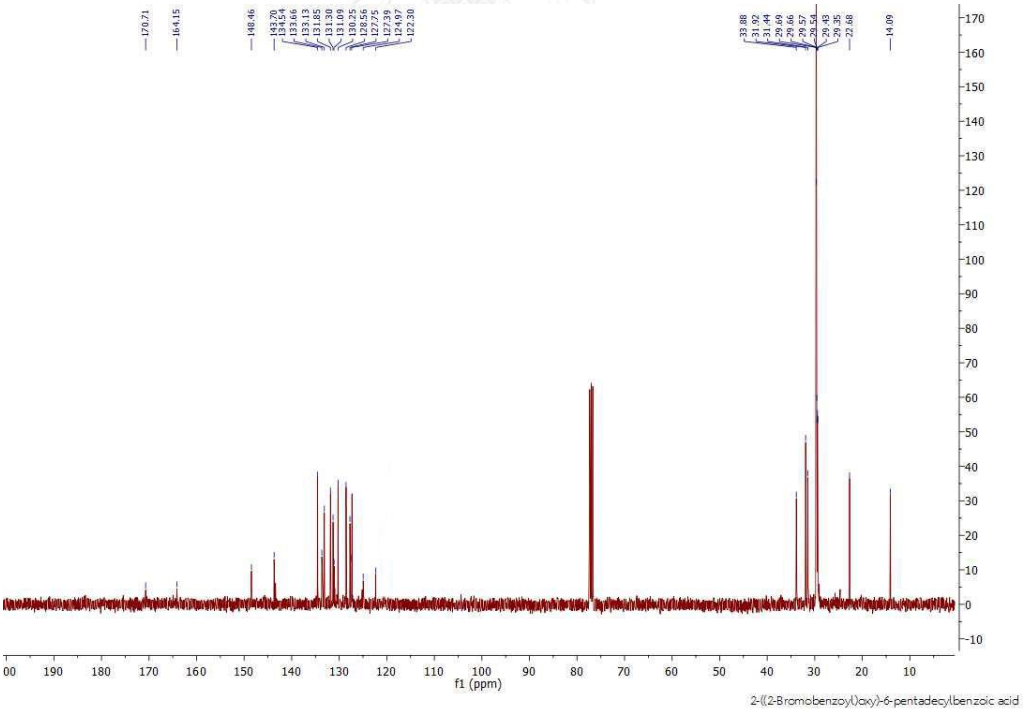


Figure 3.21 The ^{13}C NMR spectrum ($CDCl_3$) of 2-((2-bromobenzoyl)oxy)-6-pentadecylbenzoic acid **33**

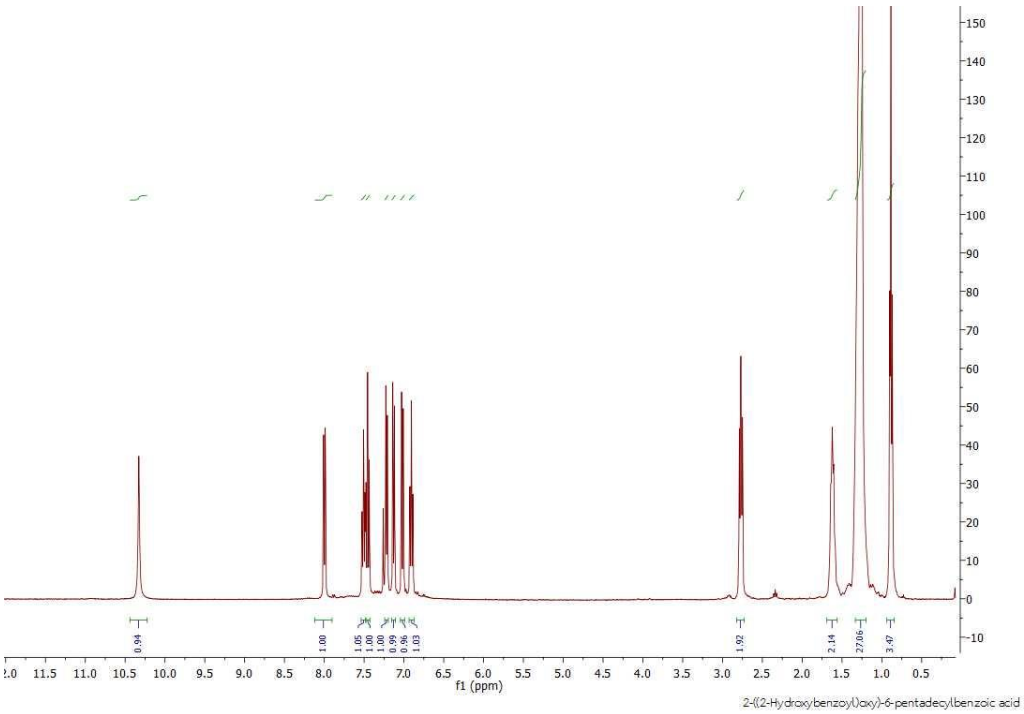


Figure 3.22 The ^{1}H NMR spectrum ($CDCl_3$) of 2-((2-hydroxybenzoyl)oxy)-6-pentadecylbenzoic acid **34**

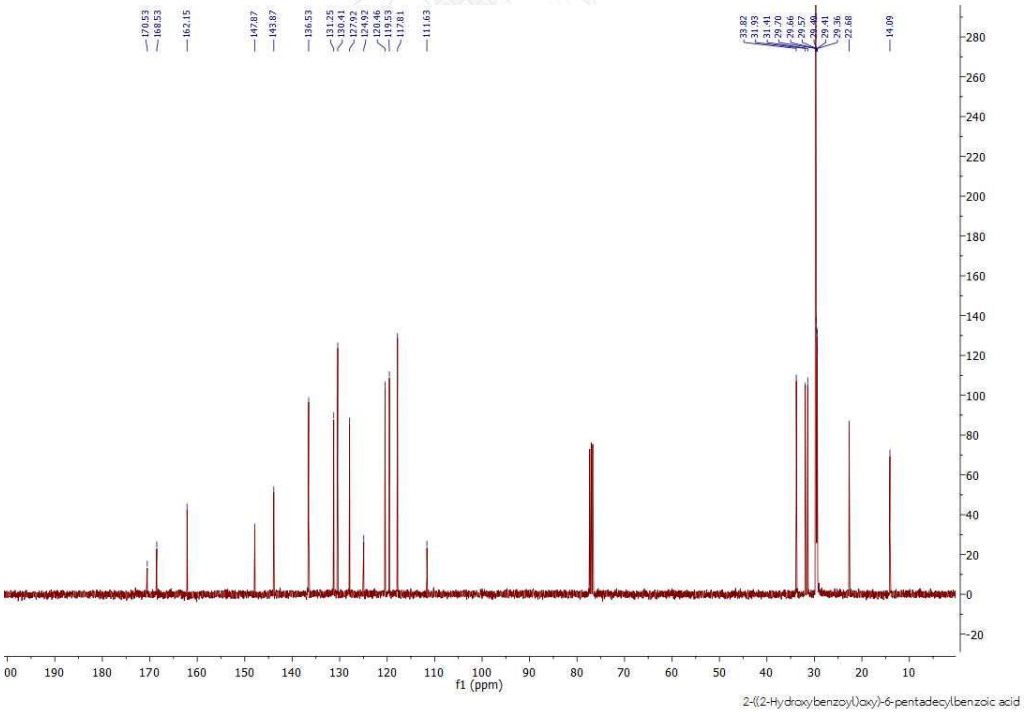
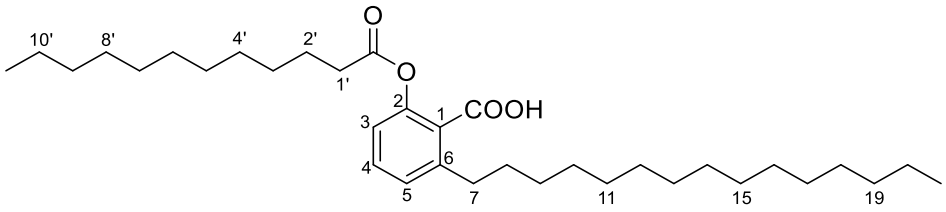


Figure 3.23 The ^{13}C NMR spectrum ($CDCl_3$) of 2-((2-hydroxybenzoyl)oxy)-6-pentadecylbenzoic acid **34**



The ^{1}H NMR spectrum of 2-acetoxy-6-pentadecylbenzoic acid **35** in Figure 3.25 presents three signals of aromatic protons at δ_H 7.40, 7.16 and 6.99 (H-3, H-5, and H-4, respectively). Two protons at δ_H 2.77 (H-7), two signals of methyl protons at δ_H 2.29 and 0.87 (H-1′ and H-21), two protons at δ_H 1.62 (H-8), and twenty four protons at δ_H 1.25 (H-9 to H-20) were observed. The ^{13}C NMR spectrum (Figure 3.25) displayed the characteristic carbonyl carbon signals of carboxyl and ester at δ_C 171.5 and 169.4, respectively. Two aromatic signals at δ_C 148.6 (C-2) and 143.6 (C-6) were detected. The signals of four aromatic carbons at δ_C 131.3 (C-4), 127.5 (C-5), 120.6 (C-3) and 115.8 (C-1) were visualized. The fourteen carbons of side chain were observed at δ_C 33.9-22.7 (C-7 to C-20), and two methyl carbon signals at δ_C 20.9 (C-1′) and 14.1 (C-21) were detected. This spectrum pattern was detected in compounds **36-38** as shown in Tables 3.6 and 3.7.

Table 3.6 The ^{1}H NMR chemical shifts of derivatives of anacardic acid **35-38**

position	Compound/ chemical shift of ^{1}H NMR (ppm)			
	35	**36**	**37**	**38**
1				
2				
3	7.40	7.38	7.39	7.38
4	6.99	6.98	6.99	6.97
5	7.16	7.14	7.15	7.14
6				
7	2.77	2.76	2.79	2.76
8	1.62	1.60	1.63	1.60
9-20	1.25	1.25	1.27	1.38
21	0.87	0.87	0.88	0.87
1′	2.29	2.53	2.56	2.54
2′		1.77	1.75	1.72
3′		1.02	1.27 (H-3′-H-6′)	1.38 (H-3′-H-10′)
4′				
5′				
6′				
7′			0.88	
8′				
9′				
10′				
11′				0.87

Table 3.7 The ^{13}C NMR chemical shifts of derivatives of anacardic acid **35-38**

Compound	Chemical shift of ^{13}C NMR (ppm)
35	171.5 (**C**OOH-), 169.4 (**C**OOR-), 148.6 (C-2), 143.6 (C-6), 131.3 (C-4), 127.5 (C-5), 120.6 (C-3), 115.8 (C-1), 33.9, 31.9, 31.5, 29.7 (4C), 29.7 (2C), 29.6, 29.6, 29.4, 29.4, 22.7, 20.9 (C-1′) and 14.1 (C-21).
36	175.7 (**C**OOH-), 171.9 (**C**OOR-), 147.7 (C-2), 143.4 (C-6), 135.2 (C-4), 122.7 (C-5), 120.5 (C-3), 115.8 (C-1), 36.4, 36.1, 32.0, 31.4, 29.7 (4C), 29.7 (2C), 29.6, 29.5, 29.4, 29.4, 22.7, 18.3, 14.1 (C-3′) and 13.6 (C-21).
37	172.1 (**C**OOH-), 171.7 (**C**OOR-), 148.5 (C-2), 143.4 (C-6), 135.2 (C-4), 122.7 (C-5), 120.5 (C-3), 115.8 (C-1), 36.4, 34.2, 33.8, 31.9, 31.6, 31.4, 29.7 (4C), 29.7 (2C), 29.6, 29.5, 29.4, 29.4, 24.8, 24.7, 22.7, 22.6, 14.1 (C-7′) and 14.0 (C-21).
38	176.1 (**C**OOH-), 172.1 (**C**OOR-), 148.6 (C-2), 143.4 (C-6), 135.2 (C-4), 122.7 (C-5), 120.5 (C-3), 115.8 (C-1), 36.4, 34.2, 34.1, 33.8, 32.0, 31.9, 31.4, 29.7 (4C), 29.7 (2C), 29.6, 29.5, 29.4, 29.4, 29.3, 29.2, 29.1, 29.1, 24.7, 22.7 (2C) and 14.1 (2C) (C-11′ and C-21).

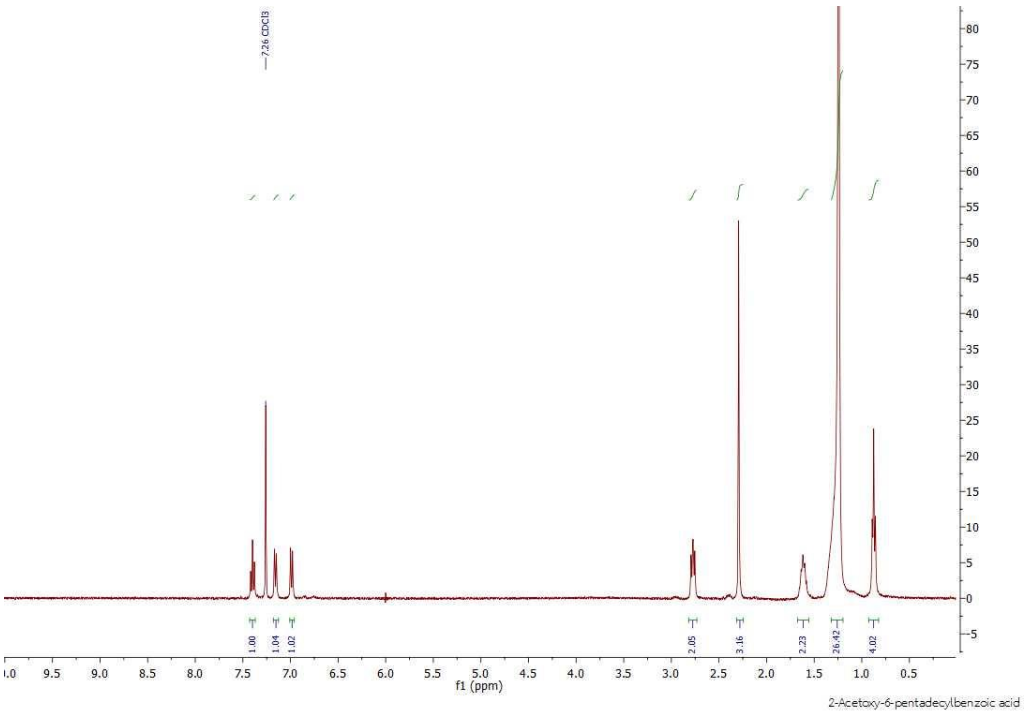


Figure 3.24 The ^{1}H NMR spectrum ($CDCl_3$) of 2-acetoxy-6-pentadecylbenzoic acid **35**

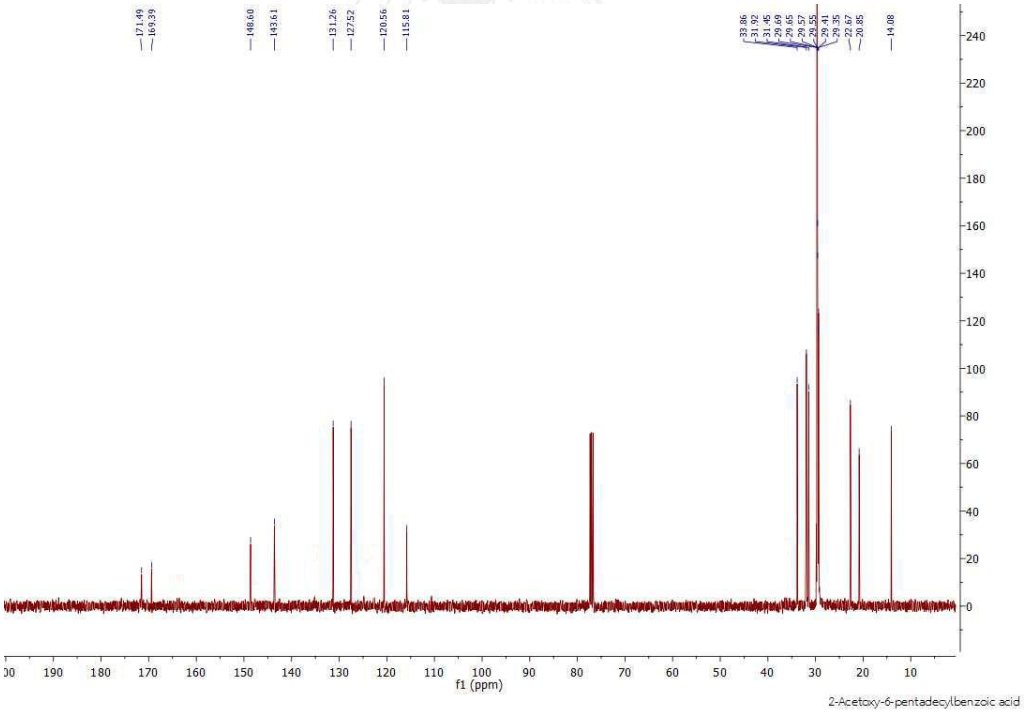


Figure 3.25 The ^{13}C NMR spectrum ($CDCl_3$) of 2-acetoxy-6-pentadecylbenzoic acid **35**

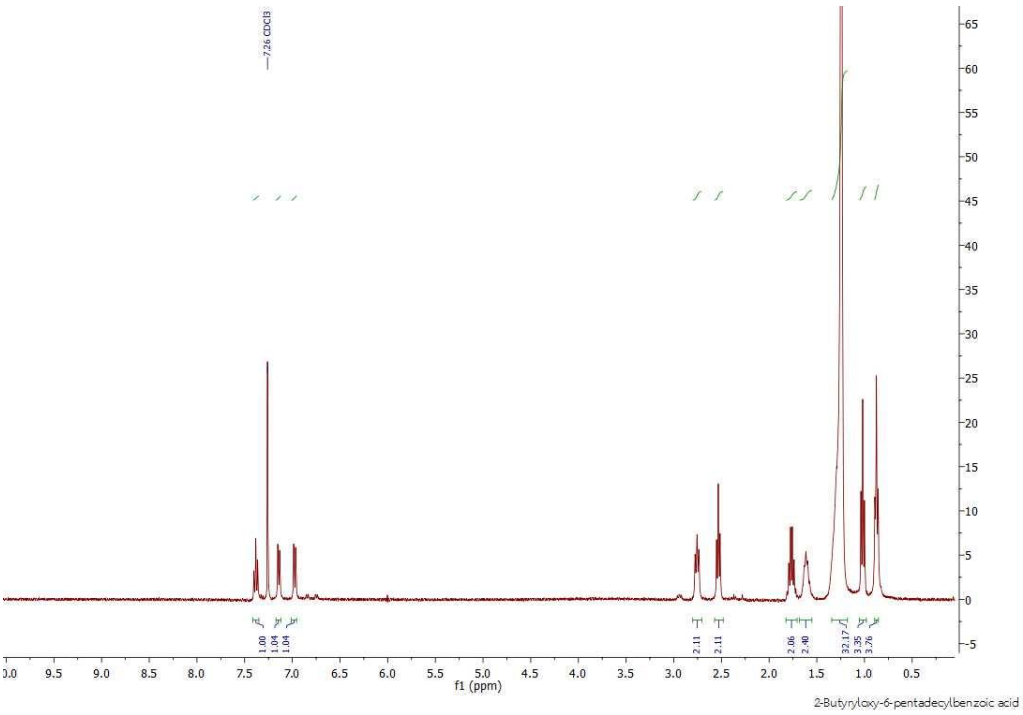


Figure 3.26 The ^{1}H NMR spectrum ($CDCl_3$) of 2-butanoyloxy-6-pentadecylbenzoic acid **36**

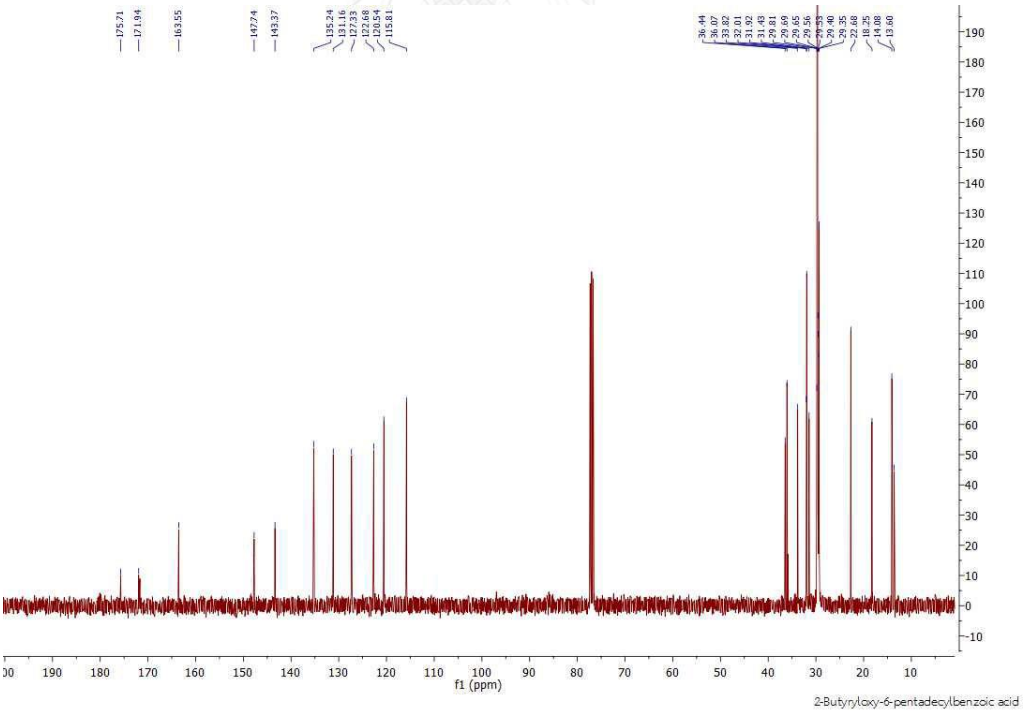


Figure 3.27 The ^{13}C NMR spectrum ($CDCl_3$) of 2-butanoyloxy-6-pentadecylbenzoic acid **36**

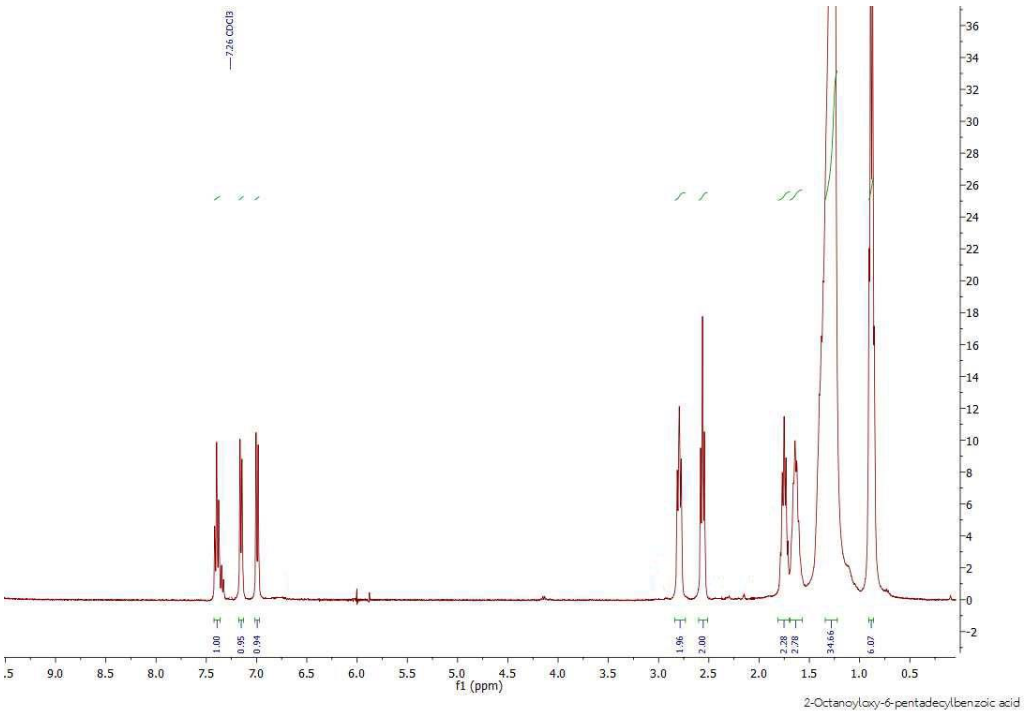


Figure 3.28 The ^{1}H NMR spectrum ($CDCl_3$) of 2-octanoyloxy-6-pentadecylbenzoic acid **37**

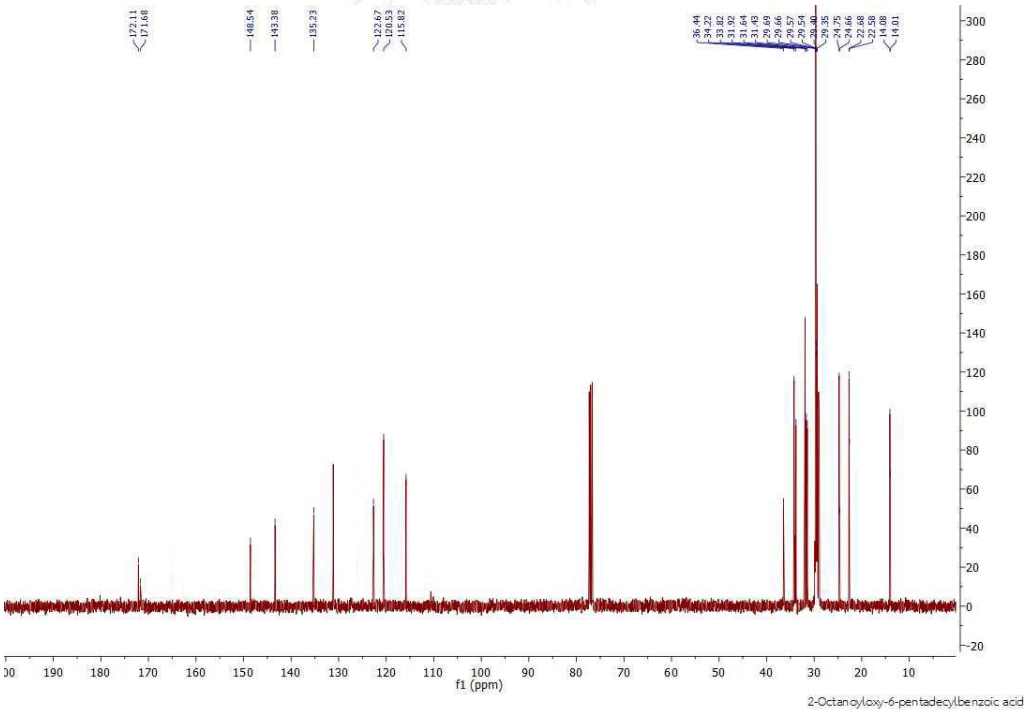


Figure 3.29 The ^{13}C NMR spectrum ($CDCl_3$) of 2-octanoyloxy-6-pentadecylbenzoic acid **37**

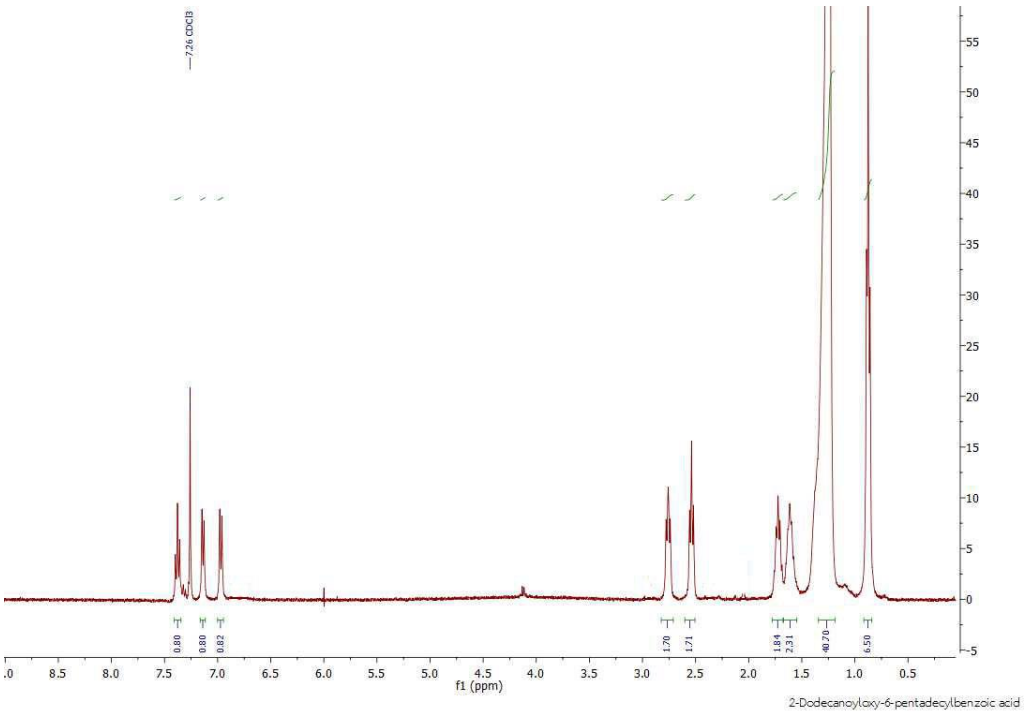


Figure 3.30 The ^{1}H NMR spectrum ($CDCl_3$) of 2-dodecanoyloxy-6-pentadecylbenzoic acid **38**

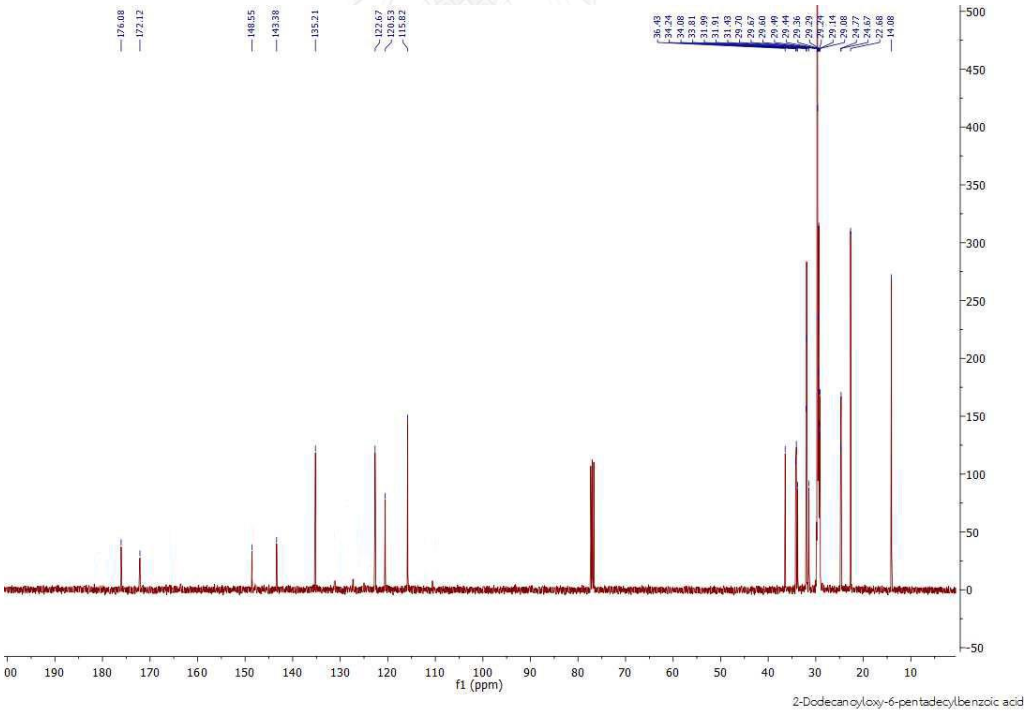


Figure 3.31 The ^{13}C NMR spectrum ($CDCl_3$) of 2-dodecanoyloxy-6-pentadecylbenzoic acid **38**

3.3.1.2 Ether derivatives

Four anacardic acid ethers **39-42** were synthesized using alkyl halide in the presence of K_2CO_3 by nucleophilic substitution reaction, and hydrogenolysis by Pd/C under H_2 atm. Two compounds **41-42** were disclosed to be new compounds. The results are indicated in Table 3.8.

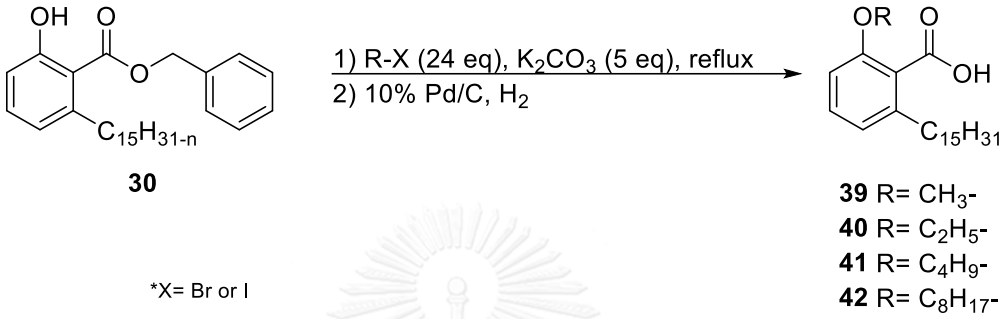
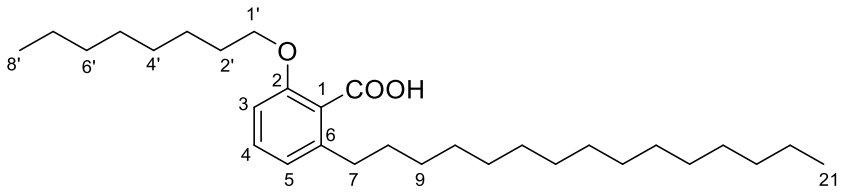


Table 3.8 Synthesis of ether derivatives **39-42** by nucleophilic substitution reaction

Entry	Alkyl halide	Compound	% Isolated yield
1	iodomethane	**39**	29
2	bromoethane	**40**	39
3	1-bromobutane	**41**	17
4	1-bromooctane	**42**	30

From Table 3.8, benzyl 2-hydroxy-6-pentadecyl(alkenyl) benzoate **30** (1 eq) was reacted with selected alkyl halide (24 eq) and K_2CO_3 (5 eq) under reflux. After worked-up, the reactions were hydrogenated using 10 %Pd/C to yield saturated side chain while benzyl group was hydrogenolyzed to carboxyl group. Compound **30** was functionalized with iodomethane, bromoethane, 1-bromobutane and 1-bromooctane to give 2-methoxy-6-pentadecylbenzoic acid **39**, 2-ethoxy-6-pentadecylbenzoic acid **40**, 2-butyloxy-6-pentadecylbenzoic acid **41**, and 2-(octyloxy)-6-pentadecylbenzoic acid **42** in 29, 39, 17, and 30 %yield, respectively (entries 1-4). All modified compounds **39-42** were characterized by ^{1}H and ^{13}C NMR. Two new derivatives **41-42** were well characterized.



The ^{1}H NMR spectrum of 2-butyloxy-6-pentadecylbenzoic acid **41** (Figure 3.32) showed the signals of aromatic protons at δ_H 7.30, 6.88 and 6.81, the ether protons at δ_H 4.08 (H-1′), two protons at δ_H 2.79 (H-7), two protons at δ_H 1.80 (H-2′), two protons at δ_H 1.60 (H-8), and two protons at δ_H 1.49 (H-3′). The twenty four protons at δ_H 1.13–1.41 (H-9 to H-20), six protons at δ_H 0.97 (H-4′) and 0.88 (H-21) were detected. The ^{13}C NMR spectrum (Figure 3.33) showed the characteristic of carboxyl carbon of at δ_C 171.1. The chemical shift of six aromatic carbons were observed at δ_C 109.8, 121.7 122.4, 131.0, 143.4 and 156.4.The ether carbon at δ_C 69.0 (C-1′), fifteen carbons at δ_C 22.7-33.9 (C-7 to C-20 and C-2′), two carbons at δ_C 19.2 (C-3′) were observed. The two carbon signals at δ_C 14.1 and 13.8 were detected and assigned for C-4′ and C-21.

The ^{1}H NMR spectrum of 2-(octyloxy)-6-pentadecylbenzoic acid **42** (Figure 3.34) showed the signals of aromatic protons at δ_H 7.29 (H-4), 6.86 (H-5) and 6.80 (H-3). The signal proton at δ_H 4.05 (H-1′) referred to methylene ether. Four signals of methylene protons at δ_H 2.77 (H-7), 1.81 (H-2′), 1.59 (H-8) and 1.44 (H-3′) were observed. Two methyl protons were monitored at δ_H 0.88. The ^{13}C NMR spectrum (Figure 3.35) presented the characteristic of carboxyl carbon at δ_C 171.2. The chemical shift of six aromatic carbons were observed at δ_C 109.8, 121.7, 122.4, 131.0, 143.4 and 156.4. The ether carbon at δ_C 69.3 (C-1′) was detected. The twenty carbons at δ_C 22.6-34.0 (C-7 to C-20 and C-2′ to C-7′) were referred to alkyl chain and at δ_C 14.1 and 14.0 were detected from two methyl carbons (C-8′ and C-21).

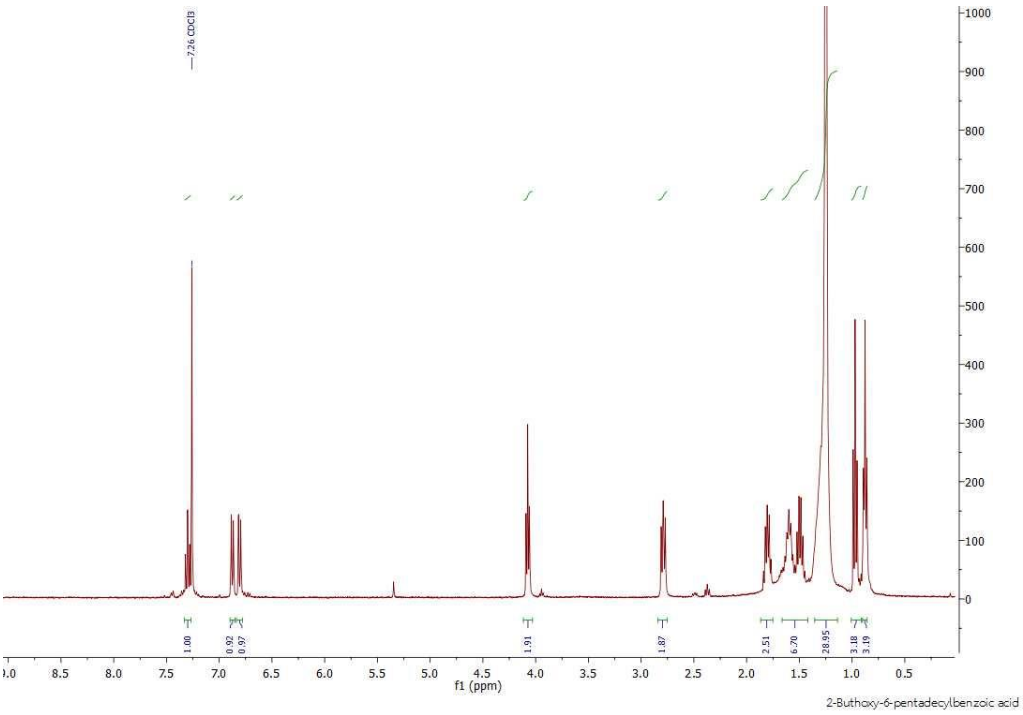


Figure 3.32 The ^{1}H NMR spectrum ($CDCl_3$) of 2-butyloxy-6-pentadecylbenzoic acid **41**

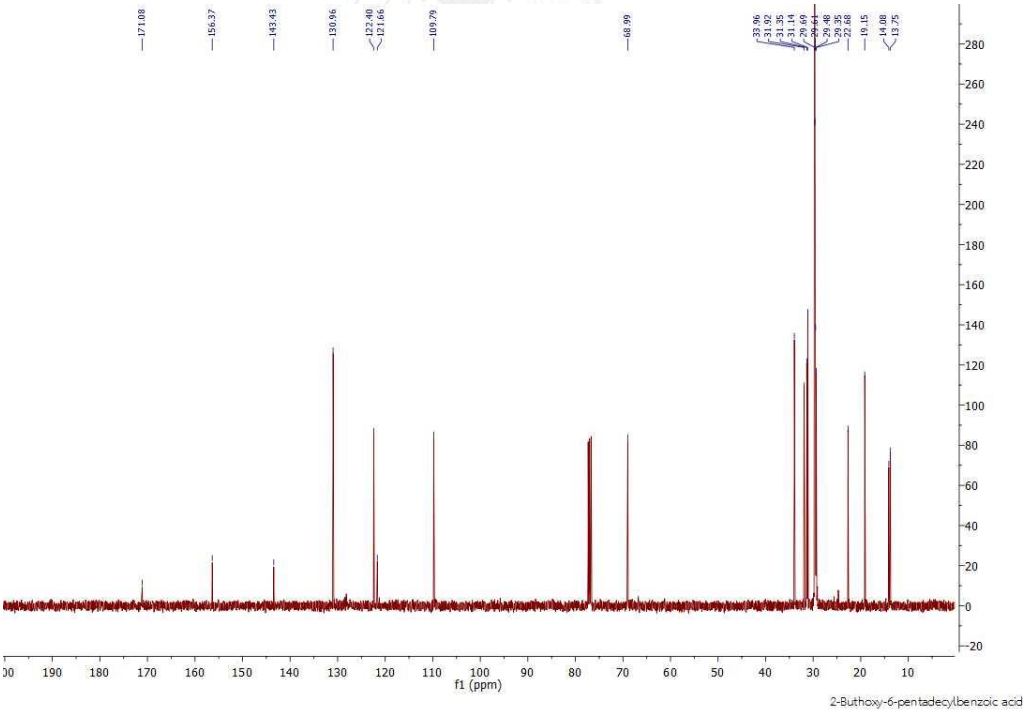


Figure 3.33 The ^{13}C NMR spectrum ($CDCl_3$) of 2-butyloxy-6-pentadecylbenzoic acid **41**

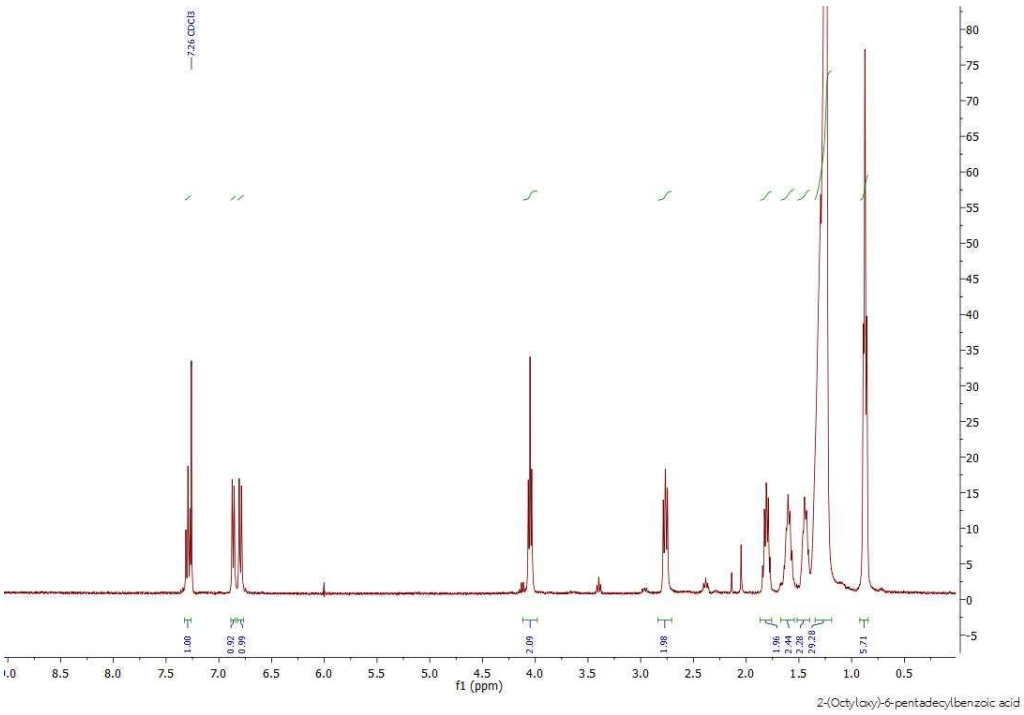


Figure 3.34 The ^{1}H NMR spectrum ($CDCl_3$) of 2-(octyloxy)-6-pentadecylbenzoic acid **42**

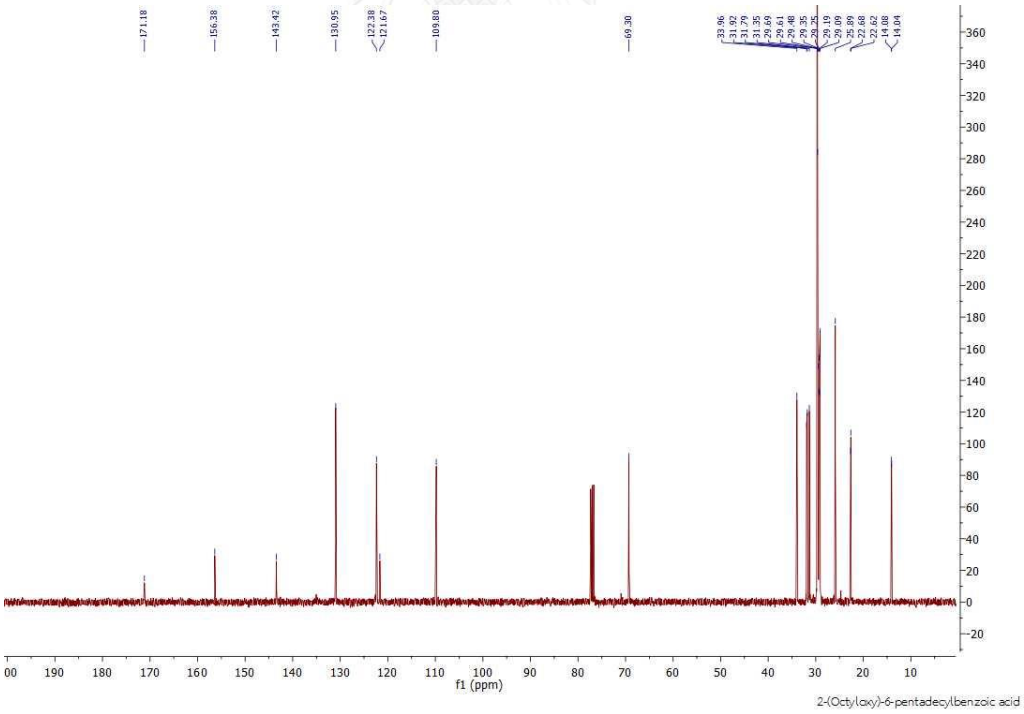
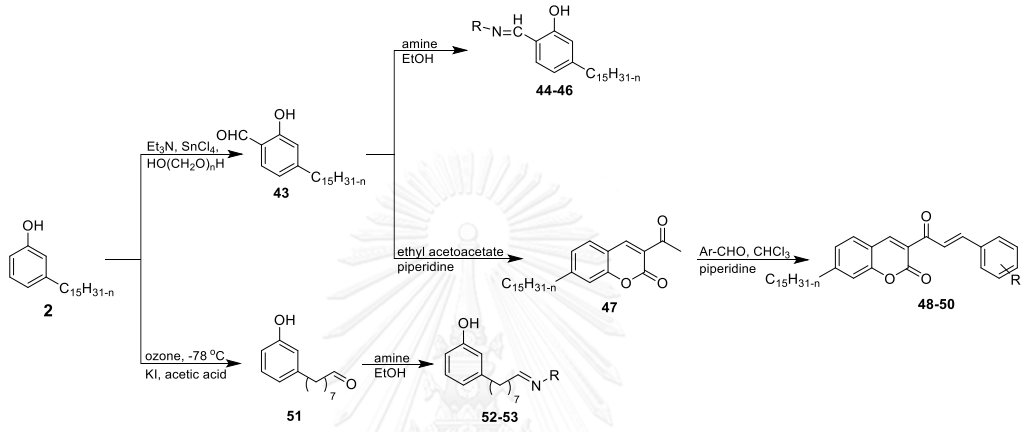


Figure 3.35 The ^{13}C NMR spectrum ($CDCl_3$) of 2-(octyloxy)-6-pentadecylbenzoic acid **42**

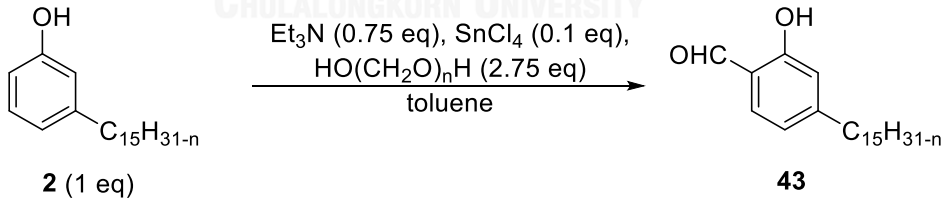
3.3.2 Synthesis and characterization of cardanol derivatives

Most of previous researches, cardanol were utilized in petrochemical industry and catalysis. This research aims to synthesize cardanol derivatives for biological activity. The first group involved imine derivatives was synthesized, while the second group, coumarin cardanol derivatives were studied. The third group dealt with ozonolyzed cardanol and their derivatives.



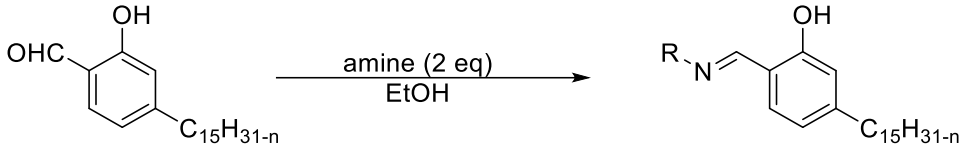
3.3.2.1 Formylated cardanol and their derivatives

Both imine and coumarin cardanol derivatives were prepared from formylated cardanol **43** which was obtained from the reaction as shown below.



Formylated cardanol **43** was prepared from cardanol **2** and paraformaldehyde with a mole ratio of 1:2.75 in the presence of Et_3N and $SnCl_4$ as catalyst. A reaction temperature of 100 °C was used and maintained for 8 h [45]. The product 72 %yield was obtained and characterized by ^1H NMR. Comparison the spectra between cardanol **2** and formylated cardanol **43**, the ^1H NMR spectrum of **43** showed additional aldehydic proton at δ_H 9.83 and hydroxyl proton at δ_H 11.06.

In addition, imine derivatives were synthesized from amine by nucleophilic addition with formylated cardanol **43** to give carbinolamine and then water was eliminated to obtain products as shown in Table 3.9.

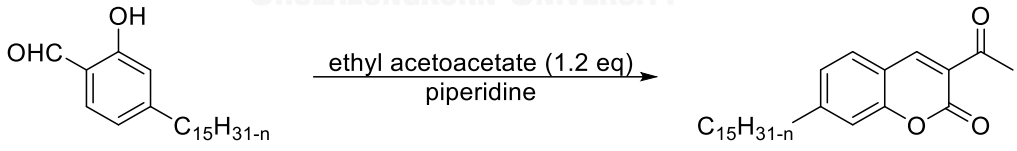


43 (1 eq) **44-46**

Table 3.9 Synthesis of imine derivatives by nucleophilic addition reaction **44-46**

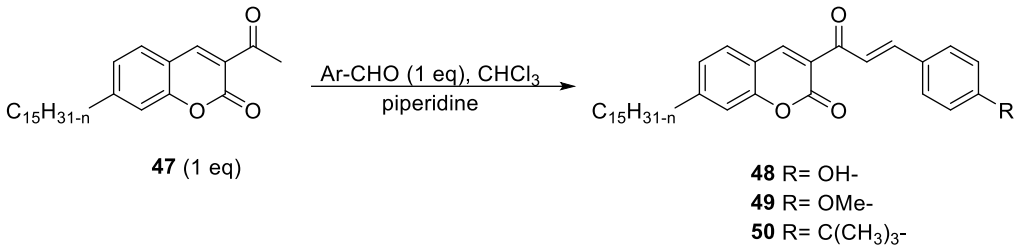
Entry	amine	Substance	% Isolated yield
1	isoniazid	**44**	27
2	thiosemicarbazide	**45**	88
3	(*E*)-1-(4-aminophenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one	**46**	52

From Table 3.9, isoniazid, thiosemicarbazide and amino-chalcone were reacted with **43** to give **44**, **45** and **46** in 25, 88 and 52 %yield. Comparison of the structures of amine employed with their %yield of products suggested that %yield increased when using amine consisting less sterically hindered substituents. The structures of synthesized substances were characterized by ^{1}H NMR.



43 (1 eq) **47**

Coumarin cardanol **47** was obtained from Knoevenagel condensation between formylated cardanol **43** and ethyl acetoacetate catalyzed with piperidine to yielding 64 %. Coumarin cardanol **47** was characterized. Its ^{1}H NMR spectrum revealed δ_H 8.49 of H at 4 position, 7.53 of H at 5 position and 7.14 of two protons at 6 and 8 positions of 3-acetylcoumarin-cardanol**.** Then ketone **47** was reacted with aromatic aldehydes to obtain coumarin-chalcones as shown in Table 3.10.



47 (1 eq)

48 R= OH-
49 R= OMe-
50 R= $C(CH_3)_3$-

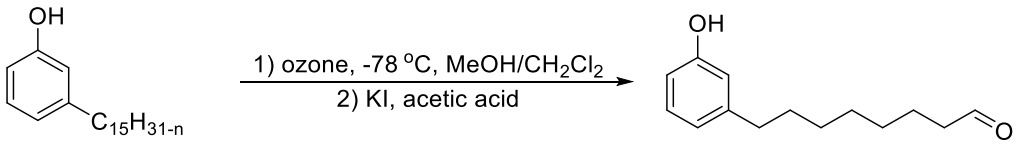
Table 3.10 Synthesis of coumarin-chalcone derivatives by Claisen-Schmidt condensation **48-50**

Entry	Aldehyde	Substance	% Isolated yield
1	4-hydroxybenzaldehyde	**48**	52
2	4-methoxybenzaldehyde	**49**	47
3	4-*tert*-butylbenzaldehyde	**50**	41

As presented in Table 3.10, three coumarin-chalcone derivatives were synthesized from Claisen-Schmidt condensation of selected aldehydes and **47**. The reaction of 4-hydroxybenzaldehyde gave the highest yield of chalcone **48** in 52 %yield. In the case of 4-methoxybenzaldehyde and 4-*tert*-butylbenzaldehyde, products **49** and **50** were obtained in 47 and 41 %yield, respectively. These results suggested that the more sterically substituents, the less amount of the desired product was afforded. All substances were characterized by ^{1}H NMR.

3.3.2.2 Ozonolyzed cardanol and its analogues

Furthermore, cardanol **2** was also functionalized to aldehyde *via* ozonolysis as shown in equation below.

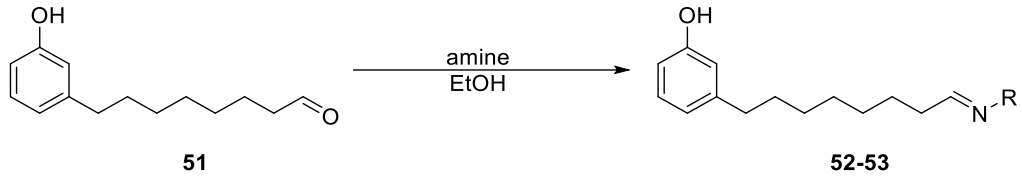


2 **51**

Cardanol **2** was ozonolyzed under ozone at -78 °C in the ratio 1:4 MeOH/CH_2Cl_2 to obtain product 34 %yield. The trace of oxygen was formed, due to decomposition of ozone, then reacted with KI to generate I_2 which separated out by extraction with

thiosulfate solution. The product **51** was characterized by ^{1}H NMR [47]. The spectrum showed a new signal of aldehyde proton at δ_H 9.72.

8-(3-Hydroxyphenyl)octanal **51** was further reacted with amine to generate imine derivatives as shown in Table 3.11.

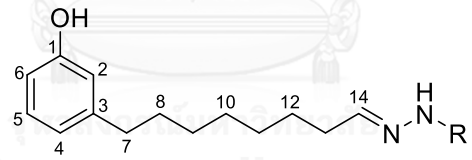


51 **52-53**

Table 3.11 Synthesis of imine derivatives from ozonolyzed cardanol **52-53**

Entry	Amine	Compound	% Isolated yield
1	isoniazid	**52**	55
2	thiosemicarbazide	**53**	41

From Table 3.11, compound **51** was reacted with two amines: isoniazid and thiosemicarbazide to give products **52** and **53** in 55 and 41 %yield. Two new compounds **52** and **53** were characterized by ^{1}H and ^{13}C NMR.



The ^{1}H NMR spectrum of (*E*)-*N*'-(8-(3-hydroxyphenyl)octylidene) isonicotinohydrazide **52** in Figure 3.36 showed the signals of –CO-N**H**-N=C protons at δ_H 11.63 (H-4), 6.86 (H-5). The hydroxyl proton was shifted from δ_H 9.24 to 9.72. Two signals at δ_H 8.72 and 7.72 were assigned for four aromatic protons on isoniazid. The signal at δ_H 7.72 could be assigned for a proton of imine (–N=C**H**-C). The signals at δ_H 7.01 and 6.54 were ascribed to four protons on aromatic ring of cardanol. Four protons of methylene groups on carbon connected to aromatic ring and imine bond were observed at δ_H 2.43 (2H) and 2.25 (2H). The ^{13}C NMR spectrum (Figure 3.37) displayed the characteristic carbonyl carbon of amide at δ_C 161.3 and imine carbon (C-14) at δ_C 154.1. The chemical shift of six aromatic carbons of cardanol were observed at δ_C 157.1 (C-1), 143.7 (C-3), 129.1 (C-5), 119.0 (C-4), 115.1 (C-2) and 112.5 (C-6). The aromatic

carbons of isoniazid ring at 150.1 (2C), 140.6 and 121.4 (2C) were detected. The seven carbons at δ_C 35.1, 31.9, 30.7, 28.5 (3C) and 25.8 (C-7 to C-13) were referred to alkyl chain.

The ^{1}H NMR spectrum of (*E*)-2-(8-(3-hydroxyphenyl)octylidene)hydrazine-1-carbothioamide **53** in Figure 3.38 displayed the signals of –CS-N**H**-N=C proton at δ_H 10.99. The hydroxyl proton and the signal of imine proton (-N=C**H**-) at δ_H 9.24 and 8.61, respectively were detected. The signal at δ_H 7.90 was assigned for two protons of amine. Four aromatic protons were observed at δ_H 6.53 and 7.39. The ^{13}C NMR spectrum (Figure 3.39) displayed the characteristic of -**C**=S of thiosemicarbazide at δ_C 177.6. The imine carbon (C-14) appeared at δ_C 147.6. The chemical shift of six aromatic carbons were detected at δ_C 157.2 (C-1), 143.6 (C-3), 129.0 (C-5), 118.9 (C-4), 115.1 (C-2) and 112.5 (C-6). Seven carbons at δ_C 35.1, 31.6, 30.8, 29.0, 28.5, 25.8 and 24.3 (C-7 to C-13) were referred to alkyl chain.

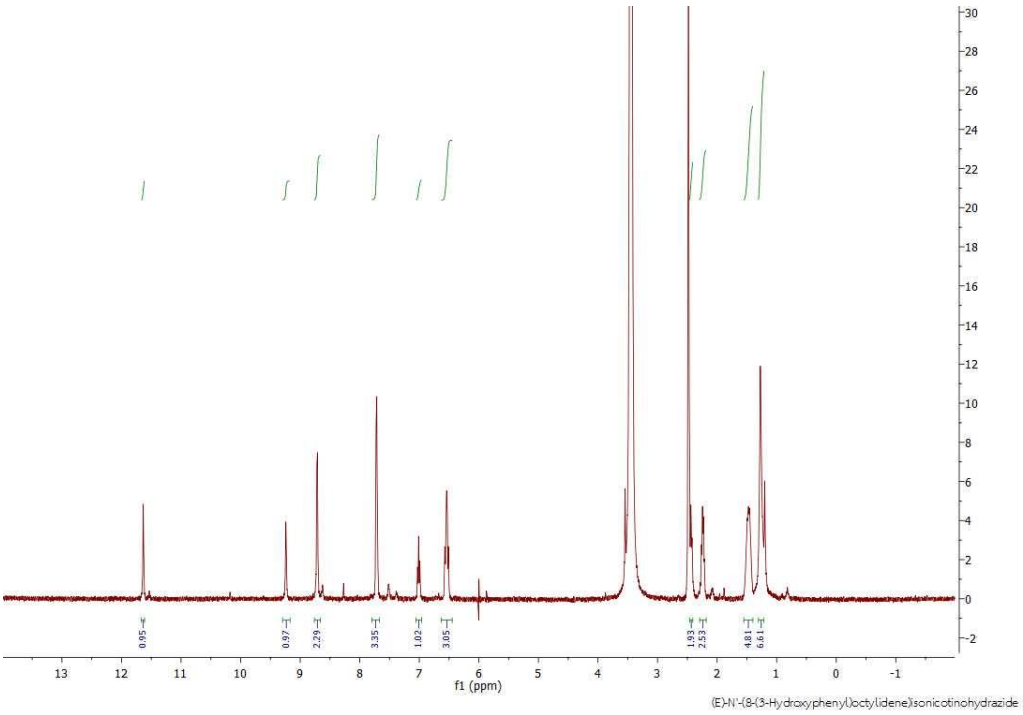


Figure 3.36 The ^{1}H NMR spectrum (DMSO-d_6) of (*E*)-*N*'-(8-(3-hydroxyphenyl)octylidene) isonicotinohydrazide **52**

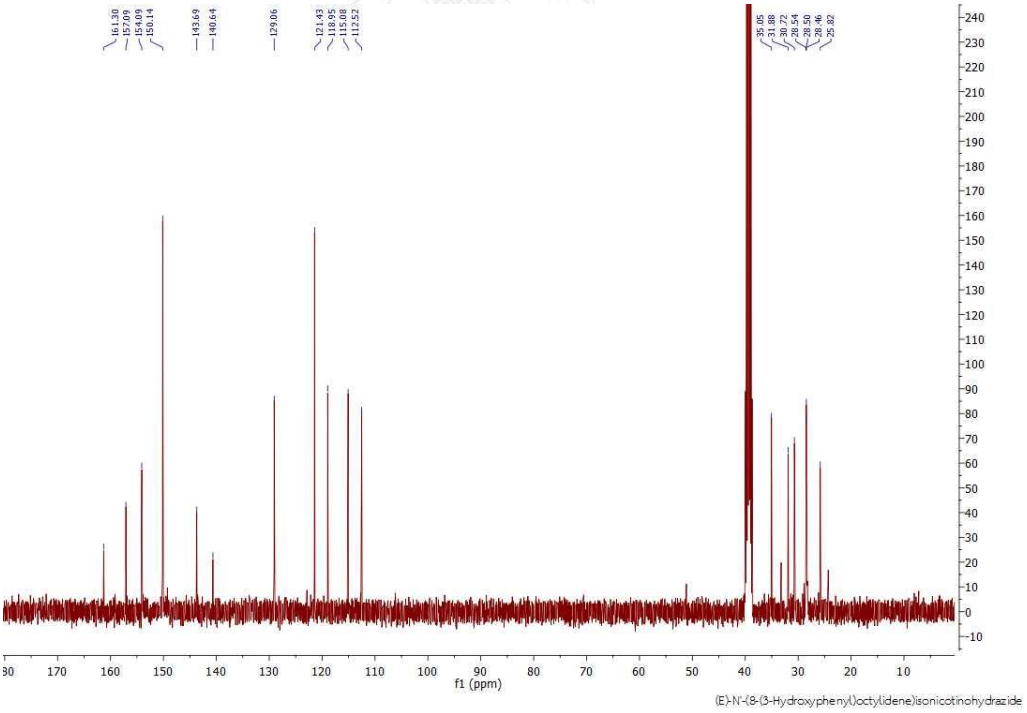


Figure 3.37 The ^{13}C NMR spectrum (DMSO-d_6) of (*E*)-*N*'-(8-(3-hydroxyphenyl) octylidene) isonicotinohydrazide **52**

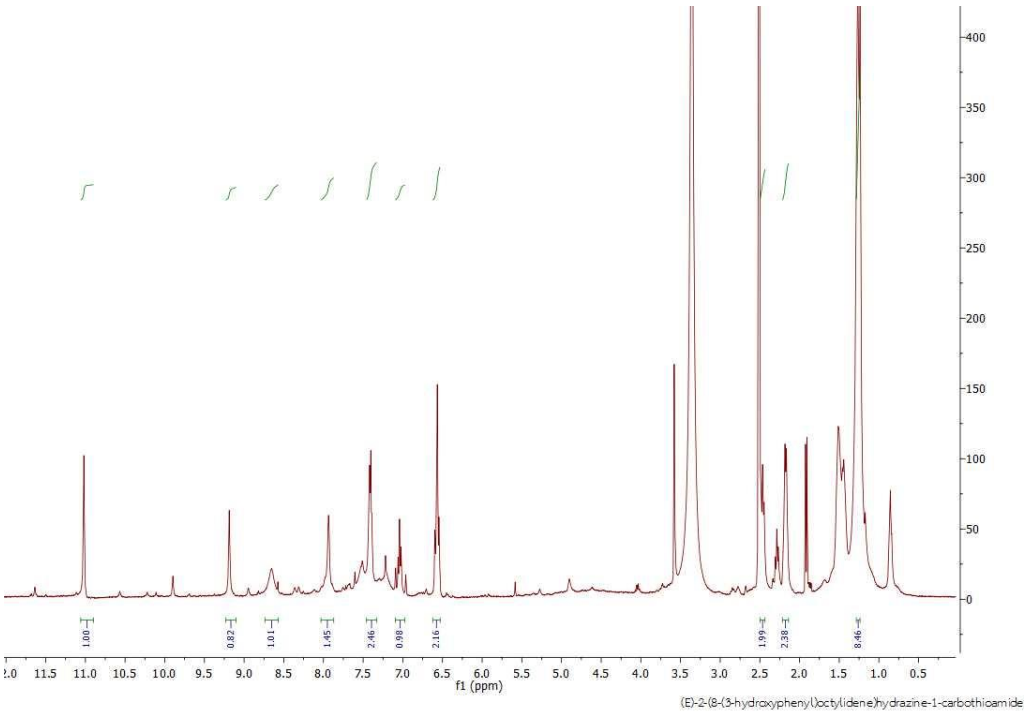


Figure 3.38 The ^{1}H NMR spectrum (DMSO-d_6) of (*E*)-2-(8-(3-hydroxyphenyl) octylidene)hydrazine-1-carbothioamide **53**

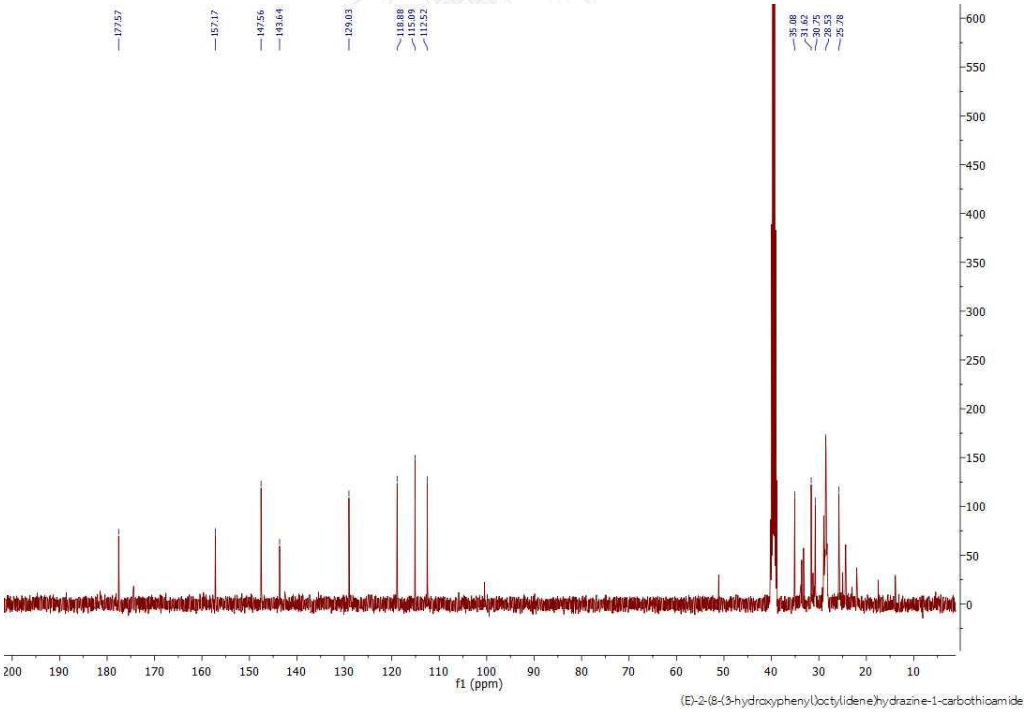


Figure 3.39 The ^{13}C NMR spectrum (DMSO-d_6) of (*E*)-2-(8-(3-hydroxyphenyl) octylidene)hydrazine-1-carbothioamide **53**

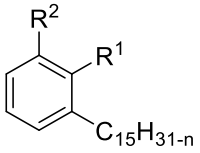
3.3.3 Antioxidant activity of anacardic acid derivatives

According to the results displayed earlier, anacardic acid **4-7** acted as antioxidant compounds better than cardol **12, 14-15** and cardanol **8-11**. Anacardic acid could scavenge superoxide anion generated by PMS-NADH system. So anacardic acid **1** was selected to modify the structure into ester and ether derivatives, and evaluated for their antioxidant activity. Two antioxidant activities: the scavenging activity of DPPH radicals, and superoxide anion radicals generated by PMS-NADH system.

3.3.3.1 DPPH radical-scavenging activity of anacardic acid and its derivatives

Fourteen anacardic acid derivatives **24-29, 35-42** (100 μM) were evaluated for scavenging DPPH radical activity compared with saturated anacardic acid **4** and two positive control agents: resveratrol (100 μM) and gallic acid (10 μM) (Table 3.12).

Table 3.12 % scavenging of DPPH radicals of anacardic acid derivatives **24-29**, **35-42**



Entry	Compound	R^1	R^2	n	% Scavenging of DPPH radicals
1	**24**	$COOCH_3$	OH	2, 4, 6	30.2 ± 0.3
2	**25**	$COOC_2H_5$	OH	2, 4, 6	29.1 ± 0.7
3	**26**	$COOC_4H_9$	OH	2, 4, 6	27.7 ± 0.1
4	**27**	$COOC_6H_{13}$	OH	2, 4, 6	27.1 ± 0.3
5	**28**	$COOC_8H_{17}$	OH	2, 4, 6	25.3 ± 0.4
6	**29**	$COOC_{12}H_{25}$	OH	2, 4, 6	24.4 ± 0.3
7	**35**	COOH	$OCOCH_3$	0	22.6 ± 2.0
8	**36**	COOH	$OCOC_3H_7$	0	14.0 ± 0.6
9	**37**	COOH	$OCOC_7H_{15}$	0	4.8 ± 0.1
10	**38**	COOH	$OCOC_{11}H_{23}$	0	1.6 ± 0.0
11	**39**	COOH	OCH_3	0	1.0 ± 0.6
12	**40**	COOH	OC_2H_5	0	5.3 ± 0.2
13	**41**	COOH	OC_4H_9	0	4.9 ± 0.3
14	**42**	COOH	OC_8H_{17}	0	1.7 ± 0.6
15	**4**	COOH	OH	0	4.3 ± 1.2
	resveratrol (100 μM)				76.1 ± 0.3
	gallic acid (10 μM)				88.5 ± 0.2

Table 3.12 shows that the ester derivatives on carboxyl **24-29** (entries 1-6) exhibited 25-30% scavenging DPPH radicals which were higher activity than the derivatives on hydroxyl **35-42** ranged 1-22% (entries 7-14) and saturated anacardic acid **4** 4.3% (entry 15). However, all modified compounds displayed low % inhibition of DPPH radicals. These results showed all derivatives were steric molecules and difficult

to transfer the electron to DPPH radical. It seemed that all functionalized anacardic acids could not scavenge DPPH radicals.

3.3.3.2 Scavenging superoxide radicals generated by PMS-NADH system of anacardic acid derivatives 24-29 and 35-42

The scavenging superoxide anion radicals of fourteen anacardic acid analogues **24-29** and **35-42** (0-200 μM) were determined by monitoring the color change of NBT. Those mixtures were measured for 60 s at 560 nm and calculated into % relative scavenging activity as shown in Figures 3.40 and 3.41.

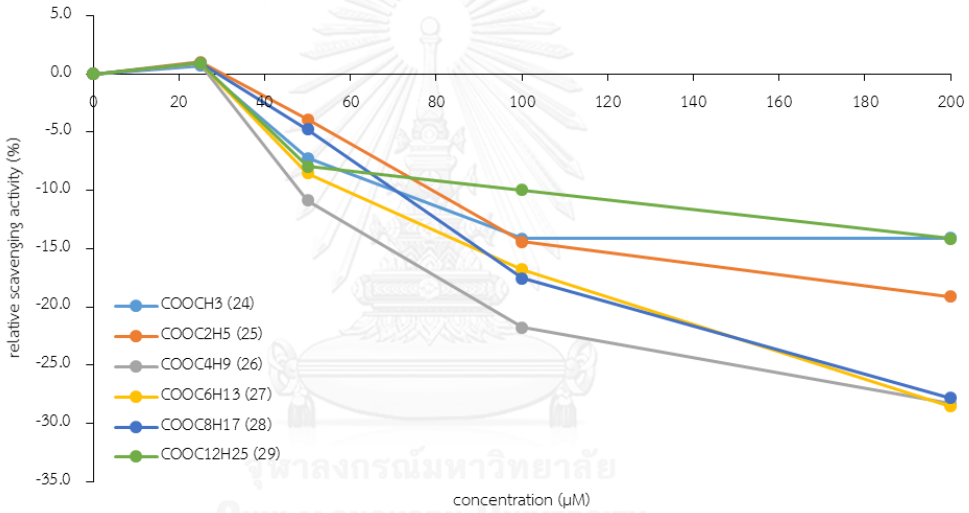


Figure 3.40 Superoxide anion radicals scavenging activity generated by PMS-NADH of ester of anacardic acid derivatives on carboxyl **24-29**

As the results presented in Figure 3.40, all alkyl anacardates **24-29** did not show scavenging superoxide anion radical activity by PMS-NADH. This pointed out that the analogues with modified carboxyl of anacardic acid to ester did not show the activity.

In addition, the scavenging superoxide anion radical activity of eight functionalized anacardic acid on hydroxyl was determined. Eight derivatives could be classified into two groups: ester **35-38** and ether **39-42**. The results are displayed in Figure 3.41.

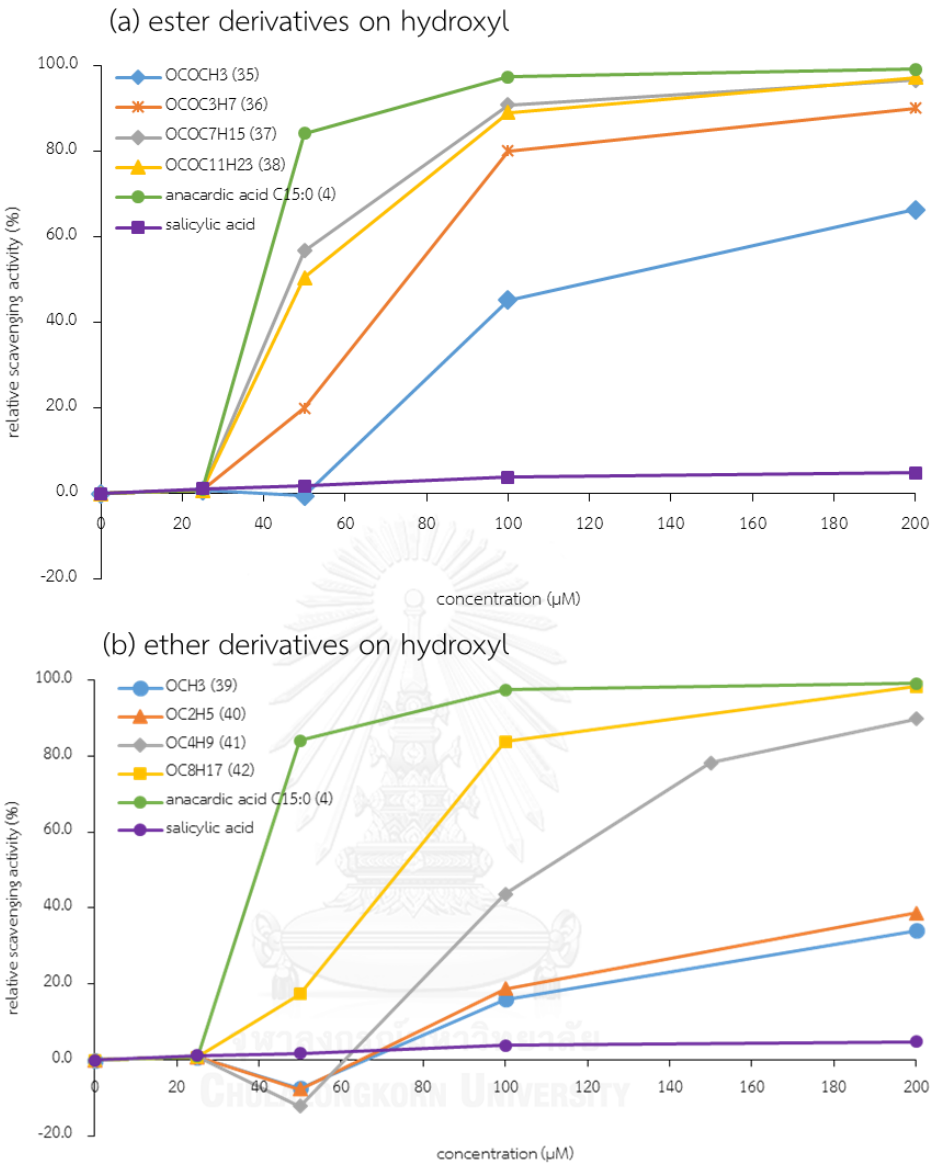


Figure 3.41 Superoxide anion radicals scavenging activity generated by PMS-NADH (a) ester derivatives **35-38**, (b) ether derivatives **39-42** on hydroxyl of anacardic acid

Figure 3.41 shows that eight functionalized anacardic acid on hydroxyl **35-42** represented lower superoxide radicals scavenging activity than saturated anacardic acid **4**, but better than salicylic acid. Both derivatives showed the relationship between the length of substituent and scavenging activity. Interestingly, the compounds which

contained eight carbon atom chain length at hydroxyl group revealed good superoxide anion radical scavenging activity of each analogues as shown in Figure 3.42.

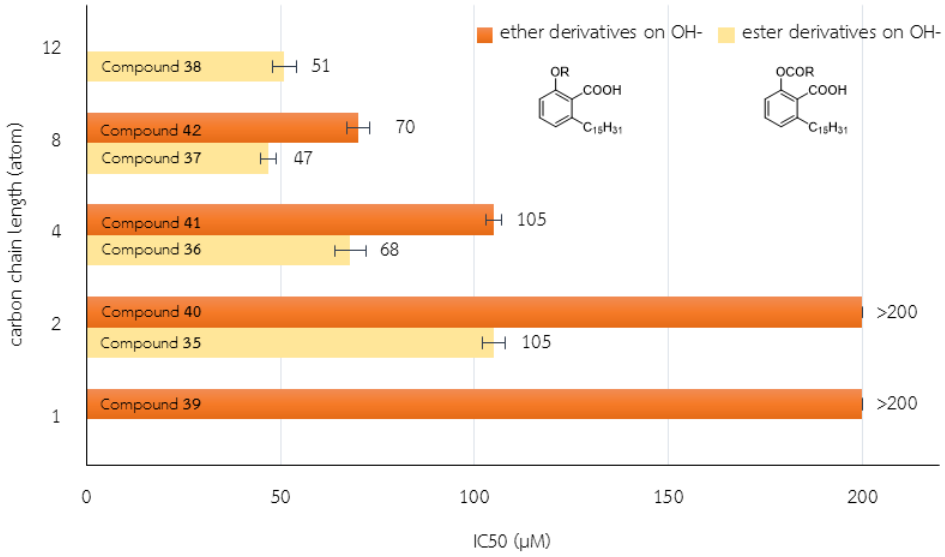
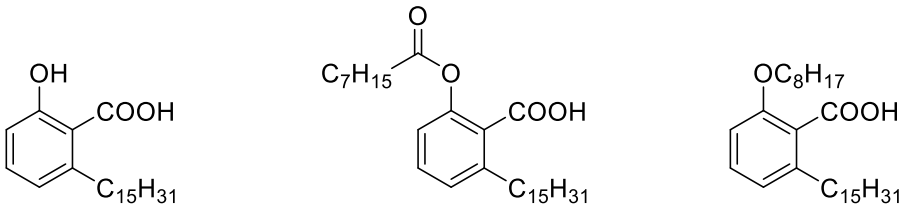


Figure 3.42 The effect of carbon chain length and modified group on scavenging superoxide anion

Figure 3.42 presents compound **37**, octanoyloxy substituted hydroxyl of anacardic acid, with IC_{50} of 47±2 μM. The decreasing activity was observed when the length of substituents was less or more than eight carbon atoms. Acetoxy **35** was the least active compound with IC_{50} of 105±3 μM, followed by butanoyloxy **36** with 68±4 μM and dodecanoyloxy **38** with 51±3 μM, the latter was a little bit less than octanoyloxy **37**. These results displayed similar trend to ether anacardic acid derivatives. The IC_{50} of compound **42**, octyloxy (70±3 μM), pointed out good activity of this analogues, followed by butyloxy **41** (105±2 μM). On the other hand, the IC_{50} of methoxy **39** and ethoxy **40** were more than 200 μM. These findings suggested that the ether substituted anacardic acid containing eight carbon atoms be the best structure. To summarize, the functionalized anacardic acids were compared with saturated anacardic acid **4.** Three best compounds for each analogues are listed in Figure 3.43.



anacardic acid $C_{15:0}$ **4** | compound **37** | compound **42**

IC_{50} 41±3 μM | IC_{50} 47±2 μM | IC_{50} 70±3 μM

Figure 3.43 The IC_{50} of three best anacardic acids possessing scavenging superoxide anion radical activity

Compared the structure of saturated anacardic acid **4** with **37**, and **42** (Figure 3.43), the ester **37** displayed better activity than ether **42**. This revealed that the carbonyl ester could promote to scavenge superoxide anion radicals. However, both analogues displayed lower activity than saturated anacardic acid **4**. This assumed that the carboxylic group and saturated side chain seemed to play an important role for this activity.

3.3.3.3 Conclusion

Anacardic acid derivatives **24-29**, **35-42** displayed low % DPPH radicals scavenging. Compounds **24-29** revealed low superoxide anion scavenging activity. In addition, compounds **37** and **42**, the substituent containing eight carbon atoms, were good scavenging superoxide anion radicals of each group. So the carboxylic group and saturated side chain were the main effect for this activity.

3.3.4 Antibacterial activity

3.3.4.1 Preliminary study on antibacterial activity of three major constituents from CNSL

Three major constituents from CNSL **1-3** and chloramphenicol, a positive control, were screened for their antibacterial activity against four Gram-positive

bacteria: *S. mutans* ATCC 25175, *S. sobrinus* KCCM 11898, *P. acnes* KCCM 41747, *S. aureus* ATCC 25923 and a Gram-negative bacteria: *S. typhi* ATCC 422. The preliminary antibacterial activity of anacardic acid **1**, cardanol **2**, and cardol **3** were performed by agar diffusion method. After incubation of the cultures at 37 °C for 24 h, the inhibition zone was measured. The results are presented in Figure 3.44 and Table 3.13.

Table 3.13 Preliminary study on antibacterial activity of anacardic acid **1**, cardanol **2**, and cardol **3** against five bacteria

Substances (1000 ppm)	Name of bacteria and inhibition zone ± standard deviation (mm)				
	S. mutans ATCC 25175	*S. sobrinus* KCCM 11898	*P. acnes* KCCM 41747	*S. aureus* ATCC 25923	*S. typhi* ATCC 422
anacardic acid **1**	16.67±0.29	14.00±0.50	15.08±0.72	11.75±0.90	13.67±0.88
cardanol **2**	8.50±0.50	9.17±0.76	12.33±0.47	8.08±0.38	9.08±0.58
cardol **3**	18.00±1.73	18.17±4.16	17.33±1.04	16.58±1.76	16.58±2.18
Chloramphenicol (0.5 mM)	21.67±0.29	20.67±0.76	26.00±0.50	26.00±0.00	21.33±1.15

Criteria of inhibition zone (mm): inhibition zone >15: excellent, 13.1-15: very good, 10.1-13: good, 8.1-10: moderate, 6.1-8: weak, ≤6: no activity

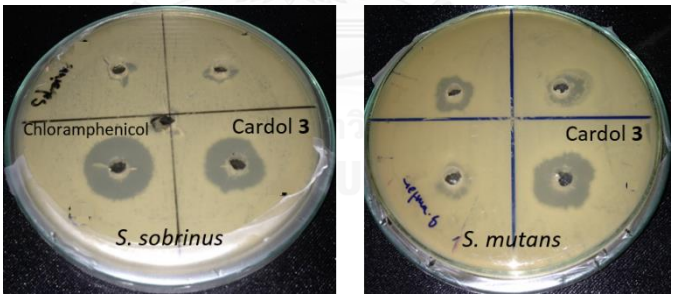


Figure 3.44 The inhibition zone of cardol **3** against (a) *S. sobrinus* and (b) *S. mutans*

According to Table 3.13, anacardic acid **1** exhibited excellent activity on *S. mutans* and *P. acnes* with the inhibition zone of 16.67±0.29 and 15.08±0.72 mm. In addition, anacardic acid **1** also revealed good to very good activity against *S. aureus*, *S. typhi* and *S. sobrinus* with the inhibition zone of 11.75±0.90, 13.67±0.88 and 14.00±0.50 mm, respectively. Cardol **3** showed the excellent activity against all five bacteria while cardanol **2** gave good activity against *P. acnes* and moderate activity against other four bacteria. These results presented that the core structure of

resorcinol (cardol **3**) was better than salicylic acid (anacardic acid **1**) and phenol (cardanol **2**), respectively. Accordingly, the MIC and MBC of anacardic acid **1** and cardol **3** were determined as shown in Table 3.14. Since, cardol **3** was obtained in low yield from the separation of CNSL, anacardic acid **1** was thus further selected to modify its structure to derivatives **24-42** and evaluated their inhibitory activity against *S. mutans*, *S. sobrinus*, and *P. acnes*. In addition, the analogues of cardanols **43-51** were prepared with the aim to explore for structure and antibacterial relationship.

Table 3.14 The MIC and MBC of anacardic acid **1** and cardol **3**

Substances	*S. mutans* ATCC 25175		*S. sobrinus* KCCM 11898		*P. acnes* KCCM 41747		*S. aureus* ATCC 25923		*S. typhi* ATCC 422	
	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MBC (μg/mL)
Anacardic acid **1**	7.81	>31.25	7.81	>31.25	3.91	7.81	7.81	>31.25	7.81	>31.25
Cardol **3**	7.81	>31.25	7.81	>31.25	7.81	7.81	7.81	>31.25	7.81	>31.25

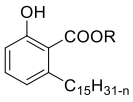
Table 3.14 shows that both anacardic acid **1** and cardol **3** were active with the MIC value of 7.81 μg/mL against four bacteria; *S. mutans*, *S. sobrinus*, *S. aureus* and *S. typhi*. In the case of antibacterial activity against *S. mutans*, the MIC value of mixtures **1** and **3** revealed higher than anacardic acid and cardol constituents with MIC 1.56-3.13 μg/mL and 0.78-1.56 μg/mL, respectively [13]. The antibacterial activity against *S. aureus* displayed that mixtures **1** and **3** (7.81 μg/mL) gave better MIC than their constituents (6.25-100 μg/mL of anacardic acid and 6.25-more than 100 μg/mL of cardol). This assumed that the combination of their constituents of each analogues were decreased the activity against *S. mutans* and increased the activity against *S. aureus*. This result may imply that they were antagonistic against *S. mutans* and synergistic on *S. aureus*. Anacardic acid **1** revealed the best activity against *P. acnes* with MIC of 3.91 μg/mL while cardol **3** displayed lower activity with MIC value of 7.81 μg/mL. These results were agreeable with the previous research, the MIC of anacardic acid constituents (0.39-1.56 μg/mL) displayed better activity than cardol constituents (0.78-50 μg/mL) [13]. In addition, the MBC of anacardic acid **1** and cardol **3** were evaluated for five bacteria. The result revealed that only *P. acnes* were killed by mixtures **1** and **3** with the MBC of 7.81 μg/mL and could point out that they were bactericidal agent due to MIC index (MBC/MIC) less than four. Anacardic acid **1** revealed

MBC against *S. mutans* more than 31.25 μg/mL which was 4-fold than its MIC (7.81 μg/mL). Thus, anacardic acid **1** was a bacteriostatic agent. These results exhibited different outcome from the previous research. Muroi reported that anacardic acid **4-6** possesed the MBC 3.13-6.25 μg/mL which were higher than MIC (1.56-6.25 μg/mL), however their ratios were not more than four [16]. So anacardic acid **4-6** were bactericidal agents. For *S. sobrinus*, *S. aureus* and *S. typhi*, anacardic acid **1** and cardol **3** revealed MBC >31.25 μg/mL against 4-fold higher than its MIC (7.81 μg/mL). Thus, they acted as bacteriostatic agents against three bacteria. According to the results, both anacardic acid **1** and cardol **3** displayed comparable trend of antibacterial activity to their constituents. Therefore, mixtures **1** and **3** should be directly utilized.

3.3.4.2 Determination of inhibition zone of anacardic acids derivatives

The antibacterial activity of three selected bacteria: *S. mutans*, *S. sobrinus*, and *P. acnes* against twenty anacardic acid derivatives **4, 24-42** and chloramphenicol was determined. The derivatives were divided into three groups: ester on carboxyl **24**-**30**, ester on hydroxyl **31-38**, and ether on hydroxyl **39-42**. The results are shown in Tables 3.15–3.17.

Table 3.15 Antibacterial activity of ester on carboxyl **24**-**30** against *S. mutans*, *S. sobrinus*, and *P. acnes*

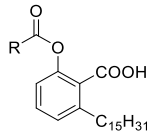
Structure		Compound (1 mM)	Name of bacteria and inhibition zone ± standard deviation (mm)		
			S. mutans ATCC 25175	*S. sobrinus* KCCM 11898	*P. acnes* KCCM 41747
	H (1000 ppm)	**1**	16.67±0.29	14.00±0.50	15.08±0.72
	CH_3-	**24**	8.33±0.47	9.00±0.00	10.00±0.00
	C_2H_5-	**25**	8.33±0.47	9.00±0.00	9.00±0.82
	C_4H_9-	**26**	8.33±0.47	9.67±0.47	10.67±0.47
	C_6H_{13}-	**27**	8.33±0.47	10.00±1.41	9.33±0.47
	C_8H_{17}-	**28**	7.67±0.94	9.33±0.47	10.00±0.00
	$C_{12}H_{25}$-	**29**	7.33±0.47	9.33±0.94	9.33±0.47
	Ph-CH_2-	**30**	9.67±0.47	9.67±0.47	7.67±0.47
Chloramphenicol (0.5 mM)			21.67±0.29	20.67±0.76	26.00±0.50

Criteria of inhibition zone (mm): inhibition zone >15: excellent, 13.1-15: very good, 10.1-13: good, 8.1-10: moderate, 6.1-8: weak, ≤6: no activity

Table 3.15 displays that alkyl anacardate **24-30** revealed weak to moderate antibacterial activity with the inhibition zone of 7.33-10.67 mm. Comparison those activities with that of anacardic acid **1** (1000 ppm), all derivatives showed lower activity. This suggested that the carboxylic acid group be necessary for antibacterial activity.

In addition, the antibacterial activity of anacardic acid esters on hydroxyl **31-38** were determined in order to study the effect of the substituents on aromatic and the carbon chain length on hydroxyl of anacardic acid **4**. The results are shown in Table 3.16.

Table 3.16 Antibacterial activity of ester on hydroxyl **31-38** against *S. mutans*, *S. sobrinus*, and *P. acnes*

Structure		Compound (1 mM)	Name of bacteria and inhibition zone ± standard deviation (mm)		
			S. mutans ATCC 25175	*S. sobrinus* KCCM 11898	*P. acnes* KCCM 41747
	Ph-	**31**	7.33±0.47	11.33±0.47	11.67±2.49
	2-Cl-Ph-	**32**	7.33±0.47	15.67±0.47	10.33±2.05
	2-Br-Ph-	**33**	9.00±1.41	12.67±1.25	8.33±1.25
	2-OH-Ph-	**34**	7.67±0.47	12.67±0.47	8.33±0.47
	CH_3-	**35**	13.33±1.04	16.83±1.89	12.25±0.87
	C_3H_7-	**36**	11.33±0.76	10.67±0.29	11.67±1.53
	C_7H_{15}-	**37**	9.00±0.50	8.50±0.50	9.00±0.00
	$C_{11}H_{23}$-	**38**	10.17±0.76	10.17±0.58	9.67±0.29
anacardic acid $C_{15:0}$		**4**	11.50±0.00	11.50±0.50	10.67±0.47
Chloramphenicol (0.5 mM)			21.67±0.29	20.67±0.76	26.00±0.50

Criteria of inhibition zone (mm): inhibition zone >15: excellent, 13.1-15: very good, 10.1-13: good, 8.1-10: moderate, 6.1-8: weak, ≤6: no activity

According to the results in Table 3.16, aryl anacardic acid ester **31-34** expressed moderate to good activity with the inhibition zone of 8.33–11.67 mm against *P. acnes*, but still less activity than saturated anacardic acid **4**. They were also evaluated against both *S. mutans* and *S. sobrinus*. Surprisingly, four derivatives revealed good to excellent activity against *S. sobrinus*, while that showed weak activity against *S. mutans*. These results presented the effect of substituents on aromatic against *S. sobrinus*. Compound **32** containing chloro substituent, exhibited excellent activity with the inhibition zone of 15.67±0.47 mm. For the others, compounds **31**, **33** and **34** having phenyl, 2-bromophenyl and 2-hydroxyphenyl also showed significant activity with the inhibition zone of 11.33±0.47, 12.67±1.25 and 12.67±0.47 mm, respectively. These results supposed that the strong withdrawing substituents on aromatic ring exhibited better activity than the weak withdrawing group and donating group or no substituent.

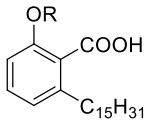
In addition, the antibacterial activity of anacardic acid esters **35-38** were evaluated. Compound **35** containing acetate displayed the best activity against *S. sobrinus* with the inhibition zone of 16.83±1.89 mm and also revealed good to very good activity against *P. acnes* and *S. mutans* with the inhibition zone of 12.25±0.87

and 13.33±1.04 mm. On the other hand, butyrate and octanoate ester on hydroxyl **36, 37** revealed that the longer carbon chain length, the decreasing activity was observed. Butyrate **36** revealed the inhibition zone of 11.33±0.76 mm against *S. mutans*, 10.67±0.29 mm against *S. sobrinus* and 11.67±1.53 mm against *P. acnes* while octanoate **37** indicated the inhibition zone of 11.33±0.76 mm against *S. mutans*, 10.67±0.29 mm against *S. sobrinus* and 11.67±1.53 mm against *P. acnes*. However, dodacanoate **38** showed better activity than **36** and **37** but less than acetate **35**.

From the results, the ester substituents on hydroxyl greatly affected on antibacterial activity, compound **35** disclosed to be the best antibacterial agent against *S. mutans*, *S. sobrinus* and *P. acnes*. The new discovery, the aromatic anacardic ester **31-34** revealed selectively against *S. sobrinus*.

Compounds **39-42** were ether derivatives on hydroxyl of anacardic acid with various carbon chain length of side chain. The antibacterial activity of these compounds were determined. The results are shown in Table 3.17.

Table 3.17 Antibacterial activity of ether on hydroxyl **39-42** against *S. mutans*, *S. sobrinus*, and *P. acnes*

Structure		Compound (1 mM)	Name of bacteria and inhibition zone ± standard deviation (mm)		
			S. mutans ATCC 25175	*S. sobrinus* KCCM 11898	*P. acnes* KCCM 41747
	CH_3-	**39**	15.50±1.80	14.83±1.76	8.08±1.01
	C_2H_5-	**40**	12.00±0.87	13.67±2.47	8.58±0.38
	C_4H_9-	**41**	8.50±0.50	9.00±0.50	7.25±0.43
	C_8H_{17}-	**42**	8.83±1.44	9.00±0.50	7.92±1.59
anacardic acid $C_{15:0}$		**4**	11.50±0.00	11.50±0.50	10.67±0.47
Chloramphenicol (0.5 mM)			21.67±0.29	20.67±0.76	26.00±0.50

Criteria of inhibition zone (mm): inhibition zone >15: excellent, 13.1-15: very good, 10.1-13: good, 8.1-10: moderate, 6.1-8: weak, ≤6: no activity

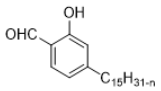
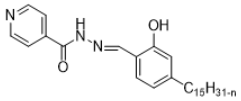
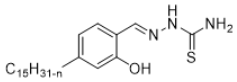
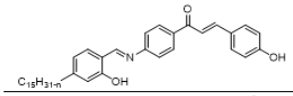
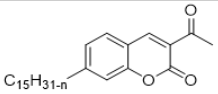
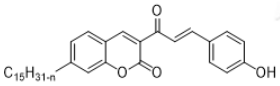
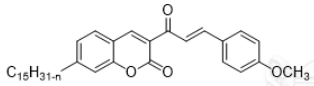
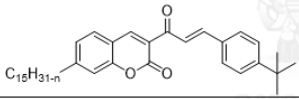
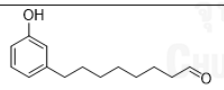
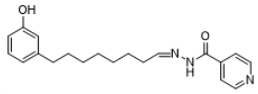
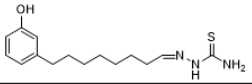
Table 3.17 displayed that compounds **39** and **40** revealed better activity against *S. mutans* and *S. sobrinus* than saturated anacardic acid **4**. Compound **39** with methoxy substituent revealed excellent and very good antibacterial activity against *S. mutans*

and *S. sobrinus* with the inhibition zone of 15.50±1.80 mm and 14.83±1.76 mm, respectively whereas the ethoxy substituent as in compound **40** presented the lower inhibition zone. Compounds **41-42** containing four and eight carbon atoms chain length displayed moderate activity against both bacteria with the inhibition zone ranged 8.50-9.00 mm. In addition, these derivatives **39-42** presented weak to moderate activity against *P. acnes*. The results pointed out that the methoxy and ethoxy groups on C-2 possessed the good structure against *S. mutans* and *S. sobrinus* while the longer chain length displayed weak activity. According to the result, the alkyl substituents more than two carbon atoms may affect steric effect on the activity. These results were agreeable with previous research that the hydroxyl was modified to methoxy or ethoxy group in their derivatives [28, 30, 32, 63].

3.3.4.3 Determination of inhibition zone of cardanol derivatives 43-53

Eleven cardanol derivatives **43-53** which could be classified into three groups as formylated cardanol derivatives **43-46**, coumarin cardanol derivatives **47-50**, and ozonolzed cardanol analogues **51-53** were evaluated for antibacterial activity against *S. mutans*, *S. sobrinus*, and *P. acnes*. The results are shown in Table 3.18.

Table 3.18 Antibacterial activity of cardanol analogues **8**, **43-53** against *S. mutans*, *S. sobrinus* and *P. acnes*

Structure	Compound (1 mM)	Name of bacteria and inhibition zone ± standard deviation (mm)		
		S. mutans ATCC 25175	*S. sobrinus* KCCM 11898	*P. acnes* KCCM 41747
cardanol	**2**	8.50±0.50	9.17±0.76	12.33±0.47
cardanol $C_{15:0}$	**8**	7.33±0.29	5.83±0.76	10.00±0.00
	**43**	7.00±0.00	7.67± 0.47	7.00±0.00
	**44**	7.00±0.00	7.33± 0.47	7.67±0.47
	**45**	7.33±0.47	8.67± 0.47	7.00±0.00
	**46**	7.00±0.00	7.33± 0.47	7.00±0.00
	**47**	9.00±0.00	7.67±0.47	9.00±1.64
	**48**	6.92±1.70	7.67±0.47	7.58±0.95
	**49**	7.75±0.50	8.00±0.00	7.58±0.38
	**50**	7.67±0.88	7.00±0.00	8.67±1.84
	**51**	9.42±0.38	12.5±0.00	9.75±1.75
	**52**	9.75±0.25	9.75±0.35	8.75±1.09
	**53**	7.50±0.71	14.00±0.00	9.00±0.71
Chloramphenicol (0.5 mM)		21.67±0.29	20.67±0.76	26.00±0.50

Criteria of inhibition zone (mm): inhibition zone >15: excellent, 13.1-15: very good, 10.1-13: good, 8.1-10: moderate, 6.1-8: weak, ≤6: no activity

Formylated cardanol and coumarin cardanol derivatives **43-50** mostly exhibited low antibacterial activity against *S. mutans*, *S. sobrinus* and *P. acnes*. For compounds **44-46**, Swamy reported the similar structure of benzohydrazide at ortho position of hydroxyl which was substituted by methoxy displayed good activity against

S. aureus [63]. This pointed out that methoxy group affected on the activity. Coumarin **47** exhibited good activity against *S. mutans* and *P. acnes*, however their derivatives **48-50** displayed weak activity. This result may imply that coumarin-chalcone could not be increasing the antibacterial activity. Ozonolyzed cardanol **51** containing eight carbon chain length presented better activity than original cardanol **2** and saturated cardanol **8** against bacteria of cavities. Compound **51** displayed moderate to good activity against *S. mutans* and *S. sobrinus* with inhibition zone of 9.42±0.38 and 12.5±0.00 mm, while cardanol **2** and saturated cardanol **8** showed weak to moderate activity. The chain length of side chain was associated with the activity as the same previous research. Kubo reported that the antibacterial activity of anacardic acid also depended on the length of side chain (eight carbons chain length is better activity than fifteen against *S. aureus*, *S. mutans* and *P. acnes*) [15]. In addition, the substituents on terminal chain of ozonolyzed cardanol **51** affected on the activity. Compound **53**, thiosemicarbazone derivative, showed very good activity with inhibition zone of 14.00±0.00 mm on *S. sobrinus*, while compound **52**, isonicotinoylhydrzone derivative, displayed moderate activity. These results indicated that cardanol containing eight carbon atoms on side chain and its thiosemicarbazone derivative revealed better antibacterial activity against *S. mutans* and *S. sobrinus*.

3.3.4.4 Conclusion

Anacardic acid **1** and cardol **3** exhibited the MIC of 3.91 and 7.81 μg/mL, and the MBC of 7.81 μg/mL against *P. acnes*. Both mixtures acted as bacteriostatic agents on *S. mutans*, *S. sobrinus*, *S. aureus* and *S. typhi*.

For anacardic acid derivatives, the anacardate derivatives **24-30** decreased the activity. The aromatic ester anacardate **31-34** revealed good activity against *S. sobrinus*. The acetate and methoxy groups on C-2 of anacardic acid **35** and **39** presented good to excellent activity against both bacteria of dental caries. Furthermore, acetate of anacardic acid **35** also showed good activity inhibiting against *P. acnes*.

Most of cardanol derivatives **43-53** expressed low antibacterial activity against *S. mutans*, *S. sobrinus* and *P. acnes*. However, thiosemicarbazone derivative **53** revealed highly active against *S. sobrinus*.

CHAPTER IV

CONCLUSION

Anacardic acid **1** is a major component from CNSL whereas cardanol **2** and cardol **3** are minor. The separation of **1-3** by semi-prep HPLC yielded nine constituents **4-7**, **9-11** and **14-15**. Saturated compounds **4, 8, 12** were prepared by hydrogenation of **1-3**. Constituents of CNSL were determined for anti-inflammatory and antioxidant activity.

For anti-inflammatory, anacardic acid **1** and cardanol **2** were not cytotoxicity on Raw 264.7 cells and showed the same potent inhibitory activity on NO production on LPS-stimulated cells. In addition, anacardic acid $C_{15:3}$ **7** exhibited the best inhibitory activity at 100 μM which could suppress the accumulation of NO production by 71%.

All eleven compounds **4-12**, **14-15** could not scavenge DPPH radicals. Anacardic acid **4-7** and cardol **12, 14-15** showed the scavenging superoxide anion radicals generated by PMS-NADH system. Anacardic acid displayed the effect of the number of double bond on activity, anacardic acid $C_{15:0}$ **4** was stronger activity than anacardic acid $C_{15:1}$ **5**, anacardic acid $C_{15:2}$ **6** and anacardic acid $C_{15:3}$ **7** with IC_{50} 41, 72, 95, and 98 μM, respectively. The result was agreeable with H_2O_2 formation. On the other hand, the number of double bonds did not affect on the activity of cardol, the IC_{50}'s of cardol $C_{15:0}$ **12**, $C_{15:2}$ **14** and $C_{15:3}$ **15** were 142, 155, and 151 μM, respectively.

Anacardic acid **1** was further transformed to ester and ether derivatives **24-42**, which were evaluated for antioxidant and antibacterial activities. For antioxidant activity, compounds **24-29** and **35-42** did not scavenge DPPH radicals. The ester and ether derivatives with eight carbon atoms **37**, **42** on hydroxyl showed best activity to scavenge superoxide anion radicals. The IC_{50} of compounds **37** and **42** were 47±2 and 70±3 μM, respectively.

For antibacterial activity, anacardic acid **1** and cardol **3** displayed good to excellent activity against five bacteria *S. mutans* ATCC 25175, *S. sobrinus* KCCM 11898, *P. acnes* KCCM 41747, *S. aureus* ATCC 25923, *S. typhi* ATCC 422. They were bactericidal

agents against *P. acnes* and bacteriostatic agents against other four bacteria. For anacardic acid derivatives, the replacement of carboxyl group of alkyl chain decreased the activity. The aromatic ester anacardic acids **31-34** displayed good to excellent activity against *S. sobrinus*. The acetate and methoxy on hydroxyl of anacardic acid **35, 39** also presented good to excellent activity against both bacteria of dental caries. In addition acetate of anacardic acid **35** revealed good activity to inhibit *P. acnes*.

Cardanols derivatives: formylated cardanol, coumarin cardanol and their analogues, mostly displayed low antibacterial activity against *S. mutans*, *S. sobrinus* and *P. acnes*. Ozonolzed cardanol **51** revealed better activity than cardanol **2** against bacteria of cavity and its thiosemicarbazone derivative also displayed very good activity against *S. sobrinus*.

The outcome from this research can be summarized as presented in Figure 4.1.

Proposed for the future work

For anti-inflammatory activity, the investigation of anacardic acid, cardanol constituents, and cardol constituents at concentration less than 50 μM on mode of action should be carried out. New derivatives of anacardic acid, cardanol and cardol should be synthesized and evaluated for antioxidant and antibacterial activities. The synergism of main constituents of CNSL with other compounds should be scrutinized.

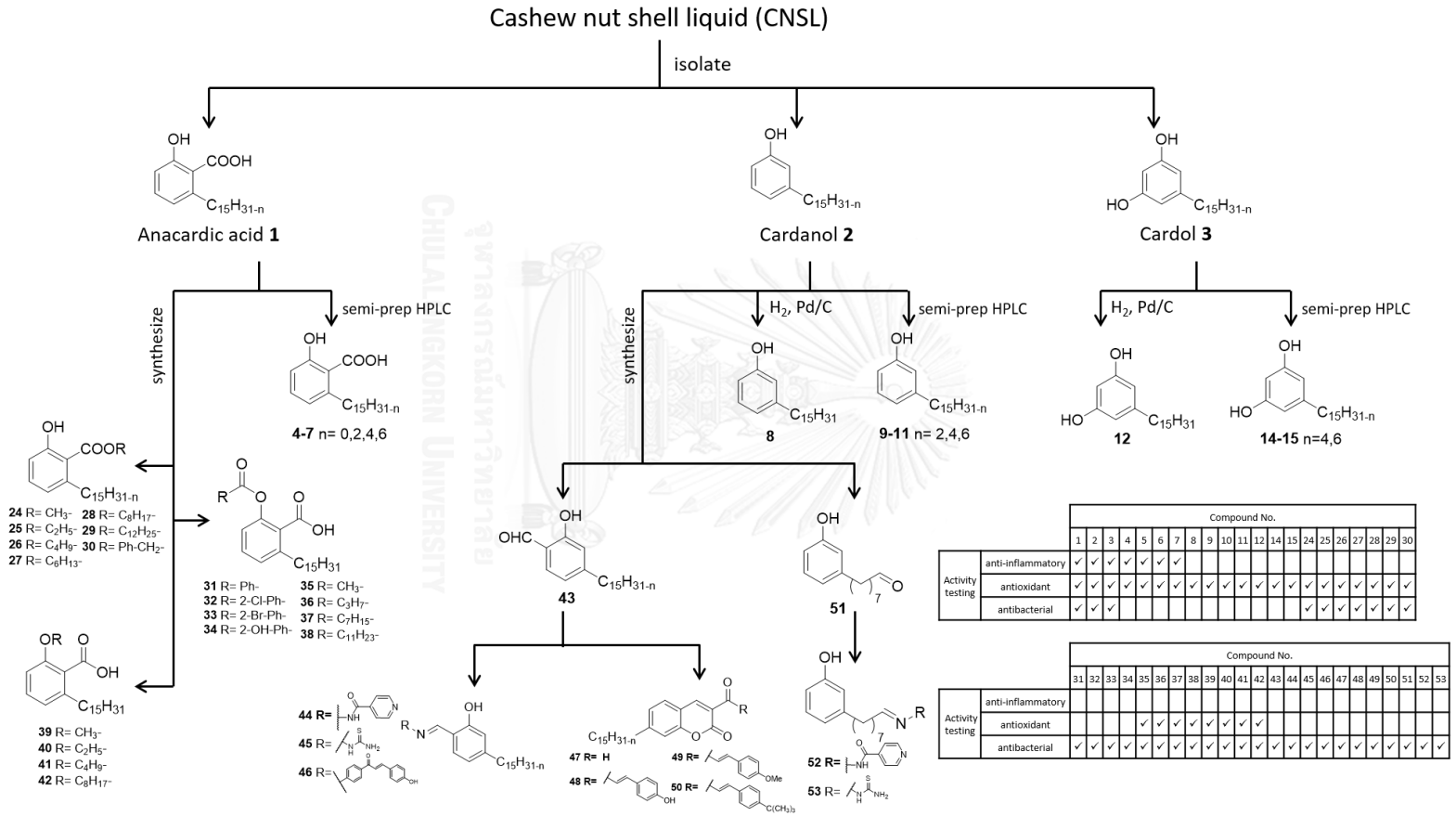


Figure 4.1 The outcome from this research

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APPENDIX

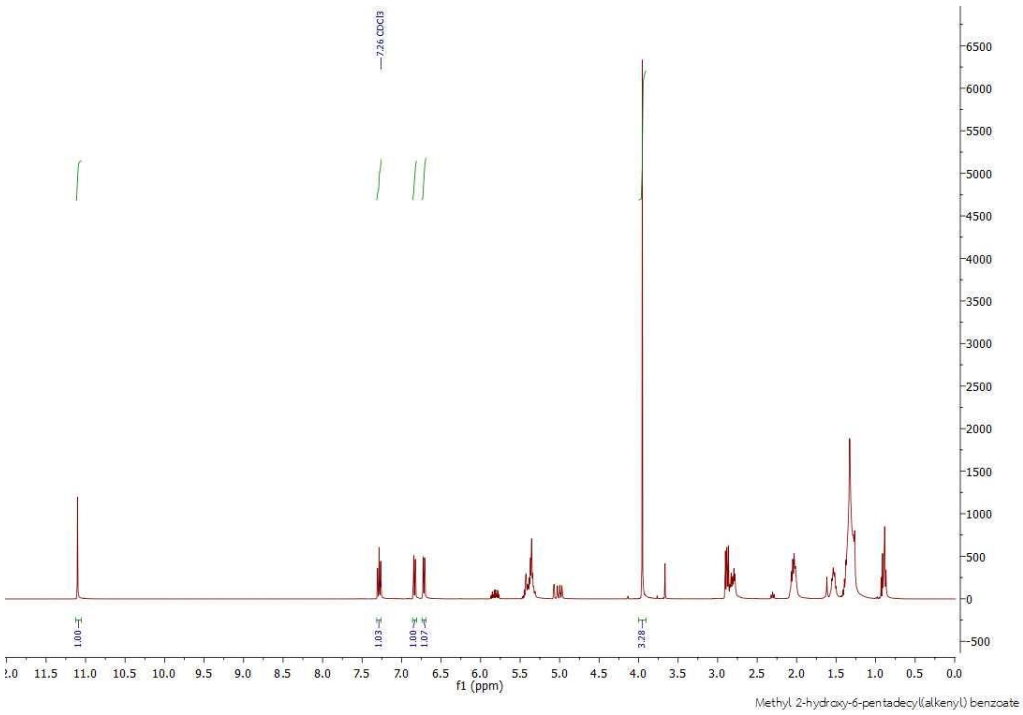

Figure A-1 The ^{1}H NMR spectrum ($CDCl_3$) of compound **24**

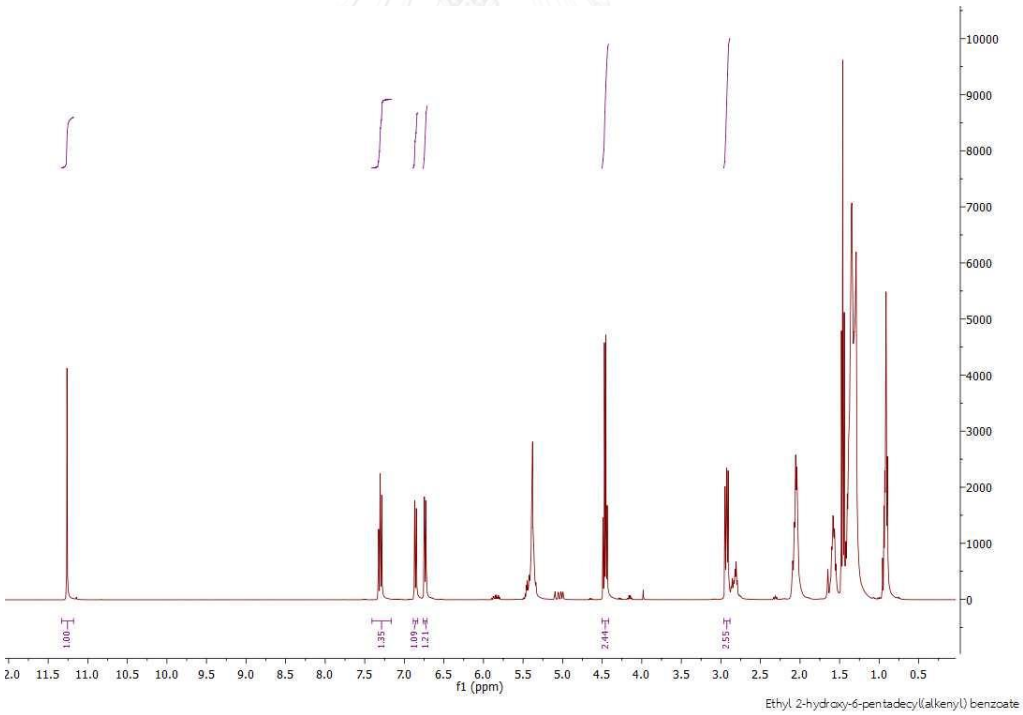

Figure A-2 The ^{1}H NMR spectrum ($CDCl_3$) of compound **25**

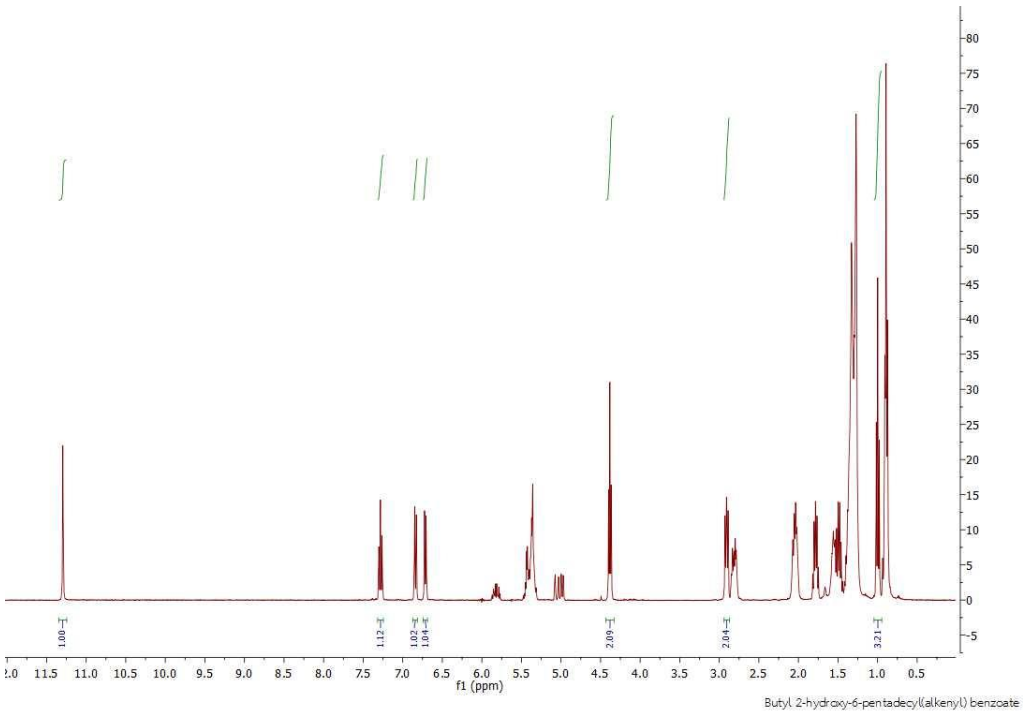

Figure A-3 The ^{1}H NMR spectrum ($CDCl_3$) of compound **26**

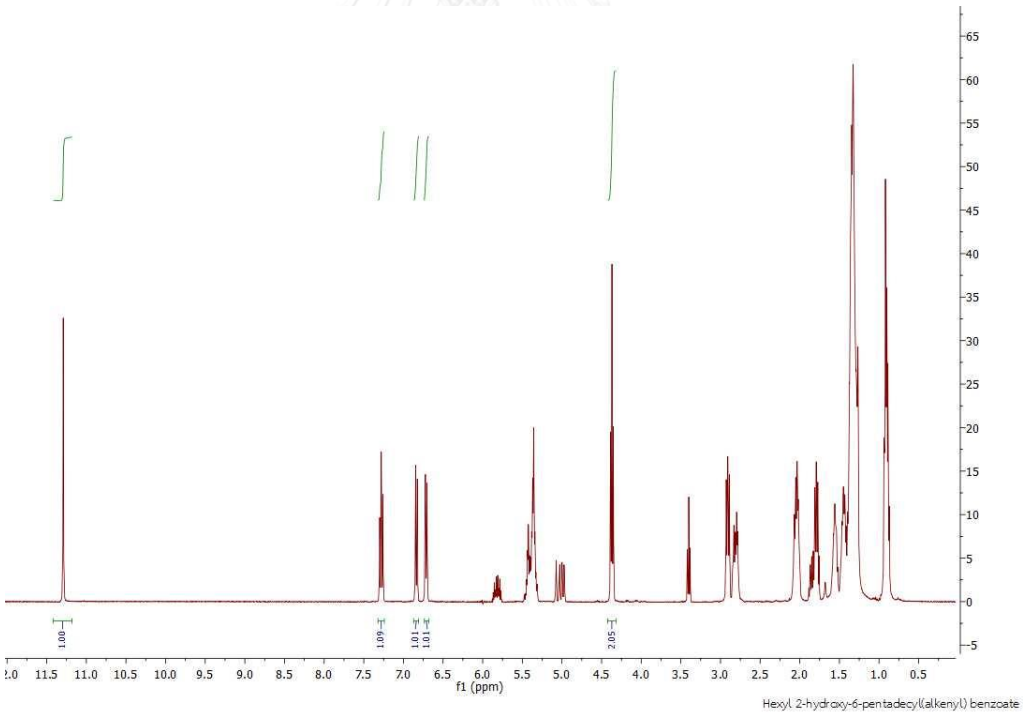

Figure A-4 The ^{1}H NMR spectrum (CDCl3) of compound **27**

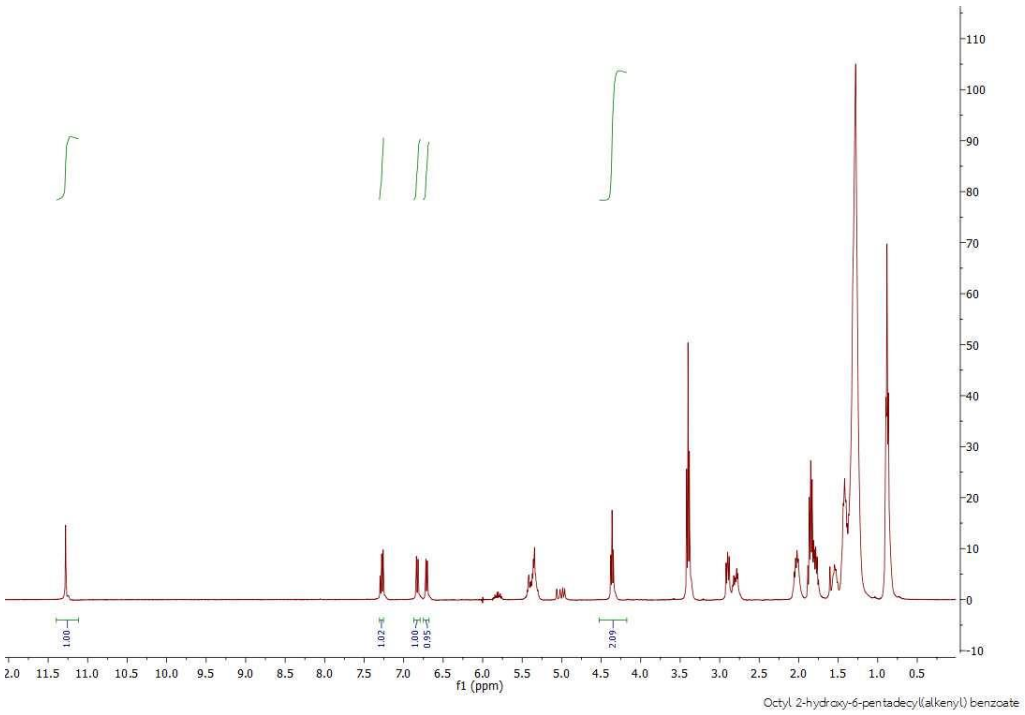

Figure A-5 The ^{1}H NMR spectrum ($CDCl_3$) of compound **28**

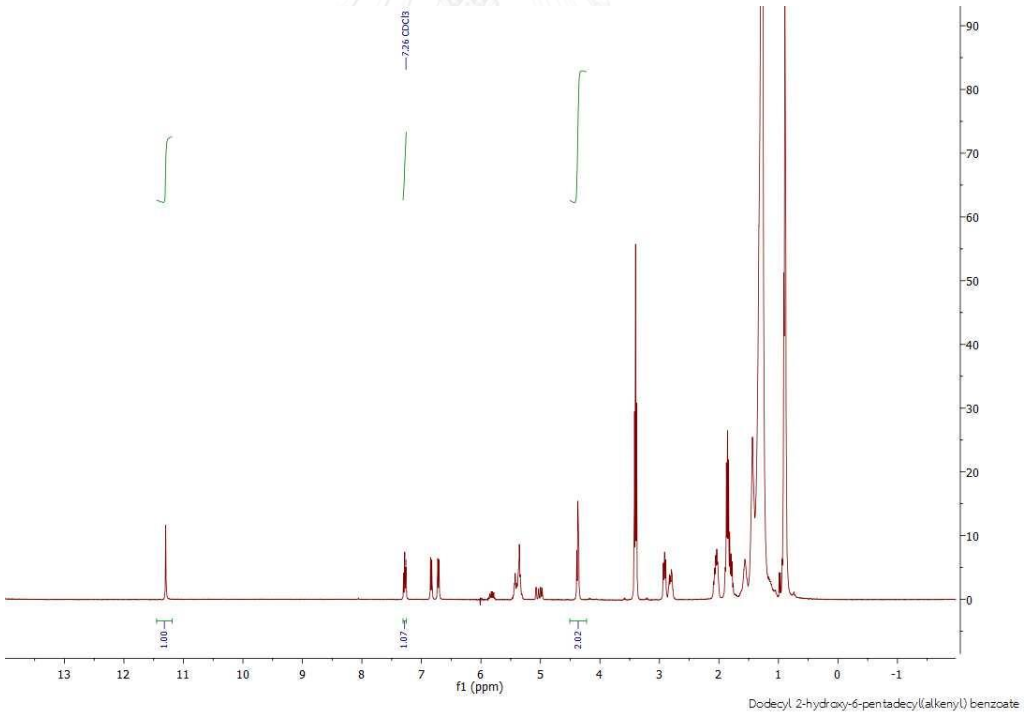

Figure A-6 The ^{1}H NMR spectrum ($CDCl_3$) of compound **29**

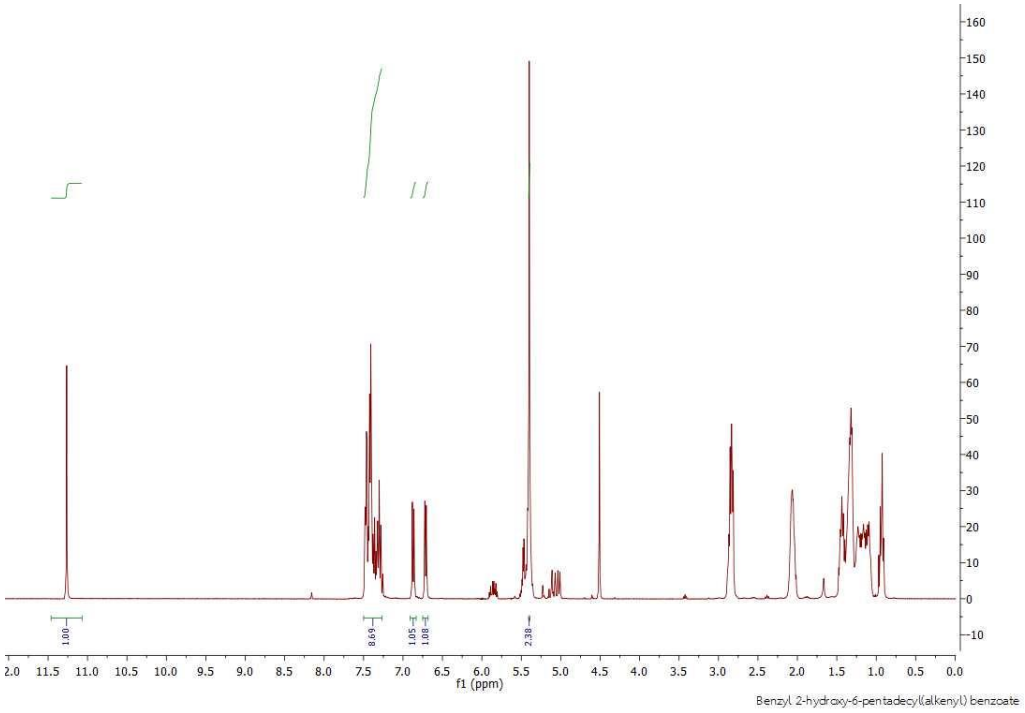

Figure A-7 The ^{1}H NMR spectrum ($CDCl_3$) of compound **30**

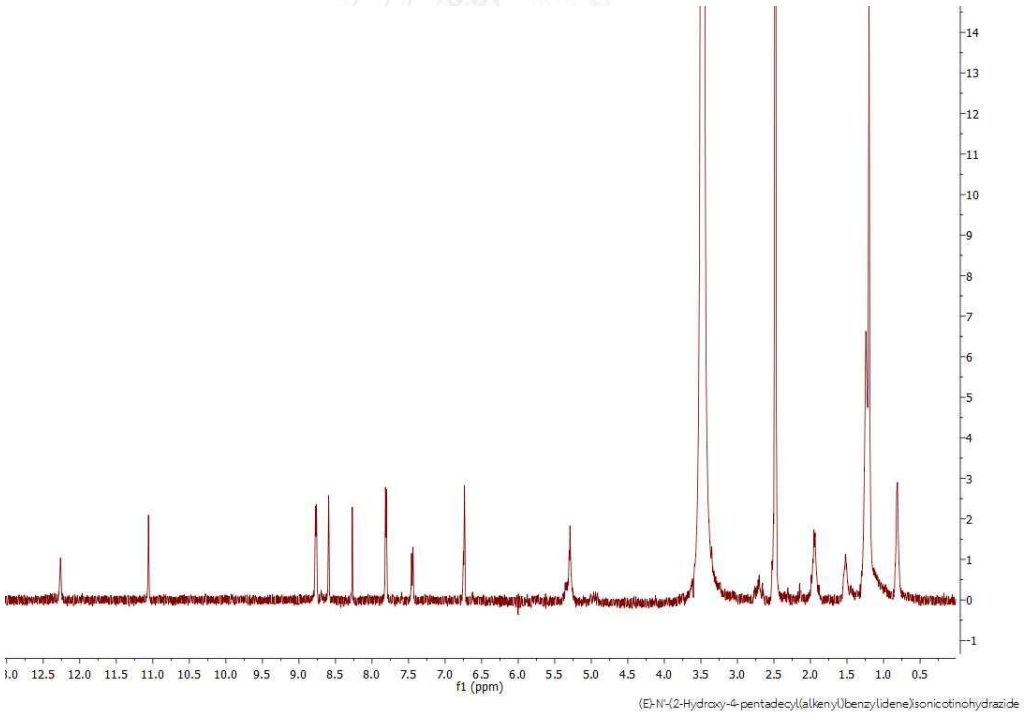

Figure A-8 The ^{1}H NMR spectrum (DMDO-d_6) of compound **44**

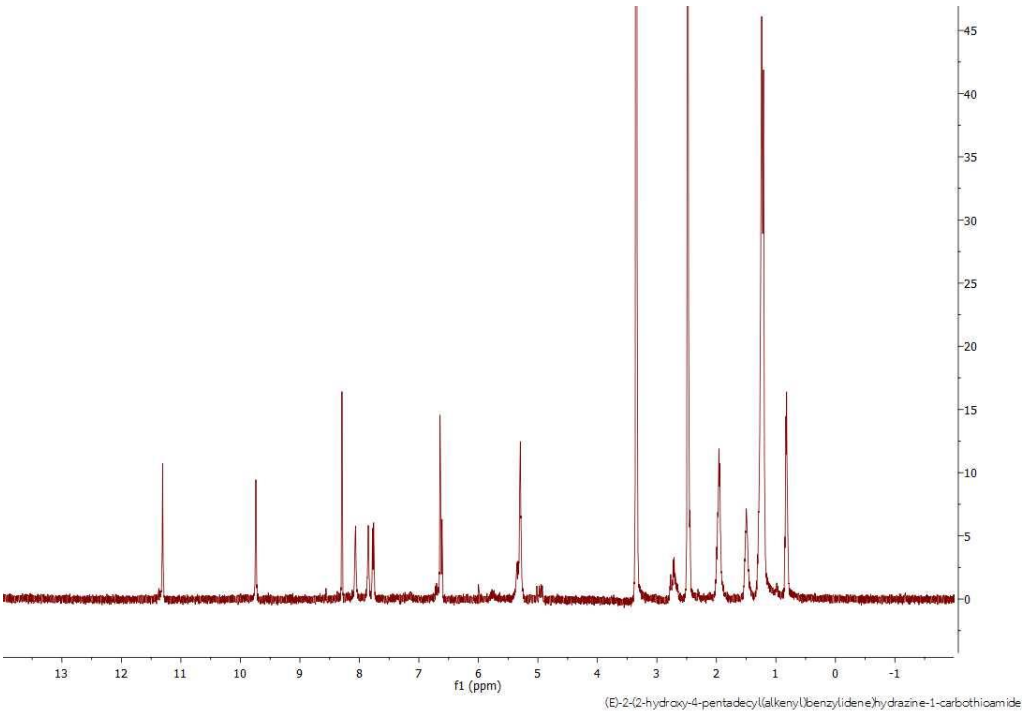

Figure A-9 The ^{1}H NMR spectrum (DMDO-d_6) of compound **45**

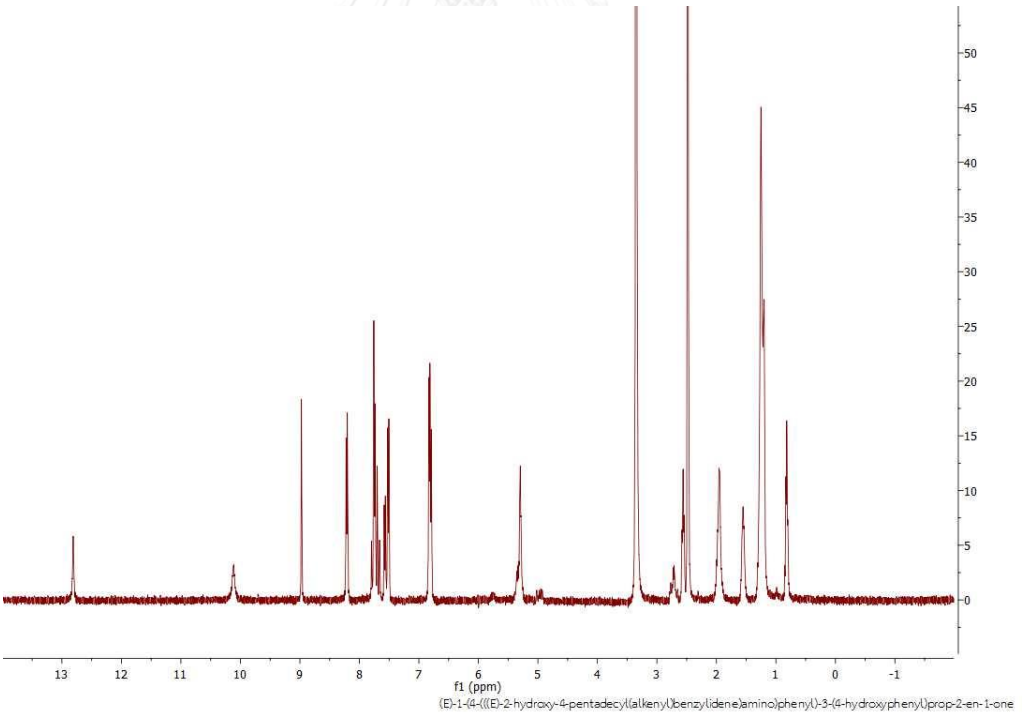

Figure A-10 The ^{1}H NMR spectrum (DMDO-d_6) of compound **46**

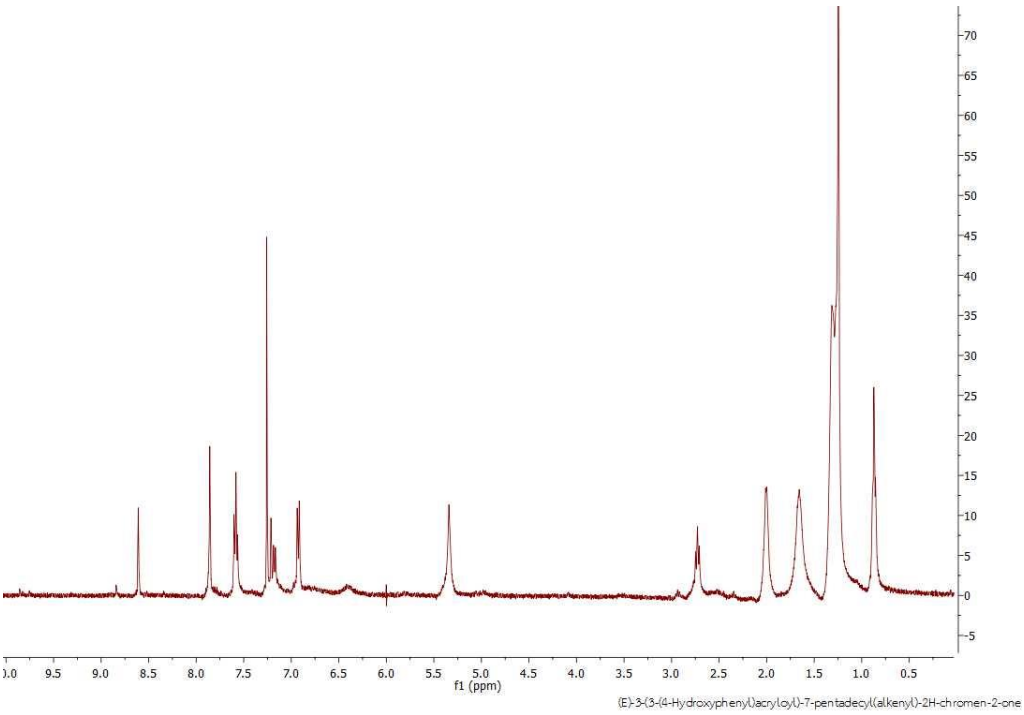

Figure A-11 The ^{1}H NMR spectrum ($CDCl_3$) of compound **48**

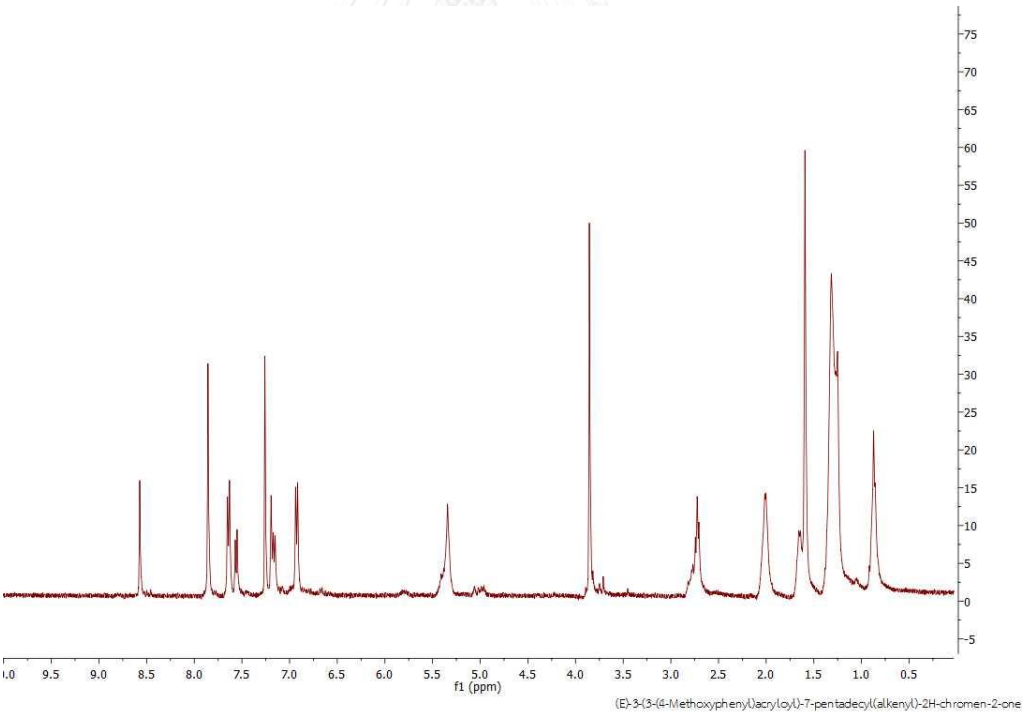

Figure A-12 The ^{1}H NMR spectrum ($CDCl_3$) of compound **49**

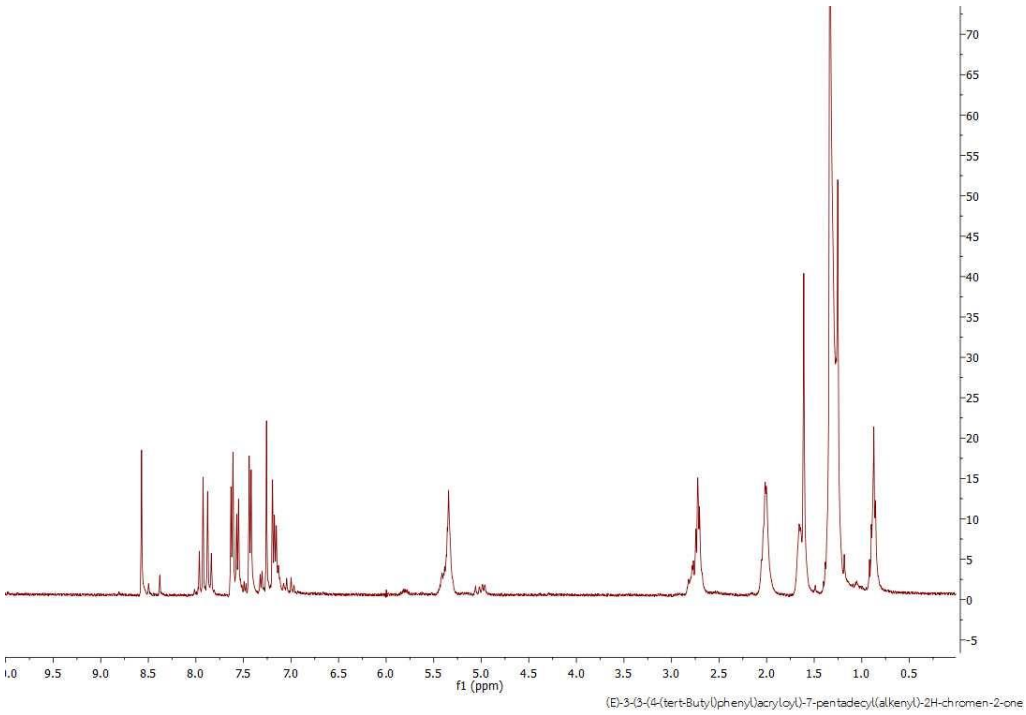

Figure A-13 The ^{1}H NMR spectrum ($CDCl_3$) of compound **50**

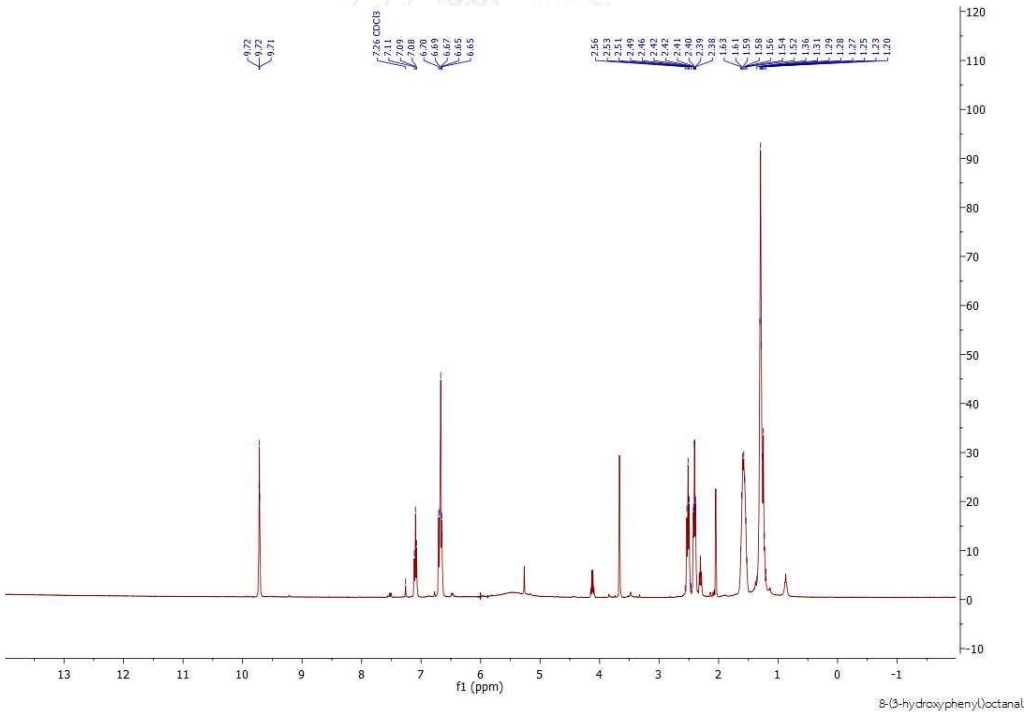

Figure A-14 The ^{1}H NMR spectrum ($CDCl_3$) of compound **51**

VITA

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