องค์ประกอบทางเคมีและฤทธิ์ยับยั้งไลเพสของมะเขือพวง *Solanum torvum*

นางสาวปวิตา ลิขิตเดชาโรจน์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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CHEMICAL CONSTITUENTS AND LIPASE INHIBITORY ACTIVITY OF *Solanum torvum*

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ปวิตา ลิขิตเดชาโรจน์ : องค์ประกอบทางเคมีและฤทธิ์ยับยั้งไลเพสของมะเขือพวง *Solanum torvum* (CHEMICAL CONSTITUENTS AND LIPASE INHIBITORY ACTIVITY OF *Solanum torvum*) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ดร.จรรยา ชัยเจริญพงศ์, 79 หน้า.

สารสกัดเอทานอลและน้ำของพืชสมุนไพร 16 ชนิด คือ กระเจี๊ยบเขียว, กระเทียม, ข่า, กระชาย, ดอกคำฝอย, ขมิ้น, ตะไคร้, ส้มแขก, กระเจี๊ยบแดง, สะระแหน่, พริกไทยดำ, ทองพันชั่ง, มะขามแขก, มะเขือพวง, ขิงและพุทราจีนถูกนำมาทดสอบฤทธิ์ยับยั้งไลเพส ในบรรดาสารสกัดเอทานอล สารสกัดของผลมะเขือพวงแสดงฤทธิ์ยับยั้งไลเพสดีที่สุดที่ 87.35 ± 2.37% ขณะที่ในกลุ่มสารสกัดของน้ำ สารสกัดใบสะระแหน่แสดงฤทธิ์ยับยั้งไลเพสดีที่สุดที่ 92.88 ± 2.99% ดังนั้นผลของมะเขือพวงจึงถูกนำมาสกัดด้วยเฮกเซน, เอทิลแอซิเตท, เมทานอล และน้ำ ตามลำดับ จากนั้นนำมาทดสอบฤทธิ์ยับยั้งไลเพสในหลอดทดลอง สารสกัดหยาบเอทิลแอซิเตทแสดงฤทธิ์ยับยั้งไลเพสที่ดีที่สุดที่ 67.23 ± 3.57% สารสกัดหยาบเฮกเซนมีฤทธิ์ยับยั้งไลเพสที่ระดับปานกลางที่ 46.00 ± 6.71% ในขณะที่สารสกัดหยาบเมทานอลและน้ำแสดงฤทธิ์ยับยั้งระดับต่ำที่ 15.82 ± 1.13% และ 8.51 ± 1.05% ตามลำดับ ดังนั้นสารสกัดหยาบเฮกเซนและเอทิลแอซิเตทถูกนำมาแยกและทำให้บริสุทธิ์ได้สาร 60 ซึ่งเป็นของผสมของ 17-(1,5-dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-ol, campesterol, stigmasterol และ β-sitosterol และสาร 61 คือ 7-hydroxy-6-methoxycoumarin หรือ scopoletin สาร 60 และ 61 แสดงฤทธิ์ยับยั้งไลเพสในระดับต่ำที่ค่า IC_{50} เท่ากับ 82.56 mg/mL และ 91.98 mg/mL ตามลำดับ ซึ่งน้อยกว่าออร์ลิสแตท ออร์ลิสแตทแสดงฤทธิ์การยับยั้งไลเพสที่ค่า IC_{50} เท่ากับ 3.79×10^{-6} mg/mL

สาขาวิชา เทคโนโลยีชีวภาพ ลายมือชื่อนิสิต .....

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The ethanol and aqueous extracts of sixteen medicinal plants including *Abelmoschus esculentus* Linn., *Allium sativum* Linn., *Alpinia galanga* (L.) Willd., *Boesenbergia rotunda* (L.) Mansf., *Carthamus tinctorius* Linn., *Curcuma longa* Linn., *Cymbopogon citrates* Stapf., *Garcinia atroviridis* Griff., *Hibiscus sabdariffa* Linn., *Metha cordifolia* Opiz., *Piper nigrum* Linn., *Rhinacanthus nasutus* (Linn.) Kurz., *Senna alexandrina* P. Miller, *Solanum torvum* Swartz., *Zingiber officinale* Roscoe., and *Ziziphus jujuba* Mill. were measured lipase inhibitory activity. Among ethanol crude extract, fruit of *S. torvum* showed the strongest lipase inhibitory activity at 87.35 ± 2.37% while among aqueous crude extract, leaf of *M. cordifola* showed the strongest lipase inhibitory activity at 92.88 ± 2.99%. Therefore, *S. torvum* fruits were extracted with hexane, ethyl acetate, methanol, and aqueous, respectively. Then, they were evaluated for lipase inhibitory activity *in vitro*. Ethyl acetate crude extract showed the strongest lipase inhibitory activity at 67.23 ± 3.57%. Hexane crude extract showed moderate lipase inhibitory activity at 46.00 ± 6.71%, whereas methanol and aqueous crude extracts exhibited weak activity at 15.82 ± 1.13% and 8.51 ± 1.05%, respectively. Therefore, hexane and ethyl acetate crude extracts were isolated and purified to give Compound 60, as a mixture of 17-(1,5-dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-ol, campesterol, stigmasterol and β-sitosterol and Compound 61, as 7-hydroxy-6-methoxycoumarin or scopoletin. Compounds 60 and 61 showed weak lipase inhibitory activity with IC_{50} values of 82.56 mg/mL and 91.98 mg/mL, respectively which were less than orlistat. Orlistat showed lipase inhibitory activity with IC_{50} value of 3.79×10^{-6} mg/mL.

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

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ABBREVIATION

δ	=	Chemical shift
ºC	=	Degree celsius
µg	=	Microgram
µL	=	Microlitre
µM	=	Micromolar
ara(f)	=	Arabinose in furanose form
A_{405}	=	Absorbance at 405 nm
BMI	=	Body mass index
^{13}C NMR	=	Carbon nuclear magnetic resonance spectroscopy
$CDCl_3$	=	Chloroform-d
CHD	=	Coronary heart disease
cm	=	Centimetre
D	=	Doublet (for NMR spectrum)
DEPT	=	Distortionless enhancement by polarization transfer
DMSO	=	Dimethyl sulfoxide
EI+	=	Electron ionization
et al.	=	And other
eV	=	Electron volt
f_{254}	=	Fluorescent indicator 254 nm
g	=	Gram
GC-MS	=	Gas chromatography-mass spectrometry
Glc	=	Glucose
^{1}H NMR	=	Proton nuclear magnetic resonance spectroscopy
H	=	Hydrogen
HMBC	=	Heteronuclear multiple bond correlation
HSQC	=	Heteronuclear single quantum correlation
Hz	=	Hertz
H_2SO_4	=	Sulfuric acid

IC_{50}	=	Half maximal inhibitory concentration
kg	=	Kilogram
L	=	Litre
m	=	Metre
M	=	Molar
mg	=	Milligram
mHz	=	Megahertz
Min	=	Minute
mL	=	Millilitre
na	=	No activity
nm	=	Nanometre
NMR	=	Nuclear magmetic resonance spectroscopy
OH	=	Hydroxyl group
PLC	=	Preparative thin layer chromatography
PNP	=	*p*-Nitrophenylpalmitate
ppm	=	Part per million
Qui	=	Quinovose
Rha	=	Rhamnose
s	=	Singlet (for NMR spectrum)
Ser	=	Serine
TLC	=	Thin layer chromatography
Tris	=	Tris(hydroxymethyl)aminomethane
Tris-HCL	=	Tris(hydroxymethyl)aminomethane hydrochloric acid
US	=	Unites States
UV/VIS	=	Ultraviolet/Visible
v/v	=	Volume by volume
WHO	=	World health organization
w/w	=	Weight by weight
Xyl	=	Xylose

CHAPTER I

INTRODUCTION

Obesity or overweight is becoming one of the greatest global health problems. Obesity is a major cause of morbidity and mortality in the United States (US). Trend in the US adults is continuing to increase in both sexes. In 2000, the prevalence of obesity (body mass index (BMI) ≥ 30) was 19.8% and increased to 20.9% in 2001, an increase of 5.6% (1). In 2007-2008, the prevalence of obesity was 32.2% and 35.5% among US adults for men and women, respectively (2). In Thailand, a cross-section population survey of Thai children and adults showed a high prevalence of overweight (BMI 25-29.9 kg/m^2) and obesity in children (3-18 years old) as 7.6% and 9.0%, respectively. Among adults, 19.0% were overweight (BMI 25-29.9 kg/m^2), 4.0% class I obesity (BMI 30.0-34.9 kg/m^2), 0.8% class II obesity (BMI 35.0-39.9 kg/m^2) and 0.1% class III obesity (BMI ≥ 40.0 kg/m^2), respectively, using the World Health Organization (WHO) definition (3). Obesity is also known to be a risk factor of atherosclerosis, cardiovascular disease, hypertension and diabetes (4). Causes of obesity are excessive food energy intake and lack of physical activity. Presently, the most common anti-obesity drug available in the market is orlistat or as a commercial name xenical. Orlistat, a hydrogenated derivative of lipstatin derived from *Streptomyces toxitricini*, is a potent inhibitor of gastric, pancreatic, and carboxyl ester lipases. It has proved to be effective for treatment of human obesity.

Lipase inhibitors in many plant species have been reported such as *Cassia nomame* (5), oolong tea (6), *Filipendula kamtschatica* (7) and *Abies sibirica* (8). Potential natural products effective for treatment of obesity could be developed as new anti-obesity drugs with minimum side effects, but they have been largely unexplored. Sixteen ethanol and aqueous crude extracts of Thai medicinal plants including fruits of *Abelmoschus esculentus* Linn., bulbs of *Allium sativum* Linn., rhizomes of *Alpinia galanga* (L.) Willd., rhizomes of *Boesenbergia rotunda* (L.) Mansf., flowers of *Carthamus tinctorius* Linn., rhizomes of *Curcuma longa* Linn., stems of *Cymbopogon citrates* Stapf., *fruits of Garcinia atroviridis* Griff., calyxs of *Hibiscus*

sabdariffa Linn., leaves of *Metha cordifolia* Opiz., fruits of *Piper nigrum* Linn., leaves of *Rhinacanthus nasutus* (Linn.) Kurz., fruits of *Senna alexandrina* P. Miller, fruits of *Solanum torvum* Swartz., *rhizomes of Zingiber officinale* Roscoe., and fruits of *Ziziphus jujuba* Mill. were used in preliminary screening (Figure 1). The ethanol crude extract of fruit of *S. torvum* showed highest lipase inhibitory activity with 87.35 ± 9.89% at concentration of 50 mg/mL.

Figure 1 Parts of plant materials for preliminary screening; a) fruits of *A. esculentus*, b) bulbs of *A. sativum*, c) rhizomes of *A. galanga*, d) rhizomes of *B. rotunda*, e) flowers of *C. tinctoriu*, f) rhizomes of *C. longa*, g) stems of *C. citrates*, *h) fruits of G. atroviridis*, i) calyxs of *H. sabdariffa*, j) leaves of *M. cordifolia*, k) fruits of *P. nigrum*, l) leaves of *R. nasutus*, m) leaves of *S. alexandrina*, n) fruits of *S. torvum*, o) *rhizomes of Z. officinale* and p) fruits of *Z. jujuba*

S. torvum or common name as turkey berry, pea eggplant or Ma Kue Phuang (Figure 2) was natived to Central and South America, Brazil, Mexico and West Indies. *S. torvum* was used in folk traditional medicine for a long time. In Thailand, the fruits used as vegetable and are regarded as an essential ingredient in Thai cuisine. Fruits and leaves are rich in alkaloids but used for medicinal purposes. In southern of China, it used to sedative, digestive, haemostatic and diuretic. In India, fruit is used in the treatment of cough, liver and spleen enlargement (9). Fruit of *S. torvum* was reported to exhibit various biological activities such as antioxidant activity (10), antimicrobial (11, 12), antiviral (13), antidiabetic (14), angiotensin and serotonin receptor blocking activities (15). Nowadays, *S. torvum* is promoted to be an ingredient in commercial products for treatment of obesity in Thailand but not scientific data proved.

Objectives

1. To screen Thai medicinal plants for *in vitro* lipase inhibitory activity.
2. To isolate and characterize chemical constituents with lipase inhibitory activity from *S. torvum* fruit.

Figure 2 Drawing of leaves, flowers and fruits of *S. torvum* (16)

CHAPTER II

THEORETICAL

2.1 Lipase

Lipases are enzymes in a subclass of esterases that the body uses to digest fats in food. It includes phospholipases and triacylglycerol lipase. Phospholipase catalyzes hydrolysis of phospholipids to free fatty acids and lysophospholipids. Triacylglycerol lipases catalyze hydrolysis of triacylglycerols to free fatty acids, mono- and diacylglycerols. Free fatty acids are employed for energy production or are re-esterified for storage in adipose tissue (17, 18). Human lipase was devived into 2 parts. First, pre-duodenal part includes lingual and gastric lipases and second, extra-duodenal part consists of pancreatic, hepatic, lipoprotein and endothelial lipases (19). Pancreatic lipase is a lipolytic enzyme that synthesized and secreted by pancreas. Pancreatic lipase is a primary lipase that hydrolyzed ester linkages of dietary triglycerides to free fatty acids and monoacylglycerols in human digestive system. This enzyme is a single chain of glycoprotein of 449 amino acids and its structural suggests that Ser-152 is the nucleophilic residue essential for catalysis (20). Basic review of human digestive and metabolic lipases is shown in Figure 3.

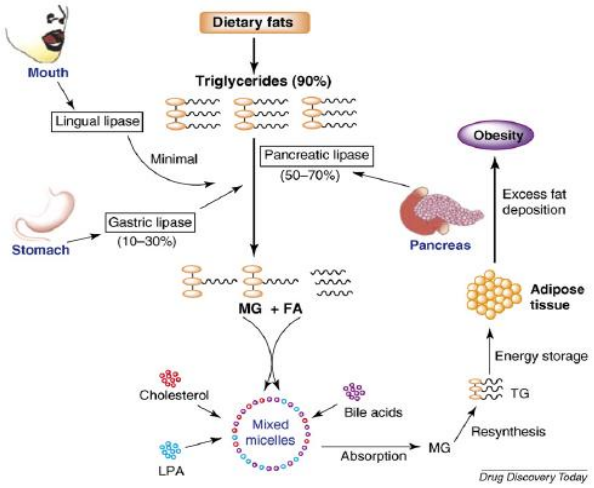


Figure 3 Physiological role of pancreatic lipase in lipid absorption (21)

2.2 Obesity

Obesity or overweight is defined as a body mass index by the World Health Organization, a person's weight (in kilograms) divided by the square of height (in meters). A person with a BMI equal to or more than 25 is considered overweight and a person with a BMI of 30 or more is generally considered obese. BMI cut-off values are ethnic-dependent and appear to be lower in certain population: a BMI of 27.5 or greater in an Asian patient is associated with comparable morbidities to those seen Caucasian patient with a BMI of 30 (22). BMI can be determined using a BMI chart as Figure 4.

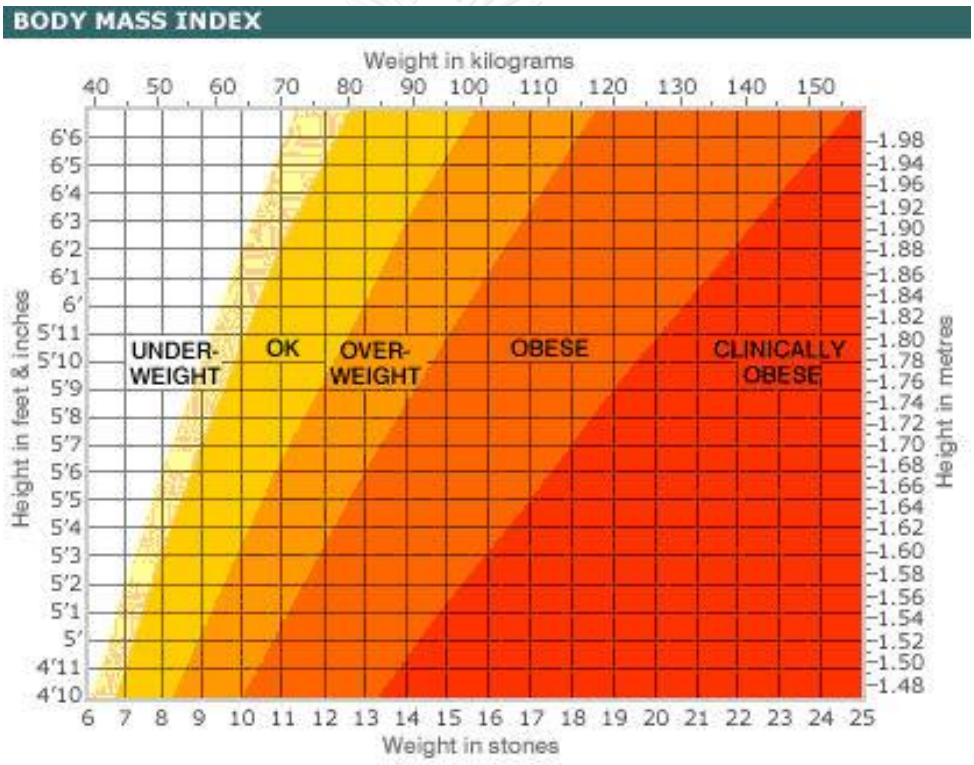


Figure 4 Body mass index chart base on World Health Organization data

The cause of obesity is a combination of various factors including high calorie foods and beverages, lacking of fruits and vegetables, full of fast foods, smoking, lack of sleeping, less active lifestyle and medical problem. In some patients, medical problem and some medication can lead to weight gain because some diseases and

medication may interfere the body's metabolism. Depakote or valproic acid which is a drug for treatment of bipolar disorder and seizures and prevention of headaches migraine, affects proteins involved in appetite and metabolites. In the research of depakote on weight gain, the weight of patients who took depakote were increased but the glucose level were decreased comparing to placebo group (23). Moreover, hormonal changes and a less active lifestyle in elder also increase risk of obesity. The muscle tends to decrease with age. Lower muscle mass leads to decrease in metabolism, also reduce calories needs and hard to keep off excess weight. During pregnancy, a woman's weight necessarily increases and difficult to lose weight after deliver a child. Weight gain may contribute to develop to obesity. Genetic is also a cause of obesity that effected on fat and body's metabolism. Obesity is related with risk factor of hypertension, coronary heart disease (CHD), type 2 diabetes, stroke, gallbladder disease, osteoarthritis, sleep apnea, respiratory problems, some types of cancer (endometrial, breast, prostate and colon), gynecological problems, erectile dysfunction and sexual health issues.

Although diet and physical exercise are great ways to lose weight but obese patients often do not succeed in controlling their diet, resulting in failure of treatment (24, 25). However, anti-obesity drugs are used in adult obese patients who do not get success in achieving a 10% weight reduction after at least three months of weight loss program by supervised balanced diet, physical exercise and lifestyle modification.

2.3 Anti-obesity drug

Two different types of anti-obesity drugs are currently available on market. First type acts on the central nervous system and second type acts on the gastrointestinal system. Anti-obesity drugs that act on nervous system to suppress appetite consist of sibutramine, fenfluramine and dexfenfluramine. They were withdrawn out of market due to many serious side effects such as insomnia, tremors, increase blood pressure and pulse rate, headache, palpitation and constipation (26). Glucomannan and orlistat are anti-obesity drug that act on the gastrointestinal system. Glucomannan is a water-soluble dietary fiber derived from *Amorphophallus*

konjac (27). It increases transit time of food and prolongs gastric emptying time, resulted in increasing of satiety, decreasing of ingestion of foods that increased cholesterol and glucose concentrations, reducing glucose in plasma, suppressing of hepatic cholesterol synthesis and increasing of the fecal elimination of cholesterol containing bile acids (28). Glucomannan is available in the market as a supplement and food products such as flour and pasta. It was well tolerated and generally considered safe but glucomannan may cause esophageal obstruction and blockage in colon. Its mild side effects are bloating, flatulence, soft stools or diarrhea on obese patients. In addition, glucomannan can reduce absorption of oral medications like sulfonylurea, a diabetes drug. Orlistat; the best selling anti-obesity drug in world wild, inhibits pancreatic and gastric lipases by decreasing triglyceride from dietary fat absorption thereby leading to weight loss.

2.4 Orlistat

Orlistat or tetrahydrolipstatin under commercial name "Xenical" is a lipase inhibitor from synthesized derivative of lipstatin, the natural product of *S. toxytricini* (29) shown in Figure 5. This drug inhibited 30% of dietary fat absorption by inhibiting pancreatic and gastric lipases. The side effects of orlistat are gastrointestinal side effects such as diarrhea, fecal incontinence, oily spotting, flatulence, bloating and dyspepsia.

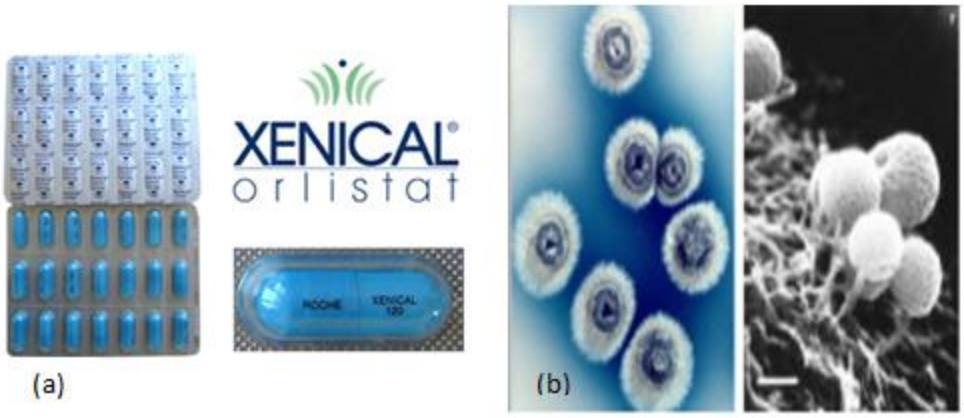


Figure 5 a) Orlistat and b) *S. toxytricini*

Orlistat and lipase form a stable complex with covalent bond. The complex induces a conformational change to a lid-like structure on lipase, hence exposing the catalytic active site. This operation leads to acylation of hydroxyl group on serine residue burden on active site of inactive enzyme. Inactive lipase is unable to digest fats to fatty acids and monoglycerides and remove with excrement (30). Two metabolites are excreted via the bile have been detected, namely: M1 (4 members lactone ring hydrolyzed) and M3 (M1 with *N*-formylleucine moiety cleaved). They found to be with no pharmacological activity in comparison to parent compound. The half-life of M1 and M3 are approximately 2 and 3 hours, respectively (31). Mechanism of action and metabolism of orlistat was showed in Figure 6. This drug prevents the hydrolysis of dietary fat into absorbable monoglyceride and free fatty acid (32). In orlistat treated patients, levels of cholesterol and low-density lipoprotein are reduced, that supports a role for triglyceride hydrolysis in cholesterol absorption (33).

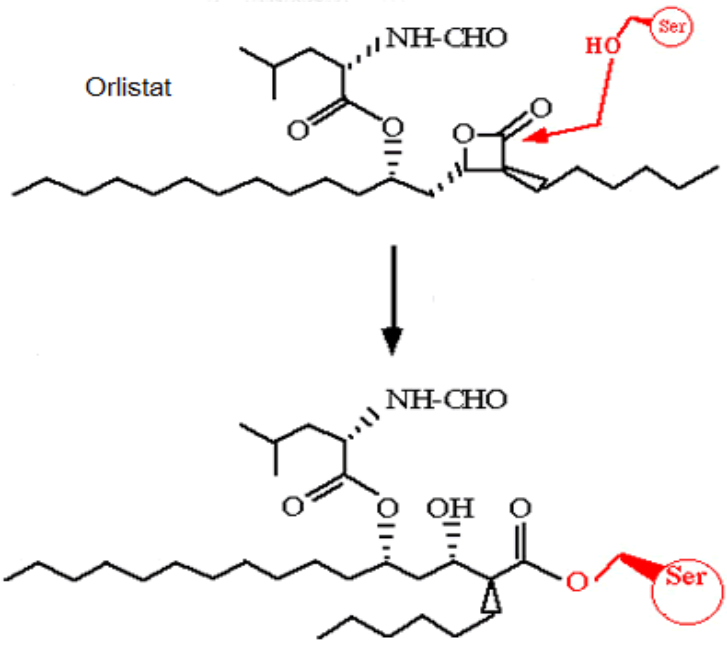


Figure 6 Mechanism on lipase inhibition of orlistat

2.5 Literature reviews

Plant extracts have been used for a variety of purposes for long times (34). Many phytochemicals from traditional medicinal plants have potent pancreatic lipase inhibitory activity (5-6, 35-53).

Table 1 Classification of pancreatic lipase inhibitors from plant sources

Type	Chemical classes	Plant source	Inhibitor	Activity	Reference
Polyphenolics	Polyphenolics	various plant materials	Luteolin **(1)**	Weak inhibitor	(35, 36)
		Rhizomes of *Alpinia officinarum*	3-Methyletherganglin **(2)**	Moderate inhibitor	(37, 38)
		Fruits of *Mangifera indica*	Mangiferin **(3)**	Moderate inhibitor	(37,38)
		Peels of *Citrus unshiu*	Hesperidin **(4)**	IC_{50} = 32 μg/mL	(37,38)
		Fruits of *Aronia melanocarpa* L.	Cyanidin-3-glucoside **(5)**	IC_{50} = 1.74 mg/mL	(39)
		Oolong tea	*(−)*-Epigallocatechin-3-*O*-gallate **(6)**	IC_{50} = 0.349 μM	(6)
			(−)-Epigallocatechin-3,5-di-*O*-gallate **(7)**	IC_{50} = 0.098 μM	(6)
			Oolonghomobisflavans A **(8)**	IC_{50} = 0.048 μM	(6)
			Oolonghomobisflavans B **(9)**	IC_{50} = 0.108 μM	(6)
			Oolongtheanin-3'-*O*-gallate **(10)**	IC_{50} = 0.068 μM	(6)
			(+)-Catechin **(11)**	IC_{50} > 20μM	(6)

Table 1 Classification of pancreatic lipase inhibitors from plant sources (continued)

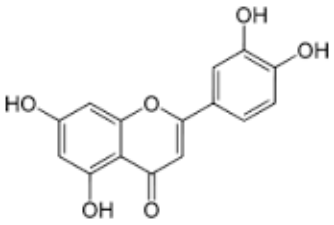
Type	Chemical classes	Plant source	Inhibitor	Activity	Reference
Polyphenolics	Polyphenolics	Oolong tea	*(–)*-Epicatechin **(12)**	IC_{50} > 20μM	(6)
			(+)-Gallocatechin **(13)**	IC_{50} > 20μM	(6)
			(–)-Epigallocatechin **(14)**	IC_{50} > 20μM	(6)
		Fruits of Cassia nomame	(2S)-3',4',7-trihydroxyflavan-(4α→8)-catechin **(15)**	IC_{50} = 5.5 μM	(5)
		Grape seed	Proanthocyanidin-rich extract	-	(40)
		Leaves and stem bark of *M. indica*	Polyphenol, including phenolic acid, phenolic ester, flavan-3-ol and mangiferin	-	(41)
		Leaves of *N. nucifera*	Phenolic constituents	IC_{50} = 0.46 mg/mL	(42)
		Shell of *Arachis hypogaea*	Coumarin derivertives and flavonoid glycosides	-	(43)
		Roots of *S. reticulata*	High concentration of polyphenol, including mangiferin, catechins and tannin	IC_{50} = 264 μg/mL	(35)
Curcumins	Curcumins	*Rhizomes of C. longa*	Erythro-1-(3-methoxy-4-hydroxy-phenyl)-propan-1,2-diol **(16)**	IC_{50} = 12.1 μM	(44)
			Threo-1-(3-methoxy-4-hydroxy-phenyl)-propan-1,2-diol **(17)**	IC_{50} = 9.1 μM	(44)

Table 1 Classification of pancreatic lipase inhibitors from plant sources (continued)

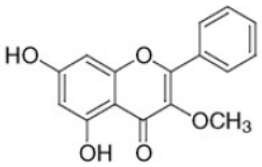
Type	Chemical classes	Plant source	Inhibitor	Activity	Reference
Saponins	Platycodin-saponins	Roots of *Platycodin gradiflorum*	Platycodin D **(18)**	-	(45)
	Scabiosaponins	Whole plant of *Scabiosa tchiliensis*	Scabiosaponin E **(19)**	-	(46)
			Scabiosaponin F **(20)**	-	(46)
			Scabiosaponin G **(21)**	-	(46)
			Hookeroside A **(22)**	-	(46)
			Hookeroside B **(23)**	-	(46)
			Scabiosaponin I **(24)**	-	(46)
			Prosapogenin 1b **(25)**	-	(46)
Saponins	Sessiloside and chiisanoside	Leaves of *Acanthopanax sessiliflorusps*	Sessiloside **(26)**	IC_{50} = 0.36 mg/mL	(47)
			Chiisanoside **(27)**	IC_{50} = 0.75 mg/mL	(47)
	Cyclocarisides	Leaves of *Cyclocarea paliurus*	Cyclocarioside A **(28)**	IC_{50} = 9.1 mg/mL	(48)
			Cyclocarioside II **(29)**	-	(48)
			Cyclocarioside III **(30)**	-	(48)
	Chikasetsusapo nins	Root of Ginseng (*Panax japonicus*)	Chikusetsusaponin III **(31)**	Active at concentration 125-500 mg/mL	(49)
			Chikusetsusaponin IV **(32)**		(49)
			28-Deglucusyl-chikusetsusaponin IV **(33)**		(49)
			28-Deglucusyl-chikusetsusaponin V **(34)**		(49)
	Dioscin and derivatives	Root of *Dioscorea nipponica*	Dioscin **(35)**	IC_{50} = 20 µg/mL	(50)
			Diosgenin **(36)**	IC_{50} = 28 µg/mL	(50)

Table 1 Classification of pancreatic lipase inhibitors from plant sources (continued)

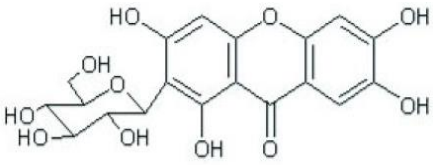
Type	Chemical classes	Plant source	Inhibitor	Activity	Reference
Saponins	Escins	Japanese horsechestnut (*Aesculus turbinate*) and Eupopean horsechesnut (*Aesculus hippocastanum*)	Gracillin **(37)**	IC_{50} = 28.9 µg/mL	(50)
			Escins Ib **(38)**	IC_{50} = 24 µg/mL	(51)
			Escins IIb **(39)**	IC_{50} = 14 µg/mL	(51)
			Escins Ia **(40)**	IC_{50} = 48 µg/mL	(51)
			EscinsIIa **(41)**	IC_{50} = 61 µg/mL	(51)
Terpenes	Carnosic acid	Leaves of *Salvia offinalis*	Carnosic acid **(42)**	IC_{50} = 12 µg/ml	(52)
			Carnosol **(43)**	IC_{50} = 4.4 µg/ml	(52)
			Roylenoic acid **(44)**	IC_{50} = 35 µg/ml	(52)
			7-Methoxyrosmanol **(45)**	IC_{50} = 32 µg/ml	(52)
			Oleanolic acid **(46)**	IC_{50} = 83 µg/ml	(52)
	Crocin and crocetin	Fruits of *Gardinia jasminoids*	Crocin **(47)**	IC_{50} = 28.63 µg/ml	(53)
			Crocetin **(48)**	-	(53)



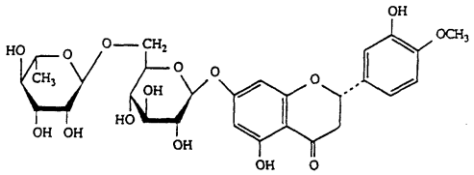
Luteolin **(1)**



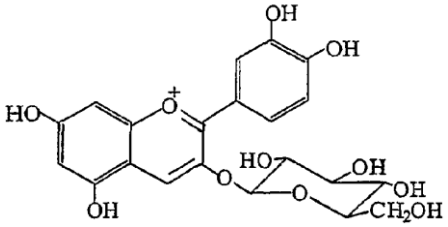
3-Methylethergangliη **(2)**



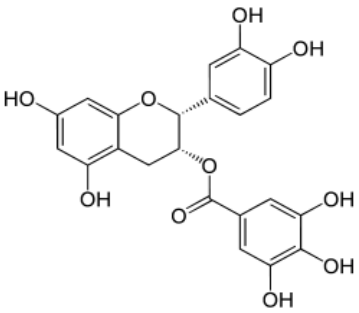
Mangiferin **(3)**



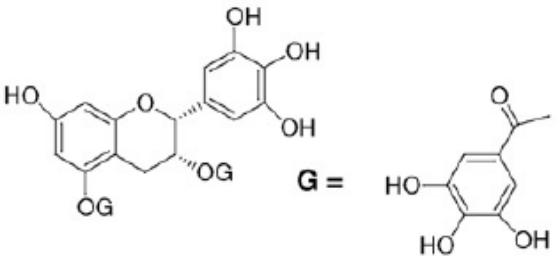
Hesperidin **(4)**



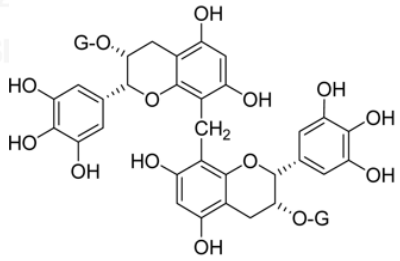
Cyanidin-3-glucoside **(5)**



(−)-Epigallocatechin-3-O-gallate **(6)**

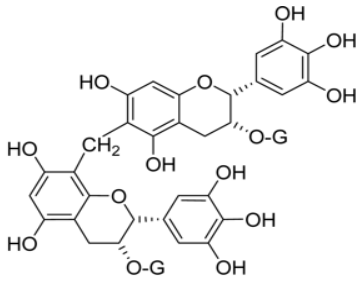


(−)-Epigallocatechin-3,5-di-O-gallate **(7)**

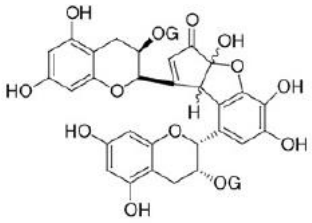


Oolonghomobisflavans A **(8)**

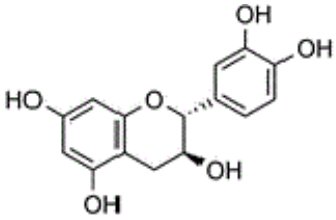
Figure 7 Structures of pancreatic lipase inhibitors



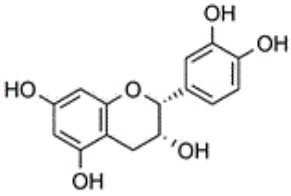
Oolonghomobisflavans B **(9)**



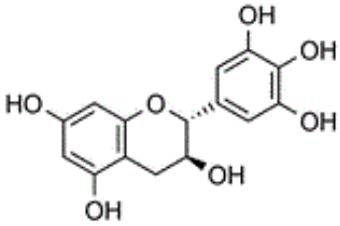
Oolongtheanin-3'-*O*-gallate **(10)**



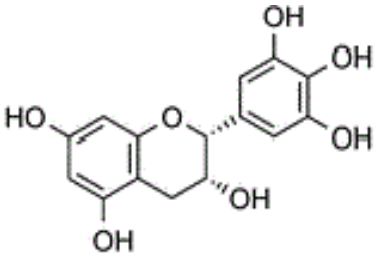
(+)-Catechin **(11)**



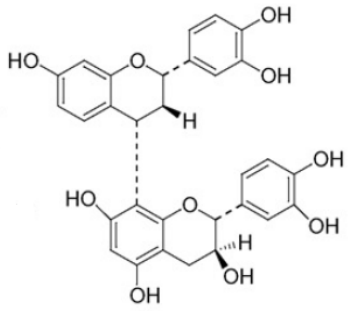
(−)-Epicatechin **(12)**



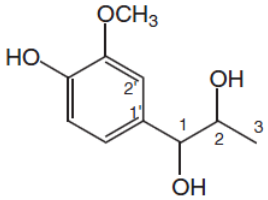
(+)-Gallocatechin **(13)**



(−)-Epigallocatechin **(14)**



(2S)-3',4',7-Trihydroxyflavan-(4$\alpha\rightarrow$8)-catechin **(15)**



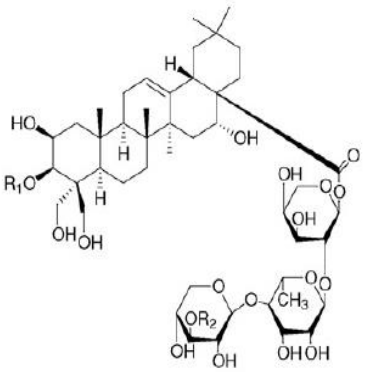
Erythro-1-(3-methoxy-4-hydroxy-phenyl)-propan-1,2-diol **(16)**

1,2-erythro

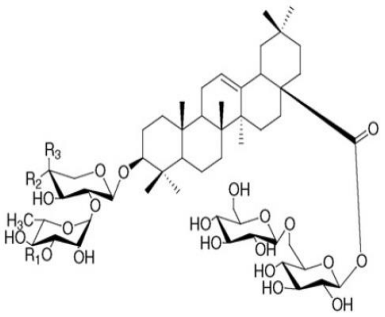
Threo-1-(3-methoxy-4-hydroxy-phenyl)-propan-1,2-diol **(17)**

1,2-threo

Figure 7 Structures of pancreatic lipase inhibitors (continued)



Platycodin D **(18)**



Scabiosaponin E **(19)**

R_1 = Xyl R_2 = OH R_3 = H

Scabiosaponin F **(20)**

R_1 = Glc R_2 = OH R_3 = H

Scabiosaponin G **(21)**

R_1 = Glc(1-4)Glc R_2 = OH R_3 = H

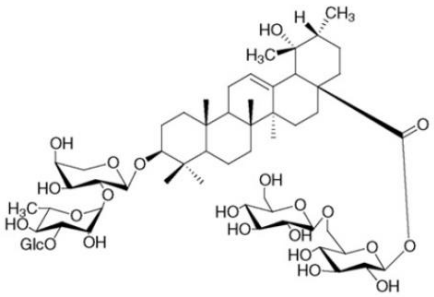
Hookeroside A **(22)**

R_1 = Glc(1-4)Xyl R_2 = OH R_3 = H

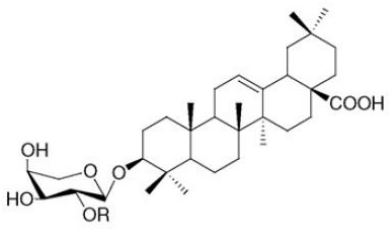
Hookeroside B **(23)**

R_1 = Glc(1-4)Glc(1-4)Xyl R_2 = OH R_3 = H

Glc = **β**-D-glucopyranosyl: **α**-L-arabinofuranosyl

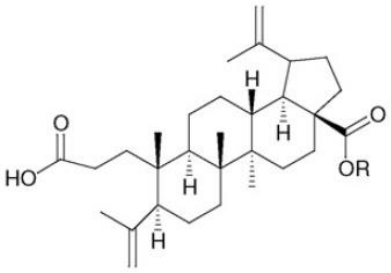


Scabiosaponin I **(24)**

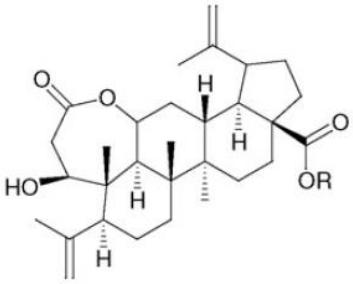


Prosapogenin 1b **(25)** R = Glc(1-4)Xyl(1-3)Rha

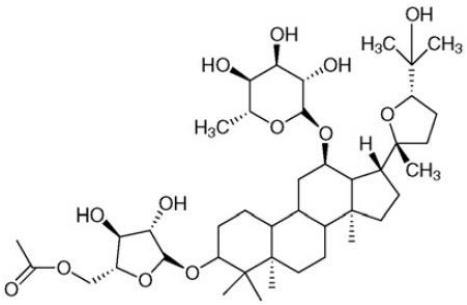
Figure 7 Structures of pancreatic lipase inhibitors (continued)



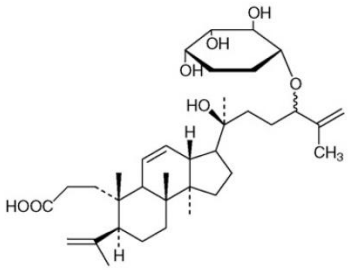
Sessiloside **(26)** R = **α**-L-Rha(1-4)-**β**-D-Glc(1-6)-**β**-D-Glc



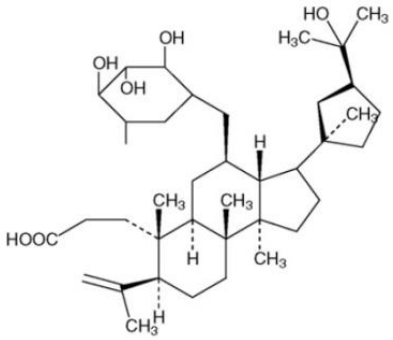
Chisanoside **(27)** R = **α**-L-Rha(1-4)-**β**-D-Glc(1-6)-**β**-D-Glc



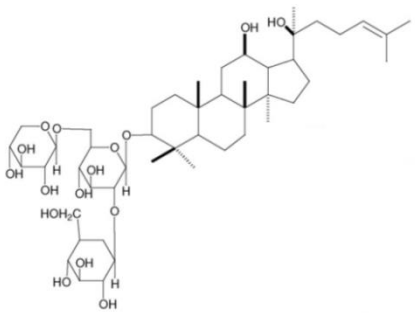
Cyclocarioside A **(28)**



Cyclocarioside II **(29)**

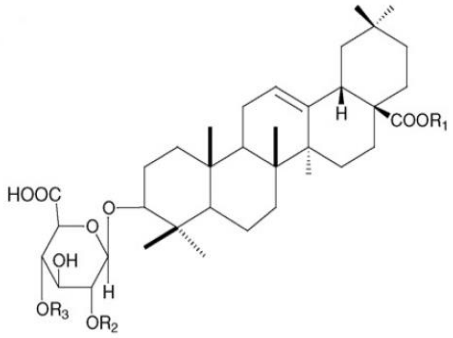


Cyclocarioside III **(30)**



Chikusetsusaponin III **(31)**

Figure 7 Structures of pancreatic lipase inhibitors (continued)



Chikusetsusaponin IV **(32)**

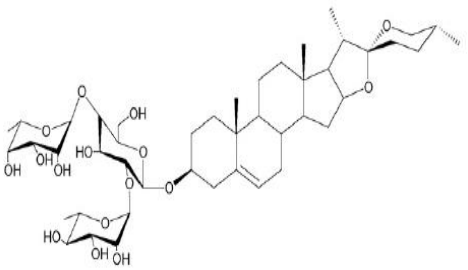
R_1 = Glc R_2 = H R_3 = Ara(f)

28-Deglucusyl- chikusetsusaponin IV **(33)**

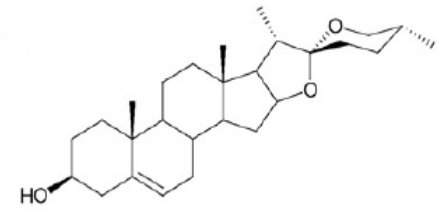
R_1 = H R_2 = Glc R_3= Ara(f)

28-Deglucusyl- chikusetsusaponin V **(34)**

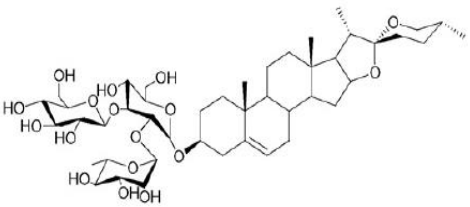
R_1 = H R_2 = Glc R_3 = H



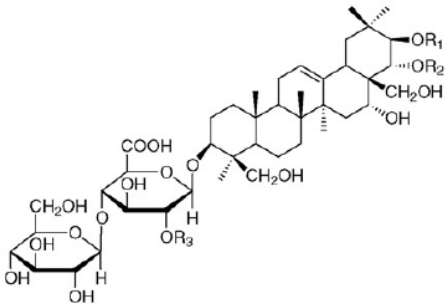
Dioscin **(35)**



Diosgenin **(36)**



Gracillin **(37)**



Escins Ib **(38)**

R_1 = Angeloyl R_2 = Aetal R_3 = D-Glucopyranosyl

Escins IIb **(39)**

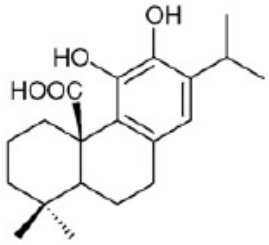
R_1 = Angeloyl R_2 = Aetal R_3 = D-Xycopyranosyl

Escins Ia **(40)**

R_1 = Trigloyl R_2 = Aetal R_3 = D-Glucopyranosyl

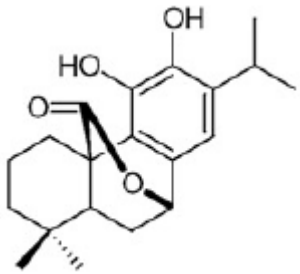
Escins IIa **(41)**

R_1 = Trigloyl R_2 = Aetal R_3 = D-Xycopyranosyl)

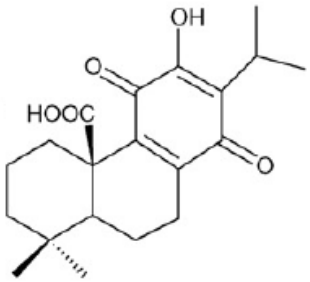


Carnosic acid **(42)**

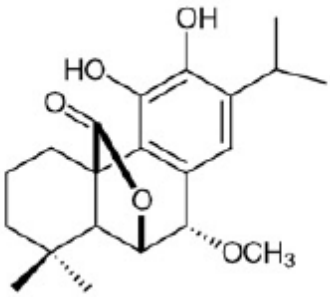
Figure 7 Structures of pancreatic lipase inhibitors (continued)



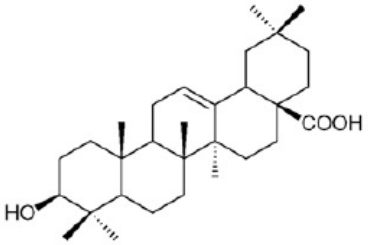
Carnosol **(43)**



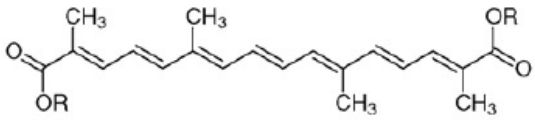
Roylenoic acid **(44)**



Triterpeneoleanolic acid **(45)**



Oleanolic acid **(46)**



Crocin **(47)** R = H

Crocetin **(48)** R = β-gentiobiosyl

Figure 7 Structures of pancreatic lipase inhibitors (continued)

2.6 *Solanum torvum*

2.6.1 General description

S. torvum is a small evergreen tree which belongs to the genus Solanum of family Solanaceae. This plant is erect spiny shrub or small tree that can grow up to 3.0 m. Stem barks are smooth and gray with raised lenticels that scatter on stem and leaves surface. Leaves are opposite, broadly ovate with the boarder entire or deeply lobed mostly 10.0-15.0 cm long, 8.0-10.0 cm wide and broadly triangular. Petioles are usually 2.0-5.0 cm long. The flowers are white, tubular with 5 pointed lobes and grouped in corymbiform cymes. Peduncles are 1.0-2.5 cm long and pedicels are 0.5-1.0 cm long, slightly longer in fruit. Calyxs are 0.3-0.5 cm long. Lobe apiculates are 0.2-0.3 cm long and 0.5-0.7 cm long of anthers. Fruits are globular with diameter about 1.0 cm, green unripe and yellow ripe fruits. They are thin-fleshed and contain numerous flat, round and brown seeds. The characteristic of aerial part, leaves, flowers and fruits of *S. torvum* show in Figure 8.

2.6.2 Distribution

S. torvum is native to the West Indies (including Bahamas, Greater Antilles and the Lesser Antilles), Mexico, Central America and northern and western South America through Brazil. It is widely cultivated in tropical for edible plants.

2.6.3 Chemical constituents of *S. torvum*

Chemical constituents of *S. torvum* have been previously isolated and reported with many bioactivities. The chemical constituents and bioactivities of *S. torvum* are shown in Table 2 (14-15, 54-56).

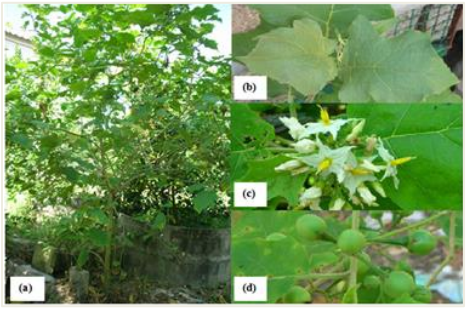
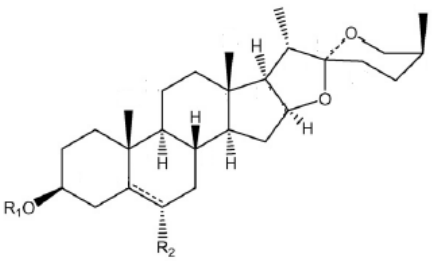


Figure 8 a) Arial part, b) leave, c) flower and d) fruit of *S. torvum*

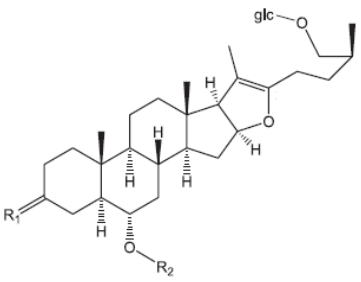
Table 2 Chemical constituents and bioactivities of *S. torvum* extracts

Part	Bioactivity	Chemical classes	Chemical constituents	Reference
Fruits	Cytotoxic activity with human cancer cell lines	Steroidal glycosides	Torvosides M **(49)**	(54)
			Torvosides N **(50)**	
	Cytotoxic activity against the human melanoma cell line A375		25(S)-26-*O*-β-D-glucopyranosyl-5α-furost-22(20)-en-3β,6α,26-triol-6-*O*-[α-L-rhamnopyranosyl-(1→3)-*O*-β-D-quinovopyranoside] **(51)**	(55)
			25(S)-26-*O*-β-D-glucopyranosyl-5α-furost-22(20)-en-3-one-6α,26-diol-6-*O*-[α-L-rhamnopyranosyl-(1→3)-*O*-β-D-quinovopyranoside] **(52)**	(55)
			25(S)-26-*O*-β-D-glucopyranosyl-5α-furost-22(20)-en-3β,6α,26-triol-6-*O*-β-D-quinovopyranoside **(53)**	(55)
			5α-Pregn-16-en-20-one-3β,6α-diol-6-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-quinovopyranoside **(54)**	(55)
	Angiotensin-converting enzyme inhibitor	Methyl salicylate glycoside	*(E)*-2,3-Dihydroxycyclopentyl-3-(3′,4′-dihydroxyphenyl)acrylate **(55)**	(15)
	α-Glucosidase inhibitor, anti-hyperglycemic activity, anti-diabetic	Hydroxycinnamic acid	Methyl caffeate **(56)**	(14)
Aerial part	Anti-neutrophilic inflammatory activity	Steroidal glycosides	Neochlorogenin6-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-quinovopyranoside] **(57)**	(56)
			Neochlorogenin6-*O*-[β-D-xylopyranosyl-(1→3)-β-D-quinovopyranoside] **(58)**	(56)



Torvosides M (**49**) Δ^5 R_1 = β-D-glu-(1→6)-*O*-β-D-glu- R_2 = H

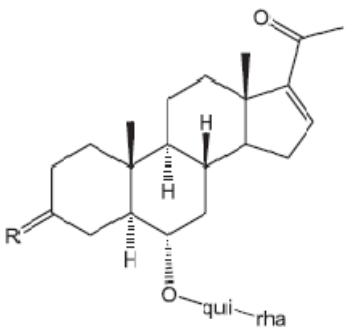
Torvosides N (**50**) 5α-H R_1 = β-D-glu-(1→6)-*O*-β-D-glu- R_2 = OH



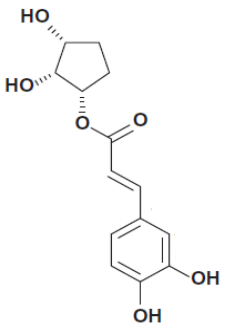
25(S)-26-*O*-β-D-glucopyranosyl-5α-furost-22(20)-en-3β,6α,26-triol-6-*O*-[α-L-rhamnopyranosyl-(1→3)-*O*-β-D-quinovopyranoside] (**51**) R_1 = β-OH, H R_2 = qui 3 rha

25(S)-26-*O*-β-D-glucopyranosyl-5α-furost-22(20)-en-3-one-6α,26-diol-6-*O*-[α-L-rhamnopyranosyl-(1→3)-*O*-β-D-quinovopyranoside] (**52**) R_1 = O R_2 = qui 3 rha

25(S)-26-*O*-β-D-glucopyranosyl-5α-furost-22(20)-en-3β,6α,26-triol-6-*O*-β-D-quinovopyranoside (**53**) R_1 = β-OH, H R_2 = qui

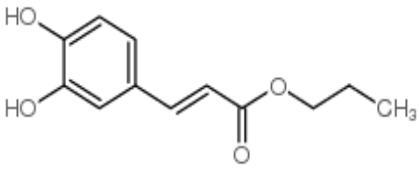


5α-Pregn-16-en-20-one-3β,6α-diol-6-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-quinovopyranoside (**54**) R = β-OH, H

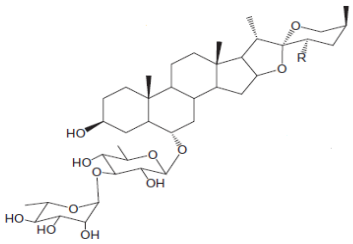


(*E*)-2,3-Dihydroxycyclopentyl-3-(3′,4′-dihydroxyphenyl)acrylate (**55**)

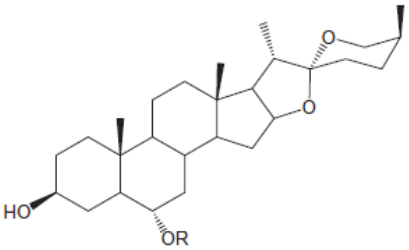
Figure 9 Structures of chemical constituents of *S. torvum* extracts



Methyl caffeate **(56)**



Neochlorogenin6-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-quinovopyranoside] **(57)** R = H



Neochlorogenin6-*O*-[β-D-xylopyranosyl-(1→3)-β-D-quinovopyranoside] **(59)** R = S^1

Figure 9 Structures of chemical constituents of *S. torvum* extracts (continue)

CHAPTER III

MATERIALS AND METHODS

3.1 Source of plant materials

3.1.1 Preliminary screening

Sixteen plant materials were purchased from Pak Kret market, Pak Kret, Nonthaburi province, Thailand. The scientific name, common name and part used of sixteen plants are shown in Table 3.

3.1.2 Fruits of *S. torvum*

The fresh fruits of *S. torvum* were collected at Bang Bua Thong, Nonthaburi province, Thailand, in October 2013. The samples were authenticated by a botanist and deposited at the Bangkok Forest Herbarium, Royal Forest Department, Bangkok, Thailand with voucher specimen BKF No. 18771.

Table 3 Uses of plant for preliminary screening

Scientific name	Common name	Part
A. esculentus	Lady's finger	Fruit
A. sativum	Garlic	Bulb
A. galanga	Galanga	Rhizome
B. rotunda	Finger root	Rhizome
C. tinctorius	Safflower	Flower
C. longa	Turmaric	Rhizome
C. citrates	Lemon grass	Stem
G. atroviridis	Garcinia	Fruit
H. sabdariffa	Roselle	Calyx
M. cordifolia	Kitchen mint	Leaf
P. nigrum	Black peper	Fruit
R. nasutus	White crane flower	Leaf
S. alexandrina	Alexandria senna	Leaf
S. torvum	Turkey berry	Fruit
Z. officinale	Ginger	Rhizome
Z. jujuba	Chinese date	Fruit

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3.2 Instruments and equipments

3.2.1 Analytical thin layer chromatography (TLC)

Technique : One dimension, ascending

Absorbent : Silica gel 60 F_{254} pre-coated plate (Merck)

Layer thickness : 0.2 mm

Developing distances : 4.5 cm

Temperature : Room temperature (30-35 °C)

Detection : a. Observe under Ultraviolet light at wavelength of 254 and 356 nm

b. Dip in 10% H_2SO_4 in aqueous ethanol and heat on hot plate

3.2.2 Conventional column chromatography

Absorbent : Silica gel 60 (Merck) partition sizes 0.063-0.200 nm. (70-230 mesh ASTM)

Packing method : Wet packing

Detection : All fractions were detected by TLC technique

3.2.3 Preparative thin layer chromatography (PLC)

Technique : One dimension, ascending

Absorbent : PLC silica gel 60 F_{254} pre-coated plate (Merck)

Layer thickness : 1 mm

Developing distance : 15 cm

Temperature : Room temperature (30-35 °C)

Detection : a. Observe under ultraviolet light at wavelength of 254 and 356 nm

b. Dip in 10% H_2SO_4 in aqueous ethanol and heat on hot plate

3.2.4 Hot air oven

The WiseVen hot air oven was used at 60 °C for drying the plant materials.

3.2.5 Rotary evaporator

The Eyela rotatory evaporator model N-1 was used for rapid removal of large amount of volatile solvents.

3.2.6 Water bath

The Memmert water bath was used for aqueous extraction and incubation sample for testing lipase inhibitory activity.

3.2.7 Spectroscopic techniques

3.2.7.1 UV/VIS spectrophotometer

Instrument name : Microplate reader Multiscan GO (Thermo Fisher Scientific)

Softwere : SkanIT 3.2

Incubate : 30.2 ℃

Wavelenght : 405 nm

3.2.7.2 Gas chromatograph-mass spectrometer (GC-MS)

Instrument name : Triple Quadrupole GC/MS (GC-QQQ) (Agilent Technologies)

GC model : Agilent 7890B GC system

MSD model : Agilent 7000C GC/MS Triple Quad

GC column : HP-5ms part no. 19091S-433 (30 m × 0.25 mm, 0.25 µm)

3.2.7.3 Nuclear Magnetic Resonance Spectrometer (NMR)

The ^{1}H and ^{13}C nuclear magnetic resonance spectra of isolated compounds were recorded at 300 MHz and 75.5 MHz, respectively, on a Bruker model Fourier 300 spectrometer in chloroform-d ($CDCl_3$).

3.3 Chemicals and reagents

3.3.1 All organic solvents (hexane, dichloromethane, ethyl acetate, acetone and methanol) were of commercial grade and were distrilled prior to use.

3.3.2 Acetonitrile, J.T. Baker, USA

3.3.3 Chloroform-d 99.8 atom % D, Sigma, Germany

3.3.4 Dimethyl sulfoxide (DMSO), ACS reagent, Fluka, Switzerland

3.3.5 Ethanol, Merck, Germany

3.3.6 Hydrochloric acid, Merck, Germany

3.3.7 Lipase from porcine pancreas Type II, crude, Promega, USA

3.3.8 *p*-Nitrophenylpalmitate, Sigma, Germany

3.3.9 Tris(hydroxymethyl)aminomethane (Tris-base), MB Grade, USB, USA

3.4 Methods

3.4.1 Preliminary screening

3.4.1.1 Dry plant materials

Six dry plant materials (flowers of *C. tinctorius*, *fruits of G. atroviridis*, calyxs of *H. sabdariffa*, fruits of *P. nigrum*, fruits of *S. Alexandrina* and fruits of *Z. jujuba*) were disposed dust and gravel. Then, they were sliced or crushed to small pieces. The plant materials were subjected to ethanol and aqueous extraction, respectively, at room temperature. The extract was filtrated with Whatman no.1 filter paper and evaporated *in vacuo* to give the crude extract. Then, the crude extracts were measured for lipase inhibitory activity.

3.4.1.2 Fresh plant materials

Ten fresh plant materials (fruits of *A. esculentus*, bulbs of *A. sativum*, rhizomes of *A. galanga*, rhizomes of *B. rotunda*, rhizomes of *C. longa*, stems of *C. citrates*, leaves o f *M. cordifolia*, leaves of *R. nasutus*, fruits of *S. torvum* and *rhizomes of Z. officinale*) were dried at 60 ℃ in hot air oven and ground to rough powder. The powdered materials were subjected to ethanol and aqueous extraction, respectively, at room temperature. The extract was filtrated with Whatman no.1 filter paper and evaporated *in vacuo* to give the crude extract. Then, the crude extracts were measured for lipase inhibitory activity.

3.4.2 Extraction of *S. torvum* fruits

Fruits of *S. torvum* (26.83 kg) were dried at 60 ℃ in hot air oven and ground to rough powder. Dry fruit powdered (4.26 kg) was extracted with hexane (20 L×3), for 27 hours at room temperature for 3 times. The extract was filtrated with Whatman no.1 filter paper and evaporated *in vacuo*. After that the residue was extracted with ethyl acetate (20 L×3), for 72 hours at room temperature for 3 times. The extract was filtrated with Whatman no. 1 filter paper and then evaporated *in vacuo*. The residue was extracted with methanol (20 L×3), for 72 hours at room temperature for 3 times and then filtrated with Whatman no. 1 filter paper and evaporated *in vacuo*. Then the residue was extracted with aqueous (20 L×3) at 60 ℃ for an hour and then

filtrated with Whatman no. 1 filter paper and evaporated *in vacuo*. Four crude extracts of *S. torvum* fruit were tested lipase inhibitory activity.

3.4.3 Isolation of *S. torvum* fruits

3.4.3.1 Separation of hexane crude extract

The hexane crude extract (81.71 g) was separated by quick column chromatography on silica gel (600 g) and eluted sequentially with hexane (15 L), hexane:ethyl acetate (1:1 v/v)(7 L) and ethyl acetate (4 L) to give three fractions (A-C). Then, washed down with ethyl acetate:methanol (9:1 v/v) (4 L) to give fraction D. Each fraction was evaluated for lipase inhibitory activity. Then active fractions were further separated by silica gel column chromatography.

The fraction B which was to eluted with hexane:ethyl acetate (1:1 v/v), showed strong activity. Fraction B was separated by silica gel column chromatography, using hexane, hexane:ethyl acetate (1:1 v/v), ethyl acetate, ethylacetate:methanol (9:1 v/v) and ethyl acetate:methanol (4:1 v/v) to give eight fractions (BA-BH). Each fraction was tested lipase inhibitory activity.

The fraction BA showed strong lipase inhibitory activity. Fraction BA was further separated by silica gel column chromatography, using hexane, hexane:ethyl acetate (9:1 v/v) and ethyl acetate to give four fraction as fractions BAA-BAD. Then, four fractions were tested lipase inhibitory activity. Fraction BAA was subjected to column chromatography on silica gel, using hexane and dichloromehane to give four fractions (BAAA-BAAD) and washed down with ethyl acetate to give fraction BAAE. Then, all fractions were elucidated for lipase inhibitory activity. The fraction BAAA was separated by silica gel column chromatography, using heptane, heptane:hexane (1:1 v/v), hexane and dichloromethane to give four fractions (BAAAA-BAAAE). Then, fraction BAAAD was subjected to column chromatography on silica gel, using hexane, hexane:dichloromethane (1:1 v/v), hexane:dichloromethane (3:2 v/v) to give four fractions (BAAADA-BAAADD) and washed down with dichloromethane to give fraction BAAADE. All of them were not pure compounds.

The fraction BB showed the strongest inhibitory activity. Fraction BB was subjected to column chromatography on silica gel, using hexane,

hexane:dichloromethane (1:1 v/v), dichloromethane, ethyl acetate and washed down with ethyl acetate:methanol (9:1 v/v) to give five fractions (BBA-BBE). All fractions were elucidated for lipase inhibitory activity. The fraction BBC showed the strongest lipase inhibitory activity. Then, fraction BBC was further separated by silica gel column chromatography, using hexane, hexane:dichloromethane (4:1 v/v) and dichloromethane to give three fractions (BBCA-BCCC) and then washed down with ethyl acetate to give one fraction as BBCD. Fraction BBCC gave a major violet spot on TLC plate. It was separated by silica gel column chromatography, using hexane, hexane:dichloromethane (7:3 v/v), hexane:dichloromethane (3:2 v/v), ethyl acetate and ethyl acetate:methanol (9:1 v/v) to give seven fractions (BBCCA-BBCCG). Fraction BBCCD gave a major violet spot on TLC plate. It was separated by silica gel column chromatography, using hexane, hexane:acetone (1:49 v/v) and hexane:acetone (1:19 v/v) to give seven fractions (BBCCDA-BBCCDG). Fraction BBCCDF was white crystal but not pure. Fraction BBCCDF was separated by prep-TLC plate using 19:1 v/v of dichloromethane:ethyl acetate as developing solvent to give white crystal of Compound **60** (0.0376 g).

The fraction BC showed strong lipase inhibitory activity as same as fraction BA. Then, fraction BC was further separated by silica gel column chromatography, using hexane, hexane ethyl acetate (4:1 v/v) and hexane:ethyl acetate (3:2 v/v) to give seven fractions (BCA-BCG) and washed down with ethyl acetate to give two fractions (BCH-BCI). These nine fractions were not pure compounds.

Fraction BE showed strong lipase inhibitory activity. This fraction was subjected to column chromatography on silica gel, using dichloromethane, diclorometha ne:acetone (19:1 v/v), dichloromethane:acetone (9:1 v/v), dichloromethane:acetone (4:1 v/v), dichloromethane:hexane (1:1 v/v) and acetone to give twelve fractions (BEA-BEL). Fraction BEA was further separated by silica gel column chromatography using, heptane, heptane:hexane (1:1 v/v), hexane, hexane:dichloromethane (9:1 v/v), hexane:dichloromethane (4:1 v/v) and dichloromethane to give nine fractions (BEAA-BEAI). Fraction BEC was subjected to column chromatography on silica gel, using hexane, hexane:ethyl acetate (19:1 v/v),

hexane:ethyl acetate (9:1 v/v) and hexane:ethyl acetate (1:1 v/v) to give eight fractions (BECA-BECH).

3.4.3.2 Separation of ethyl acetate crude extract

The ethyl acetate crude extract (75.74 g) was separated by quick column chromatography on silica gel (400 g) and eluted sequentially with hexane (9 L), hexane:ethyl acetate (1:1 v/v) (13 L), ethyl acetate (8 L) and ethyl acetate:methanol (9:1 v/v) (5 L) to give four fractions (E-H). Then, washed down with ethyl acetate:methanol (4:1 v/v) (8 L) to give one fraction (I). Each fraction was elucidated for lipase inhibitory activity.

The fraction F was the strongest lipase inhibitory activity among fractions E-I. Then, fraction F was further separated by silica gel column chromatography, using dichloromethane, dichloromethane:ethyl acetate (9:1 v/v), dichloromethane:ethyl acetate (1:1 v/v), ethyl acetate to give eight fractions (FA-FH) and then, washed down with ethyl acetate:methanol (4:1 v/v) to give two fractions (FI and FJ). Each fraction was tested lipase inhibitory activity.

Fraction FE showed the strongest lipase inhibitory activity. The fraction FE was separated by silica gel column chromatography, using hexane, hexane:ethyl acetate (19:1 v/v), hexane:ethyl acetate (9:1 v/v), hexane:ethyl aceate (4:1 v/v), hexane:ethyl acetate (3:2 v/v) and ethyl acetate to give nine fractions (FEA-FEI) and washed down with ethyl acetate:methanol (4:1 v/v) to give last fraction (FEJ).

Fraction FEE showed a major yellow spot when observe by dipped TLC-plate in 10% H_2SO_4 in aqueous ethanol and heat on hot plate. Fraction FEE was further separated by silica gel column chromatography, using dichloromethane, dichloromethane:ethyl acetate (1:1 v/v) and ethyl acetate to give three fractions (FEEA-FEEC). Fraction FEEA was separated by silica gel column chromatography, using hexane, hexane:acetone (19:1 v/v) and hexane:acetone (1:1 v/v) to give four fractions (FEEAA-FEEAD). Fraction FEEAC was light yellow solid but it still was a mixture. Fraction FEEAC was further separated by silica gel column chromatography, using hexane, hexane:dichloromethane (4:1 v/v), dichloromethane and ethyl acetate to give nine fractions (FEEACA-FEEACI). The fifth fraction of FEEAC (FEEACE) was light yellow crystal but it still was a mixture. This fraction was separated by prep-TLC

plate using 4:1v/v of heptane:acetone as developing solvent to give light yellow crystal but it was not pure compound.

Fractions FEH and FEI were light yellow crystal and green liquid. These two fractions showed a major bright spot with same R_f value when observed under ultraviolet light at wavelength 356 nm. Fractions FEH and FEI were crystallized using dichloromethane. But the crystal was not pure. So, they were combined as fraction FEH-I and further separated by silica gel column chromatography, using dichloromethane, dichrolomethane:ethyl acetate (1:1 v/v) and ethyl acetate to give Compound **61** (0.0231 g).

3.4.4 Gas Chromatography-Mass Spectrometry (GC-MS) analysis of Compound **60**

GC-MS is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances with a standard samples. In this study, the condition for analysis the Compound **60** was followed the study of Sheng and Chen (57). The mobile phase for GC separation was Helium gas (1.2 mL/min) with split ratio 10:1. Injector temperature was 280 °C. The initial column temperature was 240 °C, then rose up to 265 °C at the rising speed of 10 °C/min and remained at 300 °C for 3 minutes. The coupled mass spectrometer was operated in ionization mode as EI+ with electron energy 70 eV. Interface temperature and ion source temperature were 280 °C with sample loading 0.5 **µ**L.

3.4.5 Measurement of lipase inhibitory activity *in vitro*

The method of Slanc *et al*. (58) was employed with some modifications. *p*-Nitrophenylpalmitate (PNP) was used as substrate. The test sample was dissolved in DMSO and water at a concentration of 50 mg/mL. Porcine pancreatic lipase was dissolved in Tris-HCl buffer (pH 8.5) to a final concentration 5 mg/mL. Reaction mixtures, containing 32 µL test sample, 280 µL Tris-HCl pH 8.5, 40 µL 6.66 mM PNP and 48 µL lipase, were incubated at 37 °C for 25 minutes and stoped reaction by 600 µL ethanol. The absorbance of released *p*-nitrophenol was measured at 405 nm using a microplate reader. Blank was added ethanol before enzyme. The extract was replaced by DMSO or water for the control. The absorbances of samples were corrected by subtracting the absorbance of the blanks. The activity assay was

performed in triplicate for each crude extract. The results were averaged and expressed with standard deviations. A solution of orlistat was prepared in DMSO as a positive control at a concentration 0.01 µg/mL. The absorbance at 405 nm (A_{405}) of liberated *p*-nitrophenol was measured with *p*-nitrophenol per minute under assay condition.

% inhibition = [(A-a)-(B-b)/(A-a)]x100

When A = absorbance of control (without crude extract)

a = absorbance of control (without crude extract and lipase)

B = absorbance of sample

b = absorbance of sample (without lipase)

3.4.6 Determination of IC_{50} value of isolated compounds

The IC_{50} value of isolated Compounds **60** and **61** was determined by dissolving in DMSO (3.125, 6.25, 12.5, 25, 50, 80 and 100 mg/mL). Then all prepared solutions were investigated for lipase inhibitory activity comparing with orlistat, as a positive control, (0.01, 0.005, 0.0025, 0.00125, 0.000625 and 0.0003125 µg/mL) according to the procedure 3.4.5.

CHAPTER IV
RESULTS AND DISSCUSSION

4.1 Extraction of plant materials for preliminary screening

Result of plant extracts with different solvent extraction showed different characteristic and quantities of the extracts. Percentage yields and characteristic of plant extracts are shown in Table 4.

4.2 The lipase inhibitory activity for preliminary screening

The ethanol and aqueous crude extracts of sixteen plants were preliminary evaluated for lipase inhibitory activity. The lipase inhibitory activity is shown in Table 5. Ethanol crude extracts, at a concentration of 50 mg/mL, of fruit of *S. torvum*, fruit of *P. nigrum*, rhizome of *C. longa*, stem of *C. citrates,* leaf of *M. cordifolia*, leaf of *S. alexandrina* and fruit of *G. atroviridis* exhibited strong inhibition at 87.35 ± 9.90%, 76.22 ± 12.18%, 75.42 ± 1.29%, 71.76 ± 2.88%, 66.40 ± 8.36%, 62.75 ± 12.55% and 60.74 ± 12.06%, respectively. Fruit of *A. esculentus*, bulb of *A. sativum* and flower of *C. tinctorius* exhibited moderate inhibition at 56.75 ± 2.84%, 51.50 ± 1.51% and 42.87 ± 12.56%, respectively. Rhizome of *A. galanga,* leaf of *R. nasutus*, rhizome of *Z. officinale* and fruit of *Z. jujuba* showed weak inhibition at 37.63 ± 3.86%, 31.03 ± 3.54%, 18.97 ± 2.37% and 14.83 ± 12.11%, respectively. The ethanol extract of rhizome of *B. rotunda* and calyx of *H. sabdariffa* showed no lipase inhibitory activity.

Aqueous extract, at a concentration of 50 mg/mL, from leaf of *M. cordifolia,* calyx of *H. sabdariffa,* rhizome of *A. galanga,* bulb of *A. sativum,* fruit of *Z. jujuba,* fruit of *G. atrovirids*, rhizome of *B. rotunda*, leaf of *S. alexandria* and rhizome of *Z. officinale* exhibited strong inhibition at 92.88 ± 2.99%, 91.90 ± 1.32%, 88.38 ±1.74%, 85.34 ± 10.74%, 77.73 ± 10.79%, 77.33 ± 7.56%, 71.78 ± 5.81%, 68.26 ± 8.16 and 67.69 ± 9.14, respectively. Fruit of *A. esculentus* showed moderate inhibition at 52.01 ± 10.98%. Aqueous extracts of fruit of *P. nigrum* and flower of *C. tinctorius* exhibited weak inhibition at 24.90 ± 10.14% and 23.51 ± 3.58%. Aqueous of rhizome of *C. longa* showed no lipase inhibitory activity. The aqueous extract of leaf of *M. cordifolia* showed the strongest lipase inhibitory activity. Orlistat showed pancreatic

lipase inhibitory activity with 93.60 ± 2.36% inhibition at a concentration of 1×10^{-5} mg/mL. Therefore, it was interested to isolate lipase inhibitor from ethanol extract of *S. torvum* fruit.

Table 4 Percentage yields and characteristics of plants for preliminary screening

Plant materials			Crude extract characteristic		Yield of crude extract (%)	
Scientific name	Common name	part	Ethanol	Aqueous	Ethanol	Aqueous
A. esculentus	Lady's finger	Fruit	Green gum	Brown solid	6.29	2.53
A. sativum	Garlic	Bulb	Brown wax	Brown solid	5.51	3.66
A. galanga	Galanga	Rhizome	Brown gum	Brown gum	8.57	4.46
B. rotunda	Finger root	Rhizome	Brown gum	Brown gum	5.11	0.21
C. tinctorius	Safflower	Flower	Yellow gum	Brown gum	6.15	2.24
C. longa	Turmaric	Rhizome	Yellow solid	Brown gum	10.10	4.62
C. citrates	Lemon grass	Stem	Brown gum	Brown solid	6.77	3.34
G. atroviridis	Garcinia	Fruit	Brown solid	Brown solid	7.09	8.85
H. sabdariffa	Roselle	Calyx	Red gum	Brown solid	7.47	8.19
M. cordifolia	Kitchen mint	Leaf	Brown gum	Brown solid	8.81	1.60
P. nigrum	Black peper	Fruit	Brown solid	Brown solid	6.12	4.53
R. nasutus	White crane flower	Leaf	Brown gum	Brown gum	4.80	0.55
S. alexandrina	Alexandria senna	Leaf	Brown solid	Brown solid	4.63	2.24
S. torvum	Turkey berry	Fruit	Brown gum	Brown solid	9.66	0.67
Z. officinale	Ginger	Rhizome	Brown wax	Brown solid	5.75	4.79
Z. jujuba	Chinese date	Fruit	Brown solid	Brown solid	8.14	7.57

*Percent yield of crude extract (%) = (weight of crude extract / weight of dried plant) × 100

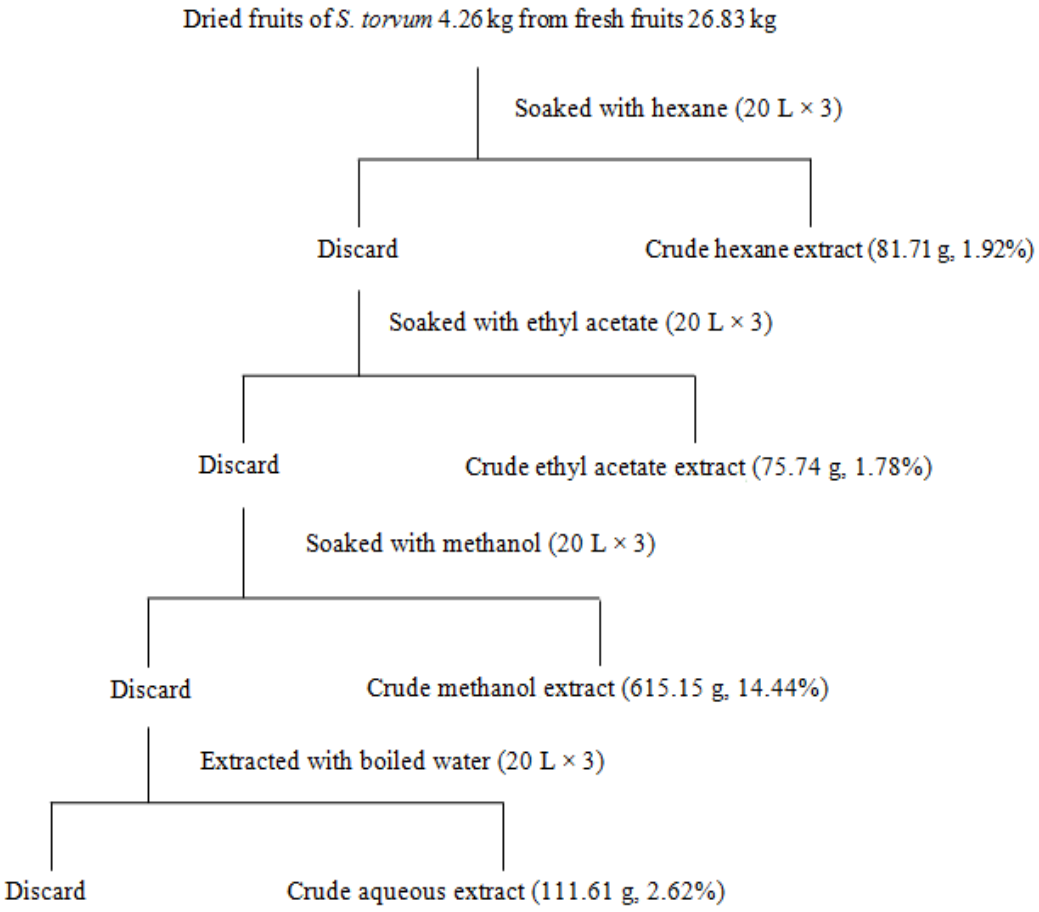
Table 5 Preliminary screening for lipase inhibitory activity

Plant materials			% inhibition	
Sciencetific name	Common name	Part	Ethanol	Aqueous
A. esculentus	Lady's finger	Fruit	56.75 ± 2.84	52.01 ± 10.98
A. sativum	Garlic	Bulb	51.50 ± 1.51	85.34 ± 10.74
A. galanga	Galanga	Rhizome	37.63 ± 3.86	88.38 ± 1.74
B. rotunda	Finger root	Rhizome	na	71.78 ± 5.81
C. tinctorius	Safflower	Flower	42.87 ± 12.56	23.51 ± 3.58
C. longa	Turmaric	Rhizome	75.42 ± 1.29	na
C. citrates	Lemon grass	Stem	71.76 ± 2.88	4.23 ± 9.64
G. atroviridis	Garcinia	Fruit	60.74 ± 12.06	77.33 ± 7.56
H. sabdariffa	Roselle	Calyx	na	91.90 ± 1.32
M. cordifolia	Kitchen mint	Leaf	66.40 ± 8.36	92.88 ± 2.99
P. nigrum	Black peper	Fruit	76.22 ± 12.18	24.90 ± 10.14
R. nasutus	White crane flower	Leaf	31.03 ± 3.54	76.07 ± 10.82
S. alexandrina	Alexandria senna	Leaf	62.75 ± 12.55	68.26 ± 8.16
S. torvum	Turkey berry	Fruit	87.35 ± 9.90	7.96 ± 9.06
Z. officinale	Ginger	Rhizome	18.97 ± 2.37	67.69 ± 9.14
Z. jujuba	Chinese date	Fruit	14.83 ± 12.11	77.73 ± 10.79
Orlistat				93.60 ± 2.36*

*at a concentration of 1×10^{-5} mg/mL, na = no activity

4.3 Extraction of *S. torvum* fruits

Dry *S. torvum* fruit (4.26 kg) was extracted with hexane, ethyl acetate, methanol, and aqueous, respectively. Each extract was evaporated under reduce pressure to give the hexane crude extract as light green wax (81.71 g, 1.92%), ethyl acetate crude extract as dark green wax (75.74 g, 1.78%), methanol crude extract as dark brown gum (615.15 g, 14.44%) and aqueous crude extract as dark brown solid (111.61 g, 2.62%), respectively.



Scheme 1 Extraction of *S. torvum* fruits

Four crude extracts of *S. torvum* fruit were tested lipase inhibitory activity. The ethyl acetate crude extract showed the strongest lipase inhibitory activity with 67.23 ± 3.57%, while hexane crude extract exhibited moderate activity with 46.00 ± 6.71%. The methanol and aqueous crude extracts showed weak activity with 15.82 ± 1.13%

and 8.51 ± 1.05%, respectively. The results are shown in Table 6. Therefore, hexane and ethyl acetate crude extracts were further purified by chromatographic techniques and evaluated for lipase inhibitory activity *in vitro*.

Table 6 Lipase inhibitory activity of crude extracts of *S. torvum* fruit

Solvent extract	Appearance	Weight (g)	% w/w of the dried plant	% Inhibition
Hexane	Light green wax	81.71	1.92	46.00 ± 6.71
Ethyl acetate	Dark green wax	75.74	1.78	67.23 ± 3.57
Methanol	Dark brown gum	615.15	14.44	15.82 ± 1.13
Aqueous	Dark brown solid	111.61	2.62	8.51 ± 1.05
Orlistat				93.62 ± 1.12*

*at a concentration of 1×10^{-5} mg/mL

4.4 Isolation of *S. torvum* fruits

4.4.1 Seperation of hexane crude extract

The hexane crude extract (81.71 g) was separated by quick column chromatography on silica gel and eluted sequentially with hexane, hexane:ethyl acetate (1:1 v/v), ethyl acetate and ethyl acetate:methanol (9:1 v/v) to give fractions A-D. Each fraction was tested lipase inhibitory activity. The results are shown in Table 7. Fraction C of hexane crude extract showed the strongest lipase inhibitory activity with 50.33 ± 6.12%. Fractions B and A were exhibited moderate inhibition with 48.78 ± 2.26% and 47.97 ± 7.25%, respectively. Fraction D of hexane crude extract showed weak lipase inhibitory activity with 11.71 ± 5.74%. Fraction B was further isolated to find lipase inhibitor.

Fraction B was separated by silica gel column chromatography, using hexane, hexane:ethyl acetate (1:1 v/v), ethyl acetate, ethyl acetate:methanol (9:1 v/v) and ethyl acetate:methanol (4:1 v/v) to give fractions BA-BH. Each fraction was tested lipase inhibitory activity. The results are shown in Table 8. Fractions BB, BC, BA, BF,

BD, BG and BE exhibited strong inhibition at 96.35 ± 1.30%, 94.11 ± 6.89%, 92.34 ± 2.37%, 82.85 ± 3.71%, 75.00 ± 7.08%, 70.80 ± 6.81% and 65.90 ± 2.30%, respectively. Fraction BH showed weak lipase inhibitory activity at 32.07 ± 7.14%. Oristat showed pancreatic lipase inhibitory activity with 93.15 ± 0.29% inhibition at a concentration of 1×10^{-5} mg/mL.

Table 7 Lipase inhibitory activity of fractions A-D

Fraction	Weight (g)	% Inhibition
A	15.20	47.97 ± 7.25
B	68.16	48.78 ± 2.26
C	0.85	50.33 ± 6.12
D	0.38	11.71 ± 5.74
Orlistat		93.62 ± 1.16*

*at a concentration of 1×10^{-5} mg/mL

The fraction BA showed strong lipase inhibitory activity with 92.34 ± 2.37% at a concentration of 4 mg/mL. Fraction BA was further separated by silica gel column chromatography, using hexane, hexane:ethyl acetate (9:1 v/v) and ethyl acetate to give four fractions as fractions BAA-BAD. Then, four fractions were tested lipase inhibitory activity. The results are shown in Table 9, all fractions, BAA-BAD showed strong lipase inhibitory activity. Comparing with oristat, it showed pancreatic lipase inhibitory activity with 92.13 ± 4.69% inhibition at a concentration of 1×10^{-5} mg/mL.

Table 8 Lipase inhibitory activity of fractions BA-BH

Fraction	Weight (g)	% Inhibition
BA	9.82	92.34 ± 2.37
BB	35.20	96.35 ± 1.30
BC	14.17	94.11 ± 6.89
BD	4.49	75.00 ± 7.08
BE	1.56	65.90 ± 2.30
BF	1.03	82.85 ± 3.71
BG	0.56	70.80 ± 6.81
BH	1.07	32.07 ± 7.14
Orlistat		93.15 ± 0.29*

*at a concentration of 1×10^{-5} mg/mL

Table 9 Lipase inhibitory activity of fractions BAA-BAD

Fraction	Weight (g)	% Inhibition
BAA	2.5887	88.41 ± 2.78
BAB	3.8912	87.53 ± 1.75
BAC	0.4616	87.97 ± 13.40
BAD	0.1288	94.17 ± 7.22
Orlistat		92.13 ± 4.69*

*at a concentration of 1×10^{-5} mg/mL

The fraction BAA was subjected to column chromatography on silica gel, using hexane and dichloromethane to give four fractions (BAAA-BAAD) and washed down with ethyl acetate to give fraction BAAE. Then, all fractions were elucidated for lipase inhibitory activity. The results are shown in Table 10.

Table 10 Combined fractions of fraction BAA

Fraction	Weight (g)
BAAA	0.0985
BAAB	0.2328
BAAC	1.4724
BAAD	0.2469
BAAE	0.0802

Fraction BAAA was separated by silica gel column chromatography, using heptane, heptane:hexane (1:1 v/v), hexane and dichloromethane to give four fractions (BAAAA-BAAAD). The results are shown in Table 11.

Table 11 Combined fractions of fraction BAAA

Fraction	Weight (g)
BAAAA	0.0044
BAAAB	0.0009
BAAAC	0.0032
BAAAD	0.0020

Then, fraction BAAAD was subjected to column chromatography on silica gel, using hexane, hexane:dichloromethane (1:1 v/v), hexane:dichloromethane (3:2 v/v) to give four fractions (BAAADA-BAAADD) and washed down with dichloromethane to give fraction BAAADE. These five fractions of BAAAD were not pure compound. The results are shown in Table 12.

Table 12 Combined fractions of fraction BAAAD

Fraction	Weight (g)
BAAADA	0.0004
BAAADB	0.0007
BAAADC	0.0001
BAAADD	0.0002
BAAADE	0.0005

Then, fraction BB was subjected to column chromatography on silica gel, using hexane, hexane:dichloromethane (1:1 v/v), dichloromethane, ethyl acetate and washed down with ethyl acetate:methanol (9:1 v/v) to obtain fractions BBA-BBE. Then, each fraction was tested lipase inhibitory activity. The results are shown in Table 13. Fractions BBC and BBE exhibited strong inhibition with 87.90 ± 1.16% and 71.26 ± 2.09%. Fractions BBD, BBA and BBB exhibited moderate inhibition with 57.14 ± 9.38%, 55.00 ± 3.66% and 55.00 ± 3.66%, respectively. Comparing with oristat, it showed pancreatic lipase inhibitory activity with 93.15 ± 0.29% inhibition at a concentration of 1×10^{-5} mg/mL.

The fraction BBC was the strongest lipase inhibitory activity with 87.90 ± 1.16%. Then, fraction BBC was further separated by silica gel column, using hexane, hexane:dichloromethane (4:1 v/v) and dichloromethane to give fractions BBCA-BCCD. The results of each fraction are shown in Table 14.

Table 13 Lipase inhibitory activity of fractions BBA-BBE

Fraction	Weight (g)	% Inhibition
BBA	8.71	55.00 ± 3.66
BBB	9.79	55.00 ± 3.66
BBC	4.98	87.90 ± 1.16
BBD	10.69	57.14 ± 9.38
BBE	0.69	71.26 ± 2.09
Orlistat		93.15 ± 0.29*

*at a concentration of 1×10^{-5} mg/mL

Table 14 Combined fractions of fraction BBC

Fraction	Weight (g)
BBCA	0.0126
BBCB	0.0584
BBCC	0.4511
BBCD	3.9751

Fraction BBCC gave a major violet spot on TLC plate. So, it was further separated by silica gel column chromatography, using hexane, hexane:dichloromethane (7:3 v/v), hexane:dichloromethane (3:2 v/v), ethyl acetate and ethyl acetate:methanol (9:1 v/v) to obtain fractions BBCCA-BBCCG. The results are shown in Table 15.

Table 15 Combined fractions of fraction BBCC

Fraction	Weight (g)
BBCCA	0.0023
BBCCB	0.0037
BBCCC	0.0023
BBCCD	0.1080
BBCCE	0.0104
BBCCF	0.0554
BBCCG	0.0792

Fraction BBCCD gave a major violet spot on TLC plate, were separate by silica gel column chromatography, using hexane, hexane:acetone (1:49 v/v) and hexane:acetone (1:19 v/v) to give fractions BBCCDA-BBCCDG. The results are shown in Table 16.

Fraction BBCCDF was white crystal but not pure. Fraction BBCCDF was further separated by prep-TLC using 19:1 v/v of dichloromethane:ethyl acetate as developing solvent to give white crystal of compound 60 (0.0376 g, 0.0009%).

The fraction BC showed strong lipase inhibitory activity with % inhibition value of 94.11 ± 6.89% at a concentration of 4 mg/mL. Then, fraction BC was further separated by silica gel column chromatography, using hexane, hexane ethyl acetate (4:1 v/v), hexane:ethyl acetate (3:2 v/v) and ethyl acetate to give nine fractions (BCA-BCI). Fractions BCA-BCI were not pure compounds. The results are shown in Table 17.

Table 16 Combined fractions of fraction BBCCD

Fraction	Weight (g)
BBCCDA	0.0004
BBCCDB	0.0009
BBCCDC	0.0023
BBCCDD	0.0047
BBCCDE	0.0054
BBCCDF	0.0876
BBCCDG	0.0014

Table 17 Combined fractions of fraction BC

Fraction	Weight (g)
BCA	0.0033
BCB	0.0233
BCC	0.0141
BCD	0.1252
BCE	0.8039
BCF	0.5433
BCG	0.3470
BCH	1.0017
BCI	3.0134

Fraction BE showed strong lipase inhibitory activity. This fraction was subjected to column chromatography on silica gel, using dichloromethane,

dicloromethane:acetone (19:1 v/v), dichloromethane:acetone (9:1 v/v), dichloromethane:acetone (4:1 v/v), dichloromethane:hexane (1:1 v/v) and acetone to give twelve fractions (BEA-BEL). The results are shown in Table 18.

Fraction BEA was further separated by silica gel column chromatography using, heptane, heptane:hexane (1:1 v/v), hexane, hexane:dichloromethane (9:1 v/v), hexane:dichloromethane (4:1 v/v) and dichloromethane to give nine fractions (BEAA-BEAI). But all fractions were not pure compounds. The results are shown in Table 19.

Table 18 Combined fractions of fraction BE

Fraction	Weight (g)
BEA	0.0145
BEB	0.0122
BEC	0.0632
BED	0.0219
BEE	0.0126
BEF	0.0286
BEG	0.0846
BEH	0.0374
BEI	0.0323
BEJ	0.0087
BEK	0.0892
BEL	0.4030

Fraction BEC was subjected to column chromatography on silica gel, using hexane, hexane:ethyl acetate (19:1 v/v), hexane:ethyl acetate (9:1 v/v) and hexane:ethyl acetate (1:1 v/v) to give eight fractions (BECA-BECH). All fractions of BECA-BECH were not pure compounds. The results are shown in Table 20.

Table 19 Combined fractions of fraction BEA

Fraction	Weight (g)
BEAA	0.0007
BEAB	0.0013
BEAC	0.0012
BEAD	0.0005
BEAE	0.0015
BEAF	0.0014
BEAG	0.0011
BEAH	0.0009
BEAI	0.0021

Table 20 Combined fractions of fraction BEC

Fraction	Weight (g)
BECA	0.0007
BECB	0.0003
BECC	0.0019
BECD	0.0023
BECE	0.0085
BECF	0.0118
BECG	0.0181
BECH	0.0224

4.4.2 Separation of ethyl acetate crude extract

The ethyl acetate crude extract (75.74 g) was separated by quick column chromatography on silica gel and eluted sequentially with hexane (9 L), hexane:ethyl acetate (1:1 v/v) (13 L), ethyl acetate (8 L) and ethyl acetate:methanol (9:1 v/v) (5 L) to give fractions E-I. Each fraction was tested lipase inhibitory activity. The results of each fraction are shown in Table 21. Fractions F, G, H and I exhibited strong inhibition with 82.77 ± 8.17%, 80.37 ± 4.52%, 67.91 ± 5.15% and 62.11 ± 2.77%, respectively. Whereas, fraction E of ethyl acetate crude extract showed weak lipase inhibitory activity at 38.09 ± 14.92%.

Table 21 Lipase inhibitory activity of fractions E-I

Fraction	Weight (g)	% Inhibition
E	30.70	38.09 ± 14.92
F	27.66	82.77 ± 8.17
G	6.69	80.37 ± 4.52
H	4.92	67.91 ± 5.15
I	3.86	62.11 ± 2.77
Orlistat		93.62 ± 1.16*

*at a concentration of 1 $\times 10^{-5}$ mg/mL

Then, fraction F was further separated by silica gel column chromatography, using dichloromethane, dichloromethane:ethyl acetate (9:1 v/v), dichloromethane:ethyl acetate (1:1 v/v), ethyl acetate to give fractions FA-FJ. Then, each fraction was tested lipase inhibitory activity. The results are shown in Table 22. Fractions FE, FI and FD exhibited strong inhibition with 77.43 ± 6.83%, 68.81 ± 6.63% and 66.74 ± 1.61%, respectively. Fractions FC, FH and FB exhibited moderate inhibition with 54.56 ± 2.56%, 54.20 ± 7.31% and 49.29 ± 4.94%, respectively. Fractions FF and FG exhibited weak inhibition at 23.61 ± 5.33% and 6.11± 2.85%. Just

fraction FA did not showed lipase inhibitory activity. Oristat showed pancreatic lipase inhibitory activity with 92.37 ± 0.50% inhibition at a concentration of 1×10^{-5} mg/mL.

Fraction FE was the strongest lipase inhibitory activity. The fraction FE was further separated by silica gel column chromatography, using hexane, hexane:ethyl acetate (19:1 v/v), hexane:ethyl acetate (9:1 v/v), hexane:ethyl aceate (4:1 v/v), hexane:ethyl acetate (3:2 v/v) and ethyl acetate to give fractions FEA-FEJ. The results are shown in Table 23.

Fraction FE was the strongest lipase inhibitory activity. The fraction FE was further separated by silica gel column chromatography, using hexane, hexane:ethyl acetate (19:1 v/v), hexane:ethyl acetate (9:1 v/v), hexane:ethyl aceate (4:1 v/v), hexane:ethyl acetate (3:2 v/v) and ethyl acetate to give fractions FEA-FEJ. The results are shown in Table 24.

Fraction FEE was further separated by silica gel column chromatography, using dichloromethane, dichloromethane:ethyl acetate (1:1 v/v) and ethyl acetate to give three fractions (FEEA-FEEC). The results are shown in Table 25.

Fraction FEEA showed a major yellow spot on TLC plate when observe by dipped in 10% H_2SO_4 in aqueous ethanol and heat on hot plate. Fraction FEEA was separated by silica gel column chromatography using hexane, hexane:acetone (19:1 v/v) and hexane:acetone (1:1 v/v) to give four fractions (FEEAA-FEEAD). The results are shown in Table 26.

Table 22 Lipase inhibitory activity of fractions FA-FJ

Fraction	Weight (g)	% inhibition
FA	0.26	na
FB	0.99	49.29 ± 4.94
FC	0.36	54.56 ± 2.56
FD	1.09	66.74 ± 1.61
FE	3.19	77.43 ± 6.83
FF	2.84	23.61 ± 5.33
FG	2.67	6.11± 2.85
FH	1.36	54.20 ± 7.31
FI	3.49	68.81 ± 6.63
FJ	1.34	70.23 ± 8.46
Orlistat		92.37 ± 0.50*

*at a concentration of 1×10^{-5} mg/mL, na = no activity

Fraction FEEAC was light yellow solid. This fraction was not pure compound. Fraction FEEAC was further separated by silica gel column chromatography, using hexane, hexane:dichloromethane (4:1 v/v), dichloromethane and ethyl acetate to give nine fractions (FEEACA-FEEACI). The results are shown in Table 27.

Table 23 Combined of fractions of fraction FE

Fraction	Weight (g)
FEA	0.1210
FEB	0.0027
FEC	0.0055
FED	0.6922
FEE	0.8184
FEF	0.4437
FEG	0.3408
FEH	0.0802
FEI	0.0780
FEJ	0.1558

Table 24 Combined of fractions of fraction FEE

Fraction	Weight (g)
FEEA	0.3859
FEEB	0.3012
FEEC	0.0072

Fraction FEEACE was light yellow crystal but it was not pure compound. This fraction was separated by prep-TLC plate using 4:1 v/v of heptane:acetone as developing solvent to give light yellow crystal but still a mixture.

Table 25 Combined of fractions of fraction FEEA

Fraction	Weight (g)
FEEAA	0.0076
FEEAB	0.0416
FEEAC	0.3036
FEEAD	0.0195

Table 26 Combined of fractions of fraction FEEAC

Fraction	Weight (g)
FEEACA	0.0036
FEEACB	0.0007
FEEACC	0.0028
FEEACD	0.0034
FEEACE	0.0067
FEEACF	0.0094
FEEACG	0.0475
FEEACH	0.0541
FEEACI	0.0826

Fractions FEH and FEI were a mixture of light yellow crystal and green liquid and showed a major bright spot with same R_f value when observed under ultraviolet light at wavelength 356 nm. Fraction FEH-I was new fraction from combining fractions FEH and FEI, then was separated by silica gel column, using dichrolomethane, dichrolomethane:ethyl acetate (1:1 v/v) and ethyl acetate to give Compound **61** (0.0231 g, 0.0005%).

4.5 Identification of Compound 60

Compound **60** was obtained as colorless needles (0.0376 g, 0.0009%) from fraction BBCCDF of the hexane crude extract. The ^{1}H NMR spectrum was indicated three olefinic methine protons at **δ** 5.02 (dd, **δ** = 8.7, 8.4 Hz), 5.14 (dd, **δ** = 8.7, 8.4 Hz) and 5.35 (d, **δ** = 5.1 Hz); a carbinyl proton at **δ** 3.53 (m) which are characteristic resonances of sterol of two olefins and an alcohol. The ^{1}H NMR spectrum is shown in Figure 16.

Compound **60** was further analyzed by GC-MS. Its GC-MS spectrum consisted of four peaks as acquisition time at 12.358 min, 15.333 min, 16.398 min and 18.424 min (Figure 17). The GC-MS spectrum was identified by comparison of their mass spectra with those of the Wiley and Nist standard chart library. It was found that Compound **60** consisted of four compounds and identified as 17-(1,5-dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[***α***]phenanthren-3-ol (1.18%), campesterol (19.40%), stigmasterol (33.14%) and ***β***-sitosterol (46.29%) as showed as Table 27. Figure 10 showed structure of compound **60**. This mixture is common phytosterols widely distributed in the plant kingdom. Campesterol, stigmastrol and ***β***-sitosterol were reported to have various pharmacological activities including anti-microbial (59), anti-inflamatory (60) and anti-tumor and antioxidant (61). In addition, ***β***-sitosterol was reported to have lipase inhibitory activity with 79.1 **±** 11.3% at concentration of 1.0 mg/mL (62).

Table 27 Integration peak list of Compound **60**

Peak	Name	Acquisition time (min)	% Composition
1	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[**α**]phenanthren-3-ol	12.358	1.18
2	Campesterol	15.333	19.40
3	Stigmasterol	16.398	33.14
4	***β***-Sitosterol	18.424	46.29

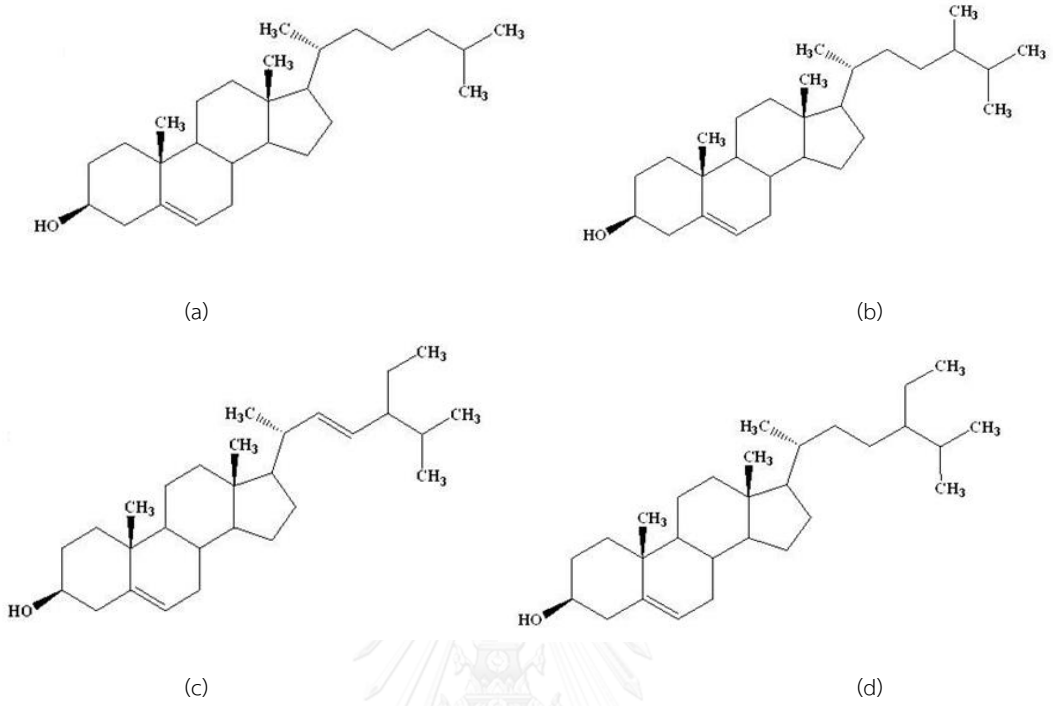


Figure 10 Structure of Compound **60;** a) 17-(1,5-dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[***α***]phenanthren-3-ol, b) campesterol, c) stigmasterol and d) ***β***-sitosterol

4.6 Identification of Compound 61

Compound **61** was obtained as light yellow crystal (0.0231 g, 0.0005%) from fraction FEH-I of the ethyl acetate crude extract. This compound gave bright spot when that observed under ultraviolet light at wavelength 356 nm.

The ^{1}H NMR spectrum was indicated hydroxyl and methoxyl protons at **δ** 6.15 (s) and 3.95 (s), respectively. The ^{1}H NMR spectrum showed doublet protons at **δ** 7.59 and 6.26 ppm and two aromatic protons at **δ** 6.92 and 6.85 ppm. ^{1}H-NMR spectrum is shown in Figure 18. The ^{13}C NMR spectrum indicated ten carbon atoms. The signal at **δ** 161.42 ppm was sp^2 carbon of carbonyl functional group of coumarin compound. The signal at **δ** 56.40 ppm was sp^3 carbon linked to oxygen atom and was probably a methoxy group (O-CH_3). ^{13}C NMR spectrum is shown in Figure 19. From DEPT 90 and DEPT 135 spectrums, the signal at **δ** 103.19, 107.47, 113.43 and

143.27 ppm were sp^2 carbons of =CH- group and the signal at **δ** 111.49, 143.98, 149.67 and 150.26 ppm were quaternary sp^2 carbons. The spectrum of DEPT 90 and DEPT 135 are shown in Figures 20 and 21. HMBC and HSQC experiments were clarified the position of the hydroxyl and methoxyl groups. The chemical shifts of all protonated carbons were assigned firmly based on the cross peaks found in HSQC and C—H long range correlation found in HMBC experiment. HMBC correlation of Compound **61** showed the correlation of hydroxyl proton with C-6, C-7 and C-8 and correlation of methoxyl protons with C-6. The HSQC and HMBC spectrums are shown in Figures 22 and 23. Therefore, Compound **61** was identified as 7-hydroxy-6-methoxycoumarin or scopoletin. The structure of Compound **61** is shown in Figure 11. HSQC and HMBC correlations of Compound **61** are shown in Figure 12.

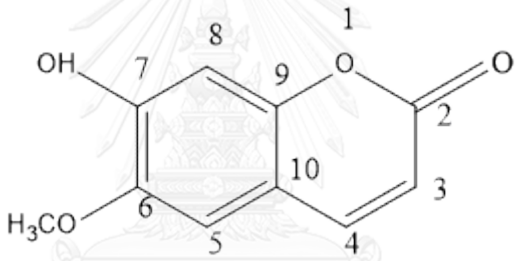


Figure 11 Structure of Compound **61** as 7-hydroxy-6-methoxycoumarin or scopoletin

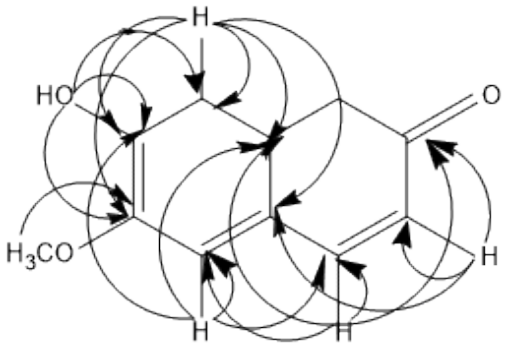


Figure 12 HSQC and HMBC correlations of Compound **61**

Table 28 ^{1}H NMR, ^{13}C NMR and HMBC correlations of Compound **61**

Position	δ_H mult. (J Hz)	δ_C	HMBC correlations
1	-	-	-
2	-	161.42	-
3	6.26 d (9.6 Hz)	113.43	C-2, C-10
4	7.59 d (9.6 Hz)	143.27	C-2, C-5, C-9
5	6.85 s	107.47	C-4, C-7, C-9
6	-	143.98	-
7	-	149.67	-
8	6.92 s	103.19	C-6, C-7, C-9, C-10
9	-	150.26	-
10	-	111.49	-
OH	6.15 s	-	C-6, C-7, C-8
OCH_3	3.95 s	56.40	C-6

7-Hydroxy-6-methoxycoumarin (Formula: $C_{10}H_8O_4$) belongs to the group of coumarins. This group also includes umbelliferone, esculetin and isoscopoletin. Scopoletin was previously isolated from several plants such as *Saussureaeo pygmae* (63)*, Morinda officinalis* (64) and *Coriaria nepalensis* (65). In family Solanaceae, scopoletin was previously isolated from *Solanum lyratum*as (66) and *Solanum ligustrinum* Lood (67).

4.7 Lipase inhibitory activity and IC_{50} of Compounds 60, 61 and orlistat

Compounds **60** and **61** exhibited weak inhibition activity with 60.67 ± 0.53% and 33.58 ± 1.36%, respectively at concentration of 4.00 mg/mL**.** Seven concentrations of Compounds **60** and **61** were prepared as 3.125, 6.25, 12.5, 25, 50, 80 and 100 mg/mL for lipase inhibitory activity assay. The lipase inhibitory activity at various concentrations of Compounds **60** and **61** are shown in Tables 29 and 30,

respectively. Comparing with orlistat, as positive control was prepared six concentrations as 0.01, 0.005, 0.0025, 0.00125, 0.000625 and 0.0003125 μg/mL, respectively. The results are shown in Table 31.

Table 29 Percentage inhibition of Compound **60**

Sample	Concentration (mg/mL)	% Inhibition
Compound **60**	100	52.11 ± 2.13
	80	49.71 ± 5.04
	50	42.55 ± 1.03
	25	28.13 ± 2.11
	12.5	12.72 ± 3.90
	6.25	6.01 ± 1.44
	3.125	2.82 ± 2.50

Table 30 Percentage inhibition of Compound **61**

Sample	Concentration (mg/mL)	% Inhibition
Compound **61**	100	54.05 ± 1.02
	80	44.40 ± 2.21
	50	33.58 ± 1.36
	25	28.14 ± 3.10
	12.5	24.05 ± 1.20
	6.25	20.85 ± 1.73
	3.125	15.35 ± 2.31

Table 31 Percentage inhibition of orlistat

Sample	Concentration (μg/mL)	% Inhibition
Orlistat	0.0003125	10.08 ± 1.54
	0.000625	13.42 ± 1.74
	0.00125	28.08 ± 1.52
	0.0025	53.43 ± 0.72
	0.005	74.82 ± 0.94
	0.01	93.80 ± 0.79

IC_{50} is the concentration of compound required to give 50% inhibition of lipase activity. From the results in Tables 29, 30 and 31, IC_{50} values were determined by graph plotting between concentration of sample (x-axis) and % inhibition (y-axis). To determined IC_{50} values, a perpendicular line was drawn from % inhibition at value of 50 at y-axis to x-axis as shown in Figures 13, 14 and 15. Compounds **60** and **61** exhibited weak inhibitory activity with IC_{50} values of 82.56 mg/mL and 91.98 mg/mL, respectively. Orlistat showed lipase inhibitory activity with IC_{50} value of 3.79×10^{-6} mg/mL.

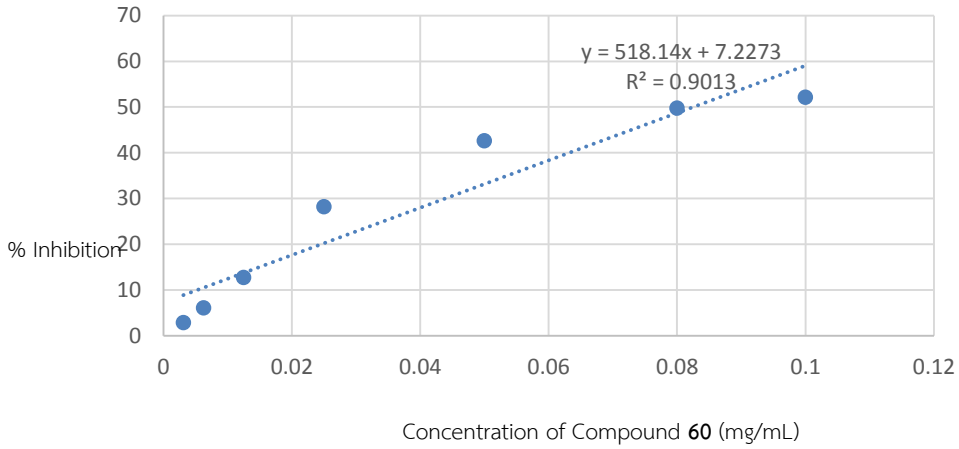


Figure 13 Effect of Compound **60** on pancreatic lipase

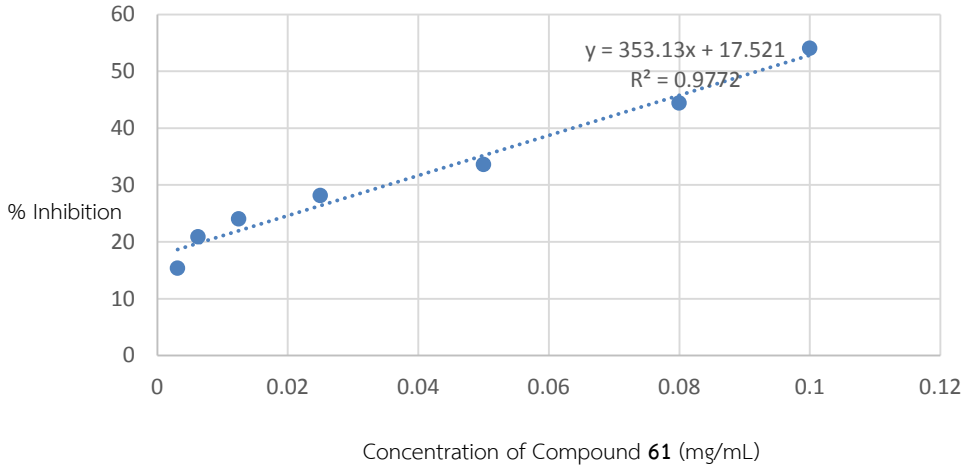


Figure 14 Effect of Compound **61** on pancreatic lipase

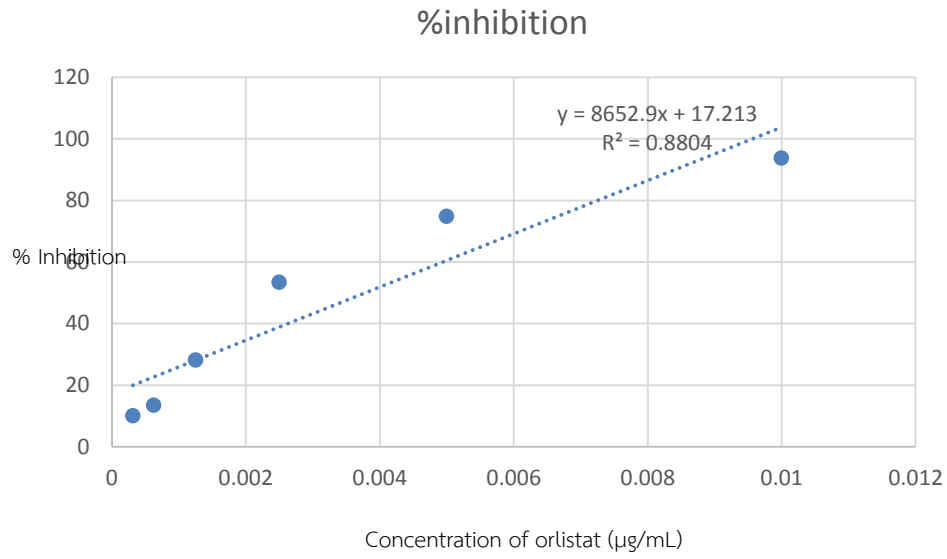


Figure 15 Effect of orlistat on pancreatic lipase

CHAPTER V
CONCLUSION

Ethanol and aqueous crude extracts of sixteen medicinal plants were screened for lipase inhibitory activity. Among ethanol crude extracts, fruit of *S. torvum* exhibited the strongest inhibition at 87.35 ± 9.90%, while the strongest of lipase inhibitory activity of aqueous extracts was leaf of *M. cordifolia* (92.88 ± 2.99%). In addition, the ethanol crude extracts of fruit of *P. nigrum*, rhizome of *C. longa*, stem of *C. citrates,* leaf of *M. cordifolia*, leaf of *S. alexandrina* and fruit of *G. atroviridis* exhibited strong inhibition at 76.22 ± 12.18%, 75.42 ± 1.29%, 71.76 ± 2.88%, 66.40 ± 8.36%, 62.75 ± 12.55% and 60.74 ± 12.06%, respectively. Including, aqueous of calyx of *H. sabdariffa,* rhizome of *A. galanga,* bulb of *A. sativum,* fruit of *Z. jujuba,* fruit of *G. atrovirids*, rhizome of *B. rotunda*, leaf of *S. alexandria* and rhizome of *Z. officinale* exhibited strong inhibition at 91.90 ± 1.32%, 88.38 ± 1.74%, 85.34 ± 10.74%, 77.73 ± 10.79%, 77.33 ± 7.56%, 71.78 ± 5.81%, 68.26 ± 8.16 and 67.69 ± 9.14, respectively. Ethanol crude extracts of fruit of *A. esculentus*, bulb of *A. sativum*, flower of *C. tinctorius* and aqueous crude extract of fruit of *A. esculentus* exhibited moderate inhibition at 56.75 ± 2.84%, 51.50 ± 1.51%, 42.87 ± 12.56% and 52.01 ± 10.98%, respectively. Ethanol crude extracts of rhizome of *A. galanga,* leaf of *R. nasutus*, rhizome of *Z. officinale* and fruit of *Z. jujuba* exhibited weak inhibition at 37.63 ± 3.86%, 31.03 ± 3.54%, 18.97 ± 2.37% and 14.83 ± 12.11%, respectively. Aqueous extracts of fruit of *P. nigrum* and flower of *C. tinctorius* exhibited weak inhibition at 24.90 ± 10.14% and 23.51 ± 3.58%, respectively. The ethanol extracts of rhizome of *B. rotunda*, calyx of *H. sabdariffa* and aqueous of *C. longa* were showed no lipase inhibitory activity. Therefore, the ethanol crude extract of *S. torvum* fruit was isolated to afford lipase inhibitors. The fruits of *S. torvum* were extracted by hexane, ethyl acetate, methanol and aqueous, respectively to give the crude extracts as light green wax, dark green wax, dark brown gum and dark brown solid, respectively. Then, they were further assayed for lipase inhibitory activity at concentration 50 mg/mL, to obtain 46.00 ± 6.71%, 67.23 ± 3.57%, 15.82 ± 1.13%,

and 8.51 ± 1.05% inhibition, respectively. Ethyl acetate crude extract showed the strongest lipase inhibitory activity. Hexane crude extract showed moderate lipase inhibitory activity. Whereas, methanol and aqueous crude extracts were exhibited weak activity. Therefore, hexane and ethyl acetate crude extracts were purified by silica gel column chromatography and prep-TLC technique to give Compound **60**, as white crystal and Compound **61**, as light yellow crystal. Compound **60** was separated from fraction BBCCDF of hexane crude extract. The Compound **60** was assigned as mixture of 17-(1,5-dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[***α***]phenanthren-3-ol, campesterol, stigmasterol and ***β***-sitosterol (0.0376 g, 0.0009%). Compound **61** was separated from fraction FEH-I of ethyl acetate crude extract and was assigned as 7-hydroxy-6-methoxycoumarin or scopoletin (0.0231 g, 0.0005%). Compounds **60** and **61** showed weak inhibitory activity with 60.67 ± 0.53% and 33.58 ± 1.36%, respectively and IC_{50} values of 82.56 mg/mL and 91.98 mg/mL, respectively, which were less than orlistat. Orlistat showed lipase inhibitory activity with IC_{50} value of 3.79×10^{-6} mg/mL. Compounds **60** and **61** are not suitable for development to be anti-obesity drug. But the ethanol crude extract of *S. torvum* fruit showed strong lipase inhibitory activity, so unpurify fractions of this crude extract may contain strong lipase inhibitors.

APPENDIX

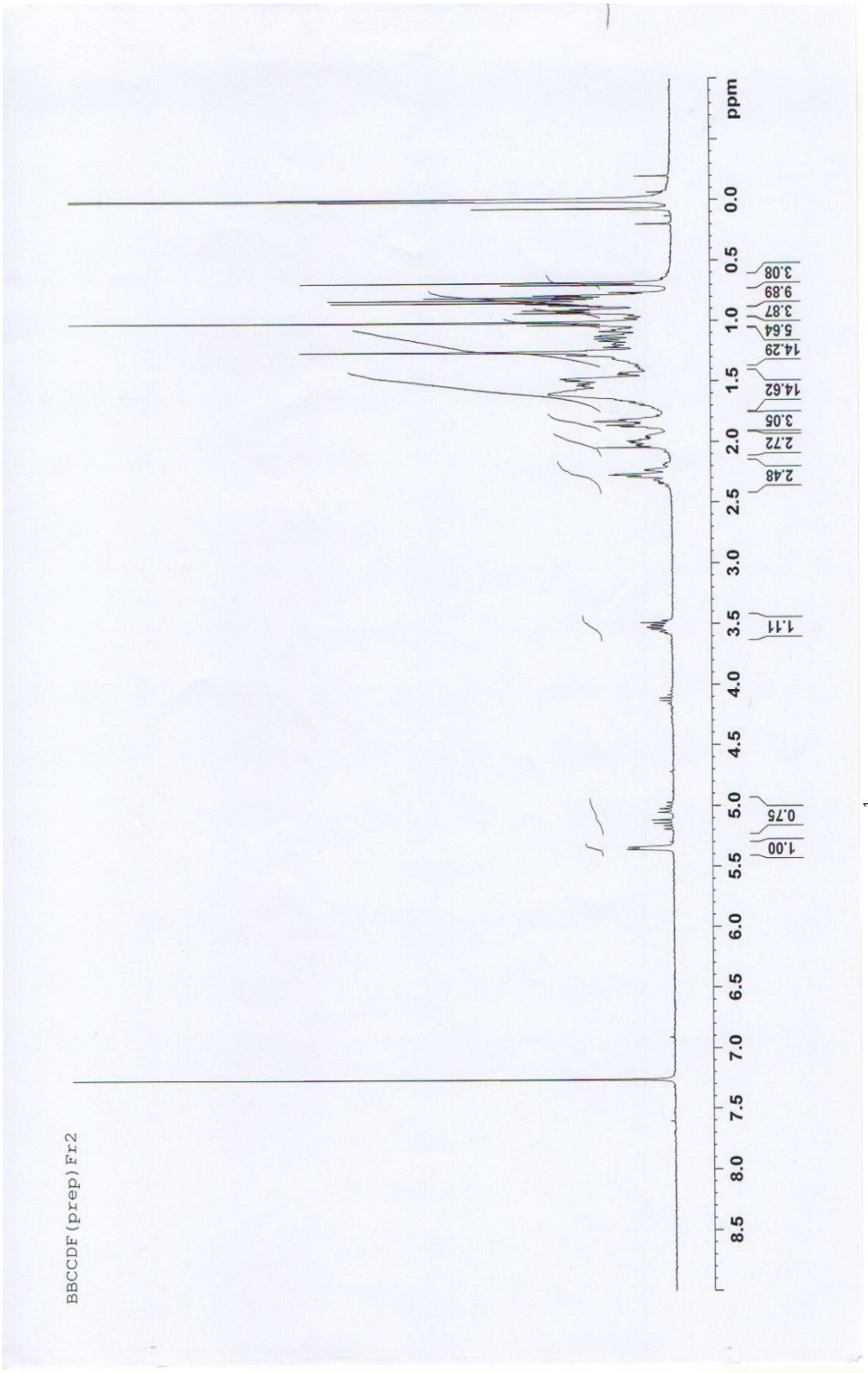


Figure 16 ^{1}H NMR spectrum of Compound **60**

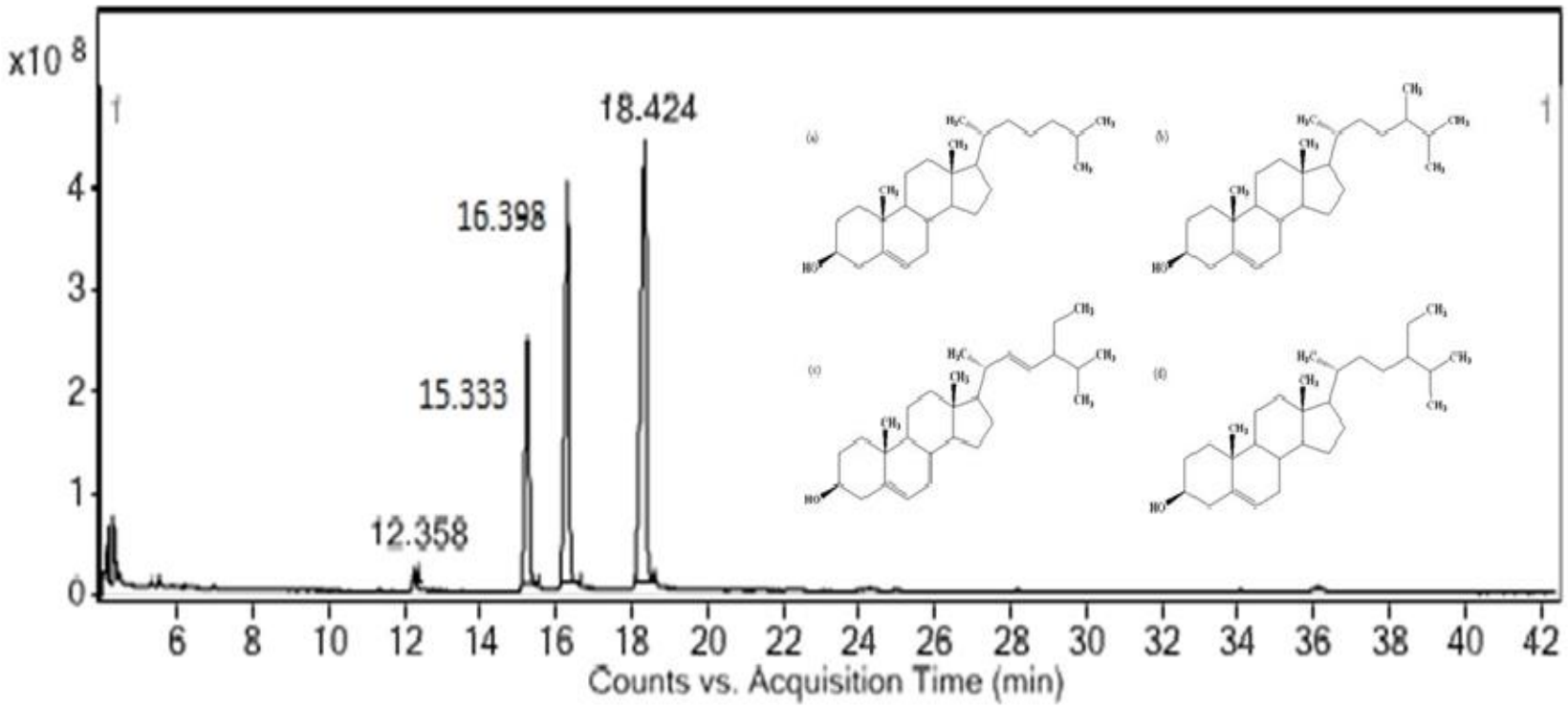


Figure 17 GC-MS spectrum of Compound **60**

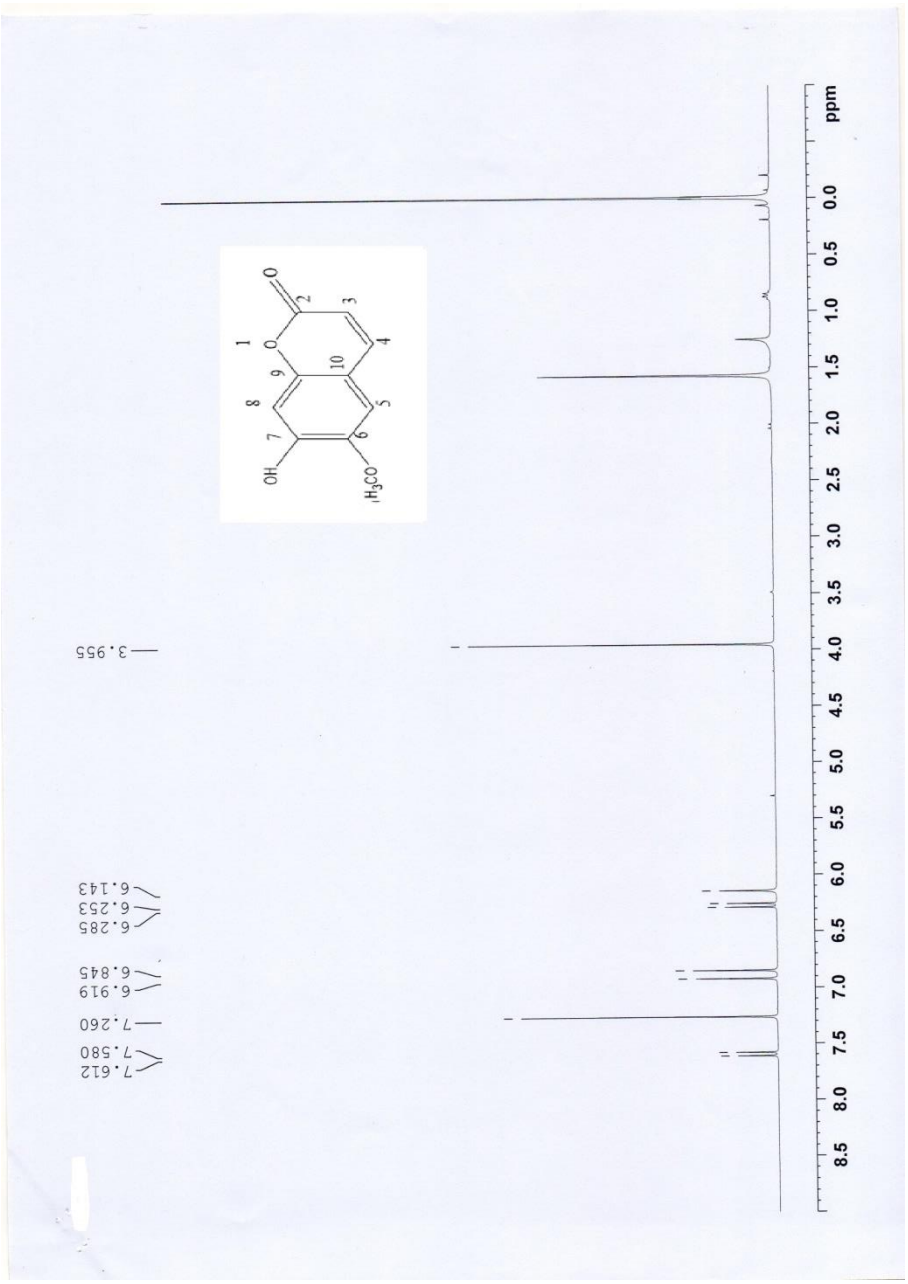


Figure 18 ^{1}H NMR spectrum of Compound **61**

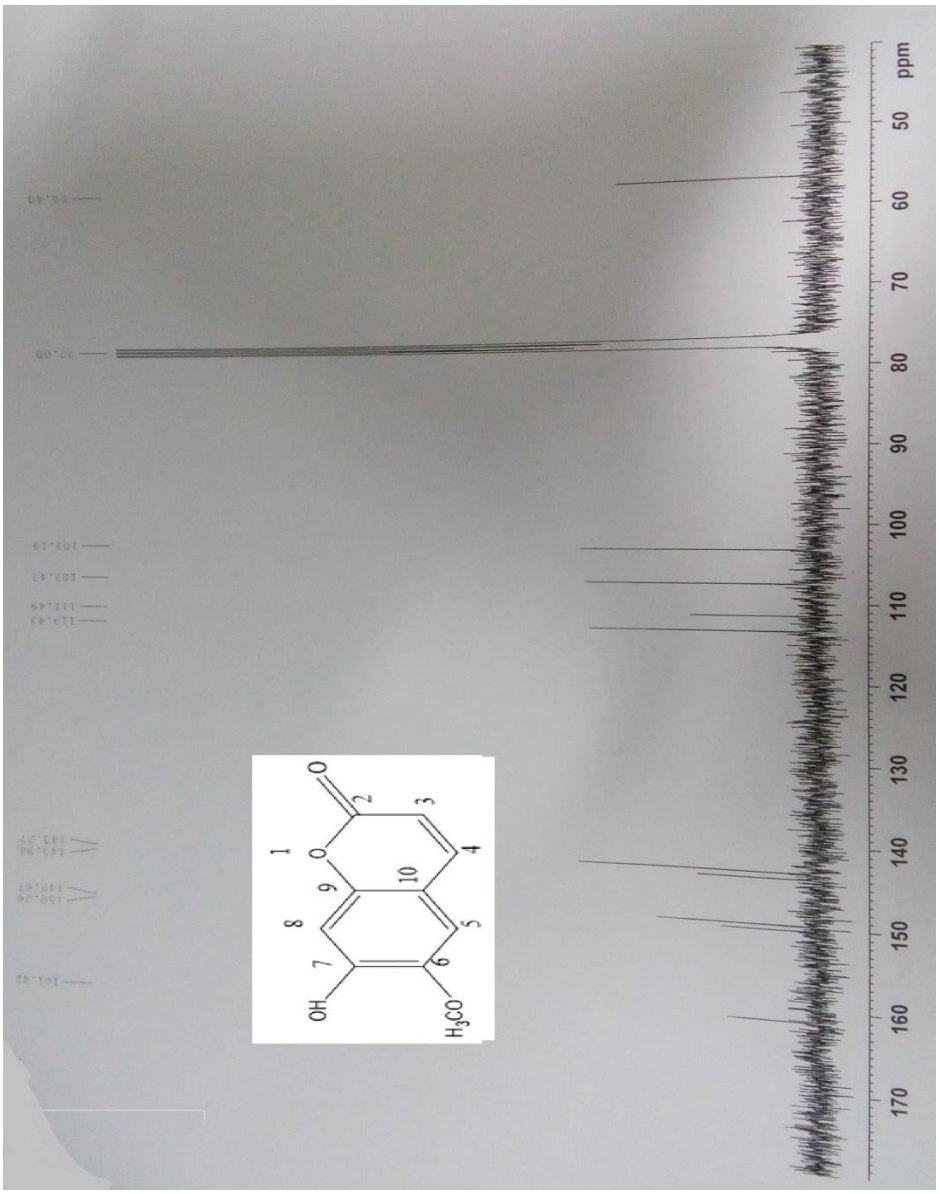


Figure 19 ^{13}C NMR spectrum of Compound **61**

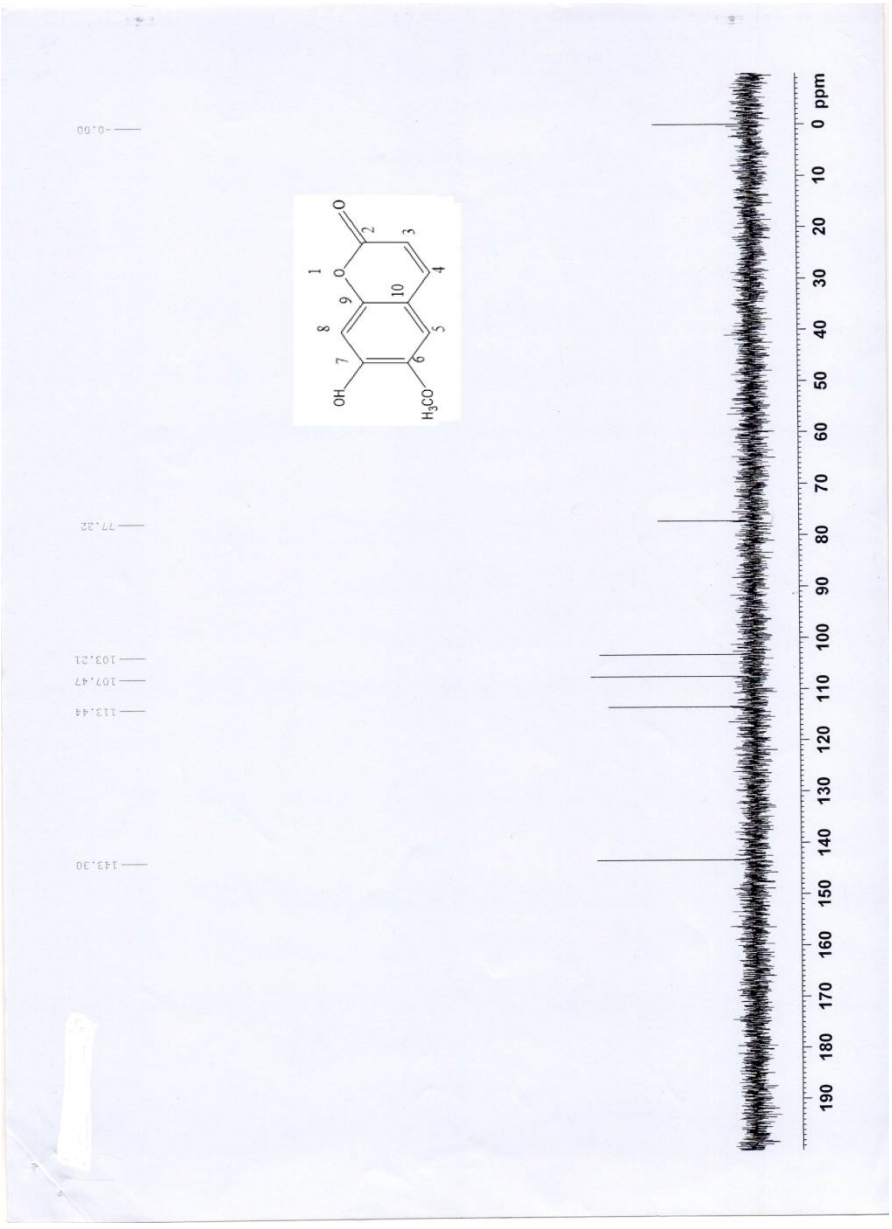


Figure 20 DEPT 90 spectrum of Compound **61**

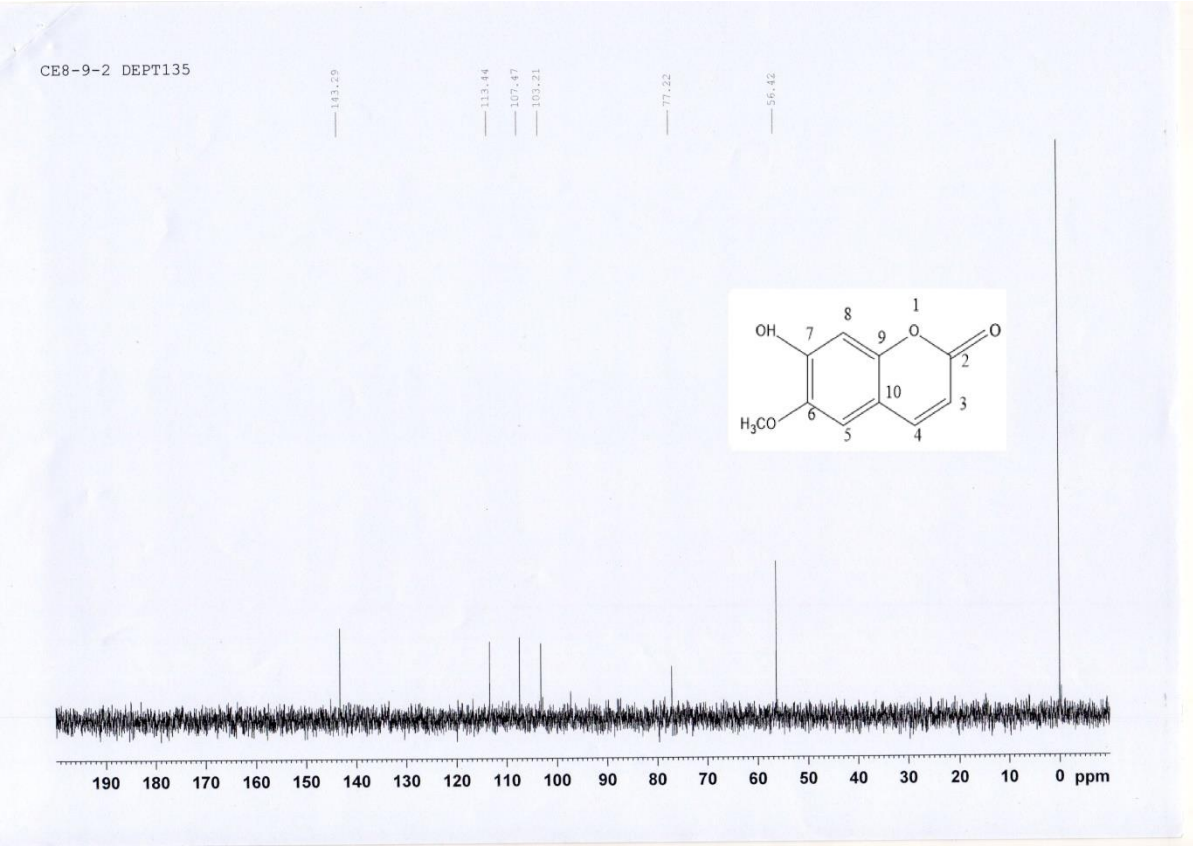


Figure 21 DEPT 135 spectrum of Compound **61**

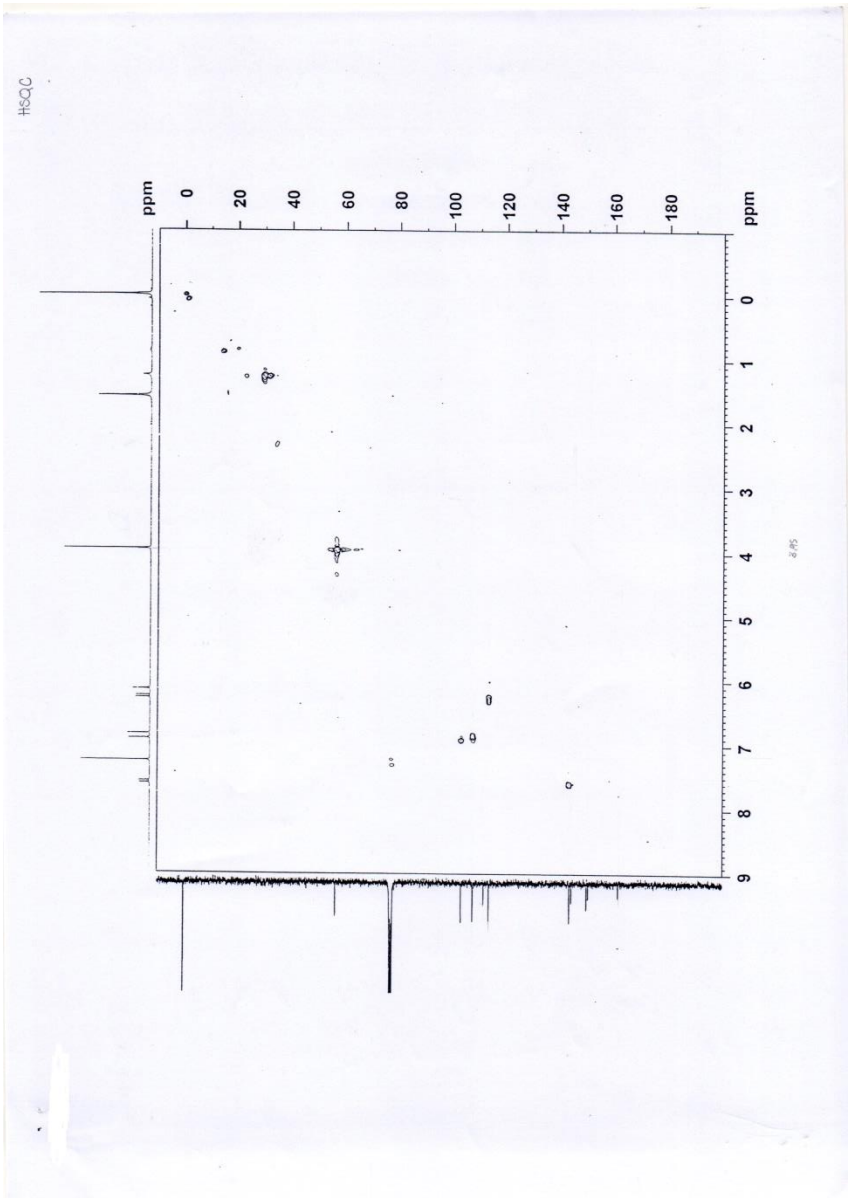


Figure 22 HSQC spectrum of Compound **61**

Figure 23 HMBC spectrum of Compound **61**

REFERENCES

1. Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS, et al. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. Journal of The American Medicinal Association. 2003;289(1):76-79.

2. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999-2008. Journal of The American Medicinal Association. 2010;303(3):235-241.

3. Jitnarin N, Kosulwat V, Rojroongwasinkul N, Boonpraderm A, Haddock C, Poston W. Prevalence of overweight and obesity in Thai population: results of the National Thai Food Consumption Survey. Eating and Weight Disorders. 2011;16(4):e242-e249.

4. Jebb S. Obesity: causes and consequences. Women's Health Medicine. 2004;1(1):38-41.

5. Hatano T, Yamashita A, Hashimoto T, Ito H, Kubo N, Yoshiyama M, et al. Flavan dimers with lipase inhibitory activity from Cassia nomame. Phytochemistry. 1997;46(5):893-900.

6. Nakai M, Fukui Y, Asami S, Toyoda-Ono Y, Iwashita T, Shibata H, et al. Inhibitory effects of oolong tea polyphenols on pancreatic lipase in vitro. Journal of Agricultural and Food Chemistry. 2005;53(11):4593-4598.

7. Kato E, Yama M, Nakagomi R, Shibata T, Hosokawa K, Kawabata J. Substrate-like water soluble lipase inhibitors from Filipendula kamtschatica. Bioorganic & medicinal chemistry letters. 2012;22(20):6410-6412.

8. Handa M, Murata T, Kobayashi K, Selenge E, Miyase T, Batkhuu J, et al. Lipase inhibitory and LDL anti-oxidative triterpenes from Abies sibirica. Phytochemistry. 2013;86:168-175.

9. Siemonsma J, Piluek K. Vegetables: Pudoc; 1993.

10. Mohan M, Kamble S, Gadhi P, Kasture S. Protective effect of Solanum torvum on doxorubicin-induced nephrotoxicity in rats. Food and chemical toxicology. 2010;48(1):436-440.

11. Ajaiyeoba E. Comparative phytochemical and antimicrobial studies of Solanum macrocarpum and S. torvum leaves. Fitoterapia. 1999;70(2):184-186.

12. Chah K, Muko K, Oboegbulem S. Antimicrobial activity of methanolic extract of Solanum torvum fruit. Fitoterapia. 2000;71(2):187-189.

13. Arthan D, Svasti J, Kittakoop P, Pittayakhachonwut D, Tanticharoen M, Thebtaranonth Y. Antiviral isoflavonoid sulfate and steroidal glycosides from the fruits of Solanum torvum. Phytochemistry. 2002;59(4):459-463.

14. Gandhi GR, Ignacimuthu S, Paulraj MG, Sasikumar P. Antihyperglycemic activity and antidiabetic effect of methyl caffeate isolated from Solanum torvum Swartz. fruit in streptozotocin induced diabetic rats. European journal of pharmacology. 2011;670(2):623-631.

15. Jaiswal B, Mohan M. Effect of Solanum torvum on the contractile response of isolated tissues preparation in fructose fed rat. International Journal Pharmaceutical Biology Science. 2012;3(3):161-169.

16. Government of South Australia DoE, Water and Natural Resources. *Solanum torvum* 2007 [updated 20101123].

17. Mayes PA, Botham KM. Metabolism of unsaturated fatty acids and eicosanoids. Harper's Biochemistry. 1996;23:232-240.

18. Mukherjee M. Human digestive and metabolic lipases—a brief review. Journal of Molecular Catalysis B: Enzymatic. 2003;22(5):369-376.

19. Phan C, Tso P. Intestinal lipid absorption and transport. Frontiers in bioscience: a journal and virtual library. 2001;6:D299-319.

20. Winkler F, d'Arcy A, Hunziker W. Structure of human pancreatic lipase. 1990.

21. Birari RB, Bhutani KK. Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug discovery today. 2007;12(19):879-889.

22. WHO EC. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004;363(9403):157.

23. Martin C, Han H, Anton S, Greenway F, Smith S. Effect of valproic acid on body weight, food intake, physical activity and hormones: results of a randomized controlled trial. Journal of Psychopharmacology. 2008.

24. Glazer G. Long-term pharmacotherapy of obesity 2000: a review of efficacy and safety. Archives of Internal Medicine. 2001;161(15):1814-1824.

25. Bray GA. Medical therapy for obesity—current status and future hopes. Medical Clinics of North America. 2007;91(6):1225-1253.

26. Kang JG, Park C-Y. Anti-obesity drugs: a review about their effects and safety. Diabetes & metabolism journal. 2012;36(1):13-25.

27. Keithley J, Swanson B. Glucomannan and obesity: a critical review. Alternative Therapies in Health and Medicine. 2005;11(6):30.

28. Shah BR, Li B, Wang L, Liu S, Li Y, Wei X, et al. Health benefits of konjac glucomannan with special focus on diabetes. Bioactive Carbohydrates and Dietary Fibre. 2015;5(2):179-187.

29. Bitou N, Ninomiya M, Tsujita T, Okuda H. Screening of lipase inhibitors from marine algae. Lipids. 1999;34(5):441-445.

30. Zhi J, Mulligan TE, Hauptman JB. Long-Term Systemic Exposure of Orlistat, a Lipase Inhibitor, and Its Metabolites in Obese Patients. The Journal of Clinical Pharmacology. 1999;39(1):41-46.

31. Kazlauskas RJ. Elucidating structure-mechanism relationships in lipases: prospects for predicting and engineering catalytic properties. Trends in biotechnology. 1994;12(11):464-472.

32. Hadvary P, Sidler W, Meister W, Vetter W, Wolfer H. The lipase inhibitor tetrahydrolipstatin binds covalently to the putative active site serine of pancreatic lipase. Journal of Biological chemistry. 1991;266(4):2021-2027.

33. Lucas CP, Boldrin MN, Reaven GM. Effect of orlistat added to diet (30% of calories from fat) on plasma lipids, glucose, and insulin in obese patients with hypercholesterolemia. The American journal of cardiology. 2003;91(8):961-964.

34. Jones FA. Herbs-useful plants. Their role in history and today. European journal of gastroenterology & hepatology. 1996;8(12):1227-1231.

35. Yoshikawa M, Shimoda H, Nishida N, Takada M, Matsuda H. Salacia reticulata and its polyphenolic constituents with lipase inhibitory and lipolytic activities have mild antiobesity effects in rats. The Journal of nutrition. 2002;132(7):1819-1824.

36. Yamamoto M, Shimura S, Itoh Y, Ohsaka T, Egawa M, Inoue S. Anti-obesity effects of lipase inhibitor CT-II, an extract from edible herbs, Nomame Herba, on rats fed a high-fat diet. International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity. 2000;24(6):758-764.

37. Shin J-E, Han MJ, Kim D-H. 3-Methylethergalangin isolated from Alpinia officinarum inhibits pancreatic lipase. Biological and Pharmaceutical Bulletin. 2003;26(6):854-857.

38. Kawaguchi K, Mizuno T, Aida K, Uchino K. Hesperidin as an inhibitor of lipases from porcine pancreas and Pseudomonas. Bioscience, biotechnology, and biochemistry. 1997;61(1):102-104.

39. Worsztynowicz P, Napierała M, Białas W, Grajek W, Olkowicz M. Pancreatic α-amylase and lipase inhibitory activity of polyphenolic compounds present in the extract of black chokeberry (Aronia melanocarpa L.). Process Biochemistry. 2014;49(9):1457-1463.

40. Moreno DA, Ilic N, Poulev A, Brasaemle DL, Fried SK, Raskin I. Inhibitory effects of grape seed extract on lipases. Nutrition. 2003;19(10):876-879.

41. Moreno DA, Ripoll C, Ilic N, Poulev A, Aubin C, Raskin I. Inhibition of lipid metabolic enzymes using Mangifera indica extracts. Journal of Food Agriculture and Environment. 2006;4(1):21.

42. Ono Y, Hattori E, Fukaya Y, Imai S, Ohizumi Y. Anti-obesity effect of Nelumbo nucifera leaves extract in mice and rats. Journal of Ethnopharmacology. 2006;106(2):238-244.

43. Moreno DA, Ilic N, Poulev A, Raskin I. Effects of Arachis hypogaea nutshell extract on lipid metabolic enzymes and obesity parameters. Life sciences. 2006;78(24):2797-2803.

44. Kim TH, Kim JK, Ito H, Jo C. Enhancement of pancreatic lipase inhibitory activity of curcumin by radiolytic transformation. Bioorganic & medicinal chemistry letters. 2011;21(5):1512-1514.

45. Han L-K, Xu B-J, Kimura Y, Zheng Y-n, Okuda H. Platycodi radix affects lipid metabolism in mice with high fat diet–induced obesity. The Journal of nutrition. 2000;130(11):2760-2764.

46. Zheng Q, Koike K, Han L-K, Okuda H, Nikaido T. New Biologically Active Triterpenoid Saponins from Scabiosa t schiliensis. Journal of natural products. 2004;67(4):604-613.

47. Yoshizumi K, Hirano K, Ando H, Hirai Y, Ida Y, Tsuji T, et al. Lupane-type saponins from leaves of Acanthopanax sessiliflorus and their inhibitory activity on pancreatic lipase. Journal of Agricultural and Food Chemistry. 2006;54(2):335-341.

48. Kurihara H, Asami S, Shibata H, Fukami H, Tanaka T. Hypolipemic effect of Cyclocarya paliurus (Batal) Iljinskaja in lipid-loaded mice. Biological and Pharmaceutical Bulletin. 2003;26(3):383-385.

49. Han L-K, Zheng Y-N, Yoshikawa M, Okuda H, Kimura Y. Anti-obesity effects of chikusetsusaponins isolated from Panax japonicus rhizomes. BMC Complementary and Alternative Medicine. 2005;5(1):9.

50. Kwon C-S, Sohn HY, Kim SH, Kim JH, Son KH, Lee JS, et al. Anti-obesity effect of Dioscorea nipponica Makino with lipase-inhibitory activity in rodents. Bioscience, biotechnology, and biochemistry. 2003;67(7):1451-1456.

51. Kimura H, Ogawa S, Jisaka M, Kimura Y, Katsube T, Yokota K. Identification of novel saponins from edible seeds of Japanese horse chestnut (Aesculus turbinata Blume) after treatment with wooden ashes and their nutraceutical activity. Journal of pharmaceutical and biomedical analysis. 2006;41(5):1657-1665.

52. Ninomiya K, Matsuda H, Shimoda H, Nishida N, Kasajima N, Yoshino T, et al. Carnosic acid, a new class of lipid absorption inhibitor from sage. Bioorganic & medicinal chemistry letters. 2004;14(8):1943-1946.

53. Lee I-A, Lee JH, Baek N-I, Kim D-H. Antihyperlipidemic effect of crocin isolated from the fructus of Gardenia jasminoides and its metabolite crocetin. Biological and Pharmaceutical Bulletin. 2005;28(11):2106-2110.

54. Lu Y, Luo J, Huang X, Kong L. Four new steroidal glycosides from Solanum torvum and their cytotoxic activities. Steroids. 2009;74(1):95-101.

55. Li J, Zhang L, Huang C, Guo F, Li Y. Five new cyotoxic steroidal glycosides from the fruits of Solanum torvum. Fitoterapia. 2014;93:209-215.

56. Lee C-L, Hwang T-L, He W-J, Tsai Y-H, Yen C-T, Yen H-F, et al. Anti-neutrophilic inflammatory steroidal glycosides from Solanum torvum. Phytochemistry. 2013;95:315-321.

57. Sheng Y, Chen X-B. Isolation and identification of an isomer of β-sitosterol by HPLC and GC-MS. Health. 2009;1(03):203.

58. Slanc P, Doljak B, Mlinarič A, Štrukelj B. Screening of wood damaging fungi and macrofungi for inhibitors of pancreatic lipase. Phytotherapy Research. 2004;18(9):758-762.

59. Peres MT, Monache FD, Cruz AB, Pizzolatti MG, Yunes RA. Chemical composition and antimicrobial activity of Croton urucurana Baillon (Euphorbiaceae). Journal of Ethnopharmacology. 1997;56(3):223-226.

60. Garcia M, Saenz M, Gomez M, Fernandez M. Topical antiinflammatory activity of phytosterols isolated from Eryngium foetidum on chronic and acute inflammation models. Phytotherapy Research. 1999;13(1):78-80.

61. Lim YM. Anti-Tumour Promoting Activity of Selected Malaysian Vegetables and Fruits, and Identification of Anti-Tumour Promoting and Antioxidant Compounds from Coleus Tuberosus, Benth (Ubi Kemili): Universiti Putra Malaysia; 2002.

62. Watanabe D, Kerakawati R, Morita T, Nakamura T, Ueno K, Kumamoto T, et al. Isolation of β-sitosterol and digalactopyranosyl-diacylglyceride from Citrus hystrix, a Thai traditional herb, as pancreatic lipase inhibitors. Heterocycles. 2009;78(6):1497-1505.

63. ZHANG B-B, Yuan D, Zhi-Xin L. Chemical constituents of Saussurea eopygmaea. Chinese Journal of Natural Medicines. 2011;9(1):33-37.

64. Zhang H-l, Zhang Q-w, Zhang X-g, Ye W, Wang Y. Chemical constituents from the root of Morinda officinalis. Chinese Journal of Natural Medicines. 2010;8:192-195.

65. Shen Y-H, Li S-H, Han Q-B, Li R-T, Sun H-D. Coumarins from Coriaria nepalensis. Journal of Asian natural products research. 2006;8(4):345-350.

66. Kang SY, Sung SH, Park JH, Kim YC. Hepatoprotective activity of scopoletin, a constituent ofSolanum Lyratum. Archives of pharmacal research. 1998;21(6):718-722.

67. Delporte C, Backhouse N, Negrete R, Salinas P, Rivas P, Cassels B, et al. Antipyretic, hypothermic and antiinflammatory activities and metabolites from Solanum ligustrinum Lood. Phytotherapy Research. 1998;12(2):118-122.

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- July 10-12, 2015: Poster presentation of The Burapha University International Conference 2015, at Bangsaen Heritage Hotel, Bangsaen, Chonburi, Thailand.