Glycation and α-glucosidase inhibitors from *Boesenbergia rotunda* and *Lansium parasiticum*

Mr. Thammatee Potipiranun

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Biotechnology
Common Course
Faculty of Science
Chulalongkorn University
Academic Year 2018

ตัวยับยั้งกลัยเคชันและเอนไซม์แอลฟากลูโคซิเดสจากกระชาย (*Boesenbergia rotunda*) และลองกอง (*Lansium parasiticum*)

นายธรรมธีร์ โพธิพีรนันท์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีชีวภาพ ไม่สังกัดภาควิชา/เทียบเท่า
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2561

Thesis Title	Glycation and α-glucosidase inhibitors from *Boesenbergia rotunda* and *Lansium parasiticum*
By	Mr. Thammatee Potipiranun
Field of Study	Biotechnology
Thesis Advisor	Associate Professor PREECHA PHUWAPRAISIRISAN, Ph.D.
Thesis Co Advisor	Associate Professor SIRICHAI ADISAKWATTANA, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirement for the Doctor of Philosophy

..... Dean of the Faculty of Science

(Associate Professor POLKIT SANGVANICH, Ph.D.)

DISSERTATION COMMITTEE

..... Chairman

(Associate Professor SEHANAT PRASONGSUK, Ph.D.)

..... Thesis Advisor

(Associate Professor PREECHA PHUWAPRAISIRISAN, Ph.D.)

..... Thesis Co-Advisor

(Associate Professor SIRICHAI ADISAKWATTANA, Ph.D.)

..... Examiner

(Associate Professor NATTAYA NGAMROJANAVANICH, Ph.D.)

..... Examiner

(Associate Professor KHANITHA PUDHOM, Ph.D.)

..... External Examiner

(Assistant Professor Wimonpan Rungprom, Ph.D.)

ธรรมธีร์ โพธิพีรนันท์ : ตัวยับยั้งกลัยเคชันและเอนไซม์แอลฟากลูโคซิเดสจากกระชาย (*Boesenbergia rotunda*) และลองกอง (*Lansium parasiticum*). (Glycation and α-glucosidase inhibitors from *Boesenbergia rotunda* and *Lansium parasiticum*) อ.ที่ปรึกษาหลัก : รศ. ดร.ปรีชา ภูวไพรศิริศาล, อ.ที่ปรึกษาร่วม : รศ. ดร.สิริชัย อดิศักดิ์วัฒนา

กลัยเคชันเป็นปฏิกิริยาที่ไม่ใช้เอนไซม์เป็นตัวเร่ง โดยเกิดจากการทำปฏิกิริยาระหว่างโปรตีนและสารตั้งต้นของปฏิกิริยากลัยเคชัน เช่น เมทิลกลัยออกซอล เกิดเป็นผลิตภัณฑ์ที่เป็นพิษต่อเซลล์ เรียกว่า advanced glycation end-products (AGEs) ซึ่งผลิตภัณฑ์ AGEs กระตุ้นให้เกิดโรคแทรกซ้อนรุนแรงหลายชนิดได้ ดังนั้นการยับยั้งการเกิดผลิตภัณฑ์ AGEs จะเป็นวิธีที่สำคัญในการบรรเทาโรคแทรกซ้อนที่เกิดจากโรคเบาหวานได้ นอกจากนี้วิธีการรักษาโรคเบาหวานที่สำคัญอีกวิธีหนึ่งคือ การยับยั้งเอนไซม์แอลฟากลูโคซิเดส เพื่อลดระดับน้ำตาลในกระแสเลือด จากที่กล่าวมาข้างต้นทำให้ผู้วิจัยสนใจที่จะศึกษาฤทธิ์ในการยับยั้งการเกิดปฏิกิริยากลัยเคชันและเอนไซม์แอลฟากลูโคซิเดส ของสารผลิตภัณฑ์ธรรมชาติ อีกทั้งยังศึกษากลไกการยับยั้งของสารดังกล่าว จากการศึกษาเบื้องต้นของสารกลุ่มฟีนอลิก พบว่าสารกลุ่ม flavanones ที่แยกได้จากกระชายสามารถยับยั้งการเกิดปฏิกิริยากลัยเคชันได้ ดังนั้นผู้วิจัยจึงเลือกกระชายมาทำการสกัดแยกสารบริสุทธิ์จากสารสกัดหยาบไดคลอโรมีเทน ได้สารกลุ่ม flavanones 3 ชนิด (pinocembrin pinostrobin และ alpinetin) สารกลุ่ม chalcones 2 ชนิด (cardamomin และ boesenbergin B) สารกลุ่ม dihydrochalcones 2 ชนิด (panduratin A และ isopanduratin A) และสารกลุ่ม kavalactone 1 ชนิด (demethoxyyangonin) จากผลการทดสอบฤทธิ์ยับยั้งการเกิดผลิตภัณฑ์ AGEs พบว่าสารบริสุทธิ์ที่แยกได้ส่วนใหญ่ให้ผลดีกว่า aminoguanidine (AG) สำหรับการศึกษาการดักจับสารเมทิลกลัยออกซอล พบว่าสารที่แยกได้มีความสามารถในการดักจับเทียบเคียงกับ AG ในการศึกษาความสัมพันธ์ระหว่างโครงสร้างสารกับการออกฤทธิ์ดักจับสารเมทิลกลัยออกซอล ได้จากการเปรียบเทียบโครงสร้างของสารในกลุ่ม flavonoids นอกจากนี้งานวิจัยนี้เป็นงานวิจัยแรกในการศึกษาการยับยั้งเอนไซม์แอลฟากลูโคซิเดสของสาร pinocembrin

ลองกองเป็นพืชอีกชนิดหนึ่งที่ผู้วิจัยนำมาศึกษา เนื่องจากพบสัญญาณที่ดีในการยับยั้งเอนไซม์แอลฟากลูโคซิเดสจากสารสกัดหยาบจากเปลือกผลลองกอง (IC_{50} 2.5-4.3 mg/mL) นอกจากนั้นลองกองยังเป็นผลไม้ที่พบมากและเป็นที่นิยมแพร่หลายในเอเชียตะวันออกเฉียงใต้ อีกทั้งยังเป็นแหล่งที่สำคัญของสารกลุ่ม triterpenoids โดยเฉพาะอย่างยิ่งโครงสร้างที่จำเพาะและหายากอย่าง onoceranoids เปลือกลองกองได้ถูกนำมาสกัดแยกและทำให้บริสุทธิ์ พบสารชนิดใหม่ 1 ชนิด คือ lamesticumin G และสารที่มีการรายงานแล้ว 4 ชนิด (lansionic acid, 3β -hydroxyonocera-8(26),14-dien-21-one, methyl lansiolate และ lansiolic acid) ซึ่งสารชนิดใหม่ lamesticumin G สามารถยับยั้งเอนไซม์แอลฟากลูโคซิเดสด้วยค่า IC_{50} 2.27 mM ขณะที่สารที่แยกได้ชนิดอื่นไม่สามารถยับยั้งเอนไซม์ได้

สาขาวิชา	เทคโนโลยีชีวภาพ	ลายมือชื่อนิสิต .....
ปีการศึกษา	2561	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5572875523 : MAJOR BIOTECHNOLOGY

KEYWORD: DIABETES, ADVANCED GLYCATION END-PRODUCTS, METHYLGLYOXAL, STRUCTURE-ACTIVITY RELATIONSHIPS, BOESENBERGIA ROTUNDA, LANSIUM PARASITICUM, α-GLUCOSIDASE

Thammatee Potipiranun : Glycation and α-glucosidase inhibitors from *Boesenbergia rotunda* and *Lansium parasiticum*. Advisor: Assoc. Prof. PREECHA PHUWAPRAISIRISAN, Ph.D. Co-advisor: Assoc. Prof. SIRICHAI ADISAKWATTANA, Ph.D.

Glycation is spontaneous non-enzymatic reaction between protein and glycation precursors; methylglyoxal (MG) that initially yields advanced glycation end-products (AGEs), which ultimately trigger to several severe complications. Therefore, the inhibition of AGEs formation is the imperative approach for alleviating diabetic complications. Moreover, one of typical diabetic therapy is α-glucosidase inhibition to reduce glucose level in bloodstream. Therefore, this research aims to study bioactivity and mechanism of natural products to inhibit glycation and α-glucosidase. From preliminary study of some phenolic compounds, flavanones from fingerroot (*Boesenbergia rotunda*) could inhibit glycation. The dichloromethane extract of fingerroot afforded three flavanones (pinocembrin, pinostrobin, and alpinetin), two chalcones (cardamomin and boesenbergin B), two dihydrochalcones (panduratin A and isopanduratin A), and one kavalactone (demethoxyyangonin). Most of isolated compounds showed higher AGEs formation inhibition than aminoguanidine (AG). Subsequent evaluation in MG-trapping assay indicated that their trapping potency was relatively comparable to AG. Their structure-activity relationships (SAR) of MG-trapping activity were investigated using the comparison of the structures of flavonoids. In addition, this is the first time that pinocembrin was tested α-glucosidase inhibitory activity. In this study, we found a promising α-glucosidase inhibition (IC_{50} 2.5-4.3 mg/mL) in the extract of *Lansium parasiticum* (syn *L. domesticum*; Meliaceae) fruit peels, which is a popular fruit in South-east Asia. It has been recognized as an important source of diverse triterpenoids, particularly a rare and unique skeleton of onoceranoids. A novel onoceranoid triterpene, named lamesticumin G along with four known compounds (lansionic acid, 3β-hydroxyonocera-8(26),14-dien-21-one, methyl lansiolate and lansiolic acid) were isolated from the ethyl acetate extract of the fruit peels of *Lansium parasiticum*. The structure of lamesticumin G was fully characterized using spectroscopic data. Lamesticumin G inhibited α-glucosidase (maltase) with IC_{50} value of 2.27 mM, while other compounds showed no inhibition.

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

Academic Year: 2018 Advisor's Signature .....

Co-advisor's Signature .....

ACKNOWLEDGEMENTS

I wish to express my deep gratitude to my advisor, Associate Professor Dr. Preecha Phuwapraisirisan and co-advisor, Associate Professor Dr. Sirichai Adisakwattana for their generous assistance, kind guidance and encouragement throughout the course of this research.

I would like to gratefully acknowledge the committees, Associate Professor Dr. Sehanat Prasongsuk, Associate Professor Dr. Nattaya Ngamrojanavanich, Associate Professor Dr. Khanitha Pudhom and Assistant Professor Dr. Wimonpan Rungprom for their comments, guidance and extending cooperation over my presentation.

I would like to express my gratitude to Program of Biotechnology and Center of Excellence in Natural Products, Faculty of Science, Chulalongkorn University for providing the chemicals and facilities throughout the course of study.

Thammatee Potipiranun

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

AG	aminoguanidine
AGEs	advanced glycation end-products
brs	broad singlet (NMR)
calcd	calculated
^{13}C NMR	carbon-13 nuclear magnetic resonance
$CDCl_3$	deuterated chloroform
CD_3OD	deuterated methanol
COSY	correlated spectroscopy
DM	diabetes mellitus
d	doublet (NMR)
dd	doublet of doublet (NMR)
2D NMR	two dimensional nuclear magnetic resonance
1D NMR	one dimensional nuclear magnetic resonance
ESIMS	electrospray ionization mass spectrometry
g	gram (s)
GO	glyoxal
^{1}H NMR	proton nuclear magnetic resonance
Hz	Hertz
HRESIMS	high resolution electrospray ionization mass spectrum
HIV	human immunodeficiency virus
IC_{50}	concentration that required for 50% inhibition in vitro
J	coupling constant
Kg	kilogram (s)

mg	milligram (s)
MG	methylglyoxal
mL	milliliter (s)
mmol	millimole (s)
m/z	mass per charge
m	multiplet (NMR)
M.W.	molecular weight
M	molar
s	singlet (NMR)
TLC	thin layer chromatography
U	unit
UV	ultraviolet
δ	chemical shift
δ_C	chemical shift of carbon
δ_H	chemical shift of proton
°C	degree Celsius
µL	microliter (s)
µM	micromolar (s)
$[\alpha]_D$	specific optical rotation

CHAPTER I

INTRODUCTION

1.1 Diabetes mellitus (DM)

Diabetes mellitus (DM) is a disease caused by a malfunction of carbohydrate metabolism, resulting in excessive blood glucose (hyperglycemia). The common symptoms of diabetes are increased appetite, thirst, and urination. This disease highly found in higher age people and the trend of patient decreased in younger people. DM was known as sweet urine. Elevated levels of blood glucose (hyperglycemia) lead to spillage of glucose into the urine. Nowadays DM is classified as non-communicable disease which has a large impact on health and has to be take care in the right way. It also plays an important role in the development of long-term diabetic complication, which is cardiovascular disease, cerebrovascular disease, diabetic neuropathy, diabetic nephropathy, diabetic ulcers, and diabetic retinopathy. The main cause of DM is a consequence of a relative or absolute deficiency of insulin secretion, resistance to insulin action, or both [1].

Insulin is a hormone produced by the pancreas. It is secreted directly into the bloodstream to regulate the blood sugar (glucose) levels in the body. After digestion, glucose is absorbed through the cell of intestine into bloodstream. When the blood glucose levels increased, the β-cell of pancreas will be stimulated to produce insulin. Thus, glucose is transported into the cells when insulin binds to insulin receptor on the cell membrane (Figure 1.1) [2]. DM is classified into two main categories: type 1 and type 2 diabetes, as illustrated in Figure 1.2 [3].

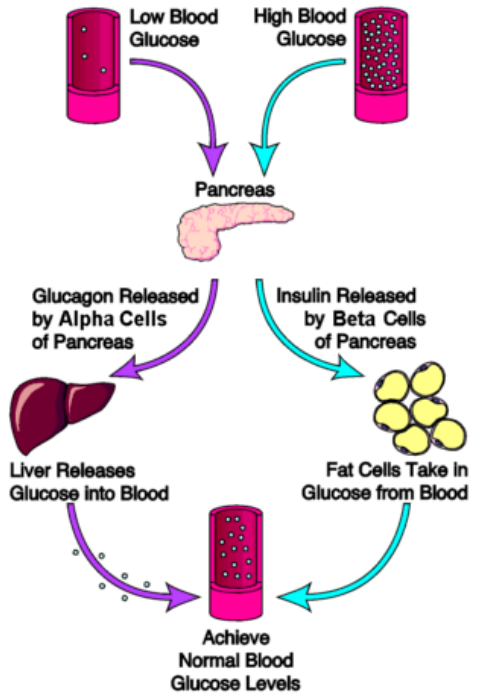

Figure 1.1 *Blood sugar regulation (www.endocrineweb.com).*

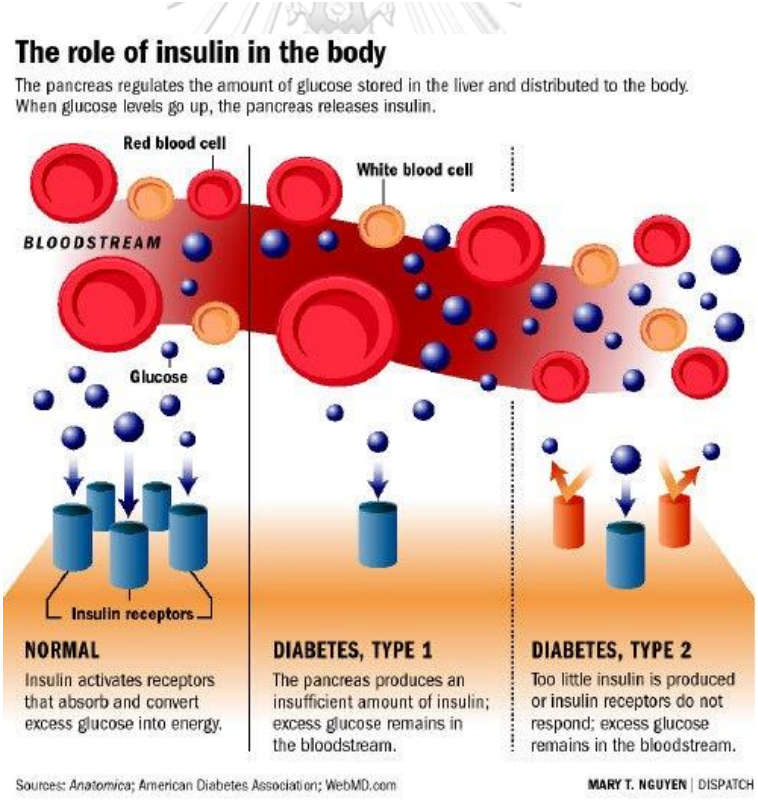

Figure 1.2 *Classification of diabetes (www.diabetes.org).*

Type 1 DM is known as insulin-dependent diabetes or juvenile-onset diabetes because this type is usually diagnosed in children and young adults [4-6]. It is

characterized by the loss of insulin-secreting capacity due to selective autoimmune destruction of the pancreatic β-cells, leading to a deficiency of insulin (Figure 1.2). Without the presence of insulin, many of the body's cells cannot take glucose from the blood and therefore the body uses other sources of energy. This type of DM comprises approximately 5% to 10% of all patients with DM. Patients with type 1 DM require insulin injection for therapy [7].

Type 2 DM, referred to as non-insulin-dependent diabetes, is characterized by a relative insulin deficiency and is associated with insulin resistance in various tissues (Figure 1.2). This type of DM comprises approximately 90% to 95% of all diabetes patients. It usually occurs in adult, in addition to young people. In all probability, the causes of type 2 DM lie in environmental and lifestyle factors. Prominence among these factors is obesity, and approximately 50% to 90% of all patients with type 2 DM are obese [8]. In 2010, there are approximately 285 million people worldwide with type 2 DM and the number of patients will increase about 54% during the next 20 years in 2030 (Figure 1.3). The highest number of type 2 DM patients is in Southeast Asia [9].

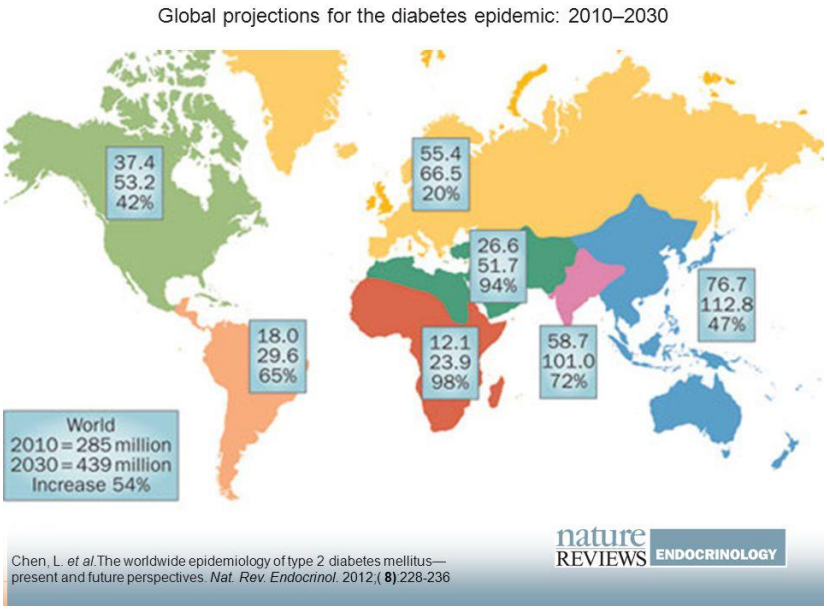

Figure 1.3 *The worldwide epidemiology of type 2 DM.*

There are several approaches for treatment of type 2 DM. Each approach works in different ways to lower blood glucose levels. In the early stages, many people with

type 2 DM can control their blood glucose levels by diet, exercise, and weight loss [10]. The next stage, various oral anti-diabetic agents were used to control hyperglycemia such as sulfonylureas (increase insulin secretion), biguannides (increase in glucose uptake) and **α**-glucosidase inhibitors (inhibit **α**-glucosidase enzyme) [11, 12]. Most importantly, an efficient therapeutic approach for treatment of type 2 DM is to delay the postprandial hyperglycemia by retarding the rate of carbohydrate digestion [10].

1.2 Glycation inhibitors

The excess glucose and other reducing sugars are high active compounds in glycation reaction. In addition, glucose that is in the blood stream for long time can be converted to many potential precursors of glycation such as glyoxal (GO), methylglyoxal (MG) and 3-deoxyglucosone (3-DG) which are more active than glucose and other reducing sugars because they are dicarbonyl compounds. Glycation precursors can be produced by many metabolic pathways such as autoxidative glycosylation and glycolysis. For example, glucose is broken down into triosephosphates (dihydroxyacetone-phosphate: DHAP and glyceraldehyde-3-phsophate: GA3P) then triosephosphates degrade to methylglyoxal (Figure 1.4) [13]. This process occurred in DM patients 2-6 times more than normal people. One of the terrible reaction, that is the reaction of dicarbonyl compounds or reducing sugars or biomolecule in body, is glycation which is spontaneous non-enzymatic reaction [14]

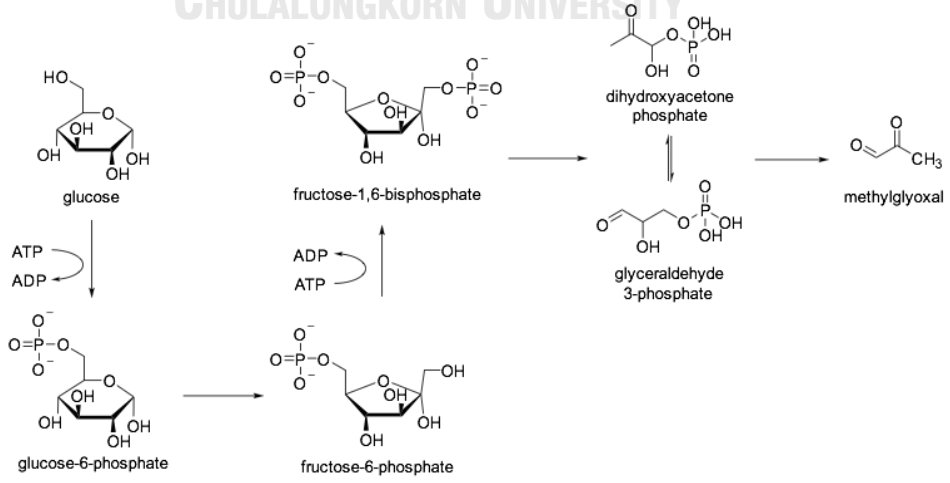

Figure 1.4 *Methylglyoxal generating via glycolysis pathway of glucose.*

Glycation or non-enzymatic glycosylation is the reaction between carbonyl group of glycation precursors or reducing sugars and amino group of amino acid or protein at arginine and histidine position [15] without catalytic enzyme. The products of Glycation are rearranged by dehydration and oxidation resulting in browning compounds or molecular crosslink, finally, they transform into irreversible huge complexes call advanced glycation end-products (AGEs). Unfortunately, AGEs are involved in the development of many health disorder such as cataract, blindness, blood vascular diseases (heart attack, hypertension and stroke), neuropathy (Alzheimer's disease), wrinkle and aging [15-17]. These symptoms are diabetic complication. Therefore, glycation inhibition is another diabetic complication alleviation.

Recently, there are two groups of glycation inhibitors which are amino compounds (Figure 1.5) and phenolic compounds (Figure 1.7). Most of amino compounds such as aminoguanidine, L-arginine, hydralazine, isoniazid and gentamicins are more toxic to diabetic patients in medicine development because they can react with carbonyl group of pyridoxal-phosphate (vitamin B6) (Figure 1.6). Hence, phenolic compounds are better candidate for glycation inhibitors [18]. There are many reports about phenolic compounds for glycation inhibitors such as pinostrobin, pinocembrin, alpinetin and panduratin A from fingerroot [19], gallic acid, *p*-coumaric acid, and epicatechin from nut grass [20], rutin from tomato [21], theaflavins from tea [22], genistein from soy bean [14], and vitein and isovitein from green bean [23] (Figure 1.7).

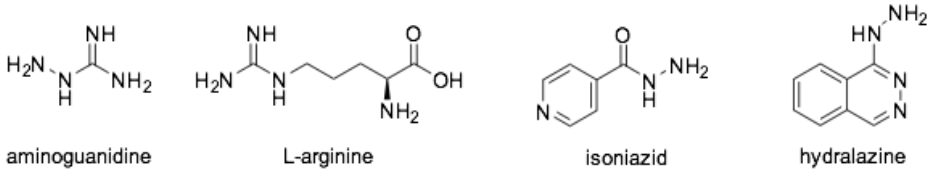

Figure 1.5 *Amino compounds as glycation inhibitors.*

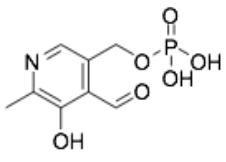

Figure 1.6 *Structure of pyridoxal-phosphate (vitamin B6).*

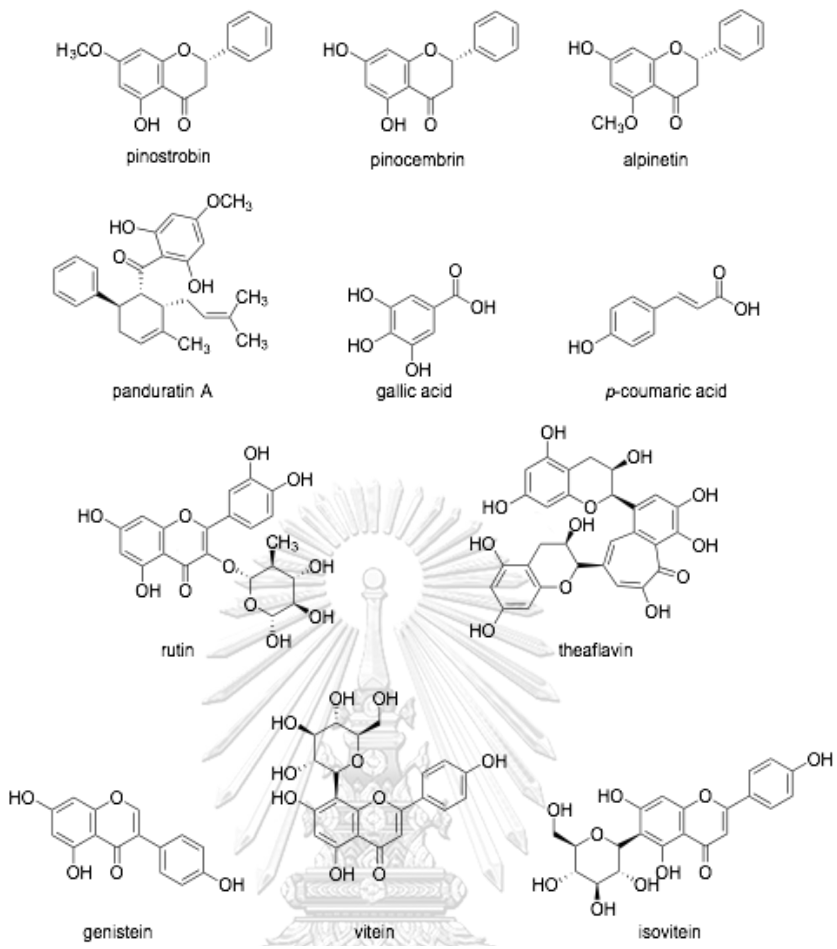

Figure 1.7 *Phenolic compounds as glycation inhibitors.*

Lv L. and co-workers (2011) studied trapping of methylglyoxal (MG) by genistein. They found that one molecule of genistein can trap two molecules of MG (Figure 1.8). It suggested that phenolic compounds have high potential for glycation inhibitors because there are more than one position at ring A of phenolic compounds that can trap MG [14]. Furthermore, there are some studies of structure-activity relationship (SAR) of glycation inhibitors. Noda Y. and Peterson D.G. (2007) study glycation inhibition of some simple phenolic compounds and epicatechin derivatives (Figure1.9) on glycation agents (methylglyoxal, glyoxal, diacetyl and 3-deoxyglucosone). They found that all flavonoids (epicatechin derivatives) can trap all glycation agents. Moreover, the meta position of hydroxy group have higher effect on glycation inhibition than the ortho position [20, 24].

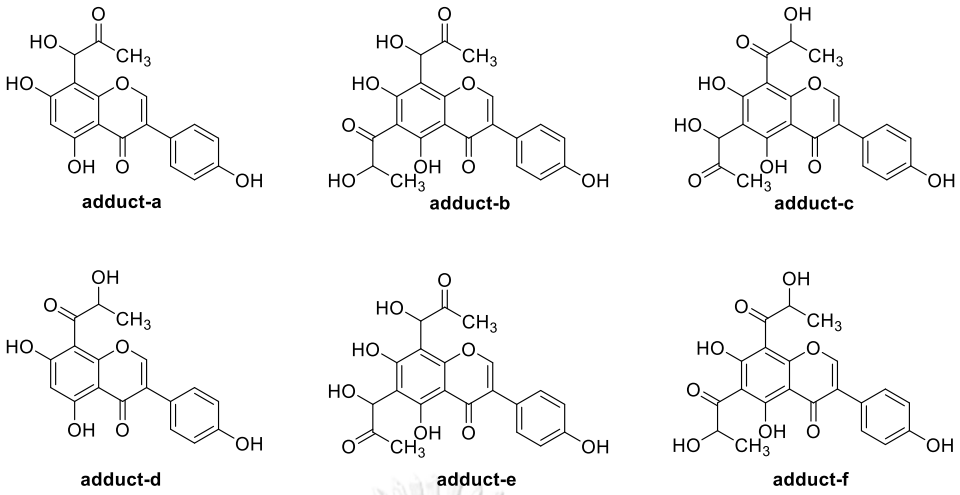

Figure 1.8 *Products (adducts) of MG and genistein glycation inhibitors.*

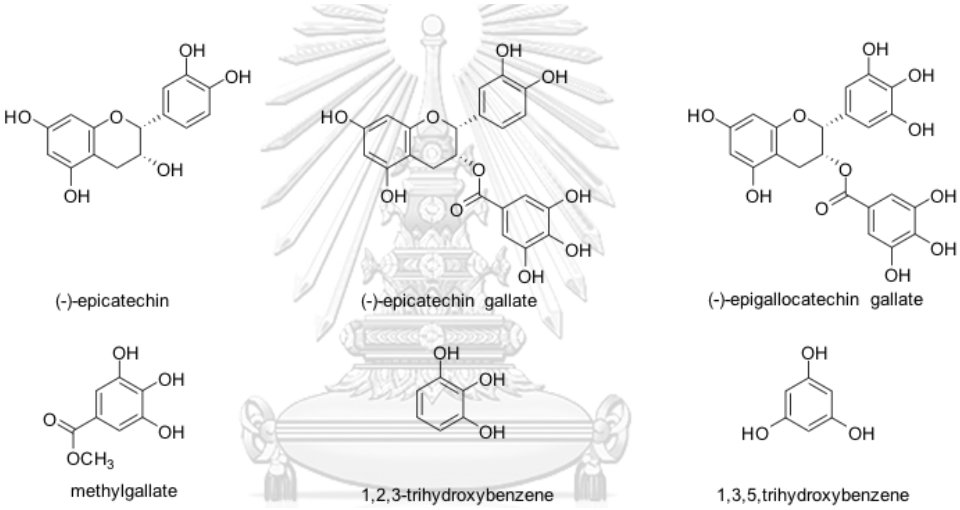

Figure 1.9 *Structures of simple phenolic compounds and epicatechin derivatives.*

1.3 α-Glucosidase inhibitors

α-Glucosidase is an enzyme (EC.3.2.1.20) located on microvillus surface of intestinal cell (Figure 1.10). It break α-1,4-glycosidic linkage of polysaccharide from dietary source to monosaccharide units (glucose) (Figure 1.11) [25, 26]. Inhibition of these enzymes by α-glucosidase inhibitors retards the rate of carbohydrates digestion and delays glucose absorption, resulting in the lower blood glucose levels.

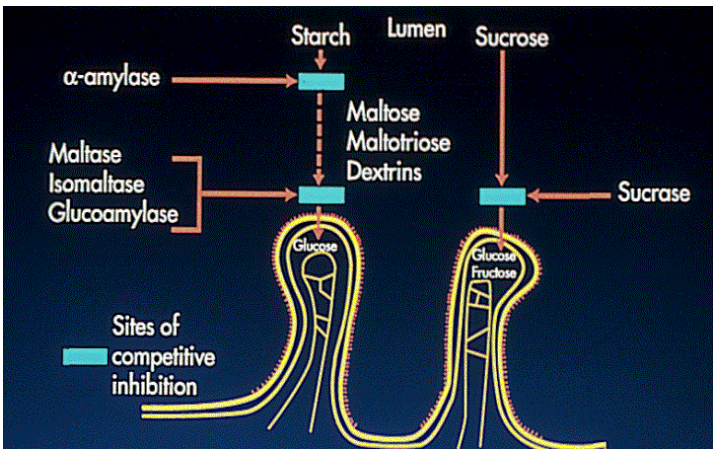

Figure 1.10 *Monosaccharide uptake in the small intestine (http://virruses.blogspot.com/2013/08/alpha-glucosidase-inhibitors.html).*

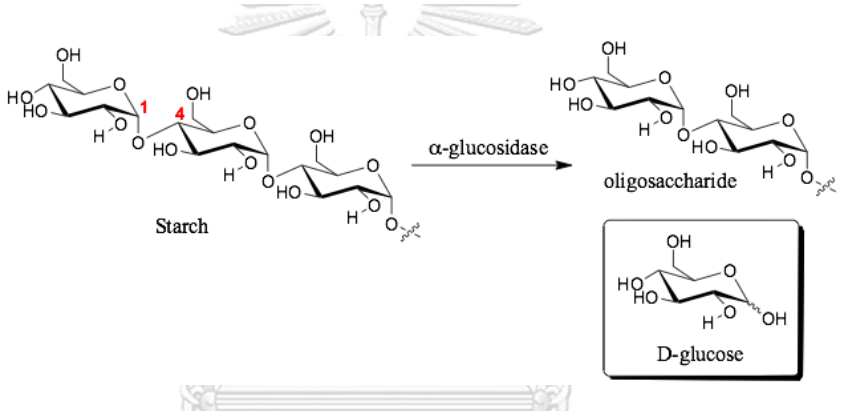

Figure 1.11 *Digestion of polysaccharide to monosaccharide by* **α***-glucosidase.*

α-Glucosidase inhibitors are classes of oral anti-diabetic agents that have been utilized for the treatment of type 2 diabetes and associated complications for more than 20 years. Currently, three drugs, which are acarbose (Precose® or Glucobay®), miglitol (Glyset®), and voglibose (Basen®), are in the market and have shown effective clinical use [27]. Moreover, the **α**-glucosidase inhibitors not only reduced blood glucose levels, but also showed anti-viral, anti-cancer and anti-HIV activities [11].

Acarbose (Precose® or Glucobay®) (Figure 1.12), a prominent **α**-glucosidase inhibitor first isolated from soil bacteria *Actinoplanes* sp [28], is a pseudotetrasaccharide containing a 4-amino-4,6-dideoxy-glucose unit connected with two glucose residues (forming maltose). Acarbose is a strong competitive inhibitor against **α**-glucosidase. It was effective in carbohydrate loading tests in rats and healthy volunteer, therefore reducing postprandial blood glucose and increasing insulin

secretion. However, long-term continuous use of acarbose may cause side effects such as flatulence, abdominal cramp, vomiting and diarrhea [26].

From a structural point of view, most of α-glucosidase inhibitor drugs are a group of aminocyclitols or carbasugars [29]. The inhibitory effect of α-glucosidase inhibitor drugs (aminocyclitols) revealed that the carbohydrate mimics containing nitrogen are protonated in the active site and act as glycoside inhibitors because of their ability to mimic the shape or charge of the presumed transition state for enzymatic glycoside hydrolysis [26, 30]. As mentioned above, α-glucosidase inhibitors therefore displayed competitive inhibition. To date, α-glucosidase inhibitors are attractive target compounds because of their therapeutic potential in the treatment of a variety of carbohydrate-mediated diseases such as cancer, viral infection, diabetes, etc [31].

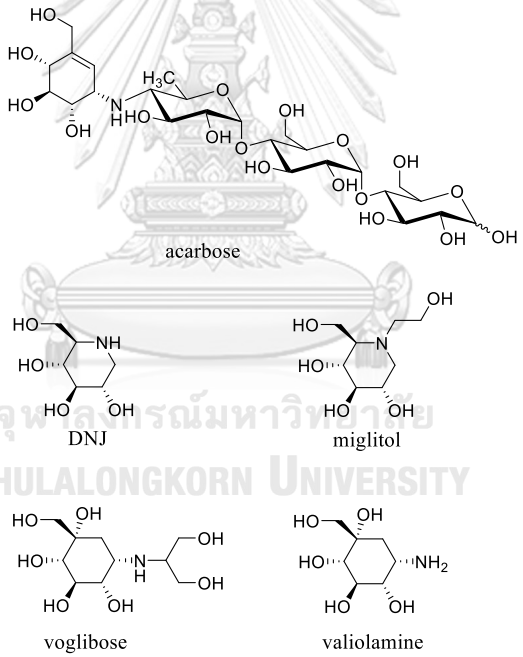

Figure 1.12 *Structures of digestive α-glucosidase inhibitors.*

In preliminary test, many compounds that have tend to inhibit glycation from edible plants (pinocembrin from fingerroot, strobopinin from rose apple, demethoxymatteucinol, chalcone, sesamolin from sesame) which were collected in the Center of Excellence in Natural Products were screened for Glycation inhibition. The highest potential inhibitor was pinocembrin from fingeroot. In addition, the crude

extract of longkong was screened for α-glucosidase inhibitory activities, and there was a promising inhibition (IC_{50} 2.5-4.3 mg/mL). These results prompted us to search for potent glycation inhibitors and antidiabetic agents from natural sources.

In this research, we study on the isolation and purification of bioactive compounds from edible plants (fingerroot and longkong). Glycation and α-glucosidase inhibitory activities of isolated compounds were evaluated. In addition, the kinetic analysis of the most active compound was investigated. Importantly, structure-activity relationships (SAR) of isolated compounds toward MG-trapping activity was also studied.

CHAPTER II

Identification of pinocembrin as anti-glycation agent and α-glucosidase inhibitor from fingerroot (*Boesenbergia rotunda*): The tentative structure-activity relationship towards MG-trapping activity.

2.1 Introduction

Diabetes mellitus is a metabolic disease caused by a malfunction of carbohydrate metabolism characterized by high blood glucose level (hyperglycemia). Chronic hyperglycemia in diabetes results in the development of long-term diabetic complication and associates with the increased risk of mortality and morbidity [32]. The excess glucose can be transformed to many potential precursors of glycation such as glyoxal (GO), methylglyoxal (MG) and 3-deoxyglucosone (3-DG). Glycation is a spontaneous non-enzymatic reaction between reducing sugars or their reactive metabolites such as MG and protein via rearrangement, oxidation, dehydration, and polymerization to generate advanced glycation-end products (AGEs). AGEs ultimately trigger several severe diabetic complications. such as cardiovascular diseases, nephropathy, neuropathy, and retinopathy [33]. Methylglyoxal (MG) is one of the reactive carbonyl species that plays an important role in glycation as a glycation precursor. Many recent studies have revealed that diabetic patients display the levels of MG higher than the normal people [34-37]. In addition, many reports indicate that MG is the most potent glycating agent among the dicarbonyl compounds [38, 39]. Aminoguanidine (AG) is a well-known anti-glycating agent that inhibits the formation of AGEs. Unfortunately, AG has been terminated in the clinical phase of medicine developing due to serious side effects such as congestive heart failure, myocardial infarction, gastrointestinal disturbance, and anemia [40]. Therefore, study on glycation inhibitors from nature has emerged as an intriguing approach to produce new candidates for the therapy of AGEs-associated diseases [41].

Boesenbergia rotunda Schltr. (*Syn*. *Boesenbergia pandurata*, *Kaempferia pandurata*), is a culinary herb of the Zingiberaceae family found in numerous Asian countries such as Indonesia, Malaysia, Thailand, India, and China. It is ordinarily utilized

as a flavoring in food such as curry, sauce and soup due to its aroma flavor, which advances craving. This herbal plant is also utilized as a conventional medicine to treat sicknesses such as muscle torment, rheumatism, gout, febrifuge, gastrointestinal disorders, flatulence, carminative, stomachache, dyspepsia, and peptic ulcer. The fresh rhizomes are utilized to treat inflammatory diseases, such as tooth decay, gum diseases, dry cough, cold, swelling, dermatitis, wounds, and diarrhea. According to Thai ethnomedicinal utilization, this plant is used as an aphrodisiac. Moreover, consumption of the leaves can relieve food allergies and poisoning. In Thailand, some AIDS patients used this plant as self-medication. In spite of the lack of scientific evidences to demonstrate the ethnomedicinal utilizations of this plant, the success of current biological researches seem possibly to clarify the significance of its traditional utilization. Several flavonoids were isolated and identified from the rhizome extract of *B. rotunda* such as flavones, flavanones, chalcones, and pimarane diterpenes [42, 43] (Figure 2.1). However, sciencefic evidences and reports related inhibitory effects against DM and AGEs have not been documented so far.

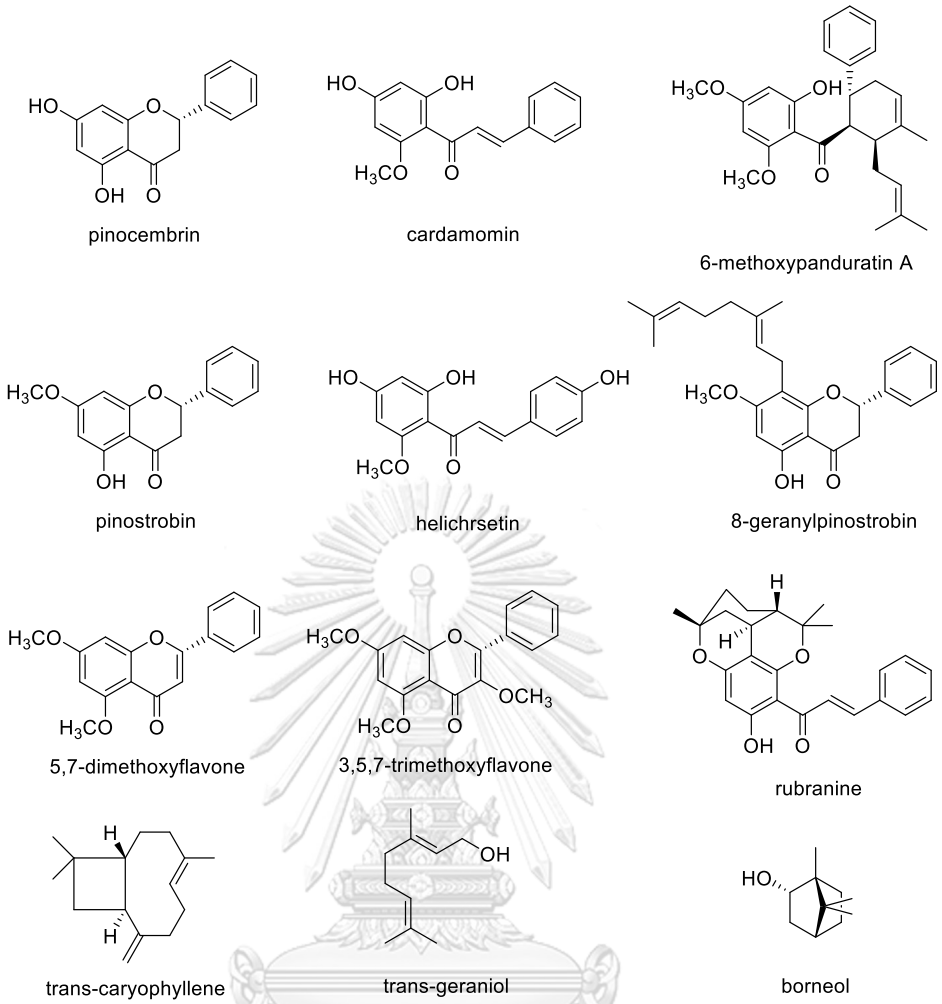

Figure 2.1 *Structures of selected flavonoids and terpenoids isolated from B. rotunda.*

In the present exploration, we applied AGEs inhibition assay to screen Thai medicinal plants. We found a promising inhibition in the CH_2Cl_2 extract of *B. rotunda* rhizomes. An attempt to identify glycation inhibitors was made and reported herein for the first time.

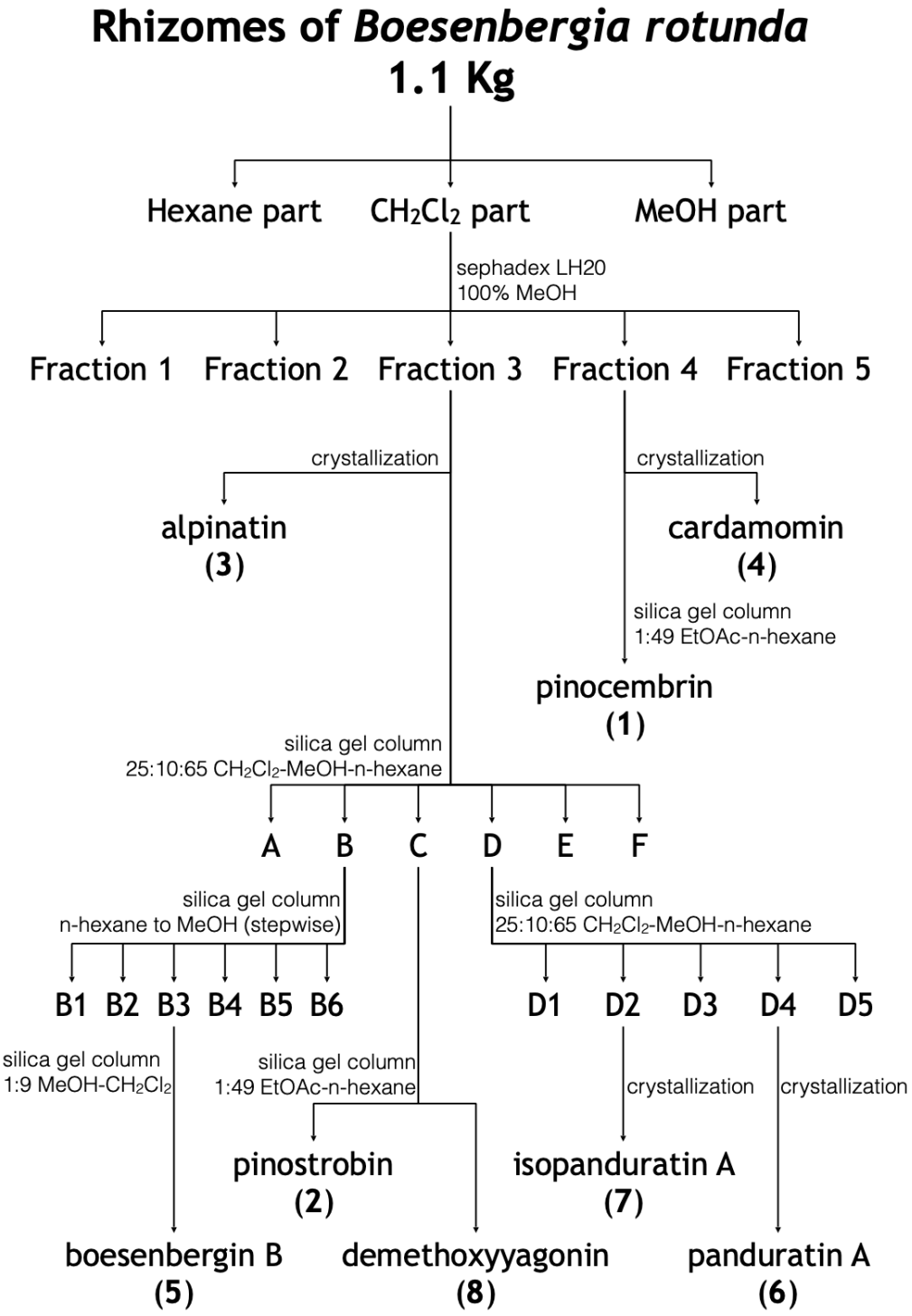

Figure 2.2 *Isolation scheme of B. rotunda rhizomes.*

2.2 Result and discussion

2.2.1 Plant isolation

The small air-dried pieces of fingerroot were submerged in hexane, dichloromethane, and methanol three times each at room temperature, respectively (Figure 2.2). The dichloromethane extract was subjected to Sephadex LH-20 column chromatography and eluted with MeOH to yield five fractions. The combined fractions 3 and 4 were subjected to silica gel column chromatography and crystallized to yield three flavanones (pinocembrin; **1**, pinostrobin; **2**, and alpinetin; **3**), two chalcones (cardamomin; **4** and boesenbergin B; **5**), two dihydrochalcones (panduratin A; **6** and isopanduratin A; **7**), and one kavalactone (demethoxyyangonin; **8**). The structures of isolated compounds (Figure 2.3) were elucidated on the basis of a detailed spectroscopic analysis including ^{1}H-NMR and ^{13}C-NMR spectroscopy techniques.

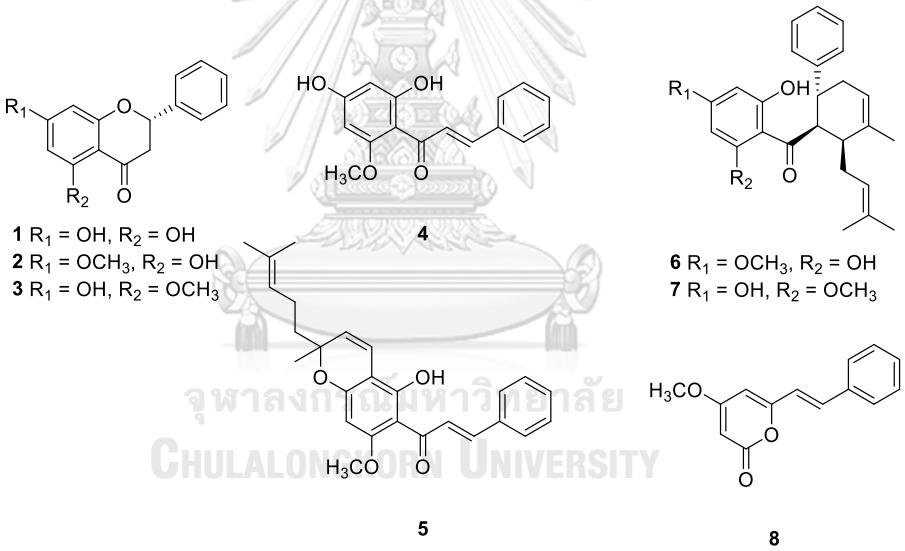

Figure 2.3 *Structures of isolated compounds (**1**-**8**).*

2.2.2. Glycation inhibition

2.2.2.1. Effect of isolated compounds on fluorescent-AGEs formation activity by MG-BSA assay

Glycation is a severe cause of diabetic complications by accumulation of AGEs along our life. Nevertheless, there are several studies indicate that glycation inhibition contributed by synthetic agents such as aminoguanidine (AG) shows serious side effects. Thus natural glycation inhibitors from edible plants are the alternative choices.

This research started with the preliminary screening of compounds from many edible plants against MG-trapping activity. Of compounds screened, pinocembrin from fingerroot revealed promising trapping activity. Therefore, fingerroot was selected for further study to search for other active components, in addition to pinocembrin (**1**).

All isolated compounds were evaluated for their inhibitory effect on the fluorescent-AGEs formation after incubation with bovine serum albumin (BSA) and MG for 24 hours (Figure 2.4). Alpinetin (**3**) showed highest inhibition (50%) against the MG-derived AGEs formation followed by demethoxyyangonin (**8**, 46%), cardamomin (**4**, 44%), panduratin A (**6**, 43%), boesenbergin B (**5**, 39%), pinocembrin (**1**, 38%), isopanduratin A (**7**, 36%), and pinostrobin (**2**, 19%), respectively. Interestingly, most isolated compounds showed higher inhibition (36-50%) against the formation of MG-derived AGEs than anti-glycating agent aminoguanidine (AG, 28%), except for pinostrobin (**2**, 19%) as shown in Figure 2.4. This result indicated that the isolated compounds might be the potent anti-glycation candidates. In addition, the results of AGEs inhibition observed in our work were comparable to those studied by Ma and coworkers [44] at the same concentration of AG (0.1 mM).

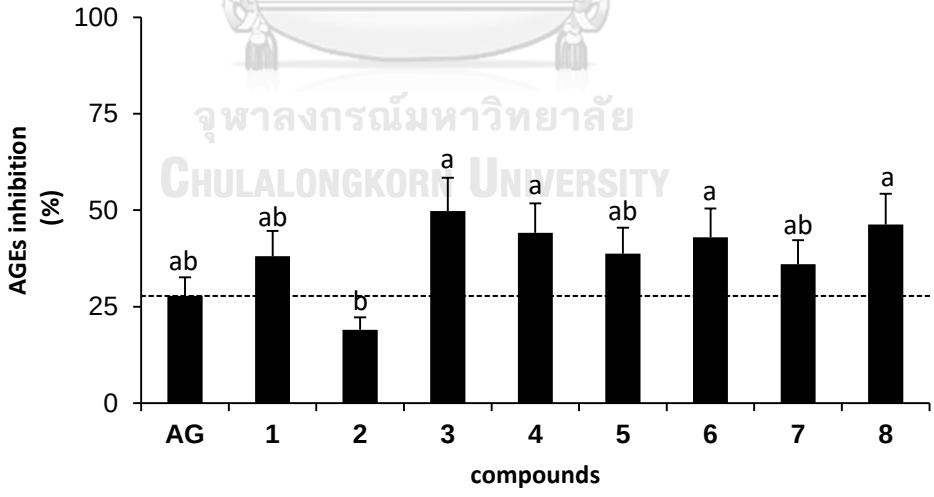

Figure 2.4 *AGEs inhibition activity of aminoguanidine (0.1 mM) and isolated compounds (0.1 mM) on the MG-derived AGEs formation inhibition in BSA. The results are presented as mean ± SEM for n = 3.* ^{ab} *Letters show significant difference that analyzed by ANOVA with Duncan's post hoc test ($p < 0.05$).*

2.2.2.2. Effect of isolated compounds on MG-trapping activity

As a result of higher inhibition of isolated compounds against AGEs formation, MG scavenging activity was subsequently examined to elucidate whether the isolated compounds inhibited AGEs formation through direct trapping of MG. Figure 2.5 demonstrates MG-trapping activity of isolated compounds, whose percentages of trapping were reported as the value related to AG (100%). Of isolated compounds, pinocembrin (**1**) demonstrated most potent trapping activity with the value of 109%, followed by panduratin A (**6**, 60%), cardamomin (**4**, 28%), demethoxyyangonin (**8**, 23%), boesenbergin B (**5**, 19%), alpinetin (**3**, 14%), isopanduratin A (**7**, 9%), and pinostrobin (**2**, 5%), respectively. It can be inferred that trapping activity of **1** was comparable to that of AG. To determine whether AGEs inhibitory effect is contributed by MG-trapping activity, Pearson's correlation analysis (Table 2.1) was carried out. The small correlation coefficient (r = 0.159) together with the p = 0.707 indicated very weak positive correlation between AGEs inhibitory effect and MG-trapping activity. In other words, the higher AGEs inhibitions of particular compounds such as alpinetin (**3**) over pinocembrin (**1**) were potentially derived from other effects.

As the small correlation coefficient from Pearson's correlation, this analysis suggested that AGEs inhibition was not mainly contributed by MG-trapping activity. It should be noted that AGEs inhibition of isolated flavonoids in this work could not be directly compared with those previously reported due to different detection methodologies such as fluorescent AGEs, non-fluorescent AGEs, glycated protein, and radical [33, 35, 45-47]. Moreover, AGEs formation inhibition is generated by many mechanism including MG-trapping activity. Therefore, the correlation could be interfered by other mechanisms. For example, Sompong [45] and Hwang [48] found that there was no correlation between % AGEs inhibition and radical scavenging activity whereas Mutsuda [49] suggested that strong AGEs inhibition activity tended to exhibit strong radical scavenging activity.

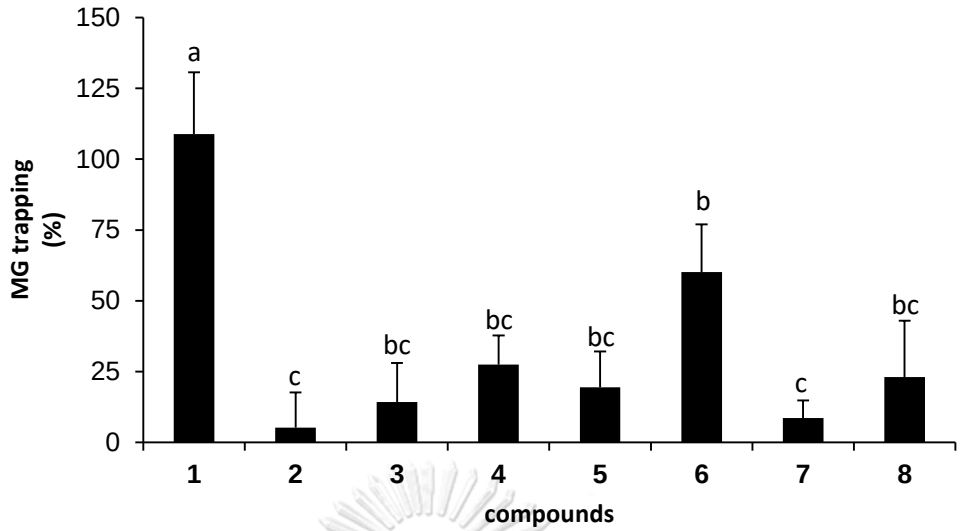

Figure 2.5 *The percentage of MG-trapping abilities of isolated compounds (0.1 mM). The results are presented as mean ± SEM for n = 3.* ^{abc} *Letters show significant difference that analyzed by ANOVA with Duncan's post hoc test ($p < 0.05$).*

Table 2.1 Pearson's correlation analysis of AGEs formation inhibition activity and MG-trapping activity of isolated compounds

Activity	MG-trapping	
	Correlation coefficient	p-value (2-tailed)
AGEs formation inhibition	0.159	0.707

Because there are variations in core structures together with types and position of substitution groups, SAR of isolated compounds on MG-trapping were carefully investigated in 6 cases. Each case focused on the structural differences as follows.

1. The presence of methoxy group on aromatic ring A of flavanone
2. The positions of methoxy group on aromatic ring A of flavanone
3. The ring C structure of flavanone and α-β unsaturated ketone structure of chalcone
4. The presence of geranyl group on chalcone
5. The positions of geranyl group on ring A (chalcone) or at α-β unsaturated ketone (dihydrochalcone)
6. The positions of methoxy group on aromatic ring A of dihydrochalcone

Of flavanones (**1** – **3**), the presence of methoxy group (OMe) on aromatic ring A apparently decreased their potency against MG-trapping (Figure 2.6). Pinocembrin (**1**), whose structure contains no methoxy group, revealed MG-trapping activity (109%) comparable to that of AG (100%). On the other hands, flavanones **2** and **3** demonstrated very low trapping activity (5 – 14%). Although methoxy and hydroxy groups are both electron donating groups and enhance electronic density to aromatic ring, a more steric hindrance of methoxy group is likely to retard aromatic ring A of flavanones **2** and **3** toward MG-trapping potency. However, the position of methoxy group on ring A showed no significant difference in the trapping activity of **2** and **3**.

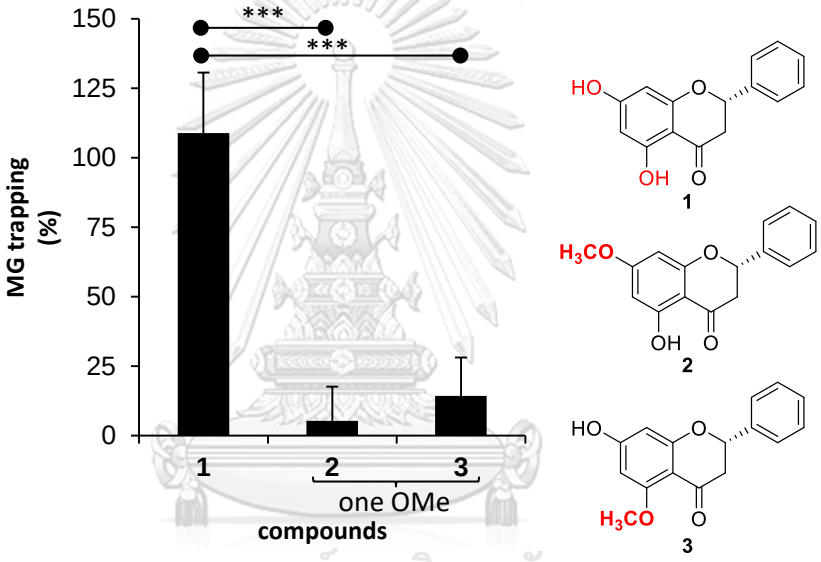

Figure 2.6 *The effect of the presence of one methoxy group substitution on aromatic ring A of flavanone on MG-trapping activity. The results are presented as mean ± SEM for n = 3. The stars (***) showed significant differences (p < 0.001) between **1** and **2**, and between **1** and **3** using paired sample t-test.*

We further analyzed the lack of ring C in chalcones and dihydrochalcones because they were dominant metabolites found in this plant. Direct comparison between alpinetin (**3**) and cardamomin (**4**) was present herein since they are structural isomers and contain similar substitution groups. Cardamomin (**4**) showed significantly higher trapping activity (28%) than alpinetin (**3**, 14%), thus indicating that the lack of ring C in chalcone did not reduce the trapping activity toward MG. Noticeably, the

presence of α-β unsaturated ketone in **4** possibly alleviated electron withdrawing potency of carbonyl group attached to ring A (Figure 2.7).

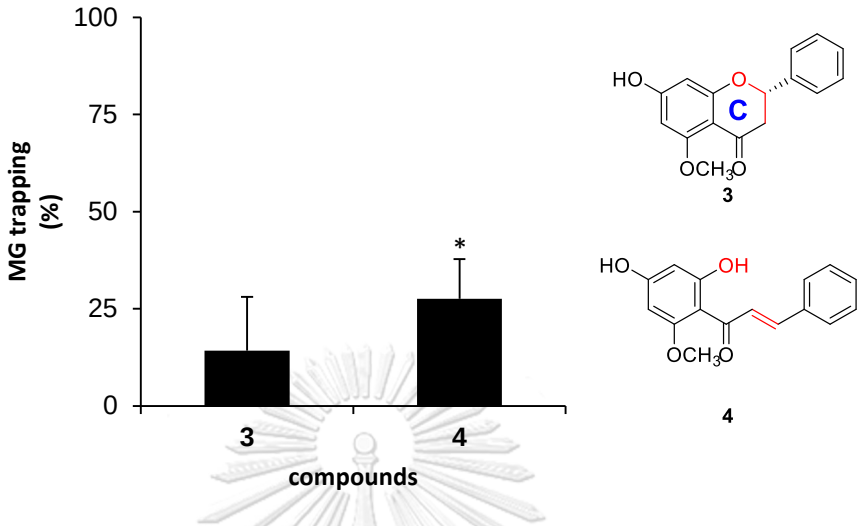

Figure 2.7 *The effect of the ring C on the structure of flavanone and the lack of ring C on the structure of chalcone against MG. The results are presented as mean ± SEM for n = 3. The star (*) showed significant difference ($p < 0.05$) between* ***3*** *and* ***4*** *using paired sample t-test.*

The presence and position of geranyl group of chalcones and dihydrochalcones toward trapping activity was also analyzed. Herein, compounds **4**, **5** and **7** were directly compared because they possessed identical pattern of hydroxy and methoxy groups on ring A. The geranyl group was likely to suppress trapping ability (Figure 2.8); however, the significantly reduced activity was found in isopanduratin A (**7**). Although the presence of geranyl group on ring A in boesenbergin B (**5**) was expected to produce steric hindrance, the decrease in trapping activity was not significantly observed.

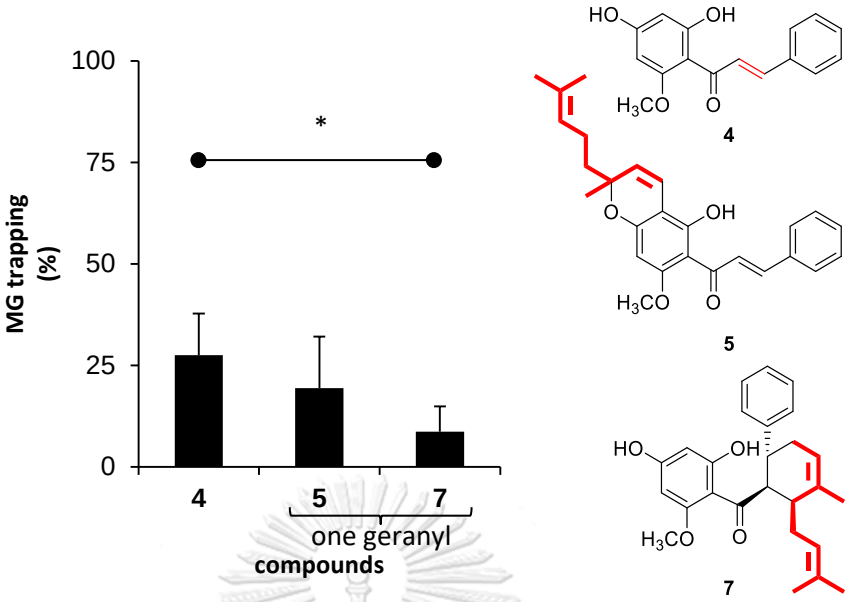

Figure 2.8 *The effect of the presence of one geranyl group on structure of chalcone on MG-trapping activity. The results are presented as mean ± SEM for n = 3. . The star (*) showed significant difference (p < 0.05) between* ***4*** *and* ***7*** *using paired sample t-test.*

The effect of the position of methoxy group of dihydrochalcone on MG-trapping activity has been investigated by direct comparison between **6** and **7**. They are structural isomers different in methoxy position; 4-OMe in **6** and 6-OMe in **7**. The activity of compound **6** (60%) was significantly higher than that of compound **7** (9%) (p = 0.020 < α = 0.05) (Figure 2.9). In addition, the presence of hydrogen bonding between 6-OH and carbonyl group at C-1 on ring A in **6** might promote the MG-trapping ability. This result was also observed in the previous reports [50, 51].

Because pinocembrin (**1**) is the most potent glycation inhibitor, it was further evaluated for half maximal effective concentration (EC_{50}) of MG-trapping activity. Compound **1** displayed high efficiency against methylglyoxal with EC_{50} value of 63.22 ± 10.12 μM.

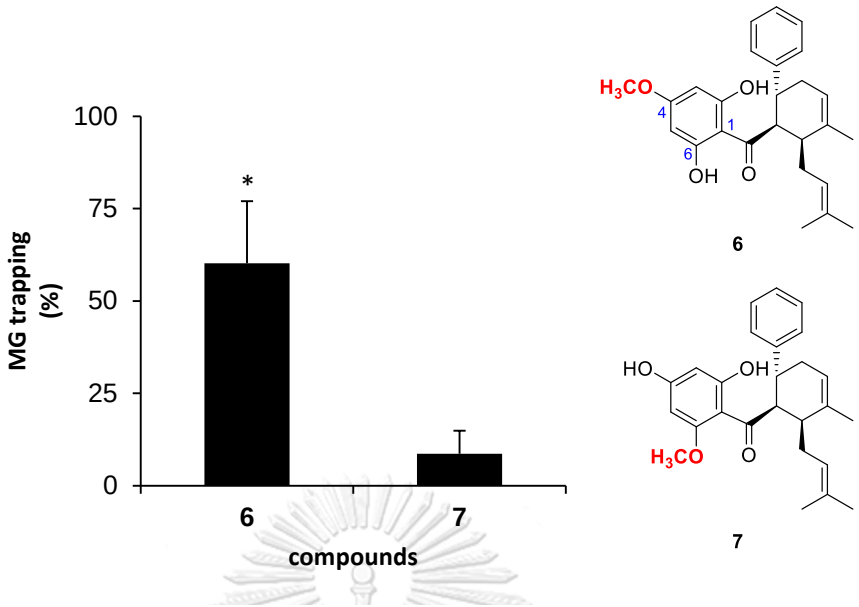

Figure 2.9 *The effect of the position of methoxy group substitution on aromatic ring A of dihydrochalcone on MG-trapping activity. The results are presented as mean ± SEM for n = 3. . The star (*) showed significant difference ($p < 0.05$) between* ***6*** *and* ***7*** *using paired sample t-test.*

2.2.3. α-Glucosidase inhibitory activity and kinetic study of pinocembrin (**1**)

For diabetes patient, the reduction of glucose uptake is very important, which can be done through suppressing carbohydrate digestion. It is of interest to evaluate α-glucosidase inhibition of **1** against rat intestinal maltase and sucrase, in hope that it would possess multifunctions beneficial to controlling diabetes and its complications. This is the first time that **1** was tested for α-glucosidase inhibitory activity. Compound **1** displayed moderate inhibition against both maltase and sucrase with IC_{50} values of 0.35 ± 0.021 and 0.39 ± 0.020 mM, respectively (supporting information).

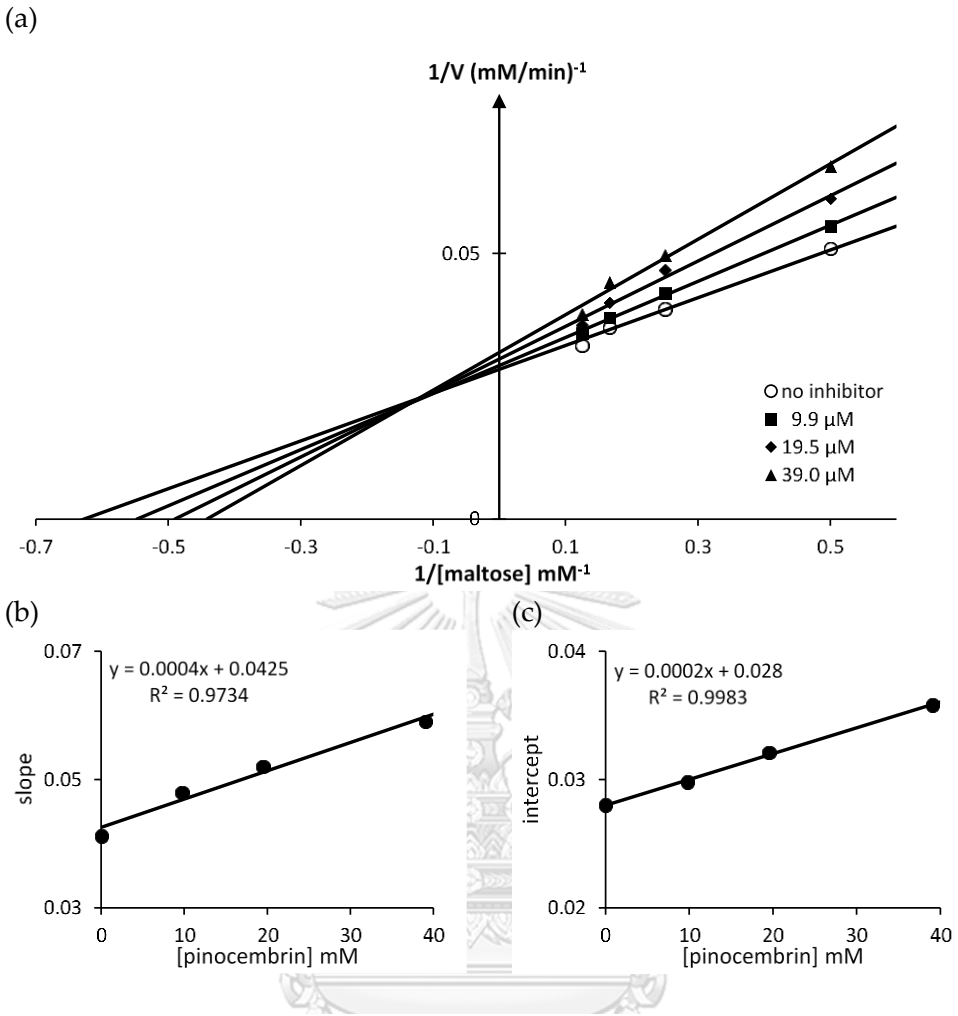

Figure 2.10 *Kinetic study plot of pinocembrin (**1**) against rat intestinal maltase; (a) Lineweaver–Burk plot; (b) secondary plot of slope and concentration of pinocembrin for the determination of Ki; (c) secondary plot of intercept and concentration of pinocembrin for the determination of Ki′.*

In order to gain further insight into how **1** interact with rat intestinal maltase and sucrase, the inhibition mode of **1** was analyzed by kinetic study. The Lineweaver-Burk plot of **1** against maltase (Figure 2.10a) displayed a series of straight lines. The intersections of all straight lines are in the second quadrant. Kinetic examination appeared that V_{max} decreased with elevated K_m in the presence of increasing concentrations of **1**. This behavior recommended that **1** inhibited maltase in a mixed-type manner comprising two diverse pathways, competitive and non-competitive. The observed result was explained by simultaneous formation of enzyme-inhibitor (EI) and

enzyme-substrate-inhibitor (ESI) complexes in competitive and non-competitive manners, respectively (Figure 2.11). We encourage examined the pathway in which **1** was preferably preceded by determining dissociation constants of EI (K_i) and ESI (K'_i) complexes (Table 2.2). Clearly, the secondary plots (Figures 2.10b and 2.10c) illustrated K_i and K'_i values of 93 and 138 μM, respectively, thus indicating that **1** was overwhelmingly bound to maltase (EI) rather than the forming ESI complex. The putative inhibitory mechanism is summarized in Figure 2.11.

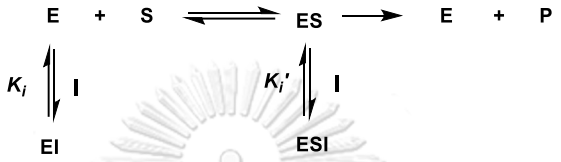

Figure 2.11 *Putative inhibitory mechanism of pinocembrin (**1**) against rat intestinal* **α***-glucosidases. E, S, I and P represent enzyme, substrates (maltose and sucrose), inhibitor (pinocembrin) and glucose, respectively.*

Table 2.2 Kinetic factors of pinocembrin (**1**) for rat intestinal **α**-glucosidase inhibition

enzyme	K_i (μM)	K'_i (μM)	Inhibition type
Maltase	93	138	Mixed
Sucrase	51	253	Mixed

The inhibitory mechanism of **1** against sucrase (Figure 2.12) was also examined utilizing the above strategy. Apparently, **1** inhibited sucrase through both competitive and non-competitive manners (mixed-type inhibition). All kinetic variables are summarized in Table 2.2.

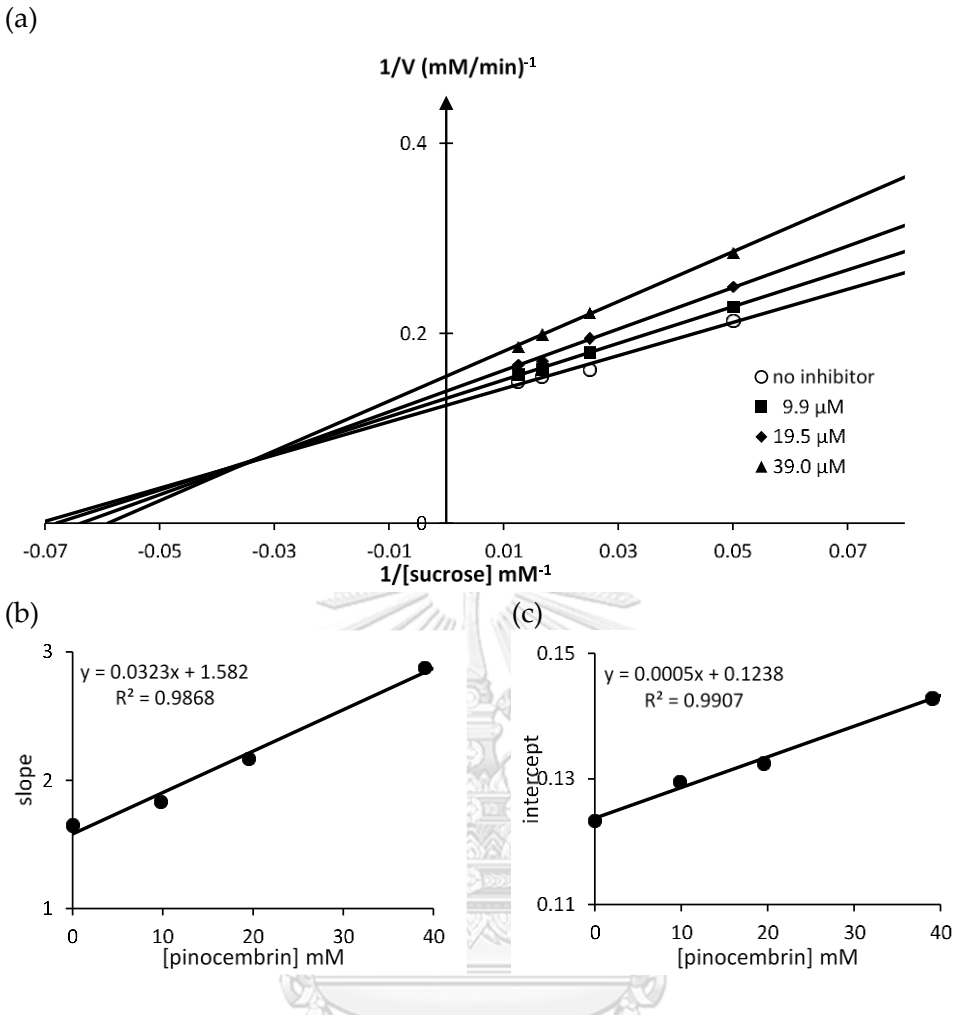

Figure 2.12 *Kinetic study plot of pinocembrin (**1**) against rat intestinal sucrase; (a) Lineweaver–Burk plot; (b) secondary plot of slope and concentration of pinocembrin for the determination of Ki; (c) secondary plot of intercept and concentration of pinocembrin for the determination of Ki′.*

2.3. Material and method

2.3.1. Plant material and isolation

Fingerroots (5 Kg) were purchased from local shop number 43 in Sam-Yan Market, Phatumwan, Bangkok, Thailand, during January, 2015. The rhizomes were cleaned and cut into small pieces before air drying. The dried plant (1.1 Kg) was submerged in hexane, dichloromethane, and methanol three time each at room temperature, respectively. The dichloromethane extract was subjected to Sephadex LH-20 column chromatography and eluted with MeOH to yield five fractions (fractions

1-5). Fraction 3 was crystallized by MeOH to yield alpinetin (**3**, 150 mg) [52], while the remaining mother liquor was subjected to silica gel column chromatography (Merck Art 7730) and eluted with 25:10:65 CH_2Cl_2-MeOH-n-hexane to give six fractions (fractions A-F). Fraction B was subjected to silica gel column chromatography and eluted in stepwise fashion with n-hexane, 1.5:8.5 CH_2Cl_2-n-hexane, 4:6 CH_2Cl_2-n-hexane, 8:2 CH_2Cl_2-n-hexane, CH_2Cl_2, 1:9 MeOH-CH_2Cl_2 and 2:8 MeOH-CH_2Cl_2 to yield six sub-fractions (sub-fractions B1-B6). Sub-fraction B3 was subjected to silica gel column chromatography and eluted with 1:9 MeOH-CH_2Cl_2, yielding boesenbergin B (**5**, 80 mg) [53]. Fraction C was subjected to silica gel column chromatography and eluted with 1:49 EtOAc-n-hexane to yield pinostrobin (**2**, 200 mg) [52] and demethoxyyangonin (**8**, 70 mg) [54]. Fraction D was subjected to silica gel column chromatography and eluted with 1:1 CH_2Cl_2-n-hexane to afford five sub-fractions (sub-fractions D1-D5). The combined sub-fractions D2 and D4 were crystallized by MeOH to yield isopanduratin A (**7**, 50 mg) [55] and panduratin A (**6**, 100 mg) [56], respectively. Fraction 4 was crystallized by MeOH to yield cardamomin (**4**, 150 mg) [52], while the remaining mother liquor was subjected to silica gel column chromatography and eluted with 1:49 EtOAc-n-hexane to yield pinocembrin (**1**, 180 mg) [52]. The structures of isolated compounds were elucidated on the basis of a detailed spectroscopic analysis including ^{1}H-NMR and ^{13}C-NMR, and compared with previous reports.

2.3.2. In vitro glycation of bovine serum albumin (BSA) by MG-BSA assay

Glycated BSA was carried out according to our previous study [46]. The mixture of bovine serum albumin (BSA, 10 mg/mL), isolated compound (0.1 mM in DMSO) and methylglyoxal (MG, 0.25 mM) in 0.1 M phosphate buffer solution (PBS, pH 7.4) was incubated at 37 °C for 24 h. The glycated BSA was monitored by a spectrofluorometer (Fluroskan Ascent FL, Thermo Scientific, Barrington, IL, USA) at the excitation wavelength of 355 nm and emission wavelength of 460 nm. Aminoguanidine hydrochloride (AG) was utilized as a positive control. The percentage inhibition of MG-derived AGE formation was calculated utilizing the formula below:

$$\text{Inhibition of AGEs formation (\%)} = \frac{(\text{FC} - \text{FCB}) - (\text{FS} - \text{FSB})}{\text{FC} - \text{FCB}} \times 100$$

Where FC and FCB were the fluorescent intensity of control with and without MG, respectively. FS and FSB were the fluorescent intensity of sample with and without MG, respectively.

2.3.3. Determination of direct MG-trapping activity by HPLC

The ability of isolated compounds to trap MG was carried out according to our previous report [45]. The reaction mixture of MG (0.1 mM), isolated compound (0.1 mM in DMSO) and 0.1 M PBS (pH 7.4) was incubated at 37 °C for 24 h. The quantitative amount of the remaining MG was determined by derivatization with *o*-phenylenediamine (*o*-PDA) to convert to 2-methylquinoxaline (2-MQ). Then, 2-MQ obtained was measured by HPLC using 5-methylquinoxaline (5-MQ) as internal standard. The mixture comprising 100 µL of *o*-PDA (20 mM) and 100 µL of 5-MQ (5 mM) was added into the sample vials and incubated at 25 °C for 30 min. HPLC analysis was performed on a Shimadzu HPLC system (binary pump; model LC-10A, an auto-injector; model SIL-10A, and a UV detector; model SPD-10A) equipped with an Inertsil ODS–3 V C18 column (4.6 × 150 mm, 5 µm particle diameter). The column was flushed with a mixture of 1:1 methanol-water at a flow rate of 1 mL/min and a 10 µL of injection volume. The overall running time was 15 min and the wavelength used for detection was 315 nm. The area under each chromatogram peak, 2-MQ and 5-MQ, was calculated with LC solution program. Peak integrality proportions of 2-MQ to 5-MQ were utilized for quantitative examination. The amount of MG was calculated by utilizing the standard curve of 2-MQ/5-MQ proportion. AG was utilized as a positive control. The percentage MG-trapping was calculated utilizing the formula below:

$$\%\mathrm{MG-trapping} = 100 - [\frac{\text{Amount of (MG in sample } - \text{ MG in positive control)}}{\text{Amount of MG in positive control}} \times 100]$$

2.3.4. α-Glucosidase inhibitory activity

α-Glucosidase (from rat intestine) inhibitory effect was evaluated utilizing our previous study [57]. Rat intestinal maltase and sucrase, a crude enzyme solution, was prepared from rat intestinal acetone powder. A solution of rat intestinal acetone powder (1 g) in 0.9% NaCl solution (30 mL) was centrifuged (12,000 rpm) for 30 min and then the aliquot obtained was used for assay. The mixture of 10 µL of isolated

compounds (1 mg/mL in DMSO), 30 μL of the phosphate buffer (0.1 M, pH 6.9), and 20 μL of the substrate solution (10 mM for maltose and 100 mM for sucrose in phosphate buffer) was added glucose assay kit (80 μL) and the crude enzyme solution (20 μL), which then was incubated at 37 °C for 10 min and 40 min for maltose and sucrose, respectively. The enzymatic activity was measured at 503 nm using microplate reader model 3550 UV. The percentage inhibition was calculated by $[(A_c-A_s)/A_c] \times 100$, where A_c is the absorbance of control and A_s is the absorbance in the presence of sample. The results were reported as IC_{50} value and the experiment was carried out in triplicate. Acarbose® was used as standard control.

2.3.5. Kinetic study of α-glucosidase inhibition

The kinetic analysis of pinocembrin was determined using increasing concentration of substrates maltose (2-10 mM) and sucrose (20-100 mM), and Lineweaver-Burk plot was performed to determine the type of inhibition. The secondary plot was utilized to determine K_i and K_i' values which replotted between slope and intercept from the Lineweaver-Burk plot vs. the various concentration of pinocembrin.

2.3.6. Statistical analysis

The data are presented as means ± standard error of mean (SEM; $n = 3$). In the experiment of MG trapping activity, paired sample *t*-test was evaluated for the significant differences that showed as the stars above the graphs, between each pairs of sample. For the significant differences that showed as the letter above the graphs, among compounds, one-way ANOVA was evaluated and Dancan's post-hoc test was used for mean comparisons. Pearson's correlation analysis was used to determine the correlation between AGEs formation inhibition activity and MG-trapping activity. A *p*-value < 0.05 was considered to be statistically significant.

2.4. Conclusion

Three flavanones (pinocembrin, pinostrobin, and alpinetin), two chalcones (cardamomin and boesenbergin B), two dihydrochalcones (panduratin A and isopanduratin A), and one kavalactone (demethoxyyangonin) were isolated from the

dichloromethane extract of fingerroots. Most of isolated compounds showed higher inhibition against the formation of MG-derived AGEs than anti-glycating agent aminoguanidine (AG, 28%). Moreover, the studies of structure-activity relationship (SAR) were investigated in this research using the comparison of the structures of flavonoids with their MG-trapping activities. The structural requirements of flavonoids on this activity were obtained and the results showed the following: 1) hydroxy group and α-β unsaturated ketone structure could facilitate the activity; 2) methoxy and geranyl groups could reduce the activity; and 3) methoxy group at C-4 position of dihydrochacone is more active than C-6 position. The small Pearson's correlation coefficient (*r* = 0.159) indicated very weak positive correlation between AGEs inhibitory effect and MG-trapping activity. Of the compounds examined, pinocembrin (**1**) demonstrated the highest trapping activity with the value of 109%. It can be inferred that trapping activity of **1** was comparable to that of AG and displayed high efficiency against methylglyoxal with EC_{50} value of 63.22 ± 10.12 μM. In addition, this is the first time that pinocembrin was tested for α-glucosidase inhibitory activity. Pinocembrin displayed moderate inhibition against both maltase and sucrase with IC_{50} values of 0.35 ± 0.021 and 0.39 ± 0.020 mM, respectively. The highly potent glycation (MG-trapping activity) and α-glucosidase and inhibitions of pinocembrin (**1**) could be beneficial in diabetes treatment as well as in preventing the onset of its complications.

Supporting information

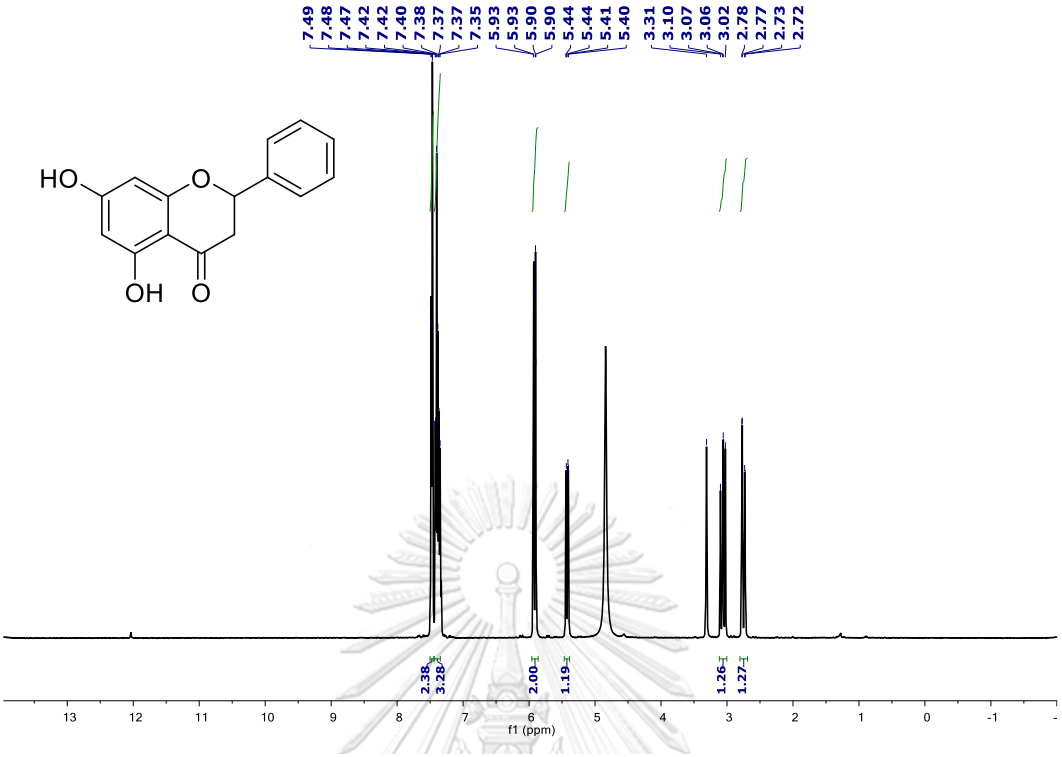

Figure 2.13 *^{1}H NMR spectrum (400 MHz, CD_3OD) of pinocembrin (**1**).*

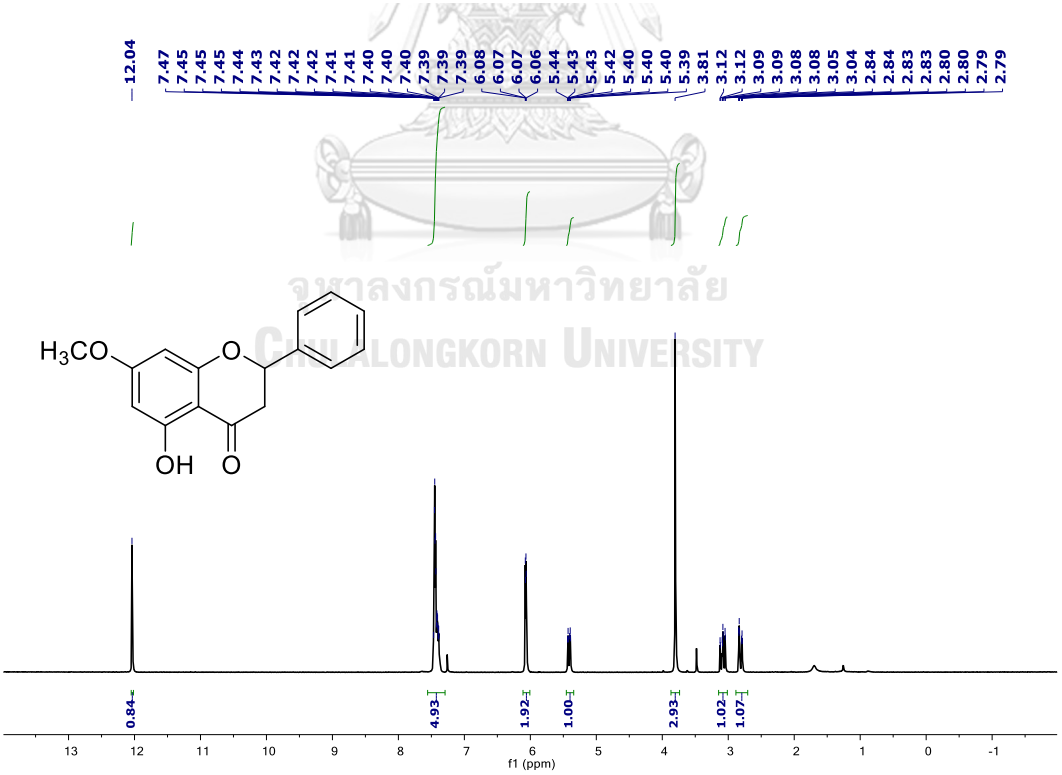

Figure 2.14 *^{1}H NMR spectrum (400 MHz, $CDCl_3$) of pinostrobrin (**2**).*

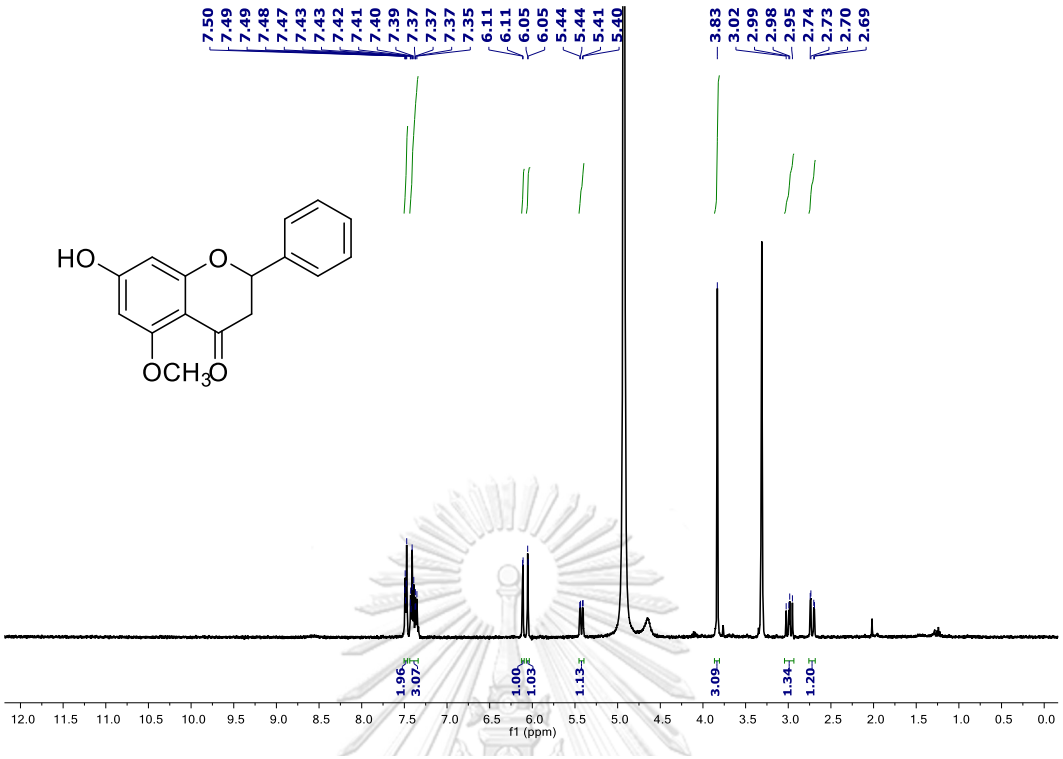

Figure 2.15 *^{1}H NMR spectrum (400 MHz, CD_3OD) of alpinetin (**3**).*

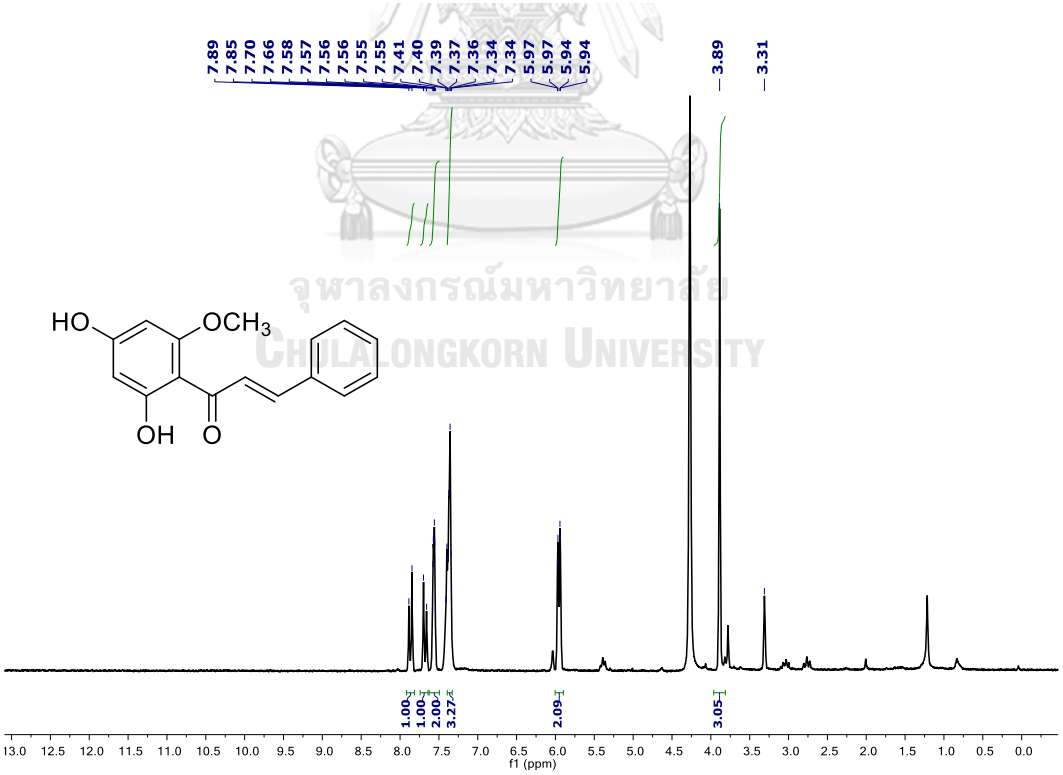

Figure 2.16 *^{1}H NMR spectrum (400 MHz, CD_3OD) of cardamomin (**4**).*

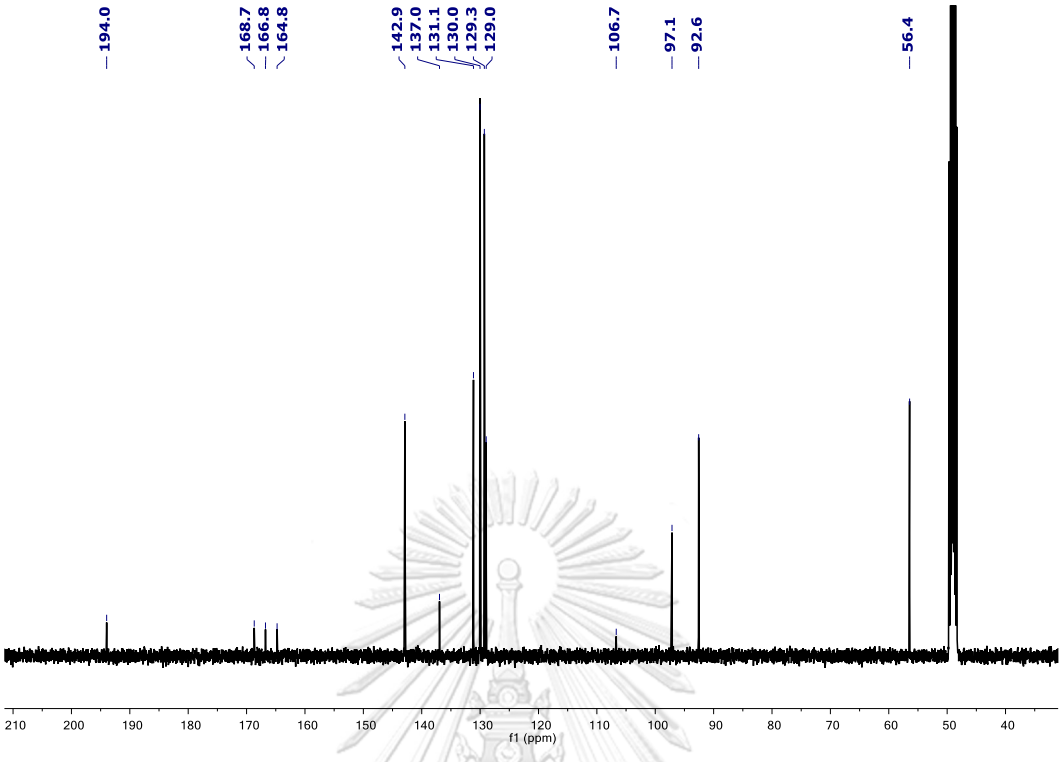

Figure 2.17 *^{13}C NMR spectrum (400 MHz, CD_3OD) of cardamomin (**4**).*

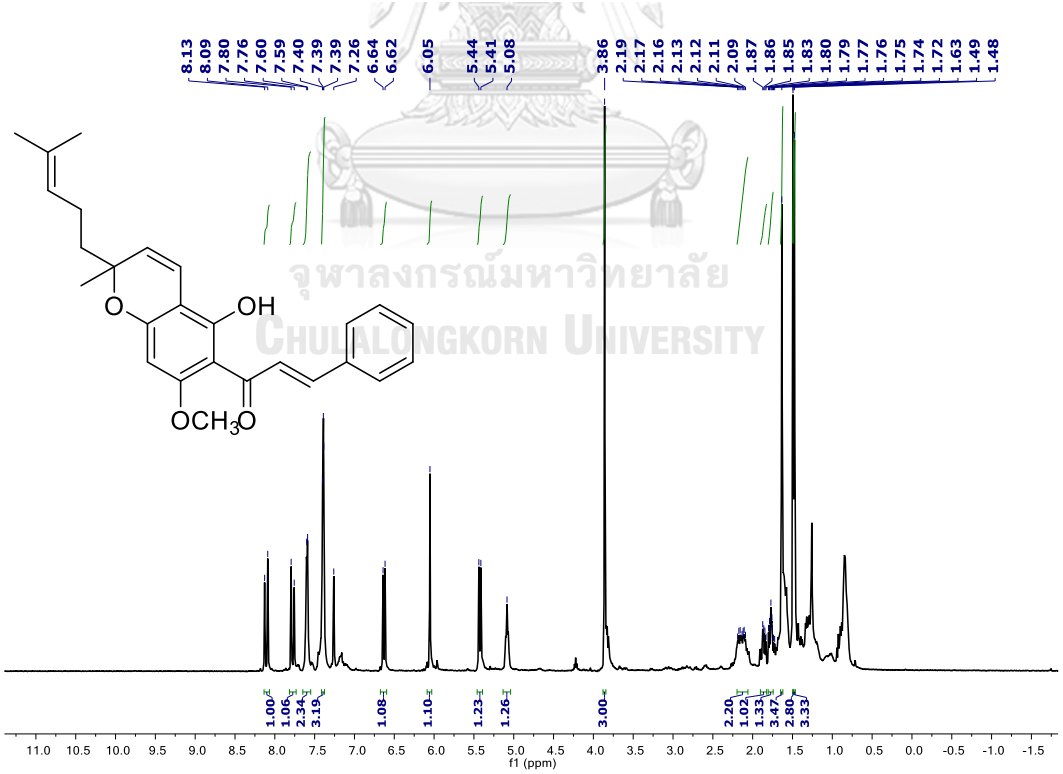

Figure 2.18 *^{1}H NMR spectrum (400 MHz, $CDCl_3$) of boesenbergin B (**5**).*

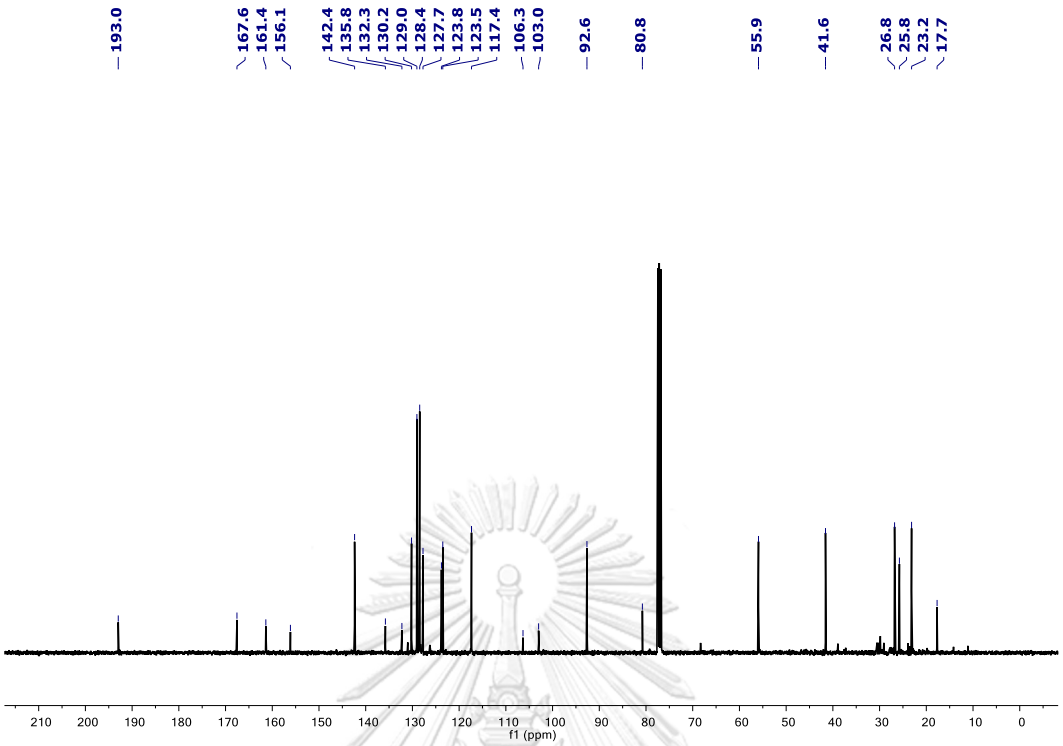

Figure 2.19 *^{13}C NMR spectrum (400 MHz, $CDCl_3$) of boesenbergin B (**5**).*

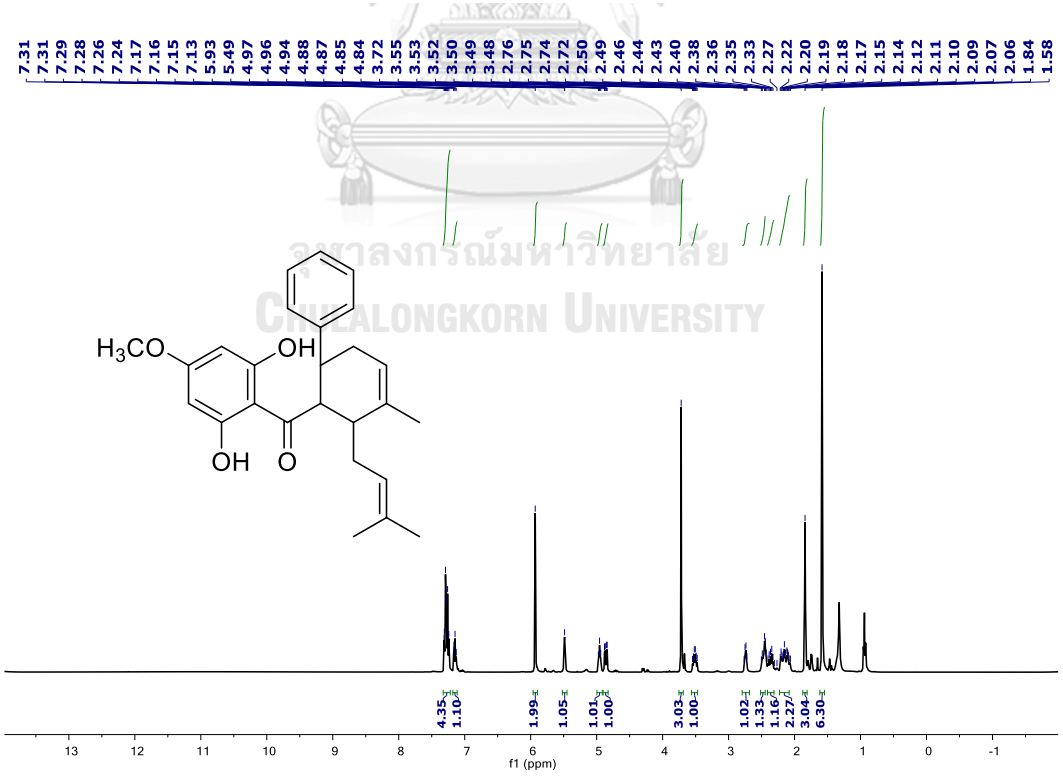

Figure 2.20 *^{1}H NMR spectrum (400 MHz, $CDCl_3$) of panduratin A (**6**).*

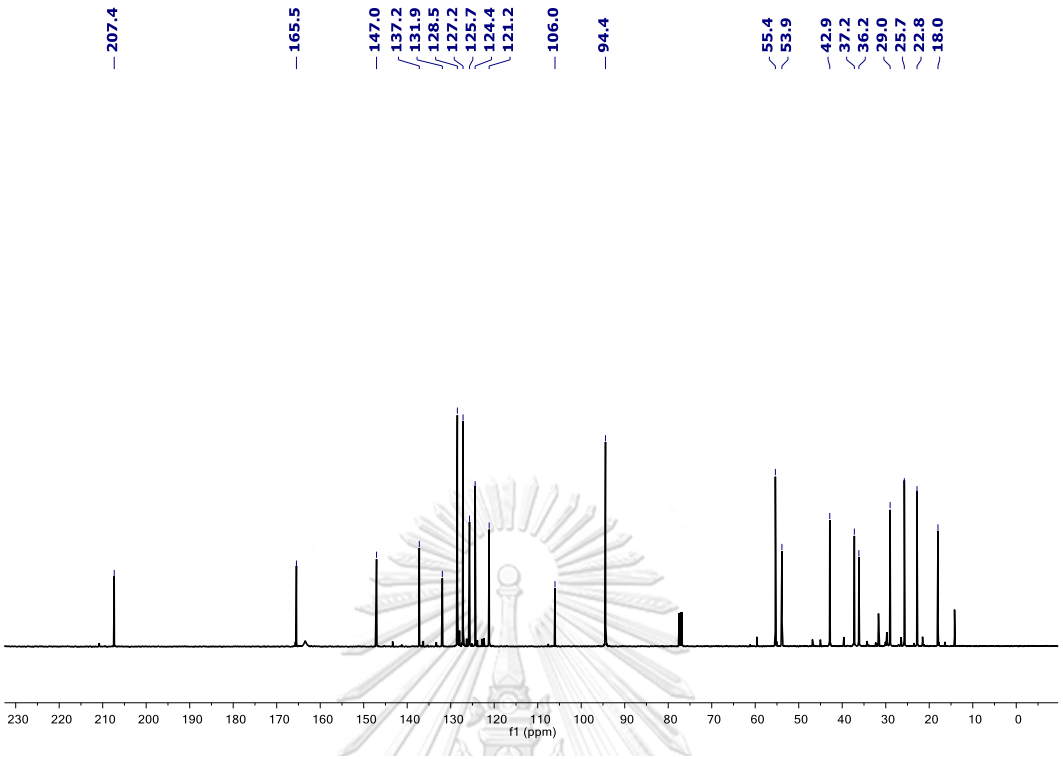

Figure 2.21 *^{13}C NMR spectrum (400 MHz, $CDCl_3$) of panduratin A (**6**).*

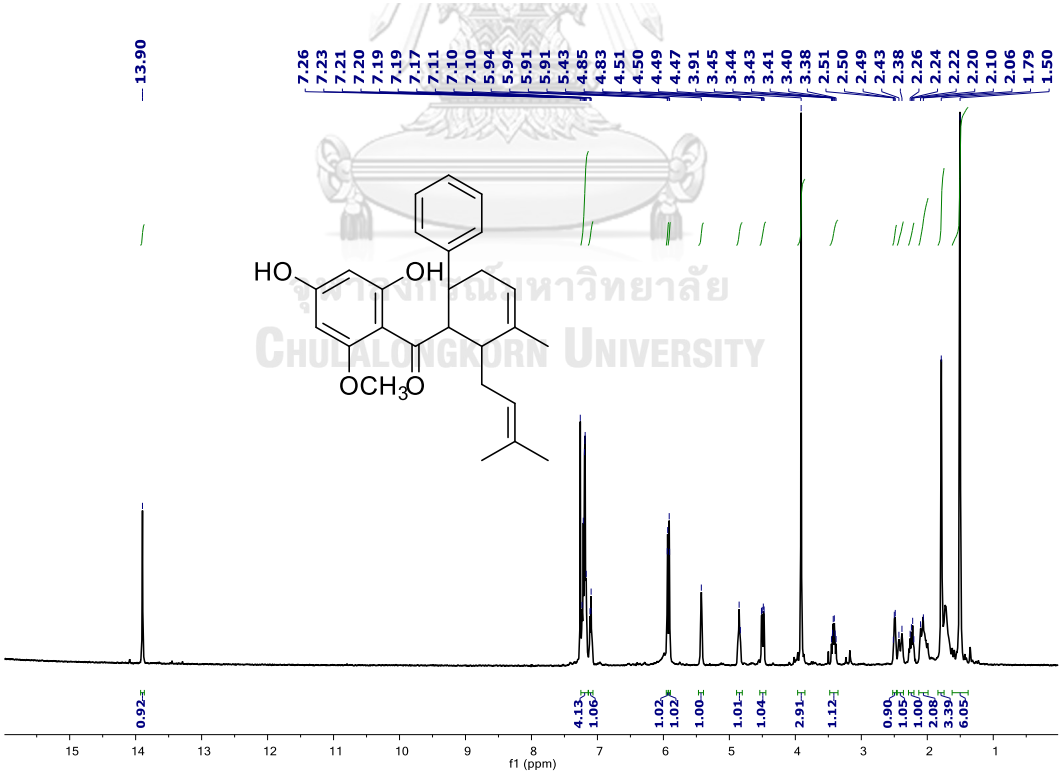

Figure 2.22 *^{1}H NMR spectrum (400 MHz, $CDCl_3$) of isopanduratin A (**7**).*

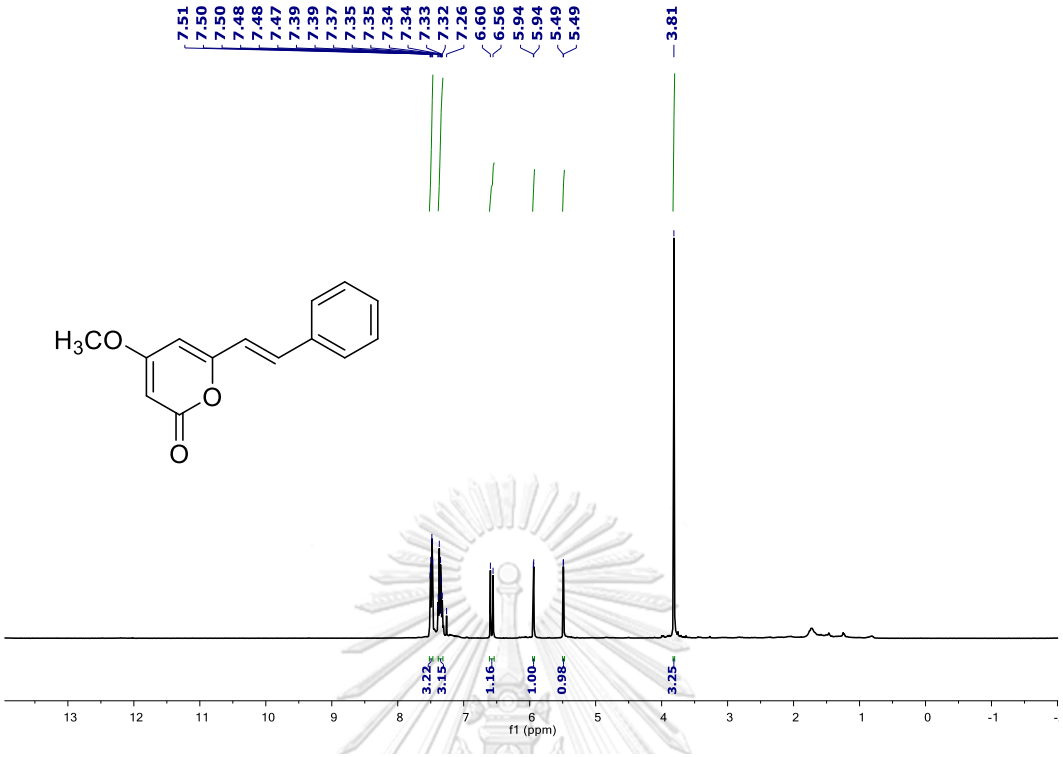

Figure 2.23 *^{1}H NMR spectrum (400 MHz, $CDCl_3$) of demethoxyyangonin (**8**).*

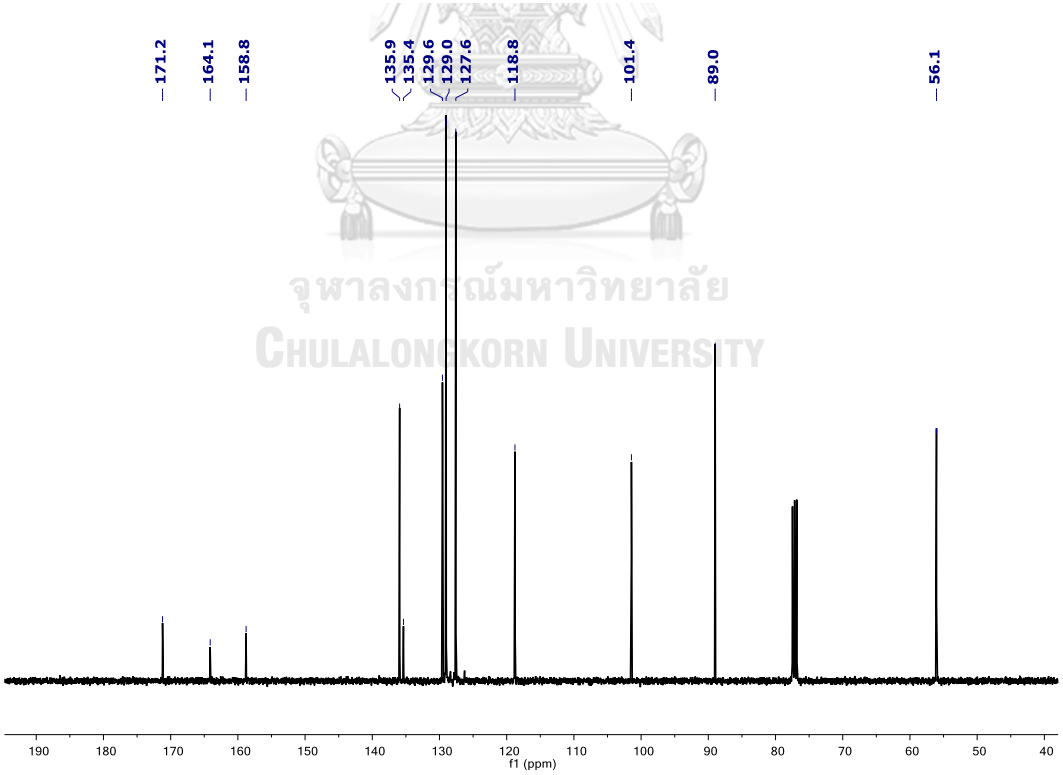

Figure 2.24 *^{13}C NMR spectrum (400 MHz, $CDCl_3$) of demethoxyyangonin (**8**).*

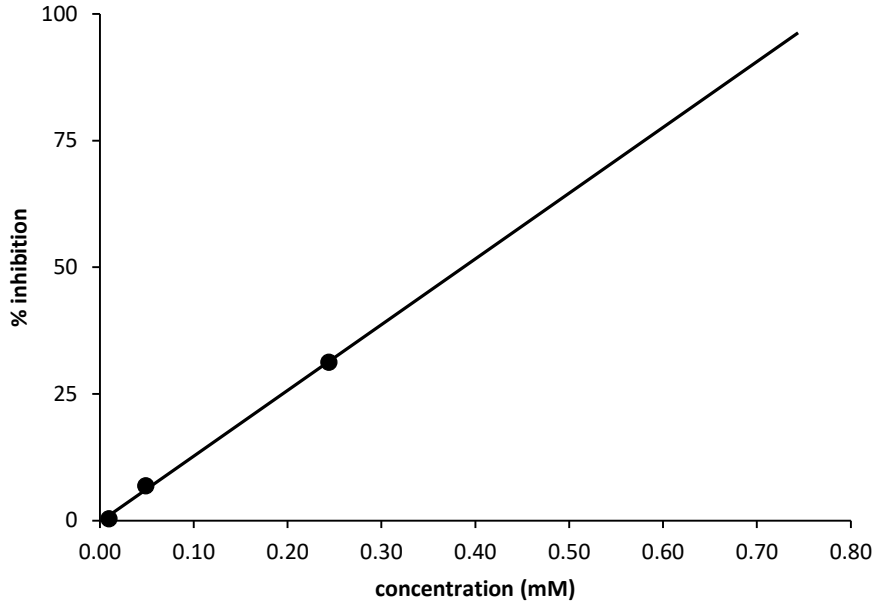

Figure 2.25 *Inhibition plot of pinicembrin against rat intestinal maltase.*

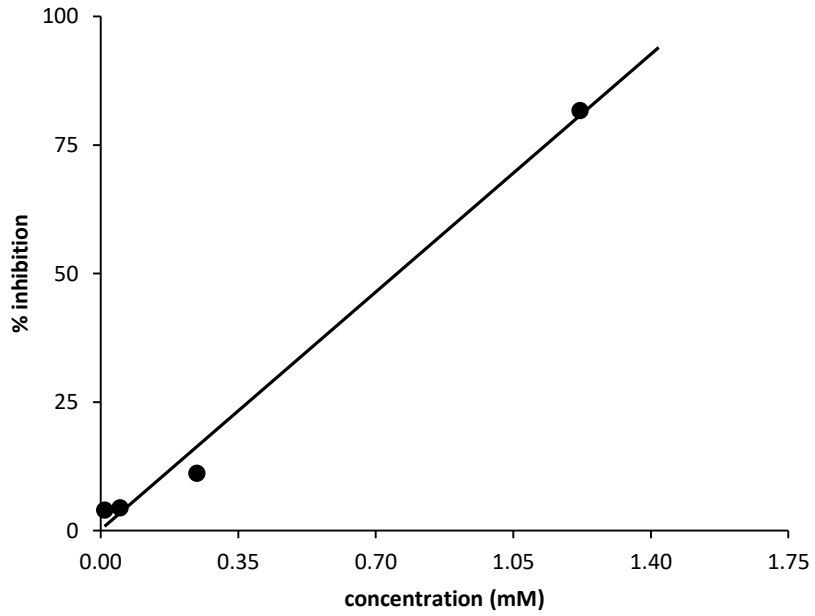

Figure 2.26 *Inhibition plot of pinicembrin against rat intestinal sucrase.*

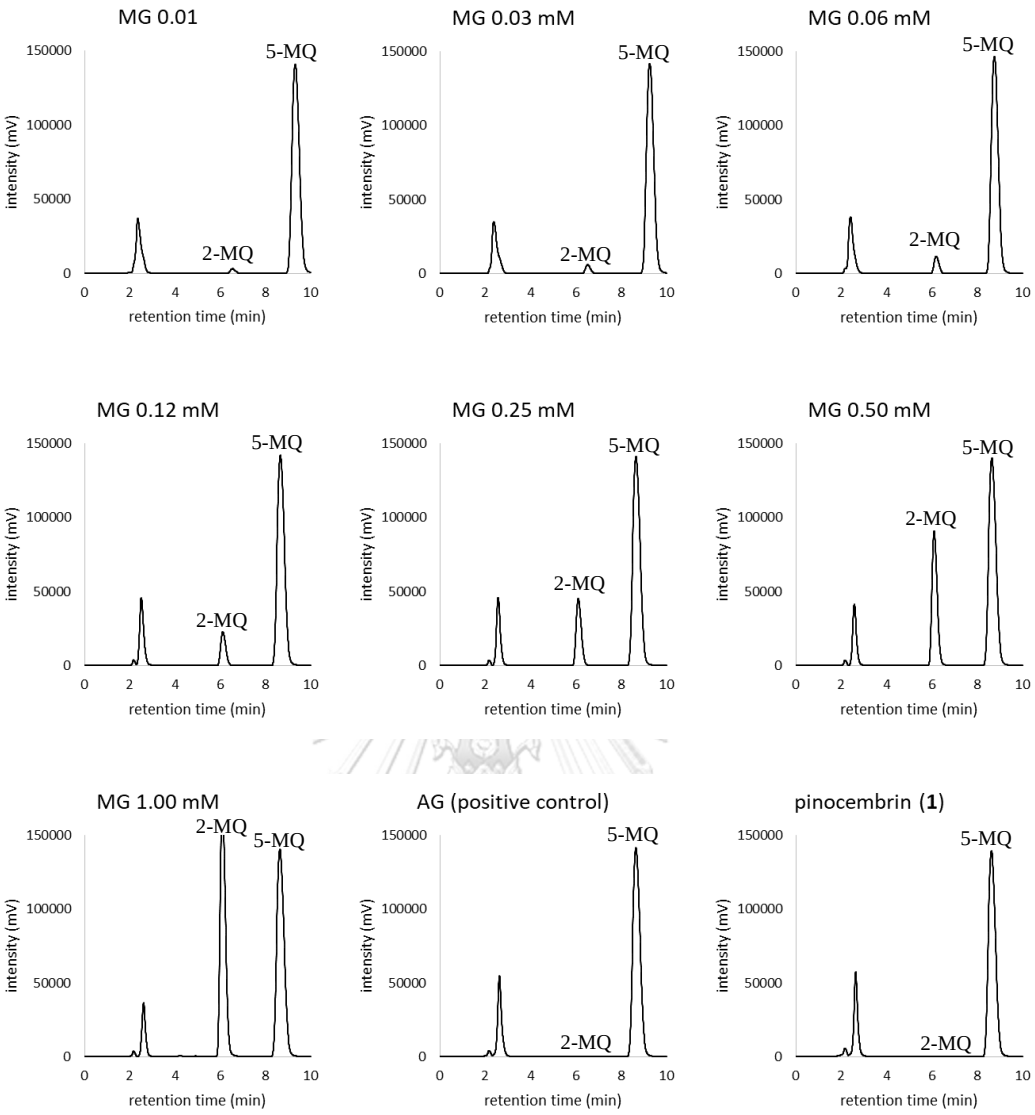

Figure 2.27 *Inhibition plot of pinicembrin against rat intestinal sucrase.*

CHAPTER III

Lamesticumin G, a new α-glucosidase inhibitor from the fruit peels of *Lansium parasiticum*

3.1 Introduction

Postprandial hyperglycaemia (PH) is an early defect of type 2 diabetes (T2D) and one of typical antidiabetic targets. Regulation of PH can be carried out by inhibiting intestinal α-glucosidase, a critical enzyme of oligosaccharide breakdown. The reduction in the amount of glucose liberated from oligosaccharide and a consequently delayed glucose absorption into the bloodstream can facilitate T2D management. To date, few drugs in this category such as Acarbose and Miglitol have been approved in the US and other countries. In our search for α-glucosidase inhibitors from nature, we have identified a variety of active secondary metabolites from medicinal plant such as *Horsfieldia macrobotrys* [57], *Piper sarmentosum* [58] and *Sesamum indicum* [59]. In the current study, we found a promising inhibition (IC_{50} 2.5-4.3 mg/mL) in the extract of *Lansium parasiticum* fruit peels. An attempt to identify α-glucosidase inhibitors was made.

Lansium parasiticum (Osbeck) Sahni & Bennet (syn *Lansium domesticum* Corrêa; family Meliacaeae) is a popular dessert in Southeast Asia. It has been recognized as an important source of diverse triterpenoids, particularly a rare and unique skeleton of onoceranoids [60-63]. Noticeably, different triterpenoids were also isolated, for example tetarnortriterpenoids from seeds [64-66] and cycloarternoid triterpenes from leaves [67] (Figure 3.1).

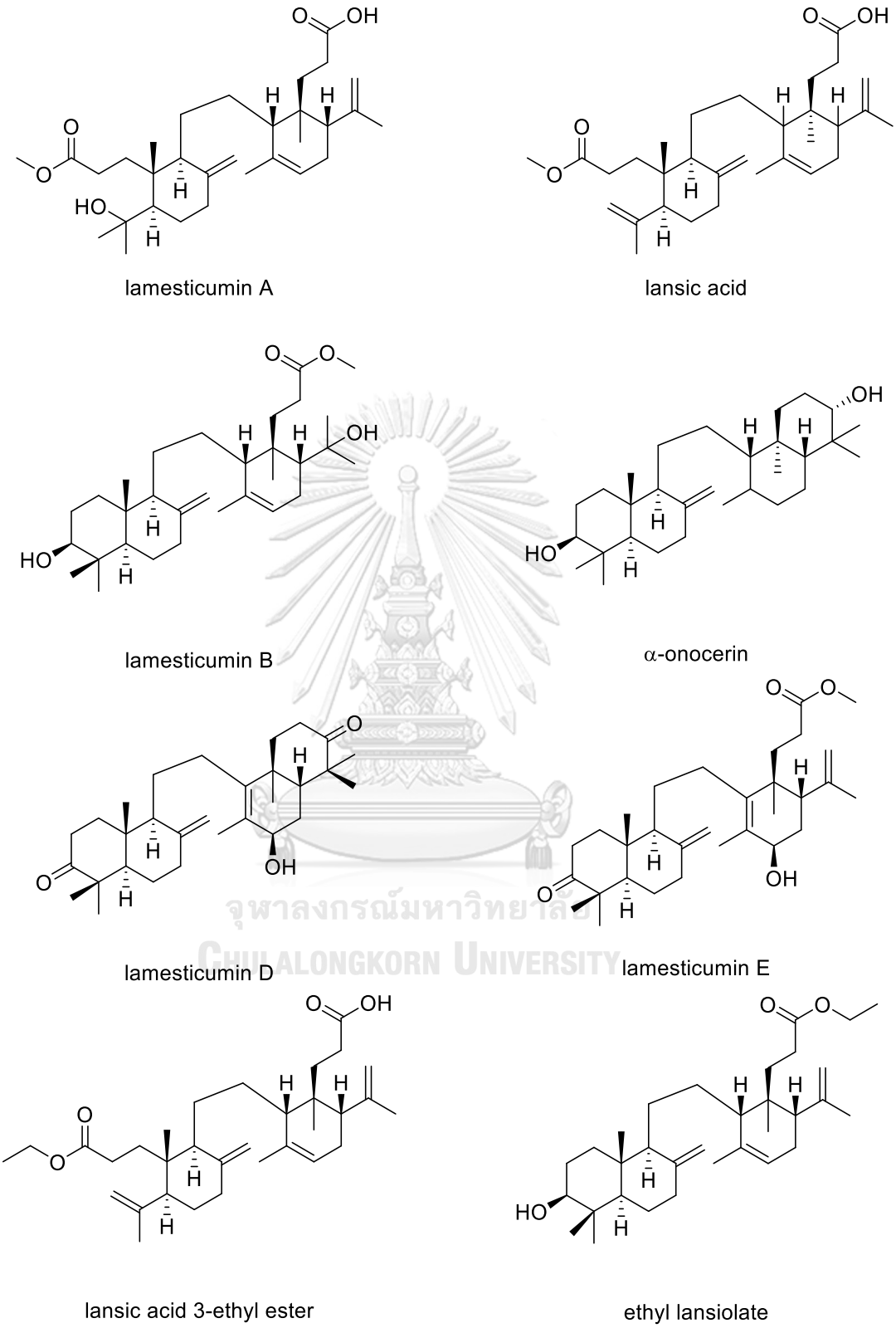

Figure 3.1 *Structures of selected onoceranoid isolated from L. parasiticum.*

3.2 Results and discussion

3.2.1 Extraction and isolation

The air-dried and finely powdered fruit peels of *L. parasiticum* were extracted with MeOH at room temperature, which were then further partitioned with n-hexane and EtOAc, respectively. The EtOAc extract was separated by the silica gel column chromatography using stepwise elution, yielding six major fractions (Figure 3.2). Fraction 2 was subjected to silica gel and Sephadex LH-20 column chromatography to obtain one new onoceranoid triterpene named lamesticumin G (**9**) and four known triterpenoids (**10**-**13**) (Figure 3.3). The structure of the new compound was elucidated on the basis of a detailed spectroscopic analysis including 1D and 2D NMR spectroscopy and mass spectrometry techniques.

3.2.2 Structure elucidation of **9**

Lamesticumin G (**9**) was isolated as amorphous powder. The molecular formula was established as $C_{30}H_{44}O_3$ by the observation of $[M+H]^+$ ion at *m/z* 453.3370, thus indicating nine degrees of unsaturation. It showed resonances assignable to seven singlet methlys (δ_H 0.83, 1.00, 1.08 (6H), 1.10, 1.23 and 1.79 ppm), in which the most downfield signal could be assigned to the methyl attached to alkene. The presence of one exocyclic alkene was suggested by the signals at δ_H 4.69 (brs) and 4.96 (brs) as well as δ_C 107.6 and 147.2.

The typical downfield signals at δ_C 216.3, 214.5 and 198.8 ppm was ascribable to the presence of three ketone, one of which (δ_C 198.8) was assigned to α,β-unsaturated ketone, which was supported by UV absorption band at 249 nm (log ε 4.43). The aforementioned data analysis tentatively suggested that **9** possessed onoceranoid-type triterpene encompassing four cyclic moieties (**A**-**D**) [63]. The core structure of **9** was further confirmed by 2D NMR, especially HMBC and COSY (Figure 3.4). The ketone group at δ_C 216.3 was assigned to C-3 of ring **A** by HMBC correlation of Me-23, Me-24 and CH_2-2 to C-3.

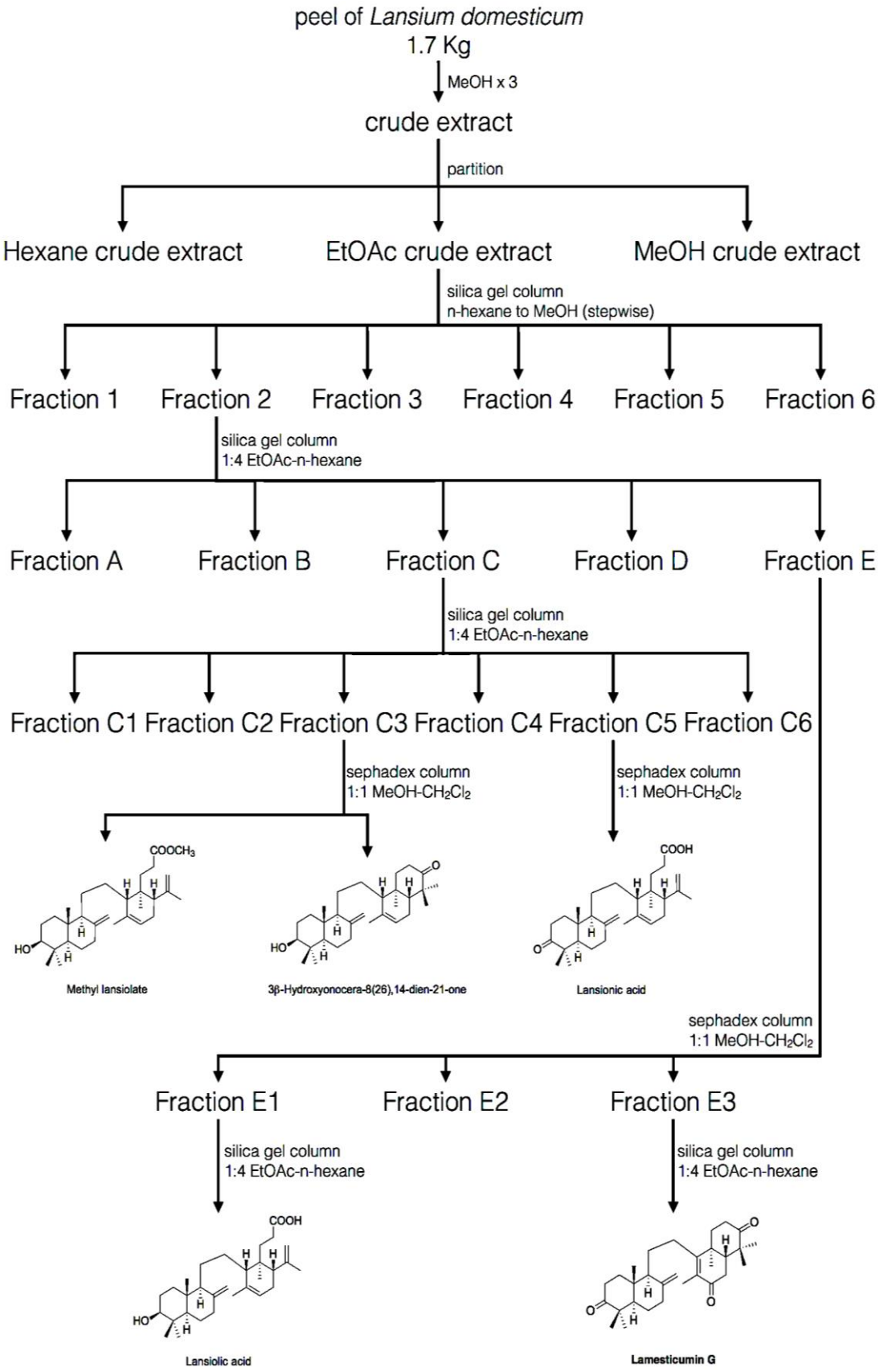

Figure 3.2 *Isolation procedure of isolated compounds.*

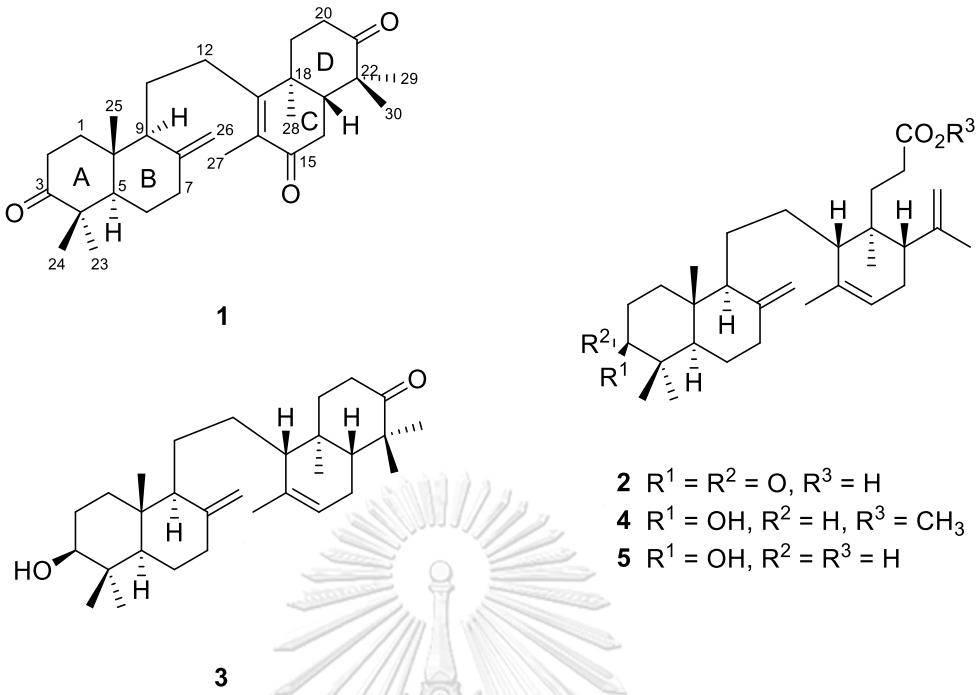

Figure 3.3 *Structures of isolated compounds.*

The exocyclic double bond was located to the C-8 of ring **B** as evident by HMBC cross peaks of CH_2-26 (δ_H 4.96 and 4.69) to the neighboring C-7 (δ_C 38.0), C-8 (δ_C 147.2), and C-9 (δ_C 58.1). The singlet methyl (δ_H 0.83 and δ_C 14.2) was accommodated at C-10 as indicated by the HMBC correlations of Me-25 to C-1 (δ_C 37.8), C-5 (δ_C 55.4), C-9 (δ_C 58.1) and C-10 (δ_C 39.7). Thus, the bicyclic **A**-**B** system was firmly established. In addition, the NMR data (particularly ^{13}C NMR) of this **A**-**B** ring were consistent with those of lansionic acid (**10**) reported by Tanaka [63].

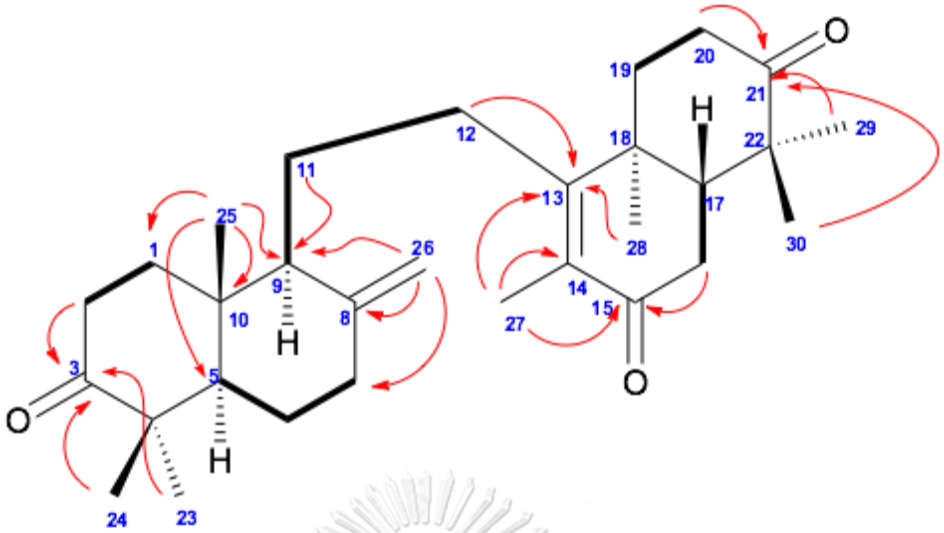

Figure 3.4 *Selected HMBC (arrow curve) and COSY (bold line) correlations of lamesticumin G (**9**).*

The other bicyclic **C**-**D** moiety was constructed based mainly on HMBC data analysis. The HMBC cross peaks of Me-29 (δ_H 1.10), Me-30 (δ_H 1.08) and CH_2-20 (δ_H 2.74 and 2.50) to carbonyl signal at δ_C 214.5 indicated that it was located at C-21 of ring **D**. The α,β-conjugated ketone (C-13 to C-15) was installed as a part of ring **C**, which was supported by HMBC correlations of CH_2-12/C-13, CH_2-16/C-15, Me-28/C-13 along with the cross peaks of Me-27 (δ_H 1.10) to C-13 (δ_C 165.7), C-14 (δ_C 130.9) and C-15 (δ_C 198.8). Therefore, the bicyclic **C**-**D** residue was established. Furthermore, the ^{13}C NMR data of the **C**-**D** moiety were closely related to those reported for lamesticumin C [60].

The bicyclic **A**-**B** and **C**-**D** systems were assembled through the CH_2-11/CH_2-12 dimethylene bridge. The HMBC crosspeaks of CH_2-11/C-9 and H_2-12/C-13 were critical evidences to connect these two bicycles via the C-9 of ring **B** to C-13 of ring **C**, thus establishing the gross structure of lamesticumin G (**9**).

With the entire structure in hand, the relative configuration of lamesticumin G (**9**) was deduced by NOESY experiment (Figure 3.5). The *trans*-fused **A**-**B** ring was assigned by two distinct correlations: Me-24/Me-25 for β-orientation while H-9/H-5/Me-23 for α-orientation. A similar *trans*-fused bicyclic was also observed for the **C**-**D** ring system, as indicated by the NOESY crosspeaks of Me-28/Me-29 and H-17/Me-30.

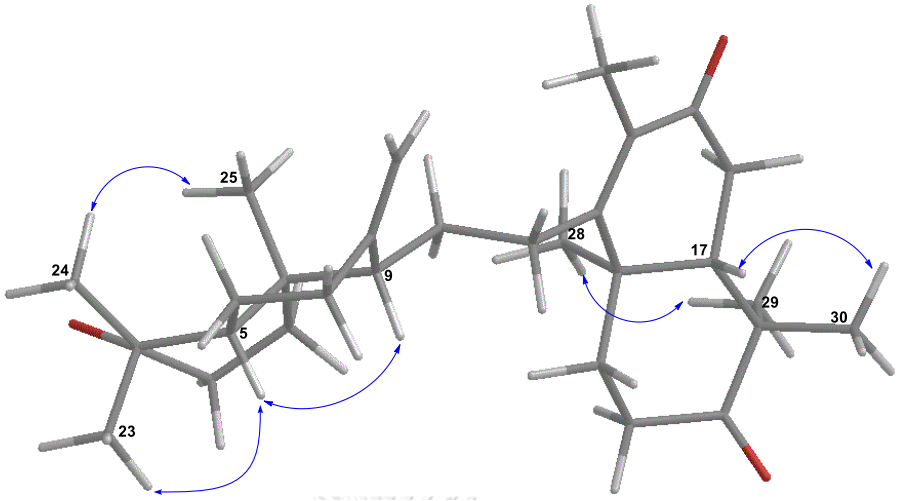

Figure 3.5 *Selected NOESY correlations of lamesticumin G (**9**).*

3.2.3 α-Glucosidase inhibitory activity

Five isolated triterpenoids (**9**-**13**) were assessed for their α-glucosidase inhibitory effect against rat intestinal α-glucosidases (maltase and sucrase) (Table 3.1). Unfortunately, only lamesticumin G (**9**) showed weak inhibition against maltase with an IC_{50} value of 2.27 mM. To our knowledge, this is the first report on α-glucosidase inhibition of onoceranoid triterpenes. The reduced inhibitory activity of isolated compounds (**9**-**13**), compared with the promising activity found in the original extract, prompted us to search for another minor triterpenes or other secondary metabolites, and the results will be published elsewhere in due course.

Table 3.1 α-Glucosidase inhibitory activity of isolated compounds

sample	IC_{50} (mM)	
	maltase	sucrase
Lamesticumin G (**9**)	2.27	NA[a]
Lansionic acid (**10**)	NA	NA
3β-hydroxyonocera-8(26), 14-dien-21-one (**11**)	NA	NA
Methyl lansiolate (**12**)	NA	NA
Lansiolic acid (**13**)	NA	NA
Acarbose®	0.0021	0.026

[a] NA, not active

3.3 Experimental section

3.3.1 General experimental procedures

Optical rotation was measured on a Perkin-Elmer 341 polarimeter using a cell with a 0.3-mL capacity and a 1 cm path length. UV-visible absorption spectra were taken on a UV-7504 spectrophotometer. 1D and 2D NMR spectra were recorded on a Bruker 400 AVANCE spectrometer. High resolution mass spectra were acquired using the Bruker micrOTOF mass spectrometer equipped with an electrospray ionization (ESI) ion source. Chromatography was performed on a Sephadex LH-20 and Merck silica gel 60 (70-230 mesh), and TLC was performed on precoated Merck silica gel 60 F254 plates (0.25 mm thick layer). Acarbose® was obtained from Bayer Vitol Leverkusen, Germany. Rat intestinal acetone powder was supplied by Sigma Aldrich. Spectrophotometric measurements for the α-glucosidase inhibition were taken on a Sunrise microplate reader spectrophotometer.

3.3.2 Plant material

Fruits of *L. parasiticum* (Osbeck) Sahni & Bennet were collected from a local farm in Ra-ngae, Narathiwat, in August 2015. The specimens (NPRU 052-2015) have been deposited at Natural Products Research Unit, Chulalongkorn University.

3.3.3 Extraction and isolation

The air-dried and finely powdered fruit peels of *L. parasiticum* (1.7 Kg) were extracted three times with MeOH at room temperature to give a crude extract. The resulting MeOH extract was suspended in water and extracted with n-hexane and EtOAc, respectively. The EtOAc extract (228 g) was subjected to silica gel column chromatography (Merck Art 7730) and eluted in stepwise fashion with n-hexane, 1.5:8.5 EtOAc-n-hexane, 4:6 EtOAc-n-hexane, 8:2 EtOAc-n-hexane, EtOAc, 1:1 MeOH-EtOAc and MeOH to yield six fractions (fraction 1-6). Fraction 2 was subjected to silica gel column chromatography and eluted with 1:4 EtOAc-n-hexane to give five fractions (fraction A-E). Fraction C was subjected to silica gel column chromatography and eluted with 1:4 EtOAc-n-hexane to yield six sub-fractions (sub-fraction C1-C6). Sub-fraction C3 was subjected to Sephadex LH-20 and eluted with 1:1 MeOH-CH_2Cl_2, yielding lansionic acid (**10**, 378 mg) and 3β-hydroxyonocera-8(26), 14-diene-21-one (**11**, 1.2 g). Sub-fraction

C5 was subjected to Sephadex LH-20 and eluted with 1:1 MeOH-CH_2Cl_2 to yield methyl lansiolate (**12**, 212 mg). Fraction E was subjected to Sephadex LH-20 and eluted with 1:1 MeOH-CH_2Cl_2 to afford three sub-fractions (sub-fraction E1-E3). Sub-fraction E1 was subjected to silica gel column chromatography and eluted with 1:4 EtOAc-CH_2Cl_2 to yield lansiolic acid (**13**, 383 mg). Sub-fraction E3 was subjected to silica gel column chromatography and eluted with 1:4 EtOAc-CH_2Cl_2 to yield lamesticumin G (**9**, 96 mg).

Lamesticumin G (**9**): white amorphous powder; $[\alpha]^{17}_{D}$ + 27.67 (c 0.45, MeOH); UV (MeOH) λ_{max} (log ε) 249 (4.43) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 4.96 and 4.69 (each 1H, brs, H-26), 2.74 and 2.50 (each 1H, m, H-20), 2.63 and 2.41 (each 1H, m, H-2), 2.56 and 1.87 (each 1H, m, H-12), 2.48 (2H, m, H-7), 2.47 (2H, m, H-16), 2.16 (1H, m, H-17), 2.15 and 1.86 (each 1H, m, H-19), 2.01 and 1.55 (each 1H, m, H-1), 1.79 (3H, s, H-27), 1.72 and 1.51 (each 1H, m, H-6), 1.71 (1H, m, H-9), 1.66 and 1.48 (each 1H, m, H-11), 1.60 (1H, m, H-5), 1.23 (3H, s, CH_3-28), 1.10 (3H, s, CH_3-29), 1.08 (3H, s, CH_3-23), 1.08 (3H, s, CH_3-30), 1.00 (3H, s, CH_3-24), 0.83 (3H, s, CH_3-25); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 216.3 (C-3), 214.5 (C-21), 198.8 (C-15), 165.7 (C-13), 147.2 (C-8), 130.9 (C-14), 107.6 (C-26), 58.1 (C-9), 55.4 (C-5), 49.7 (C-17), 47.9 (C-4), 47.1 (C-22), 40.4 (C-18), 39.7 (C-10), 38.0 (C-7), 37.8 (C-1), 35.5 (C-16), 35.1 (C-19), 34.7 (C-2), 34.5 (C-20), 29.8 (C-12), 26.1 (C-30), 25.6 (C-23), 25.3 (C-6), 23.4 (C-11), 21.7 (C-24), 21.4 (C-29), 17.9 (C-28), 14.2 (C-25), 12.0 (C-27); HRESIMS *m/z* 453.3370 $[M+H]^+$ (calcd for $C_{30}H_{45}O_3$, 453.3369).

3.3.4 α-Glucosidase inhibitory activity

Rat intestinal α-glucosidase inhibitory activity was assessed according to our previous protocol [57]. The crude enzyme solution was prepared from rat intestinal acetone powder and applied as a source of maltase and sucrase. Rat intestinal acetone powder (1 g) was initially blend in 0.9% NaCl solution (30 mL). After centrifugation (12,000 rpm) for 30 min, the supernatant was subjected to assay. The isolated compounds (1 mg/mL in DMSO, 10 µL) were mixed with 0.1 M phosphate buffer (pH 6.9, 30 µL), 20 µL of the substrate solution (10 mM maltose; 100 mM sucrose) in 0.1 M phosphate buffer, glucose kit (80 µL), and the crude enzyme solution (20 µL). The reaction mixture was subsequently incubated at 37 °C for either 10 min (for maltose) or 40 min (for sucrose). The glucose concentration liberated from the reaction was

detected by the glucose oxidase method using a glu-kit (Human, Germany). Enzymatic activity was quantified by measuring absorbance at 503 nm. The percentage inhibition was determined by $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance without the sample, and A_1 is the absorbance with the sample. The IC_{50} value was deduced from a plot of percentage inhibition versus sample concentration. Acarbose® was used as the standard control and showed the IC_{50} value of 2.1 μM (maltase) and 26 μM (sucrase). The experiment was carried out in triplicate.

3.4 Conclusion

The EtOAc extract from fruit peels of *L. parasiticum* comprised a new onoceranoid triterpene named lamesticumin G (**9**) and four related triterpenoids (**10**-**13**). Of isolated compounds, **9** showed the inhibition against maltase with IC_{50} value of 2.27 mM while compounds **10**-**13** were not active against target enzymes. The onoceranoid has been recognized as a rare triterpenoid found in nature, and its **α**-glucosidase inhibitory effect was also herein first reported.

Supporting information

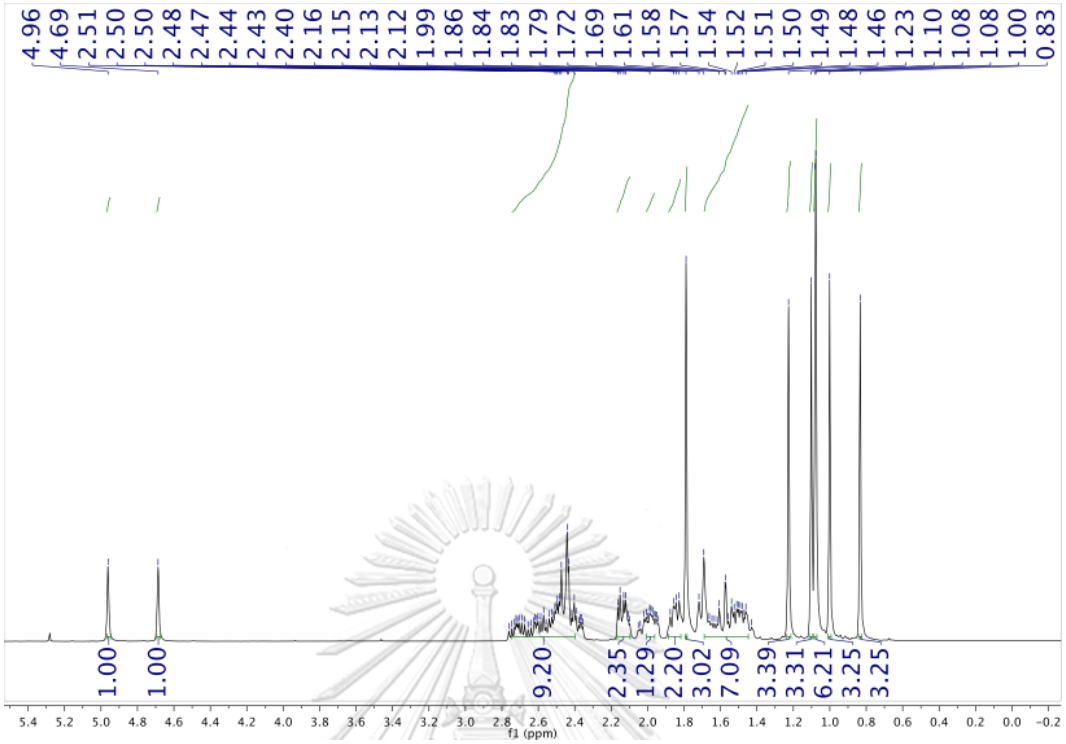

Figure 3.6 *^{1}H NMR spectrum (400 MHz, $CDCl_3$) of lamesticumin G (**9**).*

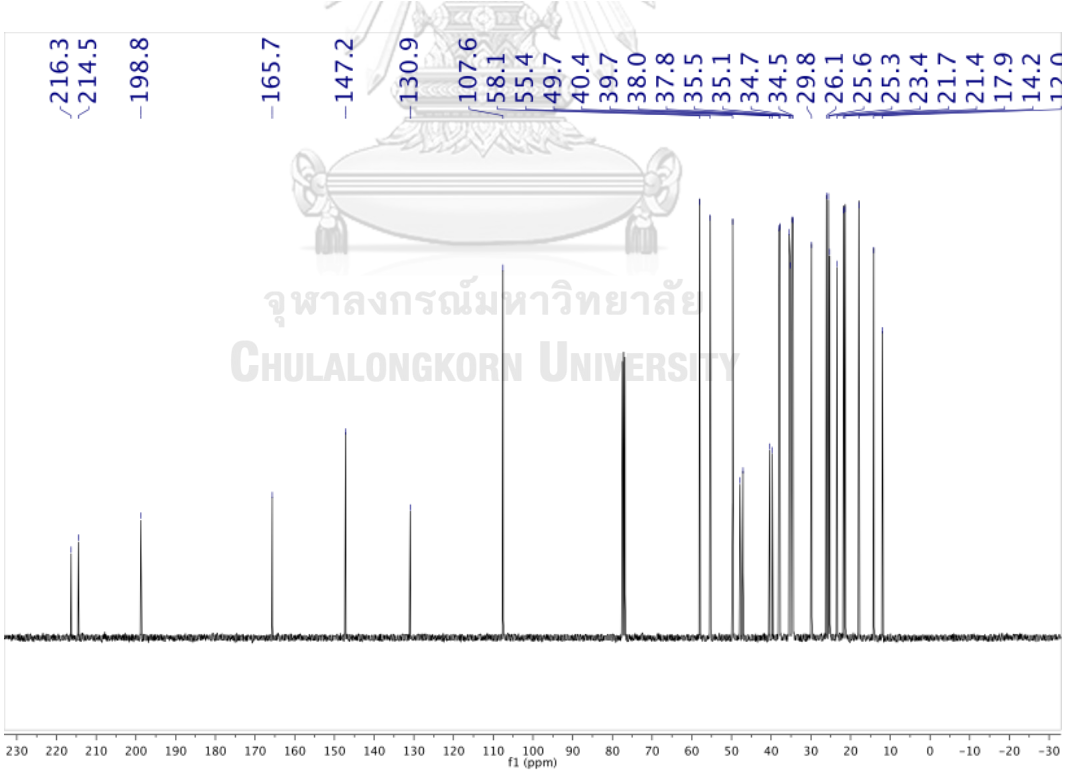

Figure 3.7 *^{13}C NMR spectrum (100 MHz, $CDCl_3$) of lamesticumin G (**9**).*

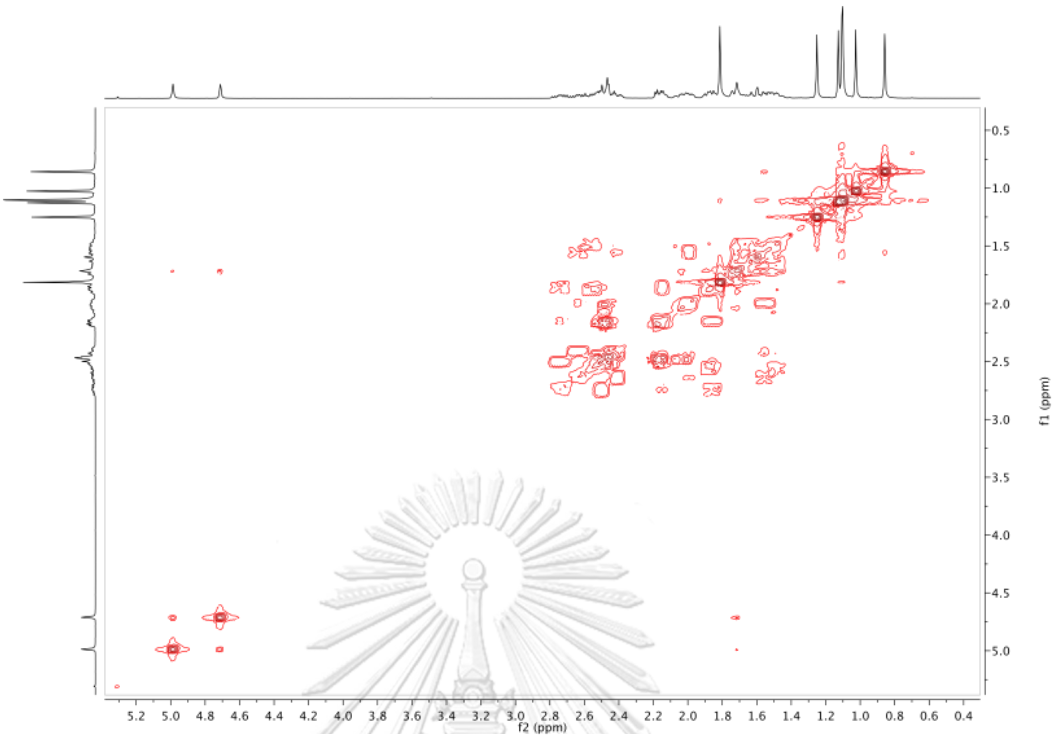

Figure 3.8 *COSY spectrum of lamesticumin G (**9**).*

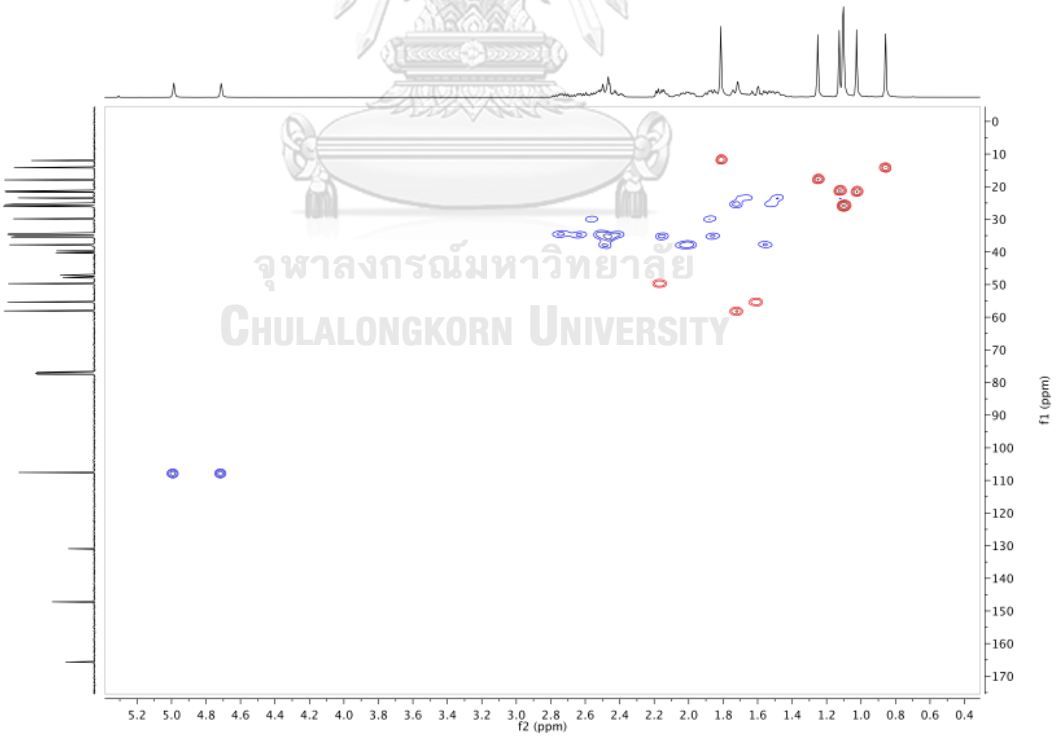

Figure 3.9 *HSQC spectrum of lamesticumin G (**9**).*

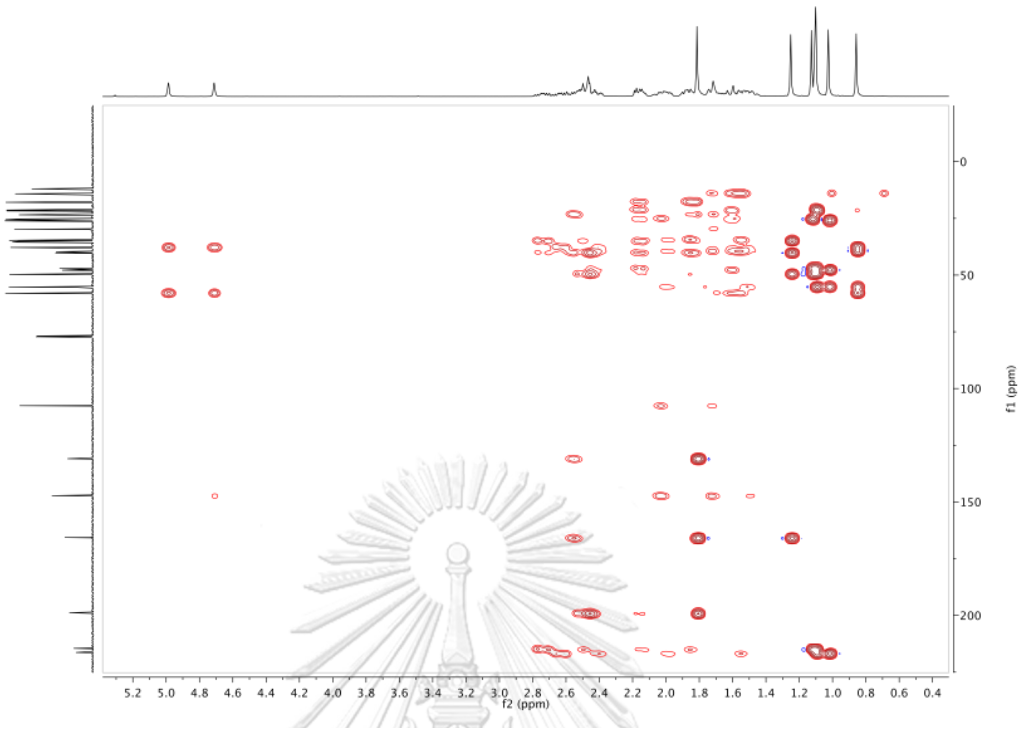

Figure 3.10 *HMBC spectrum of lamesticumin G (**9**).*

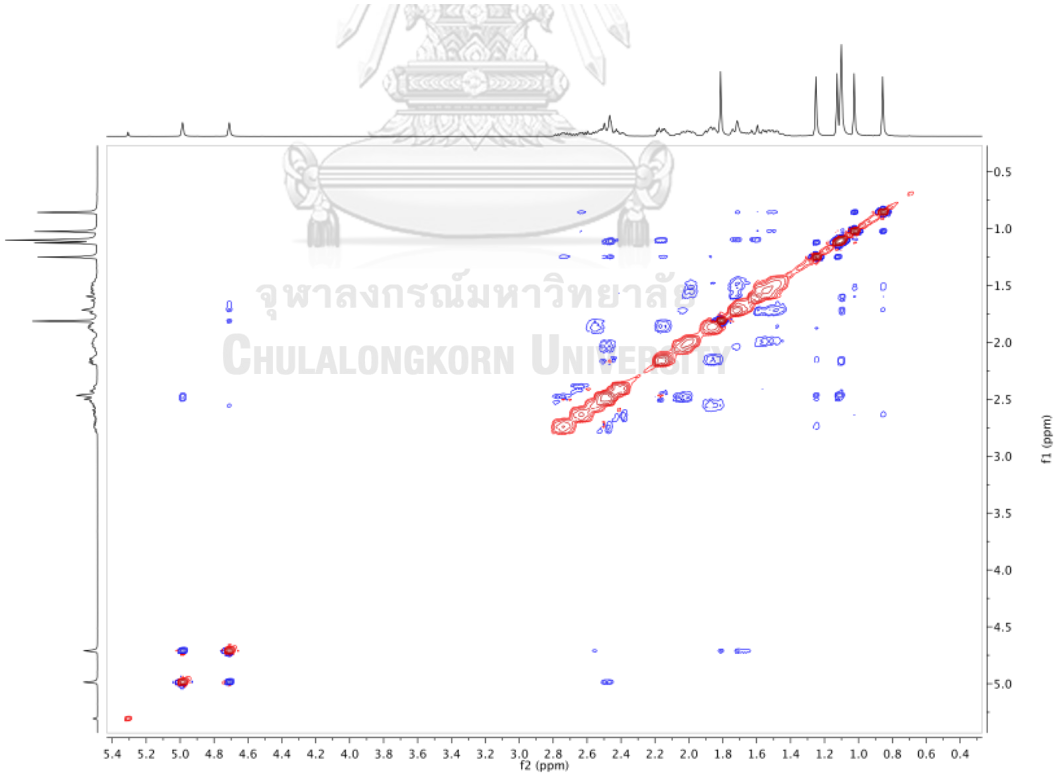

Figure 3.11 *NOESY spectrum of lamesticumin G (**9**).*

Table 3.2 α-Glucosidase inhibitory activity (maltase) of isolated compounds

sample	concentration (mg/mL)	final concentration (mg/mL)	% inhibition	IC_{50} (mM)
Lansionic acid	0.04	0.0025	-14.90	-
	0.20	0.0125	-2.95	
	1.00	0.0625	-0.92	
	5.00	0.3125	5.14	
3β-hydroxyonocera-8(26), 14-dien-21-one	0.04	0.0025	1.26	-
	0.20	0.0125	8.34	
	1.00	0.0625	4.49	
	5.00	0.3125	-14.88	
Methyl lansiolate	0.04	0.0025	-18.90	-
	0.20	0.0125	-3.60	
	1.00	0.0625	6.98	
	5.00	0.3125	-13.73	
Lansiolic acid	0.04	0.0025	-6.72	-
	0.20	0.0125	-4.68	
	1.00	0.0625	-2.14	
	5.00	0.3125	-18.72	
Lamesticumin G	0.04	0.0025	3.33	2.27
	0.20	0.0125	4.69	
	1.00	0.0625	8.80	
	5.00	0.3125	17.93	
Acarbose®	0.0016	0.0001	15	0.0021
	0.0080	0.0005	32	
	0.0400	0.0025	61	
	0.2000	0.0125	78	

Table 3.3 α-Glucosidase inhibitory activity (sucrase) of isolated compounds

sample	concentration (mg/mL)	final concentration (mg/mL)	% inhibition	IC50 (mM)
Lansionic acid	0.04	0.0025	33.00	-
	0.20	0.0125	29.28	
	1.00	0.0625	32.05	
	5.00	0.3125	26.61	
3β-hydroxyonocera-8(26), 14-dien-21-one	0.04	0.0025	-9.89	-
	0.20	0.0125	-3.59	
	1.00	0.0625	-2.71	
	5.00	0.3125	-20.69	
Methyl lansiolate	0.04	0.0025	10.26	-
	0.20	0.0125	29.01	
	1.00	0.0625	-47.84	
	5.00	0.3125	11.09	
Lansiolic acid	0.04	0.0025	30.88	-
	0.20	0.0125	20.24	
	1.00	0.0625	21.78	
	5.00	0.3125	8.43	
Lamesticumin G	0.04	0.0025	-7.25	-
	0.20	0.0125	-0.80	
	1.00	0.0625	-3.15	
	5.00	0.3125	6.58	
Acarbose®	0.008	0.0005	19	0.026
	0.040	0.0025	35	
	0.200	0.0125	46	
	1.000	0.0625	57	

CHAPTER VI
CONCLUSION

In this research, fingerroot was extracted for study the structure-activity relationship (SAR) on MG-trapping activities. Three flavanones (pinocembrin, pinostrobin, and alpinetin), two chalcones (cardamomin and boesenbergin B), two dihydrochalcones (panduratin A and isopanduratin A), and one kavalactone (demethoxyyangonin) were isolated from the dichloromethane crude extract of fingerroot rhizomes. All isolated compounds were tested for their inhibitory activity on glycation between methlyglyoxal (MG) and bovine serum albumin (BSA). Most of isolated compounds showed higher AGEs formation inhibition than antiglycating agent aminoguanidine (AG, 28%). Moreover, the studies of structure-activity relationship (SAR) were investigated in this research using the comparison of the structures of flavonoids with their MG-trapping activities, the following structural requirements of flavonoids for the activity were obtained. (1) The presence of methoxy group substitution on aromatic ring A of flavanone significantly reduces the activity. (2) The positions of methoxy group substitution on aromatic ring A of flavanone are no significant difference. (3) The activity of ring C structure of flavanone is significantly lower than that of α-β unsaturated ketone structure of chalcone. (4) The presence of geranyl group substitution on chalcone significantly decreased activity. (5) The positions of geranyl group substitution of ring A (chalcone) or at α-β unsaturated ketone (dihydrochalcone) are no significant difference. (6) The activity of methoxy group at C4 position was significantly higher than the activity of methoxy group at C6 position of dihydrochalcone. Of the compounds examined, pinocembrin (**1**) displayed the highest MG-trapping activity with the value of 9% more than AG and displayed high efficiency against methlyglyoxal with EC_{50} values of 63.22 ± 10.12 μM. In addition, this is the first time that pinocembrin was tested for α-glucosidase inhibitory activity. Pinocembrin displayed moderate inhibition against both maltase and sucrase with IC_{50} values of 0.35 ± 0.021 and 0.39 ± 0.020 mM, respectively. Furthermore, pinocembrin inhibited maltase and sucrase in a mixed-type manner comprising two different pathways, competitive and noncompetitive.

In the second part, the EtOAc extract from fruit peels of *L. parasiticum* comprised a new onoceranoid triterpene named lamesticumin G (**9**) and four related triterpenoids (**10**-**13**). Of isolated compounds, **9** showed the inhibition against maltase with IC_{50} value of 2.27 mM while compounds **10**-**13** were not active against target enzymes. The onoceranoid has been recognized as a rare triterpenoid found in nature, and its **α**-glucosidase inhibitory effect was also herein first reported.

REFERENCES

[1] Skyler, J.S. Diabetes Mellitus: Pathogenesis and Treatment Strategies. Journal of Medicinal Chemistry 47(17) (2004): 4113-4117.

[2] Sharma, D.K., Pandey, J., Tamrakar, A.K., and Mukherjee, D. Synthesis of heteroaryl/aryl kojic acid conjugates as stimulators of glucose uptake by GLUT4 translocation. European Journal of Medicinal Chemistry 85 (2014): 727-736.

[3] A., C. and P., D. Effects of insulin and other antihyperglycaemic agents on lipid profiles of patients with diabetes. Diabetes, Obesity and Metabolism 13(10) (2011): 869-879.

[4] Johansen, J.S., Harris, A.K., Rychly, D.J., and Ergul, A. Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. Cardiovascular Diabetology 4(1) (2005): 5.

[5] C., M.A., A., S.R., and B., W.J. Diabetes, oxidative stress, and antioxidants: A review. Journal of Biochemical and Molecular Toxicology 17(1) (2003): 24-38.

[6] Evans, J.L., Goldfine, I.D., Maddux, B.A., and Grodsky, G.M. Oxidative Stress and Stress-Activated Signaling Pathways: A Unifying Hypothesis of Type 2 Diabetes. Endocrine Reviews 23(5) (2002): 599-622.

[7] Tuomi, T. Type 1 and type 2 diabetes: What do they have in common? Diabetes 54(SUPPL. 2) (2005): S40-S45.

[8] Costanian, C., Bennett, K., Hwalla, N., Assaad, S., and Sibai, A.M. Prevalence, correlates and management of type 2 diabetes mellitus in Lebanon: Findings from a national population-based study. Diabetes Research and Clinical Practice 105(3) (2014): 408-415.

[9] Chen, L., Magliano, D.J., and Zimmet, P.Z. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. Nature Reviews Endocrinology 8 (2011): 228.

[10] Krentz, A.J. and Bailey, C.J. Oral Antidiabetic Agents. Drugs 65(3) (2005): 385-411.

[11] Moorthy, N.S.H.N., Ramos, M.J., and Fernandes, P.A. Studies on α-Glucosidase Inhibitors Development: Magic Molecules for the Treatment of Carbohydrate Mediated Diseases. Mini-Reviews in Medicinal Chemistry 12(8) (2012): 713-720.

[12] Asano, N. Glycosidase inhibitors: update and perspectives on practical use. Glycobiology 13(10) (2003): 93R-104R.

[13] Rabbani, N. and Thornalley, P.J. Glyoxalase in diabetes, obesity and related disorders. Seminars in Cell & Developmental Biology 22(3) (2011): 309-317.

[14] Lv, L., Shao, X., Chen, H., Ho, C.-T., and Sang, S. Genistein Inhibits Advanced Glycation End Product Formation by Trapping Methylglyoxal. Chemical Research in Toxicology 24(4) (2011): 579-586.

[15] Dukic-Stefanovic, S., Schinzel, R., Riederer, P., and Münch*, G. AGES in brain ageing: AGE-inhibitors as neuroprotective and anti-dementia drugs? Biogerontology 2(1) (2001): 19-34.

[16] Bierhaus, A., Hofmann, M.A., Ziegler, R., and Nawroth, P.P. AGEs and their interaction with AGE-receptors in vascular disease and diabetes mellitus. I. The AGE concept. Cardiovascular Research 37(3) (1998): 586-600.

[17] Marta, M., Marta, N., Vural, G., and J, M.F. Antiglycative effect of fruit and vegetable seed extracts: inhibition of AGE formation and carbonyl‐trapping abilities. Journal of the Science of Food and Agriculture 93(8) (2013): 2037-2044.

[18] Houda, M., et al. Combined anti-ages and antioxidant activities of different solvent extracts of Solanum elaeagnifolium Cav (Solanacea) fruits during ripening and related to their phytochemical compositions. EXCLI Journal 13 (2014): 1029-1042.

[19] Chong, T., et al. Boesenbergia rotunda: From Ethnomedicine to Drug Discovery. Vol. 2012, 2012.

[20] Odjakova, M., Popova, E., Al Sharif, M., and Mironova, R. Plant-Derived Agents with Anti-Glycation Activity. 2012.

[21] Pashikanti, S., de Alba, D.R., Boissonneault, G.A., and Cervantes-Laurean, D. Rutin metabolites: Novel inhibitors of nonoxidative advanced glycation end products. Free Radical Biology and Medicine 48(5) (2010): 656-663.

[22] Wang, Y. and Ho, C.-T. Flavour chemistry of methylglyoxal and glyoxal. Chemical Society Reviews 41(11) (2012): 4140-4149.

[23] Peng, X., et al. Inhibitory effect of mung bean extract and its constituents vitexin and isovitexin on the formation of advanced glycation endproducts. Food Chemistry 106(2) (2008): 475-481.

[24] Noda, Y. and Peterson, D.G. Structure–Reactivity Relationships of Flavan-3-ols on Product Generation in Aqueous Glucose/Glycine Model Systems. Journal of Agricultural and Food Chemistry 55(9) (2007): 3686-3691.

[25] McCarter, J.D. and Stephen Withers, G. Mechanisms of enzymatic glycoside hydrolysis. Current Opinion in Structural Biology 4(6) (1994): 885-892.

[26] Borges de Melo, E., da Silveira Gomes, A., and Carvalho, I. α- and β-Glucosidase inhibitors: chemical structure and biological activity. Tetrahedron 62(44) (2006): 10277-10302.

[27] Trapero, A. and Llebaria, A. A Prospect for Pyrrolidine Iminosugars as Antidiabetic α-Glucosidase Inhibitors. Journal of Medicinal Chemistry 55(23) (2012): 10345-10346.

[28] Yoon, S.-H. and Robyt, J.F. Addition of maltodextrins to the nonreducing-end of acarbose by reaction of acarbose with cyclomaltohexaose and cyclomaltodextrin glucanyltransferase. Carbohydrate Research 337(6) (2002): 509-516.

[29] Gravier-Pelletier, C., Maton, W., Dintinger, T., Tellier, C., and Le Merrer, Y. Synthesis and glycosidase inhibitory activity of aminocyclitols with a C6- or a C7-ring. Tetrahedron 59(44) (2003): 8705-8720.

[30] El Blidi, L., Ahbala, M., Bolte, J., and Lemaire, M. Straightforward chemo-enzymatic synthesis of new aminocyclitols, analogues of valiolamine and their evaluation as glycosidase inhibitors. Tetrahedron: Asymmetry 17(18) (2006): 2684-2688.

[31] Preeti, G., John, P.A.P., Suman, R.Y., and D., V.Y. Synthesis of Aminocyclitols and Trihydroxylated Indolizidinone from a D-Mannitol-Derived Common Building Block. European Journal of Organic Chemistry 2011(6) (2011): 1166-1175.

[32] Goh, S.G.K., Rusli, B.N., and Khalid, B.A.K. Evolution of diabetes management in the 21st century: The contribution of quality of life measurement in Asians. Asia Pacific Journal of Clinical Nutrition 24(2) (2015): 190-198.

[33] Poulsen, M.W., et al. Advanced glycation endproducts in food and their effects on health. Food and Chemical Toxicology 60 (2013): 10-37.

[34] Lapolla, A., et al. Glyoxal and methylglyoxal levels in diabetic patients: Quantitative determination by a new GC/MS method. Clinical Chemistry and Laboratory Medicine 41(9) (2003): 1166-1173.

[35] Lin, J.-A., Wu, C.-H., and Yen, G.-C. Perspective of Advanced Glycation End Products on Human Health. Journal of Agricultural and Food Chemistry 66(9) (2018): 2065-2070.

[36] Odani, H., Shinzato, T., Matsumoto, Y., Usami, J., and Maeda, K. Increase in Three α,β-Dicarbonyl Compound Levels in Human Uremic Plasma: Specificin VivoDetermination of Intermediates in Advanced Maillard Reaction. Biochemical and Biophysical Research Communications 256(1) (1999): 89-93.

[37] Yeh, W.J., Hsia, S.M., Lee, W.H., and Wu, C.H. Polyphenols with antiglycation activity and mechanisms of action: A review of recent findings. Journal of Food and Drug Analysis 25(1) (2017): 84-92.

[38] Price, C.L. and Knight, S.C. Advanced glycation: A novel outlook on atherosclerosis. Current Pharmaceutical Design 13(36) (2007): 3681-3687.

[39] Richard, J.P. Mechanism for the formation of methylglyoxal from triosephosphates. Biochemical Society Transactions 21(2) (1993): 549-553.

[40] Freedman, B.I., et al. Design and Baseline Characteristics for the Aminoguanidine Clinical Trial in Overt Type 2 Diabetic Nephropathy (ACTION II). Controlled Clinical Trials 20(5) (1999): 493-510.

[41] Elosta, A., Ghous, T., and Ahmed, N. Natural products as Anti-glycation agents: Possible therapeutic potential for diabetic complications. Current Diabetes Reviews 8(2) (2012): 92-108.

[42] Eng-Chong, T., et al. Boesenbergia rotunda: From ethnomedicine to drug discovery. Evidence-based Complementary and Alternative Medicine 2012 (2012).

[43] Ongwisespaiboon, O. and Jiraungkoorskul, W. Fingerroot, Boesenbergia rotunda and its aphrodisiac activity. Pharmacognosy Reviews 11(21) (2017): 27-30.

[44] Ma, H., Liu, W., Frost, L., Kirschenbaum, L.J., Dain, J.A., and Seeram, N.P. Glucitol-core containing gallotannins inhibit the formation of advanced glycation end-products mediated by their antioxidant potential. Food and Function 7(5) (2016): 2213-2222.

[45] Sompong, W. and Adisakwattana, S. Inhibitory effect of herbal medicines and their trapping abilities against methylglyoxal-derived advanced glycation end-products. BMC Complementary and Alternative Medicine 15(1) (2015).

[46] Sompong, W., Cheng, H., and Adisakwattana, S. Ferulic acid prevents methylglyoxal-induced protein glycation, DNA damage, and apoptosis in pancreatic β-cells. Journal of Physiology and Biochemistry 73(1) (2017): 121-131.

[47] Wu, C.H. and Yen, G.C. Inhibitory effect of naturally occurring flavonoids on the formation of advanced glycation endproducts. Journal of Agricultural and Food Chemistry 53(8) (2005): 3167-3173.

[48] Hwang, S.H., Wang, Z., Quispe, Y.N.G., Lim, S.S., and Yu, J.M. Evaluation of aldose reductase, protein glycation, and antioxidant inhibitory activities of bioactive flavonoids in Matricaria recutita L. and their structure-activity relationship. Journal of Diabetes Research 2018 (2018).

[49] Matsuda, H., Wang, T., Managi, H., and Yoshikawa, M. Structural requirements of flavonoids for inhibition of protein glycation and radical scavenging activities. Bioorganic & Medicinal Chemistry 11(24) (2003): 5317-5323.

[50] Rezazadeh, S., Ebrahimi, A., and Nowroozi, A. The effects of structural properties on the methylglyoxal scavenging mechanism of flavonoid aglycones: A quantum mechanical study. Computational and Theoretical Chemistry 1118 (2017): 26-38.

[51] Shao, X., Chen, H., Zhu, Y., Sedighi, R., Ho, C.-T., and Sang, S. Essential Structural Requirements and Additive Effects for Flavonoids to Scavenge Methylglyoxal. Journal of Agricultural and Food Chemistry 62(14) (2014): 3202-3210.

[52] Jaipetch, T., et al. Constituents of Boesenbergia pandurata (syn. Kaempferia pandurata): Isolation, Crystal Structure and Synthesis of (±)-Boesenbergin A. Australian Journal of Chemistry 35(2) (1982): 351-361.

[53] Itokawa, H., Morita, M., and Mihashi, S. Phenolic compounds from the rhizomes of Alpinia speciosa. Phytochemistry 20(11) (1981): 2503-2506.

[54] Ranjith W. Dharmaratne, H., Dhammika Nanayakkara, N.P., and Khan, I.A. Kavalactones from Piper methysticum, and their 13C NMR spectroscopic analyses. Phytochemistry 59(4) (2002): 429-433.

[55] Pandji, C., Grimm, C., Wray, V., Witte, L., and Proksch, P. Insecticidal constituents from four species of the zingiberaceae. Phytochemistry 34(2) (1993): 415-419.

[56] Tuntiwachwuttikul, P., Pancharoen, O., Reutrakul, V., and Byrne, L.T. (1′RS, 2′SR, 6′RS)-(2, 6-Dihydroxy-4-methoxyphenyl)-[3′-methyl-2′-(3″-methylbut-2″-enyl)-6′-phenyl-cyclohex-3′-enyllmethanone (Panduratin A)- a Constituent of the Red Rhizomes of a Variety of Boesenbergia pandurata. Australian Journal of Chemistry 37(2) (1984): 449-453.

[57] Ramadhan, R. and Phuwapraisirisan, P. New arylalkanones from Horsfieldia macrobotrys, effective antidiabetic agents concomitantly inhibiting α-glucosidase and free radicals. Bioorganic & Medicinal Chemistry Letters 25(20) (2015): 4529-4533.

[58] Damsud, T., Adisakwattana, S., and Phuwapraisirisan, P. Three new phenylpropanoyl amides from the leaves of Piper sarmentosum and their α-glucosidase inhibitory activities. Phytochemistry Letters 6(3) (2013): 350-354.

[59] Wikul, A., Damsud, T., Kataoka, K., and Phuwapraisirisan, P. (+)-Pinoresinol is a putative hypoglycemic agent in defatted sesame (Sesamum indicum) seeds though inhibiting α-glucosidase. Bioorganic & Medicinal Chemistry Letters 22(16) (2012): 5215-5217.

[60] Dong, S.H., Zhang, C.R., Dong, L., Wu, Y., and Yue, J.M. Onoceranoid-type triterpenoids from Lansium domesticum. Journal of Natural Products 74(5) (2011): 1042-1048.

[61] Nishizawa, M., Nishide, H., Hayashi, Y., and Kosela, S. The structure of Lansioside A : A novel triterpene glycoside with amino-sugar from lansium domesticum. Tetrahedron Letters 23(13) (1982): 1349-1350.

[62] Nishizawa, M., Nishide, H., Kosela, S., and Hayashi, Y. Structure of lansiosides: biologically active new triterpene glycosides from Lansium domesticum. The Journal of Organic Chemistry 48(24) (1983): 4462-4466.

[63] Tanaka, T., et al. New Onoceranoid Triterpene Constituents from Lansium domesticum. Journal of Natural Products 65(11) (2002): 1709-1711.

[64] Mayanti, T., Tjokronegoro, R., Supratman, U., Mukhtar, M.R., Awang, K., and Hadi, A.H.A. Antifeedant triterpenoids from the seeds and bark of Lansium domesticum cv kokossan (Meliaceae). Molecules 16(4) (2011): 2785-2795.

[65] Nishizawa, M., Nademoto, Y., Sastrapradja, S., Shiro, M., and Hayashi, Y. Dukunolide D, E and F: New tetranortriterpenoids from the seeds of Lansium domesticum. Phytochemistry 27(1) (1988): 237-239.

[66] Saewan, N., Sutherland, J.D., and Chantrapromma, K. Antimalarial tetranortriterpenoids from the seeds of Lansium domesticum Corr. Phytochemistry 67(20) (2006): 2288-2293.

[67] Nishizawa, M., et al. Isolation of a new cycloartanoid triterpene from leaves of Lansium domesticum novel skin-tumor promotion inhibitors. Tetrahedron Letters 30(41) (1989): 5615-5618.

VITA

NAME	Mr. Thammatee Potipiranun
DATE OF BIRTH	18 September 1983
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTIONS ATTENDED	Bachelor Degree of Science, majoring in Biology from Kasetsart University in 2005 Master Degree in Department of Biotechnology, Faculty of Science, Mahidol University in 2011
HOME ADDRESS	1150 Soi. 24 Sinthon Village, Happyland Road, Klongjan, Bangkapi, Bangkok, Thailand.
PUBLICATION	Thammatee Potipiranun, Wisuttaya Worawalai and Preecha Phuwapraisirisan. Lamesticumin G, a new α-glucosidase inhibitor from the fruit peels of Lansium parasiticum. Natural Product Research, 2018, 32, 1881-1886. Thammatee Potipiranun, Sirichai Adisakwattana, Wisuttaya Worawalai, Rico Ramadhan and Preecha Phuwapraisirisan. Identification of Pinocembrin as anti-glycation and anti-diabetic agent from fingerroot (Boesenbergia rotunda): The tentative structure-activity relationship towards MG-trapping activity. Molecules, 2018, Manuscript Submitted.