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PHYSICOCHEMICAL PROPERTIES AND BIOLOGICAL ACTIVITIES OF

MUCILAGINOUS SUBSTANCES FROM SELECTED THAI MEDICINAL PLANTS

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A Thesis Submitted in Partial Fulfillment of the Requirements

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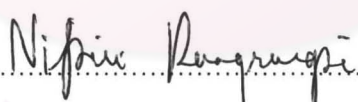Thesis Advisor Associate Professor Nijsiri Ruangrungsi, Ph.D.
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ศึกษาศักยภาพในการต้านเบาหวานชนิดที่ 2 ในหลอดทดลองของโพลีแซกคาไรด์ชนิดสาร
เมือก โดยสกัดสารเมือกจากผักปลัง (ส่วนเหนือดิน) กระเจี๊ยบเขียว (ผล) หมี่หรือหมีเหม็น (ใบ)
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การกักเก็บน้ำตาลในถุงไดอะไลซิส การยับยั้งการทำงานของเอนไซม์แอลฟากลูโคซิเดสและไลเปส
การรบกวนการละลายของไขมันในไมเซลล์ รวมทั้งการต้านอนุมูลอิสระ โดยเปรียบเทียบคุณสมบัติ
ดังกล่าวของสารเมือกที่ศึกษากับกลูโคแมนแนน นอกจากนี้ได้ศึกษาคุณสมบัติทางกายภาพของ
สารเมือก ได้แก่ การพองตัว ความหนืด น้ำหนักโมเลกุลโดยเฉลี่ย และศึกษาคุณสมบัติทางเคมีโดย
วิเคราะห์ชนิดและปริมาณของโมโนแซกคาไรด์ที่เป็นองค์ประกอบโดยวิธีแก๊สโครมาโทกราฟี
วิเคราะห์สารเมือกจากผักปลังเพิ่มเติมโดยใช้เทคนิคโปรตอนเอ็นเอ็มอาร์สเปกโทรสโคปี
ผลการศึกษาพบว่าสารเมือกจากเมล็ดแมงลักมีคุณสมบัติในการกักเก็บน้ำตาลดีกว่ากลูโคแมนแนน
และเป็นสารเมือกที่มีความหนืดสูงสุด สารเมือกจากสำรองมีคุณสมบัติในการยับยั้งการทำงานของ
เอนไซม์แอลฟากลูโคซิเดสดีที่สุด (IC_{50}=1.7 มก/มล) โดยที่ความเข้มข้นร้อยละ 0.5 สามารถยับยั้ง
เอนไซม์แอลฟากลูโคซิเดสได้ร้อยละ 82.6 ขณะที่ 1-ดีออกซีโนจิริมัยซินที่ความเข้มข้นเดียวกันยับยั้ง
การทำงานของเอนไซม์แอลฟากลูโคซิเดสได้ร้อยละ 47.6 สารเมือกจากแมงลัก ลูกซัด และกลูโคแมนแนน
ลดการละลายของคอเลสเทอรอลในไมเซลล์ได้ประมาณร้อยละ 20 สารเมือกที่ศึกษารวมทั้งกลูโค
แมนแนนลดการละลายของกรดโอเลอิกในไมเซลล์ ขณะที่สารเมือกส่วนใหญ่เพิ่มการละลายของ
กรดสเตียริกในไมเซลล์ สารเมือกที่ศึกษาไม่รบกวนการทำงานของเอนไซม์ไลเปสแต่มีผลต่อผิวสัมผัส
ของไมเซลล์ สารเมือกจากหมี่ สำรอง และกระเจี๊ยบเขียวมีคุณสมบัติต้านอนุมูลอิสระ โดยมี IC_{50}
น้อยกว่า 1 มก/มล การศึกษาคุณสมบัติทางเคมีพบกรดกาแลกทูโรนิกในสารเมือกจากผักปลัง เทียน
เกล็ดหอย และสำรอง น้ำตาลแรมโนสพบได้ในสารเมือกทุกชนิดที่ศึกษา การศึกษาสารเมือกจาก
ผักปลังพบว่าเป็นเบตากาแลกแทน และมีองค์ประกอบทางเคมี ได้แก่ อะราบิโนส (ร้อยละ 24) แรมโนส
(ร้อยละ 5) กาแลกโทส (ร้อยละ 41) กรดกาแลกทูโรนิก (ร้อยละ 13) และกลูโคส (ร้อยละ 16)

สาขาวิชา วิจัยเพื่อการพัฒนาสุขภาพ ลายมือชื่อนิสิต ....
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ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์ร่วม ....

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CHANIDA PALANUVEJ : PHYSICOCHEMICAL PROPERTIES AND BIOLOGICAL ACTIVITIES OF MUCILAGINOUS SUBSTANCES FROM SELECTED THAI MEDICINAL PLANTS. THESIS ADVISOR : ASSOC. PROF. NIJSIRI RUANGRUNGSI, Ph.D.THESIS CO-ADVISOR : SANYA HOKPUTSA, Ph.D. 135 pp.

As a continuous searching for anti-diabetic (type II) potential substances, seven mucilaginous polysaccharides from selected plants were studied as follow: aerial parts of *Basella alba* Linn., fruits of *Hibiscus esculentus* Linn., leaves of *Litsea glutinosa* (Lour.) C.B. Robinson, seeds of *Ocimum canum* Sims., seeds of *Plantago ovata* Forssk., fruits of *Scaphium scaphigerum* G. Don. and seeds of *Trigonella foenum-graecum* Linn. The bioactive properties for entrapping glucose against dialysis, inhibiting enzyme α-glucosidase, pancreatic lipase, disturbance on micellar lipids solubilization and free radical scavenger were *in vitro* studied compared to glucomannan. The physical characteristics for swelling properties, viscosity and average molecular weight were determined. The chemical characteristics were analyzed for monosaccharide composition using gas chromatography after methanolysis and TMS-derivatization. *B. alba* mucilage was further investigated by ^{1}H NMR spectroscopy. *O. canum* mucilage superiorly entrapped glucose compared to glucomannan. This activity was relevant to its highly viscous gelation. *S. scaphigerum* showed another property of α-glucosidase inhibition. Its mucilage (0.5%) inhibited the enzyme activity by 82.6%, compared to 1-Deoxynorjirimycin (by 47.6%). The IC_{50} of α-glucosidase inhibitory activity was found as 1.7 mg/ml. Mucilages from *O. canum* and *T. foenum-graecum* as well as glucomannan showed around 20% inhibition on cholesterol solubility in bile acid micelles. All mucilages decreased oleic acid micellar solubilization whilst most of them increased stearic acid micellar solubilization. The studied mucilages had no effect on the lipase activity but affected on the micellar interface. Most mucilages, except from *O. canum* and *P. ovata*, showed DPPH scavenging activity higher than glucomannan. Especially *L. glutinosa*, *S. scaphigerum* and *H. esculentus* mucilages had IC_{50} less than 1 mg/ml. Galacturonic acid was found in 3 from 7 mucilages namely *B. alba*, *P. ovata* and *S. scaphigerum*. Whereas rhamnose was common sugar found in all seven mucilages. Monosaccharide components of these mucilages were compared to another reports from distinguished techniques *B. alba* mucilage was revealed to be β-galactan with the composition of arabinose (24%), rhamnose (5%), galactose (41%), galacturonic acid (13%) and glucose (16%).

Field of Study : __Research for Health Development__ Student's Signature _Chawda Palanuvj_

Academic Year : _2009_ Advisor's Signature _Nijsiri Ruangrungsi_

Co-Advisor's Signature _Sanya Hokputsa_

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LIST OF ABBREVIATIONS

AGEs	=	Advanced glycosylated end products
AOAC	=	Association of Official Analytical Chemists
Ara	=	Arabinose
ATP III	=	Adult Treatment Panel III of the National Cholesterol Education Program
C	=	Carbon
cP	=	Centipoise
D	=	Dextro
D_2O	=	Deuterium oxide
dL	=	Deciliter
DPPH	=	1,1-Diphenyl-2-picrylhydrazyl
EC	=	Enzyme Commission number
f	=	Furanose
g	=	Gram
Gal	=	Galactose
GalA	=	Galacturonic acid
GC	=	Gas chromatography
Glc	=	Glucose
HbA1c	=	Hemoglobin A1c, glycosylated hemoglobin
HCl	=	Hydrochloric acid
HDL	=	High density lipoproteins
HPLC	=	High performance liquid chromatography
HPSEC	=	High performance size exclusive chromatography

IC_{50}	=	Concentration at 50% inhibition
IUB	=	International Union of Biochemistry
IUPAC	=	International Union of Pure and Applied Chemistry
kDa	=	Kilodalton
KGM	=	Konjac Glucomannan
L	=	Levo
L	=	Liter
LDL	=	Low density lipoproteins
M	=	Mole per liter
Man	=	Mannose
mg	=	Milligram
mM	=	Millimole per liter
MW	=	Molecular weight
NaTDC	=	Sodium taurodeoxycholate
nm	=	Nanometer
O	=	Oxygen
p	=	Pyranose
PKC	=	Protein kinase C
PVDF	=	Polyvinylidene fluoride
Rha	=	Rhamnose
TMS	=	trimethylsilyl
VLDL	=	Very low density lipoproteins
Xyl	=	Xylose
α	=	Alpha
β	=	Beta

μl	=	Microliter
μm	=	Micrometer
λmax	=	Wavelength at maximum absorption
^{13}C NMR	=	Carbon Nuclear Magnetic Resonance
^{1}H NMR	=	Proton Nuclear Magnetic Resonance

CHAPTER I

INTRODUCTION

Background and Significance of the Study

Polysaccharides or glycans are the polymer of monosaccharide or glycose units linked together with O-glycosidic bonds. Differences in the chemical properties namely the monosaccharide composition, linkage types and patterns, chain shapes and degree of polymerization dictates the polysaccharide physical properties including solubility, flow behavior, gelling potential, and/ or surface and interfacial properties. The structural diversity also dictates the unique functional properties exhibited by each polysaccharide [1]. Plants are major sources of both starch and non-starch polysaccharides. The latter polysaccharides are nominated as dietary fibers due to their resistance to digestion and absorption by human alimentary digestive enzymes and are interesting as a novel class of dietary supplements especially for diabetic prevention and care.

Diabetes is one of the global burdens of diseases that affect hundreds million of people. This disease is a life threatening condition primarily defined by high level of blood glucose. Diabetic hyperglycemia is associated with micro- and macro-vascular complications that give rise to ischemic heart disease, stroke and peripheral vascular disease. These complications reduce life expectancy as well as quality of life of people with diabetes. Ninety percent of diabetes is type 2 diabetes which is formerly called as non-insulin-dependent or adult-onset diabetes. This common form of diabetes results from insulin resistance in adults. Insulin resistance is strongly associated with dyslipidemia which then increases cardiovascular risk in diabetic type 2 patients. The World Health Organization recommends the simple lifestyle, for example healthy diet, to be effective in preventing or delaying the onset of type 2 diabetes [2-4]. The American Diabetes Association as well as Health Council of the Netherlands recommends that the general population particularly individuals at high risk for type 2 diabetes should be encouraged to intake 14 g of dietary fibers per 1,000 kilocalories (3.4 g per megajoule). Additionally, diabetic patients should be advised to increase their dietary fibers intake (3.6 to 6.0 g per megajoule or 25 to 50 g per day) [5, 6]. Dietary fibers can be categorized into water-soluble (viscous) and non-water-

soluble (non-viscous) dietary fibers. The examples of water-soluble dietary fibers types are gums and mucilages.

Gums or exudates gums are amorphous, translucent solids, insoluble in alcohol and in most organic solvents. They are, however, soluble in water to yield viscous, adhesive solutions, or are swollen by the absorption of water into a jelly-like mass. Gums are yielded by trees and shrubs belonging to a number of families. They are abnormal products, resulting from pathological conditions brought about either by injury or by unfavorable conditions of plant growth and are usually formed by changes in existing cell walls (gummosis) [7].

Mucilages are slippery, viscous polysaccharides of plant origins such as seeds, fruits, leaves, stems, bark or roots. Mucilages are similar in constitutions to gums, but are different in origination. Mucilages are not pathological products as gums, but are formed in normal plant growth. The functions of mucilages may be considered to aid in water storage, decrease diffusion in aquatic plants, aid in seed dispersal and germination and act as a membrane thickener and food reserve for plant growth [8]. Traditional or folk medicines have used plant mucilages for treatment of burns, wounds, ulcers, external and internal inflammations and irritations, diarrhea and dysentery [9]. Plant mucilages constitute a class of biopolymers with unique functional properties, such as thickening, stabilizing and gel formation. Mucilaginous polysaccharides are not soluble in water in the classical sense that micromolecules (such as sodium chloride and sucrose) are. For micromolecules, the solutions are described as homogeneous, because the two components merge into a monophase. But these polysaccharides are heterogeneous dispersions called hydrocolloids which have broad applications in food, pharmaceutical, medical and cosmetic industries [10].

The utilizations of nutritional hydrocolloids as soluble dietary fibers are increasing due to their functional properties, for example, water binding, viscosity and gelation, as well as to their bio-active role in prevention and/or treatment of certain diseases. As the primary function of hydrocolloids is to retain water, they have an important effect on stool bulking, and consequently on gut transit times as a high water-holding capacity forces the material through the gut faster. Increased stool weight can cause dilution of the intraluminal contents limiting the exposure of the gut to secondary bile acids and other toxins and potential carcinogens. The viscosity and gel formation

abilities of mucilages have the effect on glucose metabolism by reducing the rate of absorption of glucose in gastro-intestinal tract [11]. Plant mucilage such as psyllium and fenugreek are reported for their hypoglycemic effects [12-14].

In the gut of humans and monogastric animals, dietary lipids are present in the form of heterogeneous emulsified droplets. Triglyceride digestion first occurs in the stomach and is catalyzed by gastric lipase, while most fats are further hydrolyzed in the duodenum and jejunum under the action of pancreatic lipase. Lipolytic products that are generated are dispersed in the form of vesicles and mixed micelles, which interact with the intestinal mucosa to ensure the uptake of lipid moieties. A number of *in vitro* studies have shown that dietary fibers can alter the lipolysis process [15]. Gelling mucilaginous viscous fibers such as guar gum, psyllium and pectin drastically reduce the rate of lipid emulsification, lowering of the extent of fat lipolysis and absorption [16-18].

Mucilages have a long history of use as dietary fibers to promote the regulation of large bowel function, reducing symptoms of chronic constipation, diverticular disease, hemorrhoids, anti-gastric ulcer activity and may play a role in reducing the risk of certain types of cancer, such as colon cancer. Anti-oxidant property which the effect is to prevent free radical formation of some mucilages are also reported [19, 20]. Free radical species are significant factors associated to endothelial dysfunction that give rise to vascular diseases in diabetic complication [21].

Mucilages are classified as heteropolysaccharides that compose of a variety of monosaccharides. Mucilaginous polysaccharides from various sources and distinct preparations have diversities in chemical structures which influence on biological activities and functional properties. An understanding of chemical, physical and functional characteristics of each type of plant mucilages will facilitate the use of these polysaccharides in health promotion and care. It also contributes towards the sustainable use of plant resources as well. This research selects some Thai medicinal plants containing mucilages to investigate *in vitro* biological activities and also physicochemical properties compared to a well known soluble dietary fiber, glucomannan. The outcome of this present study is to provide the scientific evidence of selected plant mucilages for their potential in food supplement applications.

Objectives of the Study

1. To investigate biological activities *in vitro* of mucilaginous substances from selected Thai medicinal plants

2. To investigate physicochemical properties of mucilaginous substances from selected Thai medicinal plants

Scopes of the Study

1. Extraction of mucilages from 7 plants, namely:
   a. *Basella alba* Linn.
   b. *Hibiscus esculentus* Linn.
   c. *Litsea glutinosa* Lour.
   d. *Ocimum canum* Sims.
   e. *Plantago ovata* Forssk.
   f. *Scaphium scaphigerum* G. Don.
   g. *Trigonella foenum-graecum* Linn.

2. *In vitro* studies of the influences of the mucilages on glucose entrapment, lipid solubility, free radical scavenging activity and enzyme activity

3. Characterization of physical and chemical properties of these mucilaginous polysaccharides

4. Comparison of the biological and physico-chemical characteristics of these mucilages with Konjac glucomannan

Expected Benefits

1. This research provides scientific evidences for the *in vitro* biological activities and physicochemical properties of the mucilaginous substances from selected Thai medicinal plants.

2. This research provides the basic knowledge in mucilages as drug or food supplement development from plant-derived mucilages nutritional supplements.

CHAPTER II

LITERATURE REVIEWS

Polysaccharides

The carbohydrates can be classified according to their molecular size or degree of polymerization (number of monosaccharide units combined), into monosaccharides (or glycoses), oligosaccharides or polysaccharides. According to IUB-IUPAC Joint Commission on Biochemical Nomenclature, oligosaccharides are defined as saccharides containing between 3 and 10 sugar moieties [22]. Most of the carbohydrates found in nature occur in the form of high molecular weight polymers called polysaccharides or glycans, a general term in which large numbers of glycose are mutually joined by O -glycosidic linkages (Figure 1). The glycosidic linkage may have either α- or β-configuration, and various positions, i.e., α-1→2, α-1→3, α-1→4, or β-1→2, β-1→3, β-1→4, and so on. For example, Kojibiose, Nigerose, Maltose and Isomaltose are α-1→2, α-1→3, α-1→4 and α-1→6 D-glucose disaccharides respectively. Whilst Sophorose, Laminaribiose, Cellobiose and Gentiobiose are β-1→2, β-1→3, β-1→4 and β-1→6 D-glucose disaccharides respectively [23]. Based on chain character, polysaccharides may be linear or branched. Based on monomeric building blocks, polysaccharides can be divided into 2 classes. When polysaccharides are composed of a single monosaccharide building block, they are termed homopolysaccharides. Polysaccharides composed of more than one type of monosaccharide are termed heteropolysaccharides. Polysaccharides are high molecular weight polymers which have the degree of polymerization from a hundred to a few hundred thousand units. Based on the type of sequence of monosaccharide units, polysaccharides can be generally divided into 3 groups: periodic types or repeating pattern, interrupted types whose chains have repeating sequences separated by irregular sequences and aperiodic types which characterized by irregular sequences of monosaccharide units, linkage positions and configuration [1]. Based on nutritional purposes, the dietary polysaccharides are starch and non-starch polysaccharides.

α-D-glucopyranose

β- D-glucopyranose

α- D-glucofuranose

β- D-glucofuranose

Maltose
(α–1→4 D-glucose disaccharide)

Cellobiose
(β–1→4 D-glucose disaccharide)
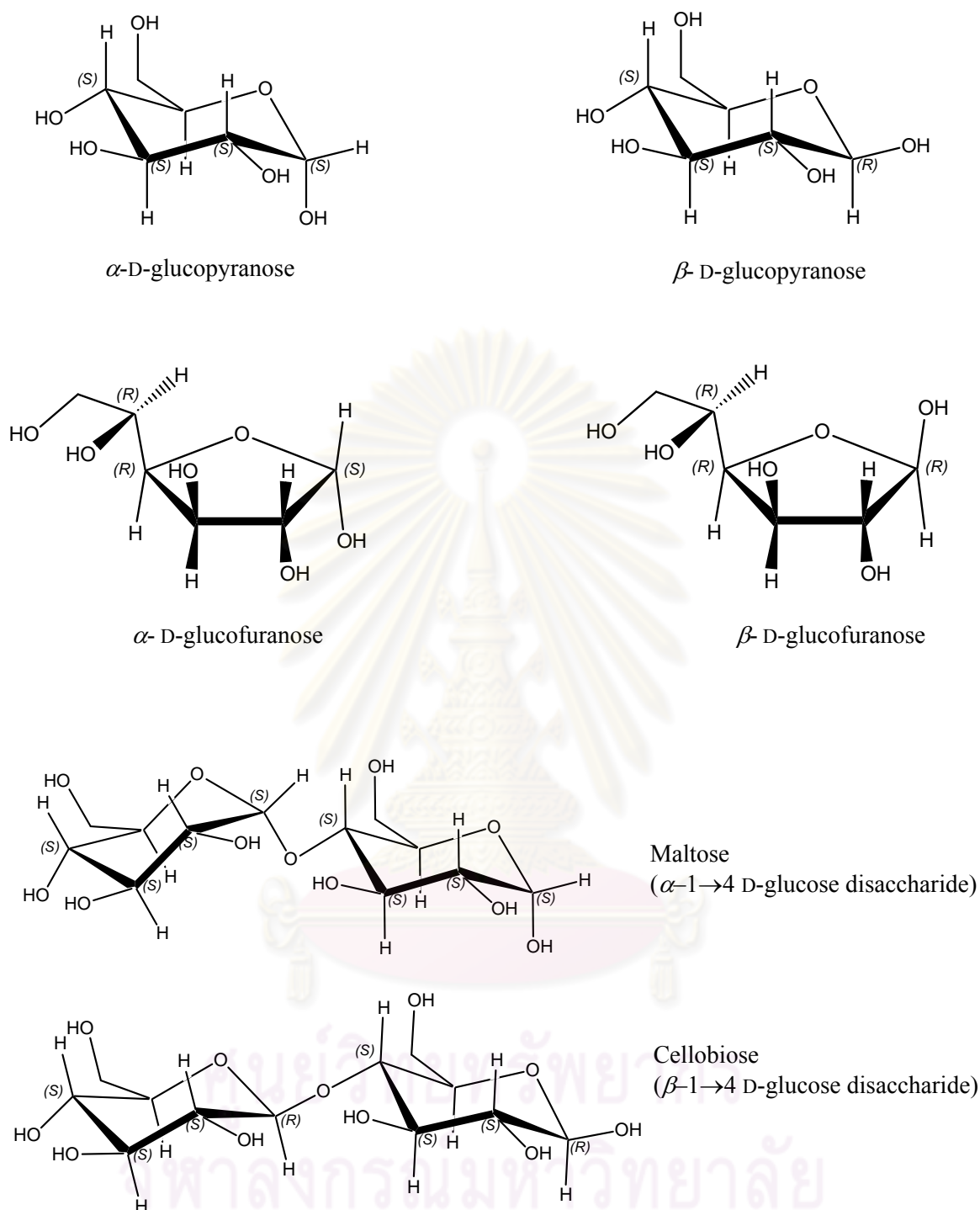
Figure 1 Ring forms of sugars and glycosidic linkage. In aqueous solution, there are the intramolecular rearrangements around the C-1 of monosaccharides (glycoses) and lead to formation of a mixture of monosaccharide anomers in equilibrium. This process is called mutarotation because of the change in optical rotation between α and β anomers. Glycans or polysaccharides are polymers in which large numbers of glycoses are mutually joined by O-glycosidic linkages [23].

Starch

Starch, the prime storage carbohydrates of almost all higher plants, provides the major share of energy in a food diet. Chemically, starch is a homopolysaccharide composed of residues of single monosaccharide D-glucose. Structurally, there are 2 types of polysaccharides in starch composition, amylose and amylopectin. Amylose has a molecular weight of around 10^5-10^6 according to the degree of polymerization between 1000 -10,000 glucose units. Amylose is mostly linear α-1$\rightarrow$4 glucopyranose chain with less than 0.5% of branched α-[1 → 6] linkages. Amylopectin is a branched polysaccharide which consists of α-1$\rightarrow$4 glucopyranose chain branching by α-1$\rightarrow$6 linkages at every 20-25 residues. Amylopectin has much higher molecular weight of about 10^8 and a degree of polymerization that may exceed one million units. Most starch contains 60–90% amylopectin. The amylose to amylopectin ratio and organization of the structure affect the physico-chemical and also biochemical characteristics of the starch [24]. The α-glycosidases enzymes [EC 3.2.1.x] are involved in the complete digestion of starch into glucose. Salivary and pancreatic α-amylases [EC 3.2.1.1] are endohydrolases that cleave the internal α-1$\rightarrow$4 bonds of starch into shorter linear and branched glucose chains. The linear products are maltose and maltotriose which contain α-1$\rightarrow$4 linkages. The other product is α-limit dextrin which is α-1$\rightarrow$6 branched oligosaccharide. These products are then further hydrolyzed by two small-intestinal brush-border α-glucosidases, human sucrase–isomaltase complex [EC 3.2.1.48 and 3.2.1.10] and human maltase–glucoamylase complex [EC 3.2.1.20 and 3.2.1.3]. These enzyme complexes are paralog exohydrolase which complementarily act at the nonreducing ends of the saccharides. Sucrase–isomaltase digests branched linkage whereas maltase–glucoamylase digests linear regions to yield glucose at the last step of starch hydrolysis. The multiplicity of α-glucosidases is an adaptation mechanism of intestinal digestive enzymes against a variety of starch [25-27]. Nevertheless, there are some types of starch that are not degraded and digested in small intestine. These are defined as resistant starch. The resistant starch is classified into 4 types as follow: type I is physically inaccessible starch *e.g.* partly milled grains and seeds; type II is resistant granules *e.g.* raw potato and green banana; type III is retrograded starch *e.g.* cooked and cooled potato, bread and cornflakes; and type IV, not found in nature, is chemically modified starch by food manufacturers [28, 29].

Non-Starch Polysaccharides

Non-starch polysaccharides are principally not α- glycosidic linked polysaccharide so they are resistant to human intestinal digestive enzymes. The most abundant of non-starch polysaccharides are the polysaccharides of plant cell wall *e.g.* cellulose, hemicelluloses and pectins. The other groups include plant storage polysaccharides *e.g.* fructans, glucomannans and galactomannans; plant gums and mucilages; bacterial polysaccharides *e.g.* xanthan gum and also algal polysaccharides *e.g.* alginate, agar, and carrageenan [28]. Non-starch polysaccharides are thus a diverse group of molecules with varying degrees of water solubility, size, and structure and are collectively designated as a class of dietary fiber. Dietary fiber is defined as "the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/ or blood cholesterol attenuation, and/ or blood glucose attenuation" [30]. Dietary fibers are further classified into water insoluble and soluble fibers based on the physico-chemical properties. Practically, soluble fibers are defined by analytical methods accepted by the Association of Official Analytical Chemists (AOAC). Digestible carbohydrates and proteins are hydrolyzed by digestive enzymes *e.g.* amylase, glucoamylase and protease. Insoluble fiber is extracted by filtration. Soluble fiber in the filtrate is precipitated by addition of four volumes of 95% ethanol then collected by filtration [31]. For non-starch polysaccharides, cellulose is insoluble fiber whereas non-cellulosic non-starch polysaccharides are mostly soluble fibers [32].

Konjac Glucomannan

Konjac Glucomannan (KGM) is a well-known soluble dietary fiber obtained from the bulbs of *Amorphophallus konjac* K. Koch (Araceae). If dissolved in water, KGM yields a high viscosity and is useful as a thickening, gelling, texturing and water binding agent. This non-starch heteropolysaccharide comprises of β-1→4 linked D-glucose and D-mannose residues [33]. The main chain is linear with degree of branching of about 8% [34, 35]. Branching point is controversy between β-1→3 glucosyl units [34] and β-1→6 glucose units [35]. In addition, there is approximately 5-10% acetylation at sixth carbon of glucose unit. Degree of acetylation is a factor

influenced on gelling agent property. Deacetylation, for example with alkali, changes sol state of GM dispersing in water into gel state. This phenomenon is due to non-covalently crosslinked network formation of GM chains (Figure 2) [36, 37]. A model of basic structure of KGM analyzed by ^{13}C NMR spectroscopy is proposed as Glc-Man-Glc-Man(2)-Glc-Man(3)-Glc(2)-Man-Glc(2)-Man(2)-Glc(2)-Man(3)-Glc(2)-Man(4)-Glc(2)-Man(5)-. There are 13 glucosyl units combined with 21 mannosyl units and the ratio of mannose to glucose is 1.6 [35]. KGM is high polymer with the molecular weight of around 1×10^6. The molecular chain is extending, semi-flexible, linear and without branching [38].
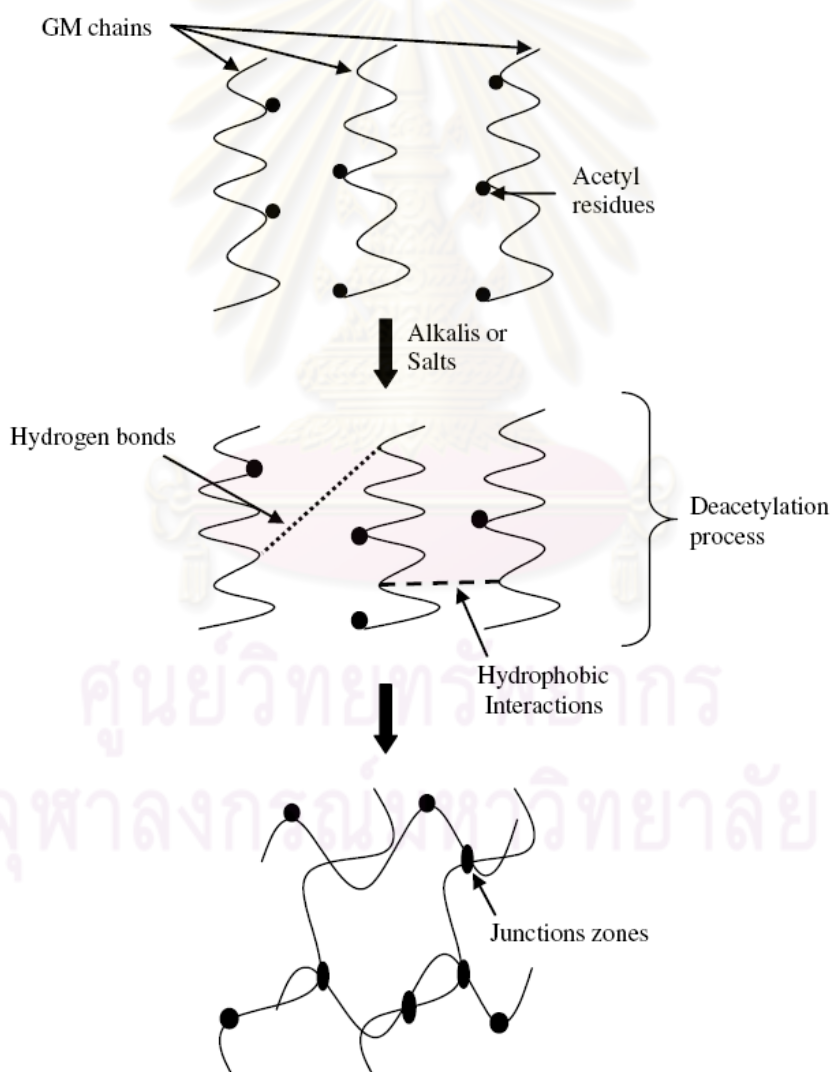
Figure 2 Gelation mechanism of glucomannan. Deacylation enhances GM chain interaction by hydrogen bonding [36].

KGM has beneficial impacts on human gastrointestinal health. The viscous gel of KGM promotes laxation by improving stool consistency, increasing stool frequency as well as stool weight, decreasing the stool transit time and reducing episodes of painful defecation in healthy and chronic constipation [39-41]. Furthermore, KGM has potential effects on blood glucose and blood cholesterol attenuation. Fourteen randomized controlled trials of KGM efficacy on the metabolic syndrome conducted during 1984-2007 were systematically analyzed. The meta-analysis including 531 subjects showed that the use of KGM appeared to statistically significantly lower total cholesterol, low density lipoproteins (LDL), triglycerides, body weight, and fasting blood sugar. However, it did not appear to alter high density lipoproteins (HDL) or either systolic or diastolic blood pressure [42]. The effect on LDL is beneficial for cardiovascular patients. The Adult Treatment Panel III (ATP III) of the National Cholesterol Education Program issued that for every 1% (1mg/dL) reduction in LDL, the relative risk for major coronary heart disease events is reduced by approximately 1% [43]. KGM was shown to reduce about 16 mg/dL of LDL therefore its effect could be clinically significant [42]. KGM is a potential food supplement aiding diabetic control [44, 45]. It has been found to decrease the serum glucose levels and also the postprandial insulin flow [46, 47]. The prebiotic effects of KGM and KGM hydrolysates on colonic health are emerged. KGM administration in both animal models and human decrease the fecal pH and increase short chain fatty acids. The growth of probiotic bacteria, for example *Bifidobacteria* and *Lactobacilli*, are promoted whereas pathogenic *Clostridium* growth is suppressed [39, 41, 48, 49]. Short chain fatty acids are proposed to involve in the detoxification of compounds associated with oxidative stress. In addition, probiotics, prebiotics and short chain fatty acids may play a synergistic role in protective mechanisms against carcinogen-enhanced colon cancer [50].

Plant Mucilaginous Polysaccharides

Plant mucilages are the other kinds of water soluble fibers. They are non-starch polysaccharides secreted in the plant cell as normal products of cell activities. Their functions may be considered to aid in water storage, decrease diffusion in aquatic plants, aid in seed dispersal and germination and act as a membrane thickener and food reserve for plant growth [8]. Mucilages and exudate gums are look-alike. Whilst mucilages are normal products in plant cells (Figure 3), gum is abnormal products, resulting from pathological conditions brought about either by injury or by unfavorable conditions of plant growth and are usually formed by changes in existing cell walls (gummosis) [7].

Mucilages are insoluble in alcohol and in most organic solvents. They are soluble in water and yield slippery, viscous or gel-like mass. Mucilages are found in many parts of plants such as seeds, fruits, leaves, stems, bark or roots. Plant mucilages have been credited as one of plant chemical constituents showing hypoglycemic and hypolipidemic activities [51-53].
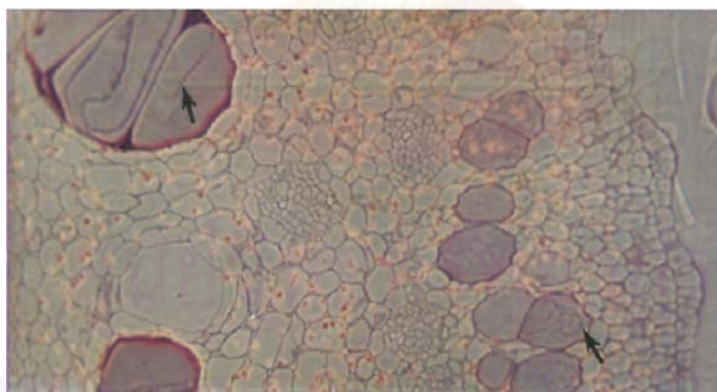
Figure 3 Plant secretory cells containing mucilages. A transverse section through the outer portion of a young okra pod stained for polysaccharide by the Periodic acid-Schiff reaction. The large secretory cells of the pericarp are conspicuous because of their size and staining reaction (x 160) [54].

Basella alba **Linn. (Basellaceae)**

Ceylon spinach (Figure 4) is commonly grown for its young shoots, which make a succulent, slightly mucilaginous vegetable. A number of medicinal applications have been reported: young leaves are used as a laxative and the red fruit juice as eye-drops to treat conjunctivitis. In Kenya the leaves are used to cure stomachache and constipation after childbirth, pulped leaves are applied as a poultice to sores. In East Africa the plant is given to livestock to increase milk production. Red forms of *B. alba* are commonly planted as ornamentals [55-57].

Figure 4 The shoots of *B. alba* (left). Drawing of flowering and fruiting shoot (right) [55].

Hibiscus esculentus **Linn. (Malvaceae)**

Okra (Figure 5) is widespread in tropical, subtropical and warm temperate regions, but is particularly popular in West Africa, India, the Philippines, Thailand and Brazil. All parts of the plant especially the pods (fruits) are mucilaginous. In Asia, okra is typically prepared as a vegetable and used in traditional medicine for the treatment of sorethroat, catarrh, gastric irritations and also used as enemata [9, 58]. Various researches have given different compositions of the okra mucilage. Whistler and Conrad reported that the polysaccharide obtained from okra pod was composed of galactose, rhamnose and galacturonic acid [59, 60]. Amins also found these three monosaccharides and arabinose. The ratio of galactose to rhamnose to galacturonic acid and to arabinose was 8.0 : 1.0 : 0.6 : 0.3 [61]. While Woolfe and colleagues found that

the okra mucilage was composed of galactose, rhamnose, galacturonic acid and glucose in the ratio of 1.00 : 0.12 : 1.30 : 0.10 [62]. The okra mucilage, isolated and purified from deseeded immature okra fruits by Tomoda *et al.*, was composed of rhamnose, galacturonic acid, and galactose in the ratio of 2.52 : 2.18 : 2.73 [63]. Hirose reported the composition of galactose, rhamnose, galacturonic acid, glucose and glucuronic acid in the ratio of 1.83 : 2.46 : 1.00 : 0.33 : 0.17 [64]. Sengkhamparn studied the alcohol insoluble polysaccharide from okra pod which was shown to be arabinose : rhamnose : xylose : mannose : galactose : galacturonic acid : glucose : glucuronic acid in the ratio of 5 : 3 : 5 : 3 : 17 : 16 : 44 : 7 [65]. The main structural of okra mucilage, studied by Tomoda *et al.*, was revealed as a repeating unit of alternating α-(1→2)-linked rhamnosyl and *β*-(1→4)-linked galacturonic acid residues with a disaccharide side chain of *β*-(1→4)-linked galactosyl units attached to O-4 of about half the L-rhamnosyl residues. The acetyl content was approximately 5.5% w/w. However, the precise position of the acetyl groups was not mentioned [63, 65].

Okra mucilage has been indicated for the abilities to lower blood glucose and cholesterol in rats [66, 67]. Cholesterol-lowering effect is related to bile acid binding capacity. The *in vitro* bile acid-binding percentage of okra was 16% compared to cholestyramine (a bile acid-binding, cholesterol-lowering drug) on equal dry matter basis [68, 69].

Figure 5 The fruiting plant of *H. esculentus* (left). Drawing of flowering shoot and fruit (right) [58, 70].

Litsea glutinosa **Lour. (Lauraceae)**

This evergreen shrub or tree (Figure 6) is a common species throughout India and also in Southeast Asia and the Philippines. The leaves are mucilaginous and are regarded in India as emollient and antispasmodic. The bark contains many mucilages, slightly balsamic and mildly astringent. Ground to a paste, it is applied as an emollient on wounds, bruises, sprains, rheumatic and other swollen joints. It contains laurotetanine, tannin and a red-brown coloring matter [9]. A water-soluble arabinoxylan (-xylose and -arabinose in the molar ratio 1.0:3.4) is isolated from the mucilaginous bark and a backbone of (1→4)-linked β-D-xylopranosyl residues substituted at both positions 2 and 3 with side chains composed of either single or (1→3)-linked arabinofuranosyl residues is indicated [71].

Figure 6 The leaves of *L. glutinosa* (left). Drawing of flowering branches and fruits (right) [72].

Ocimum canum **Sims. (Labiatae)**

This plant (Figure 7) is well-known for its mucilaginous seeds. The mucilages of the seeds is composed of D-glucose, D-galactose, D-mannose, L-arabinose, D-xylose, and L-rhamnose in the approximate ratio 8 : 5 : 2 : 1 : 1 : 2 and uronic acids (8.15%). The uronic acids were identified as D-galacturonic and D-mannuronic acids. The mucilages were fractionated into an acid-soluble fraction, which was significantly acetylated (6.6%) and composed of D-xylose (42%), L-arabinose (14%), L-rhamnose (12%), and D-galacturonic acid (30%) together with traces of D-galactose and D-glucose. The acidic polysaccharide is highly branched having a (1→4)-linked xylan backbone in which some the xylose residues carry branch points at C-2 and some at C-3, and also revealed the mode of linkage of the sugar residues present in the side chains. The acid- insoluble fraction was rich in hexosans which on further fractionation with alkali gave a glucomannan with glucose and mannose in the ratio 10 : 3 and a galacto-glucomannan with galactose, glucose, and mannose in the ratio 2 : 4 : 1 [73, 74].

There are some reported beneficial effects of *Ocimum canum* mucilage. Ingestion of swollen seeds in water showed reducing in the incidence of constipation in 53 elderly postoperative patients [75]; lowering plasma glucose in 16 patients with diabetes mellitus [76]; decreasing in serum total cholesterol and also increasing in serum HDL in 20 hyperlipoproteinemic patients [77].

Figure 7 The leaves and inflorescences of *O. canum* (left). Drawing of plant (right) [78].

***Plantago ovata* Forssk. (Plantaginaceae)**

Psyllium (Figure 8) is native to the Mediterranean region of Europe and North Africa and is cultivated extensively in Iran and India for its seeds. When the seeds are boiled in water, the thin, white seedcoat (called "husk"), greatly expands due to its 25-30% mucilages content. The separated "husk' is much used as a demulcent, mainly as a gentle bulk laxative, and for soothing the intestinal irritation or inflammation which causes diarrhea and dysentery [9]. Laidlaw and Percival revealed that psyllium mucilaginous polysaccharide composes of D-xylopyranose and L-arabofuranose [79, 80]. Further studies showed the detail of a *neutral* arabinoxylan (arabinose 22.6%, xylose 74.6%, molar basis; only traces of other sugars). With about 35% of nonreducing terminal residues, the polysaccharide is highly branched. The data are compatible with a structure consisting of a densely substituted main chain of *β*-(1 → 4)-linked D-xylopyranosyl residues, some carrying single xylopyranosyl side chains at position 2, others bearing, at position 3, trisaccharide branches having the sequence L-Ara*f*-α-(1 → 3)- D-Xyl*p*-β-(1 → 3)- L-Ara*f* [81-83].

Figure 8 The leaves and inflorescences of *P. ovata* [84].

Psyllium is notably promoted in laxative property. The studies *in vivo* and *in vitro* showed that psyllium mucilage was partially fermented in the large intestine. The mucilages in caecum helped in gelatinous or slippery stool and also increased the stool weight as well as stool viscosity [85-88]. Psyllium has been reported to produce lipid-lowering effect in hypercholesterolemic patients. Dietary psyllium supplement reduces blood total and LDL cholesterol but not HDL-cholesterol [89, 90]. However, the effect in normocholesterolemic or slightly hypercholesterolemic subjects has not been significantly shown [91]. Animal model studies have supported that the mechanism of cholesterol-modulating effect is due to increasing in hepatic bile acid synthesis from cholesterol and fecal bile acid excretion [88, 92, 93]. Psyllium improves glycemic control in type II diabetic patients as well. Psyllium intake decreases not only fasting blood glucose and glycosylated hemoglobin (HbA1c) in type II diabetic patients but also postprandial glucose and insulin in healthy individuals [12, 13, 94-96]. Psyllium mucilage seems to act on small-intestinal motility, in the manner that reduces transit time in the proximal small intestine, thereby shorten glucose contact time with the absorbing surface and thus flatten glycemic response [94, 96].

Scaphium scaphigerum G. Don. (Sterculiaceae)

Malva nuts, the fruits of *Scaphium scaphigerum* (Figure 9) are known to contain a large amount of mucilaginous substance and have been used as a traditional medicine in South-East Asia. The fruits have a yellowish black skin and very much wrinkled. When soaked in water, the layers swell up into a cloudy mass. It is used for curing fever, dry cough, phthisis, hemorrhoids, all sorts of ulcer and recovering the diseased organs. its virtue is due to being a mucilaginous drink for domestic cooling, demulcent and laxative remedy [97].

S. scaphigerum mucilage composes of 31.9% arabinose, 29.2% galactose, 29.5% rhamnose and also 6.4% uronic acid. The linkages of polysaccharide are terminal l-Ara$f$, 1,3-linked l-Ara$f$, 1,4-linked D-Gal$p$, 1,4-linked D-GalAp with small amounts of branching units: 1,2,4-linked D-Gal$p$ and 1,2,3,4-linked Rha$p$ [98].

Figure 9 The fruits (left) and tree (right) of *S. scaphigerum*

***Trigonella foenum-graecum* Linn. (Papilionaceae)**

Fenugreek (Figure 10) is a leguminous plant appeared to be indigenous to the Mediterranean. It is an annual herb with trifoliate leaves and whitish flowers followed by a beaked pod which contains 10-20 yellowish-brown color seeds. The seeds are bitter, mucilaginous, aromatic and are traditionally used as galactogogue, carminative, astringent, emollient and anaphrodisiac [99]. Monosaccharide and methylation analysis of the seeds mucilages suggest that the structural backbone is composed of a linear β-D (1→4) D -mannose units with α- D (1→6) D -galactose units attached as side chains. The fenugreek galactomannans have the ratios of galactose to mannose from 1.00:1.02 to 1.00:1.20 [100, 101].

Fenugreek seed is a well-known Indian medicinal plant traditionally used as hypoglycemic agent [102]. The seed powder was shown to improve blood as well as urinary glucose, mean area under the glucose tolerance curve, glycosylated hemoglobin and serum insulin levels in diabetic patients [103, 104]. The water extract of fenugreek seed exhibited dose dependent effects on hypoglycemic and anti-hyperglycemic activities in normal, hyperglycemic and induced diabetic rats [105, 106]. The seed

fractionation showed the involvement of the galactomannan mucilaginous component in the hypoglycemic effect [104, 107, 108]. Galactomannan from fenugreek seed acted as viscous barrier against glucose diffusion then reduced rate of glucose uptake in the small intestine [109]. Fenugreek also plays a role in lipid metabolism improvement. It has been shown the reduction in blood cholesterol, low density lipoprotein and triglyceride in human and animal model [104, 105, 107].

Figure 10 Drawing of plant, fruit and seed of *T. foenum-graecum* [110].

Type 2 Diabetes Mellitus and Complications

Diabetes mellitus means sweet urine disease. The word "diabetes" comes from the Greek word meaning "siphon" or "pass through" which is the obvious sign of the disease - excessive urination. The word "mellitus" comes from the Latin word meaning "sweet like honey" according to the observation that when a container of urine from a person with diabetes is allowed to evaporate, it appears to have a solid which is sugar (glucose) [111]. Diabetes is a metabolic disease that occurs when the pancreas does not produce enough insulin, or alternatively, when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar. Hyperglycemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels. There are 4 types of diabetes classified by etiology. Type 1 diabetes is characterized by a lack of insulin production. It is typically an autoimmune disease, resulting in destruction of pancreatic islet β-cells which secrete insulin. Type 2 diabetes is more common form of diabetes results from the body's ineffective use of insulin or known as insulin resistance. Type 3 diabetes is other specific type, for example, diseases, drugs or chemicals induced diabetes. Type 4 diabetes is any form of diabetes during pregnancy or gestational diabetes [2, 112].

Type 2 diabetes comprises 90% of people with diabetes around the world and is one of the major public health challenges of the 21st century. The number of cases worldwide in 2000 is estimated to be about 171 million and is projected to rise to 366 million in 2030 [113]. The World Health Organization indicates that diabetes-related deaths worldwide are almost 3 million cases per year and will increase to double in 2030. The estimation illustrates that in people aged 35–64 years, about 6–27% of deaths are related to diabetes. In most developing countries, almost one in ten deaths in economically productive individuals aged 35–64 years can be attributed to diabetes. Even in the poorest countries, at least one in twenty adult (35–64 years of age) deaths are diabetes related and this burden will double in the next 25 years. The situation in Thailand is shown similarly. The prevalence of diabetes in Thailand will increase from about 1.5 million in 2000 to 2.7 million in 2030 [2, 114]. This circumstance results that the demand for medical care in type 2 diabetes will continue to increase. The substantial care and cost are due to the management of complications of the

disease at both the starting point and the degree of deterioration over time. Macrovascular complications (ischemic heart disease, peripheral vascular disease, and cerebrovascular disease) has been estimated to be the largest cost component followed by microvascular complications (nephropathy, neuropathy and retinopathy) [115]. The risks of cardiovascular disease and myocardial infarction in diabetic patients are at least 4-fold higher than people without diabetes. Diabetes is reported as an independent risk factor for stroke at all age range. Occlusion of peripheral arteries can cause intermittent cramping pain of muscles which can later result in functional impairments, disabilities and amputations. Diabetes nephropathy is a leading cause of renal failure. Microvascular complication of the peripheral retina leading to visual disability and blindness increases with prolonged duration of diabetes. Peripheral neuropathy in diabetes includes sensory, focal/multifocal, and autonomic neuropathies. Loss of sensation coupled with impaired peripheral vascular function leads to foot ulceration or injury and finally amputation [116, 117].

Endothelial dysfunction is considered to be an integral component of vascular diseases. The endothelium is a singer layer of cells that line the interior wall of blood vessels. It functions as an interface between the blood and the tissues and control of the transportation across the bloodstream. Impaired endothelial function induces vasoconstriction, inflammatory and proliferative changes in the arterial wall and promotes atherosclerotic lesion growth. Prevention or normalization of endothelial function, contributes to the prevention of vascular lesion progression or destabilization [21, 117]. Hyperglycemia has been proposed to be a crucial factor inducing endothelial dysfunction. High concentration of blood glucose as well as high glucose fluctuation during postprandial period correlates with the increase in reactive oxygen species or oxidative stress. Reactive oxygen species, such as superoxide anion, hydrogen peroxide, mediates the activation of the imbalance in vasoregulating factors (vasodilators and vasoconstrictors) then affects endothelial homeostasis and triggers atherogenic changes, including increases in low-density lipoprotein oxidation, sympathetic tone, vasoconstriction, and thrombogenicity [118, 119]. The mechanisms involve overrunning of mitochondrial electron transport system due to high glucose transport through endothelial cells. This mitochondrial flux activates reactive oxygen species mediated glyceraldehyde-3-phosphate dehydrogenase inhibition. This glycolytic enzyme converts glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate,

an intermediate in glycolytic pathway. Accumulation of glyceraldehyde-3-phosphate initiates alternative pathways and by-products which activate mechanisms resulting endothelial damage.

One of the by-products is irreversible cross-linked protein derivatives called advanced glycosylated end products (AGEs) which stimulate stiffness of the endothelium. The other is diacylglycerol which further mediates activation of protein kinase C (PKC). PKC plays a role in impairment of regulating endothelial cell contraction, shape-change and intercellular adhesion which contribute to the loss of endothelial barrier function in diabetes. Proteinuria and microangiopathy which affect survival and visual outcome, for example, are diabetic complications of macromolecules leakage across the endothelial cells barrier. In addition to glyceraldehyde-3-phosphate, glucose accumulation also occurs due to normal glycolysis disruption. Accumulation of sugar alcohol *via* intracellular polyol pathway of glucose has been linked to osmotic damage to microvascular cells. Excess intracellular glucose also increases the hexosamine pathway flux which plays an important role in hyperglycemia - induced and lipid - induced insulin resistance. Moreover, the activation of the hexosamine pathway may affect gene expression and protein function of the endothelial cells which contribute to the pathogenesis of diabetic complications (Table 1) [116, 120-122].

Endothelial dysfunction can occur at any levels of the blood vessels (from aorta to capillaries) and is a risk factor for the development of atherosclerosis, the first stage to ischemic heart disease and ischemic stroke. Atherothrombosis is postulated as the mechanism leading to atherosclerosis. Blood vessels, with endothelial dysfunction, proceed LDL deposition, cell adhesion molecule expression, extracellular matrix and cytokines accumulation, macrophages and fibroblasts migration, smooth muscle cell proliferation then finally atherothrombotic plaques formation [123]. Dyslipidemia, as characterized by elevated blood triglyceride, cholesterol, LDL but lessen HDL, is commonly coincided in type 2 diabetes. Insulin signalling perturbation in the character of insulin resistance plays a role in postprandial intestinal chylomicrons overproduction resulting in *de novo* lipogenesis and cholesterogenesis as well as small dense LDL elevation [4, 124]. Small and dense LDL are more atherogenic than large and buoyant one. The patients with small dense

LDL had impaired response to acetylcholine, endothelium-dependent vasodilator more than comparable large LDL control. This may be due to the evidences that LDL with small size have higher affinity to deposit in the artery walls and moreover, have higher susceptibility to oxidative stress than large LDL particles. Around 50% of Type 2 diabetic patients have a prevalence of small dense LDL. Impaired glucose tolerance and clinical diabetes are associated to a stepwise decrease in LDL size [125, 126]. In summary, the concurrence of insulin resistance, hyperglycemia and dyslipidemia aggravate atherosclerosis and complications in type 2 diabetic patients.

Table 1 Mechanisms of hyperglycemia-induced endothelial dysfunction

Mechanisms	Effects	Complications

• Glycolytic enzymes fluctuation
  o Activation of PKC
  o Increase polyol pathway flux
  o Increase hexosamine pathway flux
	• Increase oxidative stress
	• Increase precursor of AGEs
	• Decrease vasodilator - nitric oxide
	• Increase vasoconstrictor - Endothelin-1
	• Increased vascular endothelial growth factor
	• Increase plasminogen activator inhibitor-1
	• Increase fibrinolytic inhibitor
	• Increased nuclear factor – kappa B
		• Blood-flow abnormalities
		
		• Vascular permeability
		
		• Angiogenesis
		
		• Capillary occlusion
		
		• Vascular occlusion
		
		• Pro-inflammatory gene expression
		
		• Multiple effects

• Non-enzymatic reactions between extracellular proteins and glucose o Increase AGEs	• Increased vascular endothelial growth factor • Increased nuclear factor – kappa B	

Postprandial Hyperglycemic Control

The management to minimize diabetes related morbidity and mortality is a crucial need. Accordingly, blood glucose control is an important goal to diminish the risk of long term health complications of type 2 diabetes. In addition to glycosylated hemoglobin and fasting plasma or preprandial glucose, postprandial glucose is recently recommended as essential target for diabetes management [127, 128]. Glycemic goals for clinical management of diabetes are defined as glycosylated hemoglobin <6.5%, preprandial glucose <100 mg/dl and 2-hour postprandial glucose <140 mg/dl. A plasma glucose concentration of more than 140 mg/dl at 2 hours after food ingestion is defined by The World Health Organization as postprandial hyperglycemia. Postprandial glycemic control is more important for preprandial glucose and HbA1c goal achievement. With normal glucose tolerance, postprandial glucose level typically returns to preprandial level within 2-3 hours [128]. Impairment in postprandial glycemic control is a first sign of abnormality in insulin sensitivity. Moreover, postprandial glucose fluctuation significantly correlates to oxidative stress and inflammation activations. Intervention to flatten postprandial glucose excursion is focusing as a potential preventive care for type 2 diabetes complication especially vascular failure [119, 129, 130].

After meal ingestion, the digestion of starch to glucose starts in the mouth by salivary α-amylase and completely digested in small intestine by pancreatic α-amylase in the lumen and *α*-glucosidases in the brush border of intestinal epithelial cells or enterocytes. Glucose final product then transports across enterocyte into blood by diffusion (passive transport) and glucose transporters (active transport) at brush border membrane (lumen-enterocyte interface) and basolateral membrane (enterocyte-blood interface) of enterocyte. The rate-limiting step of glucose absorption is the final glucose transport into the enterocyte. Retardation of glucose releasing and adhering to intestinal brush border during nutrient movement along the intestinal tract is up to lessen glucose absorption and flatten postprandial hyperglycemia [27, 131, 132].

Soluble dietary fibers including mucilages and gums are designated as useful functional foods according to the association with the reduced risks of diabetes and cardiovascular diseases [133, 134]. Viscosity is the ability of some polysaccharides to thicken or form gels when mixed with fluids resulting from physical entanglements

among the constituents within the fluid or solution. Soluble fibers can form thickened or viscous solutions dependent on the physico-chemical factors including molecular weight, particle size, chemical composition and structure. The concentration of soluble fiber usually has a direct, non-linear effect on viscosity of solutions at a constant temperature. High concentrations of soluble fibers such as β-glucans, alginate, gum, glucomannan and galactomannans have been associated with the ability of these fibers to form highly viscous solutions when mixed with fluid [135]. The viscous characteristics due to water-holding and gel-forming capabilities have been proposed as an important mechanistic factor to delay gastric emptying and delay absorption of glucose in gastrointestinal tract [136-138]. Intestinal contraction is the mechanism beneficial to create turbulence and convection currents which accelerates nutrient movement from the center of the lumen to the epithelial cells for diffusion or transportation of the nutrients. Dialysis tubing technique is a simple model simulated to intestinal contractions and it has been shown that viscous luminal contents retard both convection and diffusion resulting in slow nutrient movement and absorption [139].

The inhibition of α-glucosidases is one of the powerful interventions coping directly with postprandial hyperglycemia. The α-glucosidases are two enzyme complexes in the brush border of the small intestine that breakdown oligosaccharides and disaccharides from amylase digested starch and glycogen to absorbable glucose. They are human sucrase–isomaltase complex [EC 3.2.1.48 and 3.2.1.10] and human maltase–glucoamylase complex [EC 3.2.1.20 and 3.2.1.3]. Natural or synthetic α-glucosidase inhibitors are of therapeutic interest to delay postprandial hyperglycemia in type 2 diabetes. Amongst these, Acarbose, Miglitol and Voglibose which are saccharide derivatives, have been approvable for anti-diabetic drugs and widely used in the treatment of patients with type 2 diabetes [140-142]. The α-glucosidase inhibiting activity is due to the substrate mimetic structure of these drugs (Figure 11). The first discovered natural glucose mimicking α-glucosidase inhibitor is an iminosugar produced by *Streptomyces* species, Nojirimycin. This compound is unstable and usually has to be derivatized to 1-deoxynojirimycin which is a potent inhibitor of all kinds of α-glucosidases *in vitro* but has moderate efficacy *in vivo*. Natural 1-deoxynojirimycin can be biosynthesized in the mulberry trees as well as produced by many strains in the genera *Bacillus* and *Streptomyces*. There will still be

a continuous finding of carbohydrate-derived or glycomimetic drugs acting against α-glucosidases. The benefit of α-glucosidases inhibition in accordance with a reduction in diabetic complications especially cardiovascular symptoms has been reported. This protective effect of α-glucosidase inhibitors has also been recommended for pre-diabetic as well as anti-diabetic agents [143, 144].
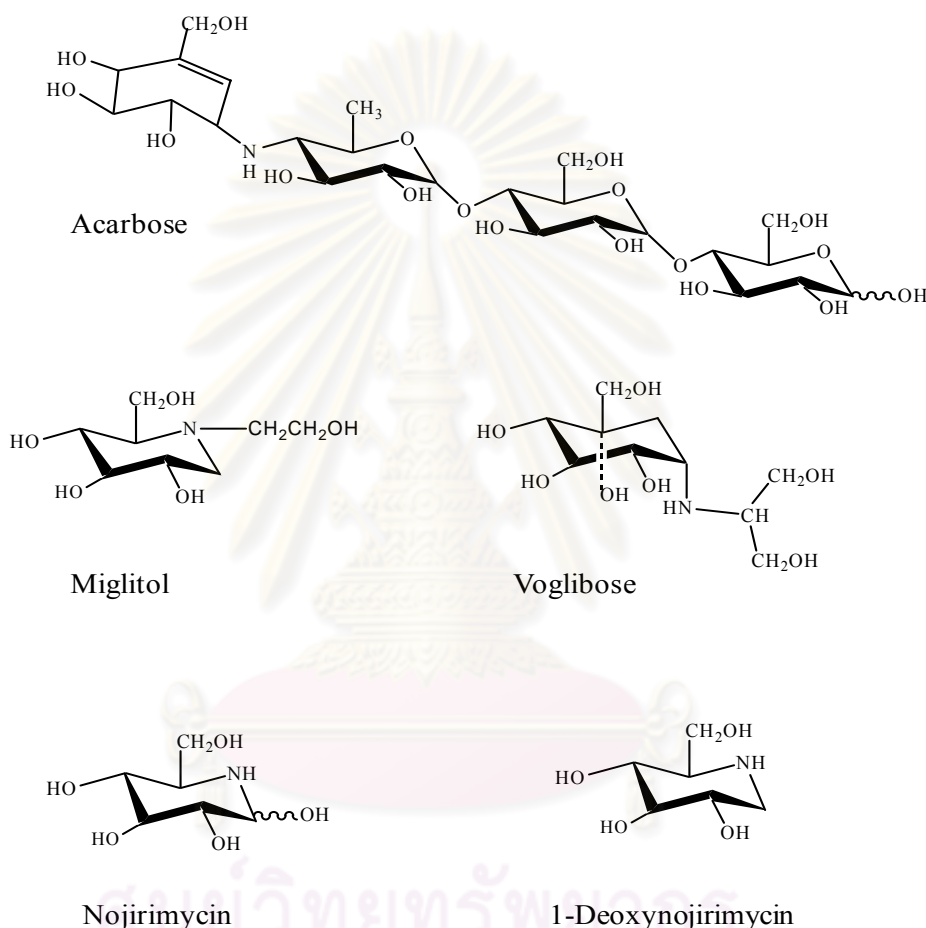
Figure 11 Carbohydrate-derived-α-glucosidase inhibitors

Postprandial Dyslipidemic Control

The postprandial state is a cluster of metabolic abnormalities that tends to be new targets for prevention and care of diabetic complications. Despite of the normal fasting lipids concentration, the metabolic abnormalities can be associated with delayed postprandial lipids clearance. In addition to postprandial hyperglycemia,

postprandial lipemia (hypertriglyceridemia and/or hypercholesterolemia) is recognized as the other risk factor for atherosclerosis process in type 2 diabetic people [124, 125]. Triglycerides or triacylglycerols are esters of 3 fatty acids and 1 glycerol molecule. These lipids play the important roles of energy source and transporter of fat-soluble agents in bloodstream. Cholesterol is an essential lipid for mammal's bodies. Its function involves cellular membrane physiology, dietary nutrient absorption, reproductive biology, stress responses, salt and water balance, and calcium metabolism. In adults, dietary lipids digestion starts in the stomach and finishes in the small intestine. Most lipids from the diets are triglycerides. After ingestion, triglycerides are firstly emulsified by mastication and gastric peristalsis. About 5-30% of triglyceride droplets are hydrolysed by gastric lipase to liberate fatty acids and diglycerides. The fatty acids released during gastric digestion promote further pancreatic lipolysis. In duodenum, emulsified tri- and diglycerides are completely hydrolysed by dual action of gastric lipase and co-lipase dependent pancreatic lipase to yield fatty acids and monoglycerides. Besides co-lipase, the adsorption of pancreatic lipase to the lipid interfaces is also facilitated by bile acids [145]. Dietary cholesterol can be in free form or esterified with various fatty acids. Cholesteryl esters are hydrolysed by pancreatic esterase before absorption. On the contrary of triglycerides, cholesterol sources are both exogenous diets and endogenous synthesis by the liver and excretion in bile. Digested lipids (fatty acids, monoglycerides and free cholesterol) are absorbed from the lumen through the enterocyte membranes of small intestine by micellar solubilization with bile acids. In the enterocytes, monoglycerides and cholesterol are re-esterified with fatty acids and assemble with specific proteins to form lipoproteins named chylomicrons which then transport *via* the thoracic duct into blood circulation [146]. During circulation, native chylomicrons (cholesterol-poor and triglycerides-rich) are disassembled by endothelial cell-bound lipoprotein lipase and apolipoprotein exchange to distribute fatty acids and glycerol to peripheral tissues and resulting in chylomicron remnants (triglycerides-poor and cholesterol-rich) which are rapidly cleared from the circulation by the liver. Accumulation of remnants leads to hypercholesterolemia, a risk factor for atherosclerosis [147, 148].

Diet therapy is one approach of non-pharmacological treatments for postprandial dyslipidemia and atherosclerosis reduction. Soluble dietary fibers

including gums and mucilages, for example, β-glucan, psyllium, pectin and guar gum have been reported that they can reduce blood cholesterol, LDL and triglycerides in animal models and clinical trials [133, 149, 150]. Postprandial triglycerides and chylomicron remnants reductions have been shown as well [151]. One mechanism in dyslipidemia improvement of soluble fibers involves in the viscosity of the lumen medium which leading to increase size of triglyceride emulsion droplets, decrease emulsion interface areas, decrease emulsified triglycerides and finally decrease triglyceride lipolysis [152]. Another mechanism includes interference in bile metabolism and lipid micellar solubilization. Bile is an aqueous solution produced in liver, stored in gallbladder and secreted into duodenum for aiding dietary lipids digestion. The major components in bile compose of bile acids, phospholipids, cholesterol, bile pigments, proteins and inorganic compounds. Bile acids (Cholic acid and Chenodeoxycholic acid) are synthesized from cholesterol in the liver and after synthesis, they are normally conjugated with the amino acids taurine and glycine. Cholic acid can be dehydroxylated by intestinal bacteria to Deoxycholic acid. In intestinal lumen, bile acids assemble triglycerides, fatty acids, cholesterol and phospholipids into dietary mixed micelles which have a relatively high diffusion rate due to their small size around 2–3 nm in radius. Mixed micellar mechanism is one of the major routes of cholesterol transport. The aqueous solubility of cholesterol (about 1 nM) can increase more than a million fold in the presence of bile-salt micelles [153-155]. Viscous soluble dietary fibers have been shown to decrease intestinal bile acids absorption, increase fecal bile acid excretion, upregulate bile acids synthesis from cholesterol resulting in blood cholesterol reduction [92, 149]. Interference of micellar solubility is one approach for regulation of intestinal uptake of dietary lipids. There have been experimental evidences for cholesterol lowering effect *via* competition in micellar solubilization [156-159]. Pancreatic lipase is the other target in postprandial dyslipidemia control. This triglyceride hydrolytic enzyme responds in digestion of about 50–70% of total dietary lipids. Inhibition of lipase activity can decrease in dietary lipid absorption and dyslipidemia. Lipase inhibitors derived from natural products including soluble fibers have also been illustrated [160-163].

CHAPTER III

MATERIALS

Plant Materials

1. Seven mucilaginous plants were studied as follows:

    1.1 *Basella alba* Linn. (Family Basellaceae)

    Malabar Spinach

    ผักปลัง

    Collected place: local market, Bangkok

    Part used: aerial parts

    1.2 *Hibiscus esculentus* Linn. (Family Malvaceae)

    Okra

    กระเจี๊ยบเขียว

    Collected place: local market, Bangkok

    Part used: fruits

    1.3 *Litsea glutinosa* (Lour.) C.B. Robinson (Family Lauraceae)

    Avocat Marron

    หมี่, หมีเหม็น

    Collected place: Princess Maha Chakri Sirindhorn Herb Garden, Rayong

    Part used: Leaves

    1.4 *Ocimum canum* Sims. (Family Labiatae)

    Hairy Basil

    แมงลัก

    Collected place: local market, Bangkok

    Part used: seeds

    1.5 *Plantago ovata* Forssk. (Family Plantaginaceae)

    Psyllium

    เทียนเกล็ดหอย

    Collected place: local market, Bangkok

    Part used: seeds

1.6 *Scaphium scaphigerum* G. Don. (Family Sterculiaceae)

Malva Nut

สำรอง, พุงทะลาย

Collected place: Thai traditional drugstore, Bangkok

Part used: fruits

1.7 *Trigonella foenum-graecum* Linn. (Family Papilionaceae)

Fenugreek

ลูกซัด

Collected place: Thai traditional drugstore, Bangkok

Part used: seeds

All plant materials were authenticated by Ruangrungsi, N.

2. Konjac glucomannan (บุก) (the Siam Konjac Co., Ltd.) was used for comparison.

All voucher specimens have been deposited at the College of Public Health Sciences, Chulalongkorn University.

Chemicals and Reagents

1. Cholesterol (Merck, Darmstadt, Germany)

2. Cholesterol Liquicolor (Human Gasellsthaft, MBH, Germany)

3. Deuterium oxide 99.9 atom % D (Sigma–Aldrich Company Co., St. Louis, MO, USA)

4. 1-Deoxynojirimycin (Sigma–Aldrich Company Co., St. Louis, MO, USA)

5. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (Sigma–Aldrich Company Co., St. Louis, MO, USA)

6. Glucose Liquicolor kit (Human Gesellschaft für Biochemica und Diagnostica MBH, Germany)

7. α-Glucosidase (EC 3.2.1.20) from *Saccharomyces cerevisiae* (Sigma Chemical Co. Ltd, St. Louis, MO, USA)

8. Hexamethyldisilazane (Supelco, Bellefont, PA, USA)

9. Lactated Ringers Buffer pH 7 (General Hospital Product Public Co., LTD.,

Thailand)

10. Lipase (EC 3.1.1.3) from porcine pancreas (Sigma Chemical Co. Ltd, St. Louis, MO, USA)

11. Methanolic HCl (Supelco, Bellefont, PA, USA)

12. Monosaccharide standards (Sigma–Aldrich Company Co., St. Louis, MO, USA)

13. *p*-Nitrophenyl-*α*-D-glucopyranoside (Sigma–Aldrich Company Co., St. Louis, MO, USA)

14. *p*-Nitrophenyl laurate (Sigma–Aldrich Company Co., St. Louis, MO, USA)

15. Oleic acid (Sigma–Aldrich Company Co., St. Louis, MO, USA)

16. Phosphorus pentoxide (Sigma–Aldrich Company Co., St. Louis, MO, USA)

17. Pullulan standard P-series (Shodex, Showa Denko KK, Japan)

18. Sodium tauro-deoxycholate hydrate (NaTDC) (Sigma–Aldrich Company Co., St. Louis, MO, USA)

19. Stearic acid (Sigma–Aldrich Company Co., St. Louis, MO, USA)

20. Trimethylchlorosilane (Supelco, Bellefont, PA, USA)

21. All other chemicals were analytical grade.

Materials

1. Dialysis tubing cellulose membrane (molecular weight cut off = 3,500 Da) (Spectrum Medical Industries, Inc., Los Angeles, CA, USA)

2. Dialysis tubing cellulose membrane (molecular weight cut off = 12,000 Da) (Sigma–Aldrich Company Co., St. Louis, MO, USA)

3. Polyethersulfone syringe membrane filter (0.22 μm x 33 mm) (Millex[®] GP, Millipore, MA, USA)

4. PVDF centrifugal membrane filter (0.45 μm) (Ultrafree-MC, Millipore, MA, USA)

5. PVDF syringe membrane filter (0.45 μm x 13 mm) (VertiPure[TM], Vertical Chromatography Co., Ltd., Bangkok, Thailand)

6. Zebron ZB-FFAP capillary GC column (30m x 0.25 mm x 0.25 μm) (Phenomenex®, CA, USA)

7. SGE BPX5 capillary GC column (30 m x 0.25 mm x 0.25 μm) (SGE Analytical Science Pty Ltd., Victoria, Australia)

8. OHpak SB-806 M HQ HPLC column (8.0 mm, i.d. x 300 mm length) (Shodex, Showa Denko KK, Japan)

9. NMR sample tube (5 mm, i.d.) (Shigemi Co., Ltd., Tokyo, Japan)

Instrumentations

1. Falling ball viscometer (HAAKE Mess-Technik GmbH u. Co, Germany)

2. Spectrophotometer T60 (PG Instruments Limited, UK)

3. Gas chromatography (GC) Varian model CP3800 equipped with flame ionization detector (Varian Inc.,USA)

4. Gas chromatography (GC) Thermo Finnigan model Trace GC Ultra equipped with Finnigan DSQ Quadrupole detector (Thermo Fisher Scientific Inc.,USA)

5. High-performance liquid chromatography (HPLC) system consisted of a Hitachi L-600 pump (Hitachi Seisakucho Co., Japan), Rheodyne 7725i loop injector (USA) and an TRD-880 refractive index detector (Shimamura Instruments Co., Japan)

6. Nuclear Magnetic Resonance (NMR) Spectrometer model JEOL JNM-GSX500A (JEOL Ltd., Tokyo, Japan)

CHAPTER IV

METHODOLOGY

Mucilages Extraction

The mucilages were extracted from the specified plant parts with warm water and concentrated by lyophilization. The lyophilized samples were re-dissolved in water, precipitated twice with 2 volume of 80% ethanol and dialyzed against distilled water in a dialysis tubing cellulose membrane (molecular weight cut off = 3,500 Da). The samples were lyophilized, ground and kept in refrigerator for further studies.

Effect on Glucose Diffusion

Glucose entrapment against diffusion was studied using dialysis tubing method [164]. The mucilages and glucomannan were dissolved in Ringers buffer. Glucose was added to make the final concentration of 2% glucose and 0, 0.5, 1 and 2 % w/v polysaccharide gel. Four milliliters of each concentration was dialyzed against 60 ml of Ringers buffer in a dialysis tubing cellulose membrane (molecular weight cut off = 12,000 Da) for 2 h under rotationally shaking at 150 rpm. The released glucose was determined using glucose oxidase which oxidized glucose to gluconic acid with the formation of hydrogen peroxide. Then hydrogen peroxide further reacted with 4-aminophenazone and phenol in the presence of peroxidase to form quinoneimine chromophore with a λmax at 510 nm. The procedure was performed using Glucose Liquicolor kit according to manufacturer's instructions with some modification. Briefly, about 10 μl of the buffer was added to 1 ml of the reagent, mixed and incubated at room temperature for 15 min. Measure the absorbance of the standard glucose solution and the sample against the reagent blank at 510 nm.

Effect on α-Glucosidase Inhibition

α-Glucosidase activity was assayed using 0.1M sodium phosphate buffer at pH 6.9 and 1 mM p-nitrophenyl-α-D-glucopyranoside was used as a substrate [165]. The concentration of α-glucosidase was 1 U/mL in each experiment. The enzyme (4 μl) was incubated in the absence or presence of various concentrations of tested polysaccharides at 37 °C. The preincubation time was specified at 10 min and the substrate (95 μL) was added to the mixture. The reaction was carried out at 37 °C for

20 min, and then 100 μL of 1M Na_2CO_3 was added to terminate the reaction. Enzymatic activity was quantified by measuring the absorbance of *p*-nitrophenol at 405 nm. 1-Deoxynojirimycin was used as the positive control. The percent inhibition was calculated by the following equation:

Inhibition (%) = (1 –B/A) x 100

Where, A was the absorbance of reactive mixture without sample or positive control and B was the absorbance of reactive mixture with test sample or positive control). IC_{50} values denoted the concentration of sample required to inhibit 50% of enzyme activity.

Effect on Cholesterol Solubility in Single Bile Micelles

The method was adapted from Matsuoka and colleagues [166]. Ten milligrams of solid cholesterol (excess for saturation) were added into a 10 mL test- tube containing 3 ml of 25 mM sodium taurodeoxycholate (NaTDC) in 15 mM phosphate buffer saline (pH 7.5). Fifteen milligrams of the mucilages were added (equal to 0.5% w/v) and the mixtures were stirred by magnetic stirrer for 24 h (to reach the equilibrium system). Separation of soluble micelles was performed by filtration through a 0.22 μm polyethersulfone membrane filter.

The cholesterol concentration in the filtrates was determined using cholesterol oxidase which oxidized cholesterol to cholestene-3-one with the formation of hydrogen peroxide. Then hydrogen peroxide further reacted with 4-aminophenazone and phenol in the presence of peroxidase to form quinoneimine chromophore with a λmax at 510 nm. The procedure was performed using Cholesterol Liquicolor kit according to manufacturer's instructions with some modification. Briefly, about 10 μl of the filtrate was added to 1 ml of the reagent, mixed and incubated at room temperature for 15 min. Measure the absorbance of the standard cholesterol and the sample against the reagent blank at 510 nm.

Effects on Fatty Acids Solubility in Single Bile Micelles

Ten milligrams of solid stearic acid or oleic acid (excess for saturation at specified concentration of NaTDC) were added into a 10 mL test- tube containing 3 ml of 25 mM or 0.3125 mM of sodium taurodeoxycholate (NaTDC) in 15 mM phosphate buffer saline (pH 7.5) respectively. Fifteen milligrams of the mucilages

were added (equal to 0.5% w/v) and the mixtures were stirred by magnetic stirrer for 24 h (to reach the equilibrium system). Separation of soluble micelles was performed by filtration through a 0.22 μm polyethersulfone membrane filter [166].

The stearic acid and oleic acid concentration in the filtrates were determined using gas chromatographic method. The filtrate (0.2 μl) was directly injected splitlessly to FFAP capillary column and detected by flame ionization detector. The oven temperature was 200°C, raised to 250°C at the rate 2.5°C/min, while the injector and the detector temperatures were set at 200 and 250°C, respectively. The helium carrier gas was controlled at 1 ml/min. The quantification was achieved by using the external standard method.

Effect on Cholesterol Solubility in Mixed Bile Micelles

Ten milligrams of solid cholesterol were added into a 10 mL test- tube containing 3 ml of 25 mM sodium taurodeoxycholate (NaTDC) in 15 mM phosphate buffer saline (pH 7.5) containing 10 mg of oleic acid. Fifteen milligrams of the mucilages were added and the experiments were performed as described above [166].

Effect on Lipolytic Activity

Lipolytic activity was determined using *p*-nitrophenyl laurate with and without pancreatic lipase. The assay was adapted from previous report [167]. *p*-Nitrophenyl laurate emulsion was prepared as 0.08 % w/v in 5 mM sodium acetate buffer (pH 5.0) containing 2% Triton X-100. Prior to use, it was heated in boiling water bath for 5 min to aid dissolution, cooled in water bath at 37°C and mixed well. Porcine pancreatic lipase was dissolved in water (10 mg/ml) and centrifuged at 12,000 rpm for 5 min. Orlistat in 1% DMSO was used as positive control for lipase inhibitor. The assay mixture containing 400 μl of 0.1 M Tris buffer (pH 8.2), 50 μl of 10 mg/ml mucilages (equal to 1.0% w/v) and 150 μl of lipase was incubated in water bath at 37°C for 5 min then 450 μl of *p*-Nitrophenyl laurate was added to start the reaction. The mixture was incubated at 37°C for 2 h then filtered through 0.45 μm PVDF membrane. The *p*-nitrophenol liberated from lipolysis was measured by spectrophotometry at 410 nm. For lipolytic activity without lipase (micellar catalytic effect), 150 μl of water was added instead of enzyme. All samples were assayed in

triplicate and a sample blank was prepared by adding acetate buffer instead of Tris buffer.

DPPH Radical-Scavenging Activity

The potential antioxidant activity of polysaccharide samples was determined on the basis of the scavenging activity of the stable DPPH free radical [168]. Various concentrations of polysaccharides samples (0.5 ml) were added to 1.5 mL of a 0.004% methanolic solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percent scavenging activity was calculated by the following equation:

Scavenging effect (%) = (1 – B/A) x 100

Where, A was the absorbance of control (DPPH solution plus water) and B was the test sample (DPPH solution plus test sample or positive control). IC_{50} values denoted the concentration of sample required to scavenge 50% DPPH free radicals.

Swelling Properties

Swelling volume (SV) and water absorption index (WAI) were determined from the ratio of the volume and weight of swollen gel to the dry weight of sample respectively [169, 170]. A 0.050 g of each mucilage was suspended in 25 ml of water in a 25-ml graduated cylinder for 2 h. The volume of swollen gel was measured. The supernatant liquid was removed, the swollen gel was weighed and SV and WAI were calculated per gram dry weight of the mucilages.

Viscosity

Viscosity of the mucilages at various concentrations was measured with a falling ball viscometer at 20°C [171, 172]. The sample solution was filled into a slightly inclined cylindrical measuring tube of an inner diameter of approximately 15.94 ±0.01 mm. The standard ball with known density and radius was placed and allowed to pass through the measuring tube. The time a ball required to fall a defined distance was recorded and calculated to the viscosity using the following equation:

Viscosity (in mPa.s) = K (ρ1 – ρ2) _ t

Where, K was ball constant (mPa.s.cm3/g.s), $\rho 1$ was density of the ball (g/cm^3), $\rho 2$ was density of the liquid (g/cm^3) at the measuring temperature and t was falling time of the ball in seconds.

Estimation of the Average Molecular Weights

The average molecular weights of the mucilaginous polysaccharides were estimated by size exclusive chromatography using HPLC system and detected by refractive index detector [173]. The separations were performed using an OHpak SB-806 M HQ HPLC column with a mobile phase of 10 mM ammonium bicarbonate eluted at a flow rate of 0.5 ml/min. The samples were prepared in 0.1 M NaCl solution and filtered through 0.45 μm PVDF centrifugal filters. Sample concentrations were 0.5% (5 mg/ml) except *O. canum* which was 0.25%. Sample injection volume was 10 μl. A calibration curve for molecular weight estimation was performed using pullulan of defined molecular weights ranging from 5.9 to 2,350 kDa as calibrated standards.

Monosaccharides Analysis

The polysaccharide samples (1 mg) were subjected to methanolysis with 4 M methanolic HCl at 80°C for 24 h in acid-washed vial. Mannitol was added as an internal standard. The samples were dried with nitrogen, methanol was added and the samples were dried again. This washing was repeated twice [174]. Prior to gas chromatographic analysis, the samples were trimethylsilylated using trimethylchloro-silane : hexamethyldisilazane : Pyridine 1:2:5 (0.4 ml) at room temperature for 30 min. Instrumentation was performed on a Finnigan Trace GC Ultra with DSQ MS detector and a split–splitless injector. The column was a SGE BPX5 capillary GC column (30 m x 0.25 mm x 0.25 μm). Helium was used as carrier gas at a flow rate of 1.0 ml/min. The injector and detector temperature were 260 and 300°C respectively. The column temperature was initially 140°C, then an increase of 1°C/min to 170°C and followed by 6°C/min to 250°C. Standard monosaccharides were performed as above procedure except for methanolysis using 1 M methanolic HCl instead.

Total Protein Analysis

The protein content of the polysaccharide samples were determined by Lowry method [175] using bovine serum albumin as protein standard. One milligram of the

polysaccharide sample in 0.5 ml of normal saline was added to 0.7 ml of Biuret reagent, mixed and incubated at room temperature for 20 min. Then 0.1 ml of folin-ciocalteu in water (1:1.2) was added, mixed, incubated at room temperature for 30 min. then read the absorbance at 750 nm. Bovine serum albumin 0-100 μg/ml was used as standard.

NMR spectroscopy of *B. alba* mucilage

One dimension ^{1}H NMR spectroscopy was performed using the conditions described previously with some modification [176]. Six milligram of *B. alba* mucilaginous polysaccharide was kept in a desiccator over phosphorus pentoxide in vacuo for 24 h at room temperature. The thoroughly dried sample was dissolved in 0.6 mL of D2O (99.9%) and filtered through 0.45 μm PVDF centrifugal cup. The filtrate was repeatedly freeze-dried and redissolved in 0.6 mL of D2O for three times, then transferred to a Shigemi NMR tube (5.0 mm O.D. × 25 cm). NMR experiment was performed on a JEOL 500 MHz instrument with standard JEOL software at 30 °C.

Statistical Analysis

The significance of differences between the mean values was determined by analysis of variance (ANOVA), followed by Dunnett's test, and a *p* value of less than 0.05 was considered statistically significant.

CHAPTER V

RESULTS

Mucilages Extraction

Seven mucilaginous plants were studied as follows: aerial parts of *Basella alba* Linn. (Basellaceae), fruits of *Hibiscus esculentus* Linn. (Malvaceae), leaves of *Litsea glutinosa* (Lour.) C.B. Robinson (Lauraceae), seeds of *Ocimum canum* Sims. (Labiatae), seeds of *Plantago ovata* Forssk. (Plantaginaceae), fruits of *Scaphium scaphigerum* G. Don. (Sterculiaceae) and seeds of *Trigonella foenum-graecum* Linn. (Papilionaceae). The mucilages from selected plants yielded range from 3.5% to 23.0% as shown in Table 1 and Figure 12.

Table 2 Mucilaginous polysaccharides from selected plants

Plants	Used parts	Yield (% w/w)	
Basella alba Linn.	aerial parts	3.5	(fresh weight)
Hibiscus esculentus Linn.	fruits	5.6	(fresh weight)
Litsea glutinosa Lour.	leaves	12.0	(fresh weight)
Ocimum canum Sims.	seeds	17.6	(dry weight)
Plantago ovata Forssk.	seeds	19.0	(dry weight)
Scaphium scaphigerum G. Don.	fruits	23.0	(dry weight)
Trigonella foenum-graecum Linn.	seeds	15.0	(dry weight)

Figure 12 Mucilaginous polysaccharides from selected plants

1) *B. alba* 2) *H. esculentus* 3) *L. glutinosa*

Figure 12 (Cont.) Mucilaginous polysaccharides from selected plants

4) *O.* canum 5) *P.ovata* 6) *S. scaphigerum* 7) *T. foenum graecum*

Effect on Glucose Diffusion

From the studied model, all mucilages showed concentration dependence (0.5, 1.0 and 2.0%w/v) on glucose entrapment activity. The percentage of glucose releasing from 2.0% mucilages were 61.6, 70.8, 71.7, 80.6, 83.4, 85.8 and 92.8 % for *O. canum, P. ovata, T. foenum-graecum, L. glutinosa, H. esculentus, B. alba* and *S. scaphigerum* respectively. While 65.4% of glucose release was obtained from the glucomannan at the same concentration (2.0%) (Figure 13).
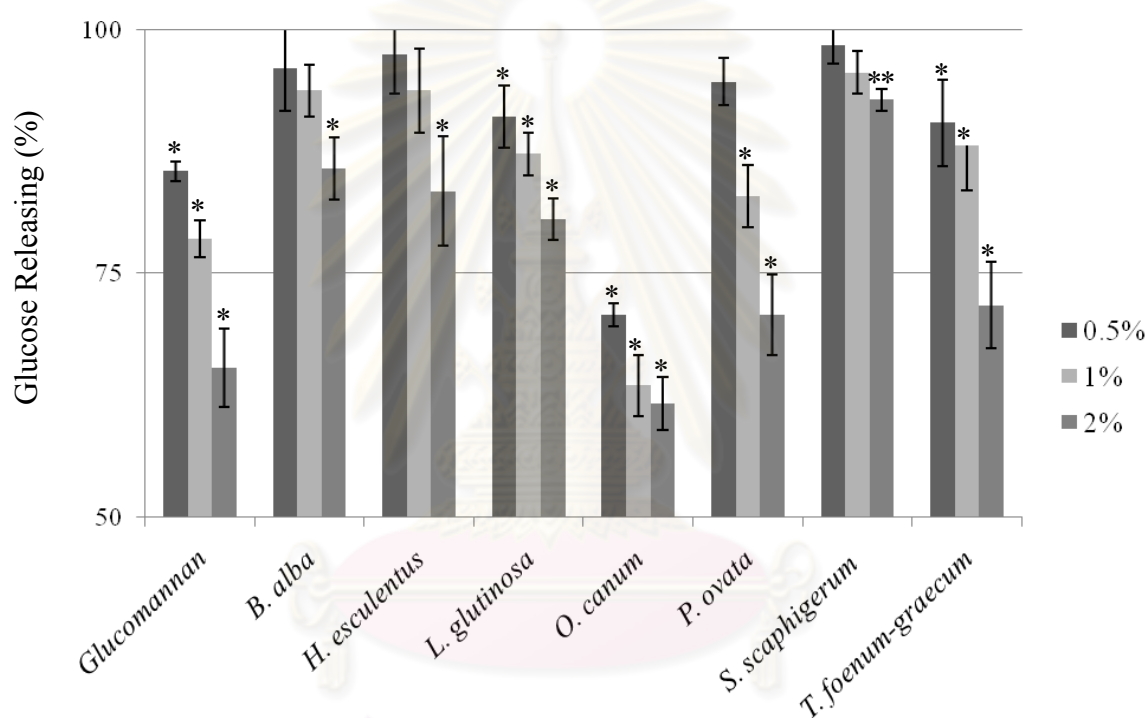
Figure 13 Percentages of glucose releasing from 0.5, 1, 2% mucilages containing 2% glucose after 2 hr dialysis (n=4). The significance of differences from the control (0% mucilages, 99.8±0.9%) was determined by ANOVA followed by Dunnett's test (*$p<0.01$, **$p<0.05$).

Effect on *α*-Glucosidase Inhibition

   Percentage of *α*-glucosidase inhibition by 0.5% mucilages isolated from, *S. scaphigerum*, *L. glutinosa*, *H. esculentus*, *O. canum*, *T. foenum-graecum*, *P. ovata*, *B. alba* and by 0.5% glucomannan showed were 82.6, 41.0, 37.6, 32.8, 30.6, 27.0, 25.0 and 19.7 % respectively, whereas 1-Deoxynorjirimycin at the same concentration showed the inhibitory activity of 47.6% (Figure 14). *S. scaphigerum* mucilage was further investigated and found that the concentration for 50% inhibition of *α*-glucosidase activity (IC_{50}) was 0.17% (1.7 mg/ml) (Figure 15).
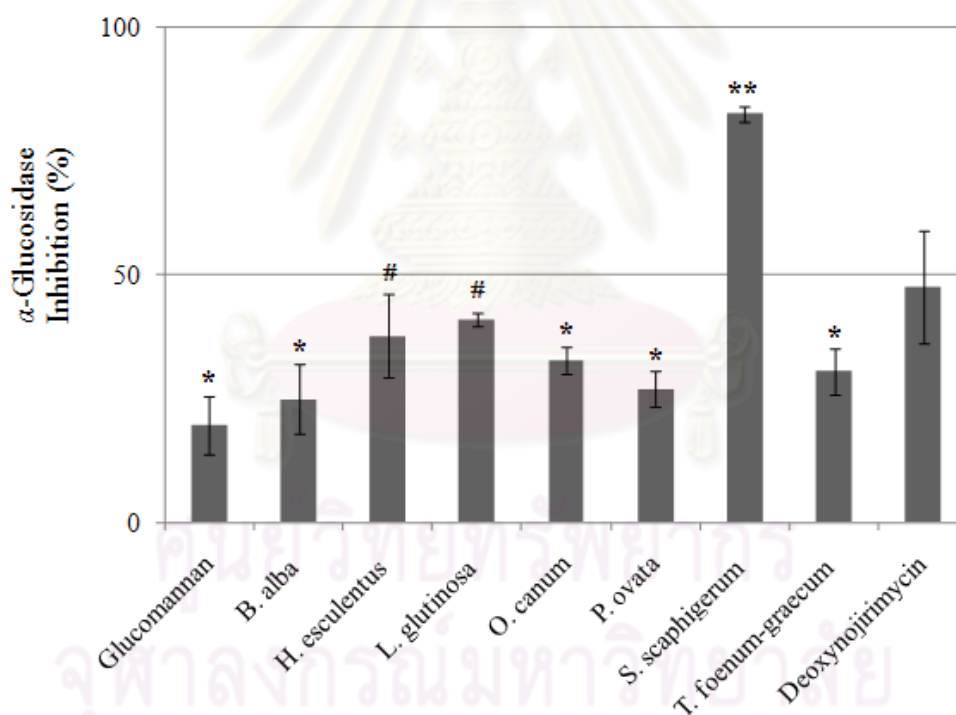
Figure 14 Percentage of *α*-glucosidase inhibition by 0.5% mucilages (n = 3). The significance of differences from the control (Deoxynojirimycin) was determined by ANOVA followed by Dunnett's test. *%inhibition was significantly lower than control ($p<0.05$), #%inhibition was not significantly different from control (Deoxynorjirimycin) ($p>0.05$), **%inhibition was significantly higher than control ($p<0.05$).
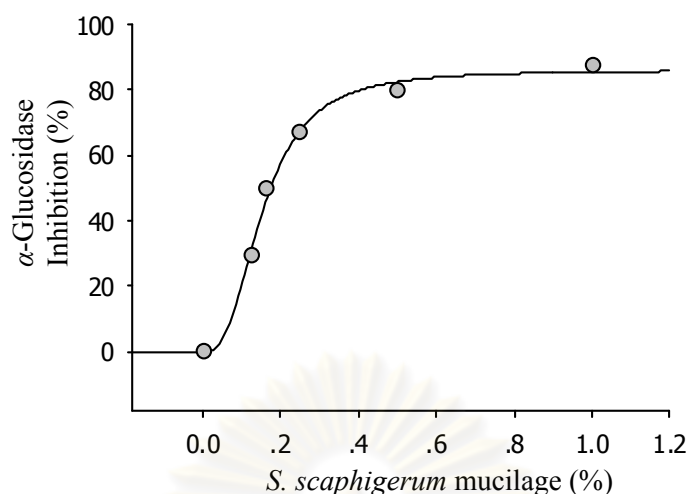
Figure 15 Percentage of *α*-glucosidase inhibition from various concentration of *S. scaphigerum* mucilage

Effect on Cholesterol Solubility in Single Bile Micelles

When mixed 0.5% of plant mucilages or glucomannan in choleterol-bile acid system, the extent of cholesterol solubility in the micelles was determined compared to bile acid alone. Mucilages from *B. alba, H. esculentus, L. glutinosa, P. ovata and S. scaphigerum* had no effect to cholesterol solubility, while mucilages from *O. canum* and *T. foenum-graecum* showed slightly inhibition as same as glucomannan (Figure 16).

Effects on Fatty Acids Solubility in Single Bile Micelles

The experiments on stearic acid (saturated fatty acid, C 18:0) showed contrary result. Most mucilages investigated and glucomannan, except mucilages from *B. alba* and *S. scaphigerum*, increased stearic acid solubilization in bile salt micelles. Mucilage from *B. alba* slightly inhibited stearic acid micellar solubilization whereas mucilage from *S. scaphigerum* had no effect on stearic acid solubility (Figure 17).

At 25 mM of NaTDC, cholesterol and stearic acid were saturated, but oleic acid was thoroughly dissolved. To reach the same saturation as cholesterol and stearic acid, bile acid was serial diluted and found that the concentration of 0.3125 mM was suitable for 10 mg of oleic acid in the study (Figure 18). At this circumstance, 0.5% mucilages especially glucomannan showed strong inhibition of oleic acid solubility in bile micelles (Figure 19).

Effect on Cholesterol Solubility in Mixed Bile Micelles

With oleic acid in the bile micelles, cholesterol solubility showed twofold increase (Figure 18). Slight inhibition of cholesterol solubility results from Glucomannan, mucilages from *O. canum* and *T. foenum-graecum* were obtained in mixed bile micelles similar to those obtained in single bile micelles, whereas mucilages from *H. esculentus, L. glutinosa, P. ovata and S. scaphigerum* had no effect on cholesterol solubility. There was an exception for the mucilage from *B. alba*. It was found that *B. alba* mucilage inhibited cholesterol solubility in mixed bile micelles only (Figure 20).
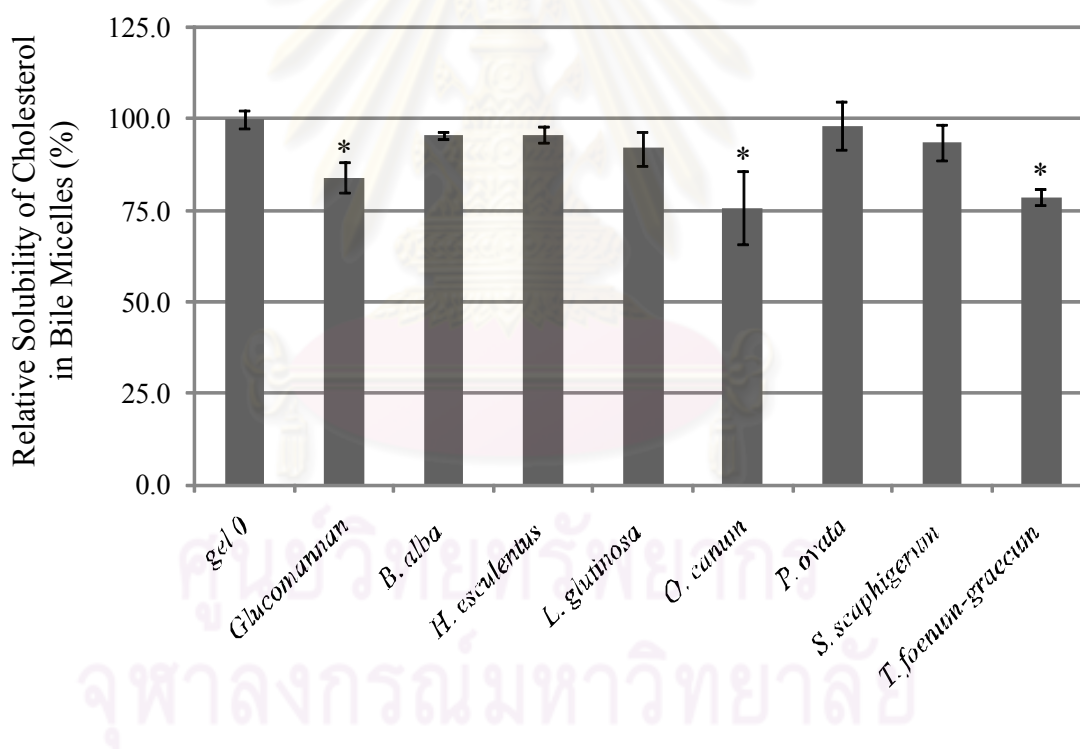
Figure 16 Percentages of micellar solubility of cholesterol in 25 mM NaTDC with 0.5 % w/v of mucilaginous polysaccharides compared to gel 0 (n=3).

The significance of differences from the control (0% mucilages) was determined by ANOVA followed by Dunnett's test (*$p<0.01$).
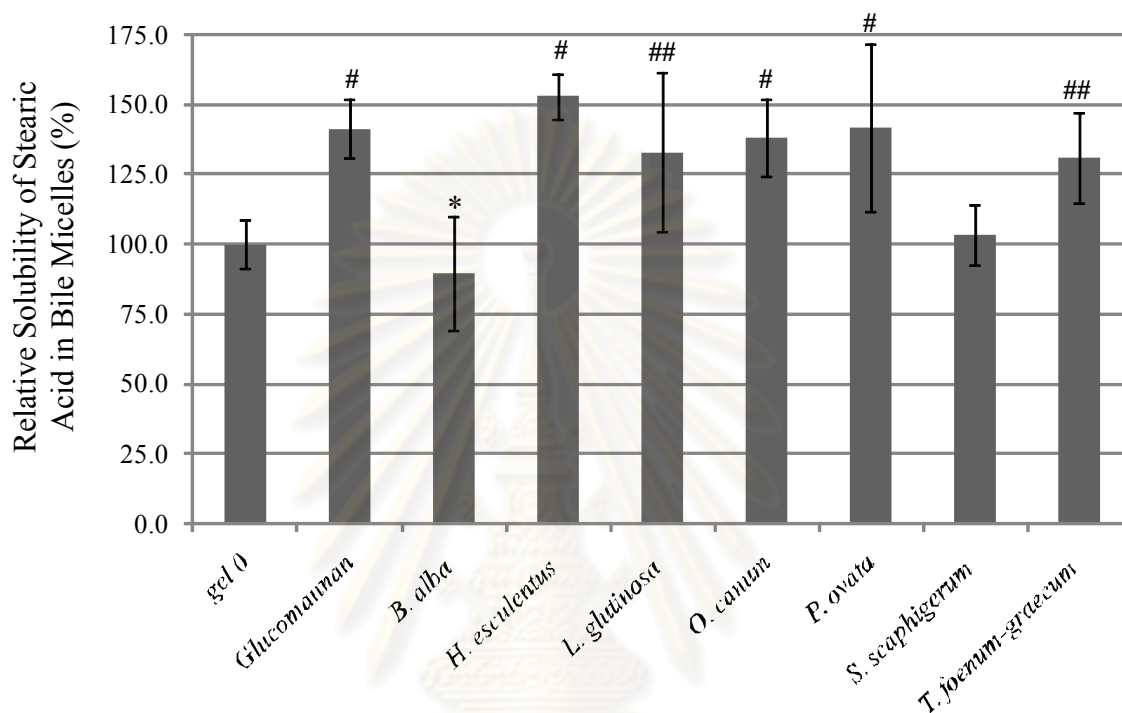
Figure 17 Percentages of micellar solubility of stearic acid in 25 mM NaTDC with 0.5 % mucilaginous polysaccharides compared to gel 0 (n=3).

*%Solubility was significantly lower than gel 0 ($p<0.05$), [#] %solubility was significantly higher than gel 0 ($p<0.01$), [##] %solubility was significantly higher than gel 0 ($p<0.05$)

The significance of differences from the control (0% mucilages) was determined by ANOVA followed by Dunnett's test.
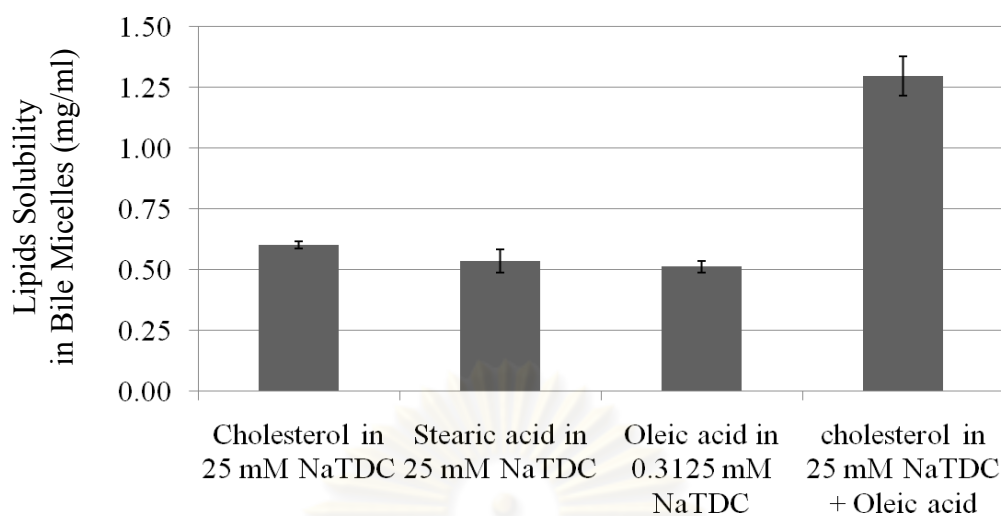
Figure 18 Concentration (mg/ml) of cholesterol, stearic acid and oleic acid in bile micelles (n=3) without mucilaginous polysaccharides (gel 0)
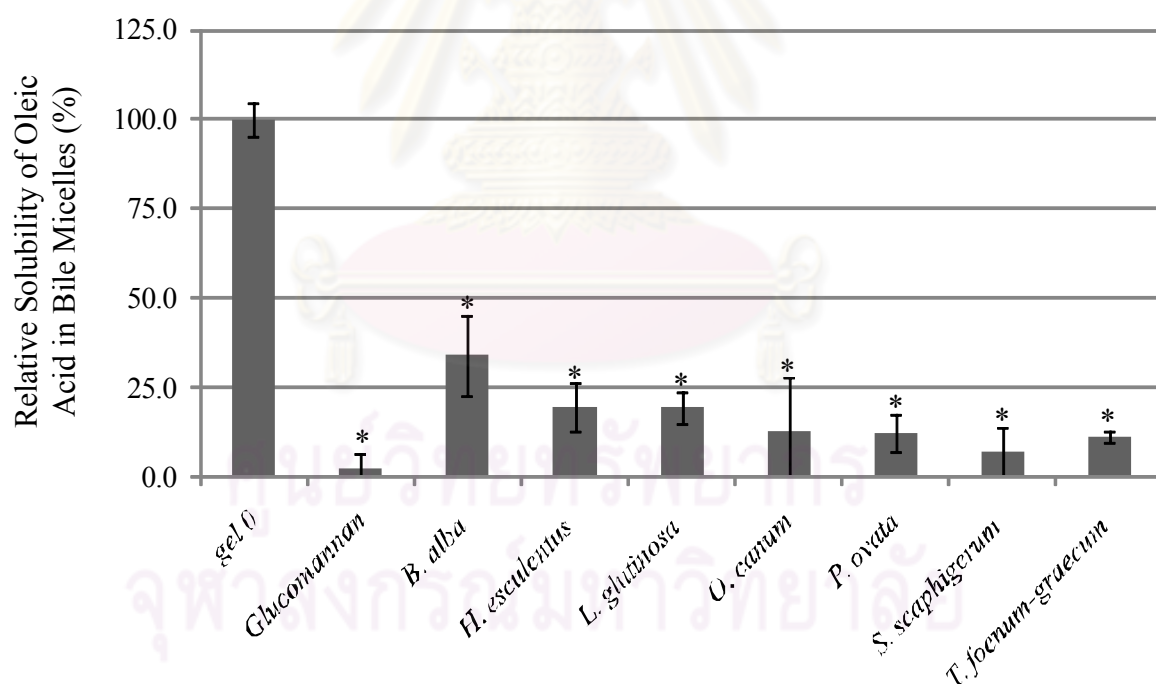
Figure 19 Percentages of micellar solubility of oleic acid in 0.3125 mM NaTDC with 0.5 % mucilaginous polysaccharides compared to gel 0 (n=3).

The significance of differences from the control (0% mucilages) was determined by ANOVA followed by Dunnett's test (*p<0.01).
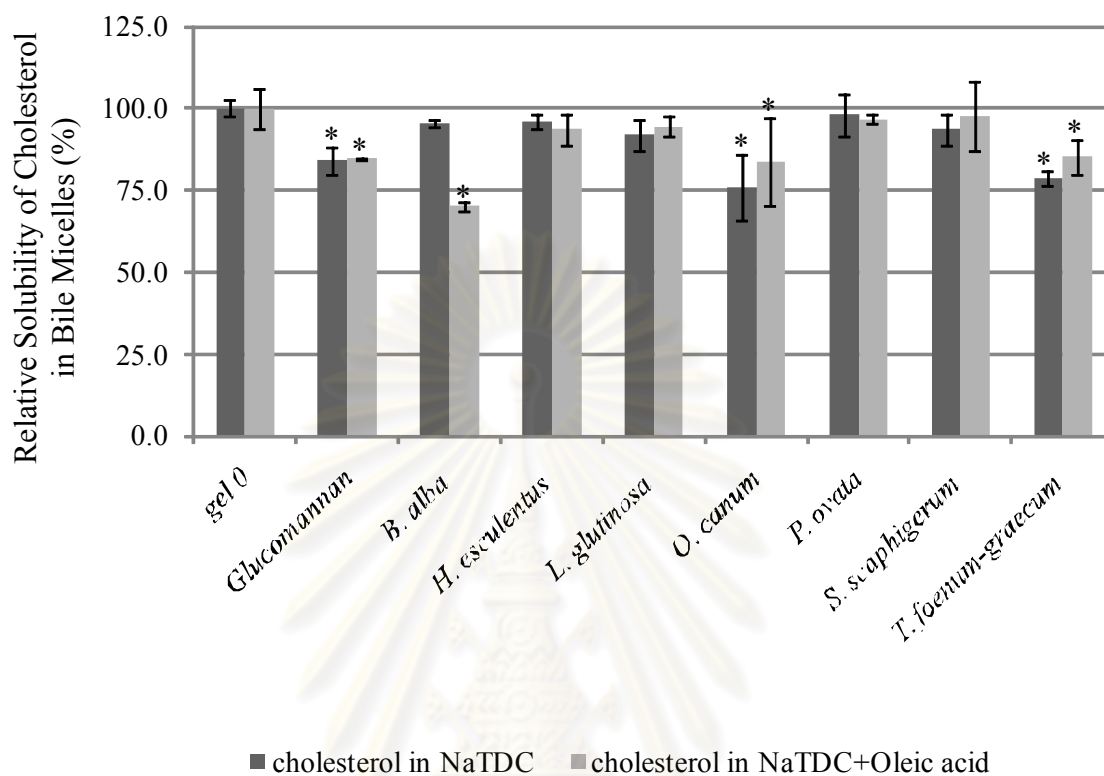
Figure 20 Percentages of micellar solubility of cholesterol in single bile micelles (25 mM NaTDC) and mixed bile micelles (25 mM NaTDC + Oleic acid) with 0.5 % w/v of mucilaginous polysaccharides compared to gel 0 (n=3).

The significance of differences from the control (0% mucilages) was determined by ANOVA followed by Dunnett's test (*$p<0.01$).

Effect on Lipolytic Activity

      Lypolysis of *p*-nitrophenyl laurate was catalyzed by pancreatic lipase. Lipolytic activity of lipase was inhibited by Orlistat with the IC_{50} of 8.4 µg/ml (Figure 21). All the studied mucilages showed no effects on the lipase activity (Figure 22). Moreover, the mucilages showed lipolytic effect on *p*-nitrophenyl laurate in the mixture without lipase (micellar catalytic effect). *O. canum* mucilage showed the strongest effect whereas glucomannan showed the weakest effect (Figure 23).
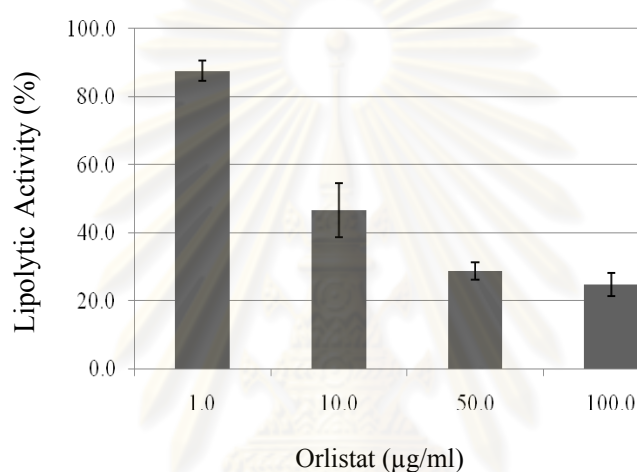
Figure 21 Percentages of lipolytic activity of pancreatic lipase with orlistat
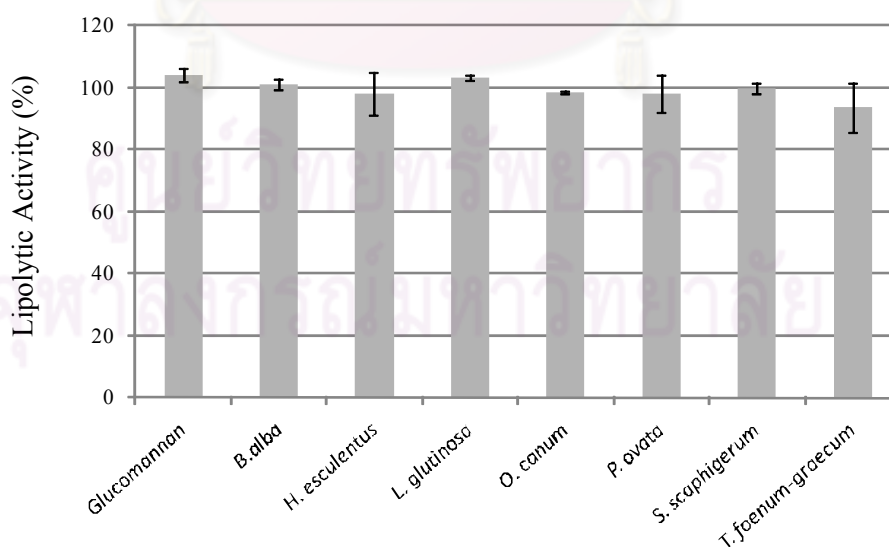
Figure 22 Percentages of lipolytic activity of pancreatic lipase with mucilages
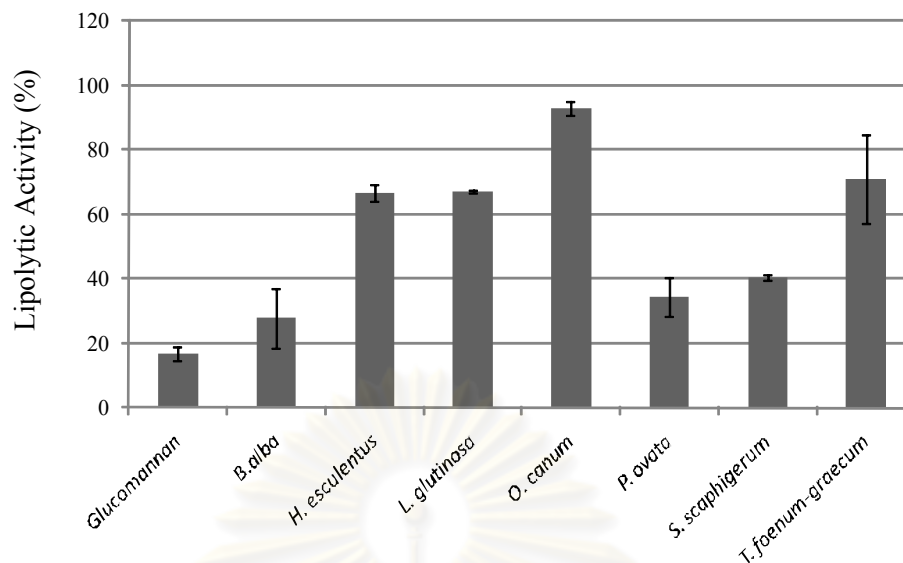
Figure 23 Percentages of lipolytic activity of mucilages without pancreatic lipase

DPPH Radical-Scavenging Activity

In the present study, the polysaccharide samples were incubated with 100 μM DPPH in methanol for 30 min using ascorbic acid as standard antioxidant (n=3). Table 3 demonstrated the scavenging activity of the polysaccharide samples on the DPPH radical. Except for mucilages from *O. canum* and *P. ovat*a, other mucilages showed scavenging activities higher than glucomannan. Especially the mucilages from *L. glutinosa, S. scaphigerum* and *H. esculentus* had IC$_{50}$ less than 1 mg/ml.

Table 3 DPPH scavenging activity as IC$_{50}$ among mucilaginous polysaccharides from selected plants

Mucilages	IC$_{50}$ (mg/ml)	Mucilages	IC$_{50}$ (mg/ml)
B. alba	2.00	*S. scaphigerum*	0.61
H. esculentus	0.70	*T. foenum-graecum*	1.52
L. glutinosa	0.49		
O. canum	>10	Glucomannan	4.15
P. ovata	>10	Ascorbic acid	0.02

Swelling Properties

Swelling properties of selected mucilages were studied (Table 4). *O. canum* and *S. scaphigerum* mucilages showed highest values of swelling volume (SV) and water absorption index (WAI) followed by *P. ovata* and *T. foenum-graecum* mucilages respectively. Other mucilages showed the same order of SV and WAI values as obtained from glucomannan.

Table 4 Swelling volume (SV) and water absorption index (WAI) among mucilaginous polysaccharides from selected plants (n=3)

Mucilages	SV (ml/g)	WAI (g/g)
Glucomannan	23.9 ± 1.9	22.1 ± 1.9
B. alba	25.7 ± 6.3	15.8 ± 3.0
H. esculentus	22.2 ± 4.6	20.7 ± 4.3
L. glutinosa	27.5 ± 8.5	20.6 ± 4.6
O. canum	$115.9 \pm 17.3^*$	$111.1 \pm 17.1^*$
P. ovata	$60.4 \pm 7.7^*$	$48.3 \pm 3.2^*$
S. scaphigerum	$210.5 \pm 4.3^*$	$102.8 \pm 1.6^*$
T. foenum-graecum	38.9 ± 1.0	19.1 ± 1.4

*The significance of differences of SV and WAI from the control (glucomannan) was determined by ANOVA followed by Dunnett's test (*p*<0.01).

Viscosity and Estimation of the Average Molecular Weights

       The viscosities of the selected mucilaginous polysaccharides using falling ball viscometry (n=3) were shown in Table 5. *O. canum* mucilage had highest viscosity value even at low concentration (0.5%), followed by Glucomannan, mucilages from *P. ovat*a, *T. foenum-graecum*, *H. esculentus*, *L. glutinosa* and *B. alba* respectively. *S. scaphigerum* mucilage formed non-homogenous gel which was broken by falling ball. Their average molecular weights (MW) as determined by high performance size exclusive chromatography (HPSEC) were shown in Table 5. *O. canum* mucilaginous polysaccharide had highest MW (~ 6,000 kDA), followed by mucilages from *T. foenum-graecum* (~ 4,000 kDA), *H. esculentus*, *S. scaphigerum*, *L. glutinisa*, Glucomannan, *P. ovat*a and *B. alba* respectively.

Table 5 Viscosity and MW among mucilaginous polysaccharides from selected plants (n=3)

Mucilages	MW (kDa)[*]	Viscosity (cP)		
		0.5%	1.0%	2.0%
Glucomannan	823 ± 99	21.1 ± 0.3	143.2 ± 8.9	$4,582.8 \pm 60.3$
B. alba	140 ± 27	2.2 ± 0.3	3.5 ± 0.4	7.0 ± 0.4
H. esculentus	$2,188 \pm 208$	7.7 ± 0.7	17.1 ± 0.9	45.1 ± 2.0
L. glutinosa	$1,532 \pm 204$	1.8 ± 0.2	5.9 ± 0.6	19.1 ± 2.6
O. canum	$> 2,350$	581.3 ± 59.1	$> 5,000$	$> 5,000$
P. ovata	408 ± 6	6.2 ± 0.3	18.5 ± 1.1	$1,575.3 \pm 57.5$
S. scaphigerum	$1,847 \pm 570$	N/A	N/A	N/A
T. foenum-graecum	$> 2,350$	6.7 ± 0.4	29.6 ± 1.9	213.0 ± 17.9

[*]Pullulan equivalent molecular weight (5.9 – 2,350 kDa)

N/A – Not Applicable

Monosaccharides and Total Protein Analysis

The selected mucilaginous polysaccharides as well as glucomannan were analyzed for the primary structures of their monosaccharide compositions as shown in Table 6 and 7. Total protein contents in these polysaccharides ranged from 2% w/w in *P. ovata* mucilage to 38% w/w in *L. glutinosa* mucilage (Table 6).

Table 6 Monosaccharide composition and total protein content (µg/mg)[a] among mucilaginous polysaccharides from selected plants

	Ara[b]	Rha	Xyl	Man	Gal	GalA	Glc	TP[c]
glucomanan				493.8 (11.6)			309.6 (3.6)	107.8 (0.04)
B. alba	43.5 (0.8)	10.7 (0.1)			88.7 (1.0)	33.7 (1.3)	34.6 (0.8)	235.5 (0.04)
H. esculentus		27.8 (0.6)			46.5 (1.4)		285.7 (1.4)	152.7 (0.04)
L. glutinosa	84.1 (1.2)	6.0 (0.2)	56.4 (0.5)	9.7 (0.1)	29.7 (0.6)		88.9 (0.5)	375.1 (0.01)
O. canum	47.8 (1.1)	28.0 (0.7)	98.4 (2.5)	37.5 (1.5)	156.2 (0.8)		66.3 (0.2)	81.8 (0.02)
P. ovata	165.2 (13.5)	52.0 (3.4)	697.5 (1.3)			51.7 (3.0)		20.3 (0.02)
S. scaphigerum	121.7 (3.4)	155.2 (4.8)	20.8 (0.7)		144.6 (0.3)	173.4 (2.9)	21.1 (0.2)	195.3 (0.03)
T. foenum-graecum		18.8 (0.4)	20.4 (0.2)	310.4 (1.2)	302.2 (1.2)		82.2 (0.5)	209.5 (0.01)

[a] mean (SD) of 3 runs. [b] monosaccharides found in these mucilages included arabinose (Ara), rhamnose (Rha), xylose (Xyl), mannose (Man), galactose (Gal), galacturonic acid (GalA) and glucose (Glc). Fucose (Fuc) and glucuronic acid (GlcA) were absent. [c] Total protein (TP).

Table 7 Monosaccharide composition (% mole ratio)[a] among mucilaginous poly-saccharides from selected plants

	Ara[b]	Rha	Xyl	Man	Gal	GalA	Glc
glucomanan				61			39
B. alba	24	5			41	13	16
H. esculentus		8			13		79
L. glutinosa	33	2	22	3	10		29
O. canum	12	6	25	8	34		14
P. ovata	18	5	74			4	
S. scaphigerum	23	24	4		23	23	3
T. foenum-graecum		3	3	42	41		11

[a] mean of 3 runs. [b] monosaccharides found in these mucilages included arabinose (Ara), rhamnose (Rha), xylose (Xyl), mannose (Man), galactose (Gal), galacturonic acid (GalA) and glucose (Glc). Fucose (Fuc) and glucuronic acid (GlcA) were absent.

NMR spectroscopy of *B. alba* mucilage

The 1D ^{1}H NMR spectra of *B. alba* mucilaginous polysaccharide was observed. The anomeric proton resonance showed chemical shifts of major *β*-linkage (4-5 ppm) and minor *α*-linkage (5-5.5 ppm). The signal at 4.20 ppm was singlet while the signals at 4.65-4.66, 5.10-5.11 and 5.24-5.25 ppm were doublet with the coupling constant of 7.8, 4.0 and 4.0 Hz respectively (Figure 24).
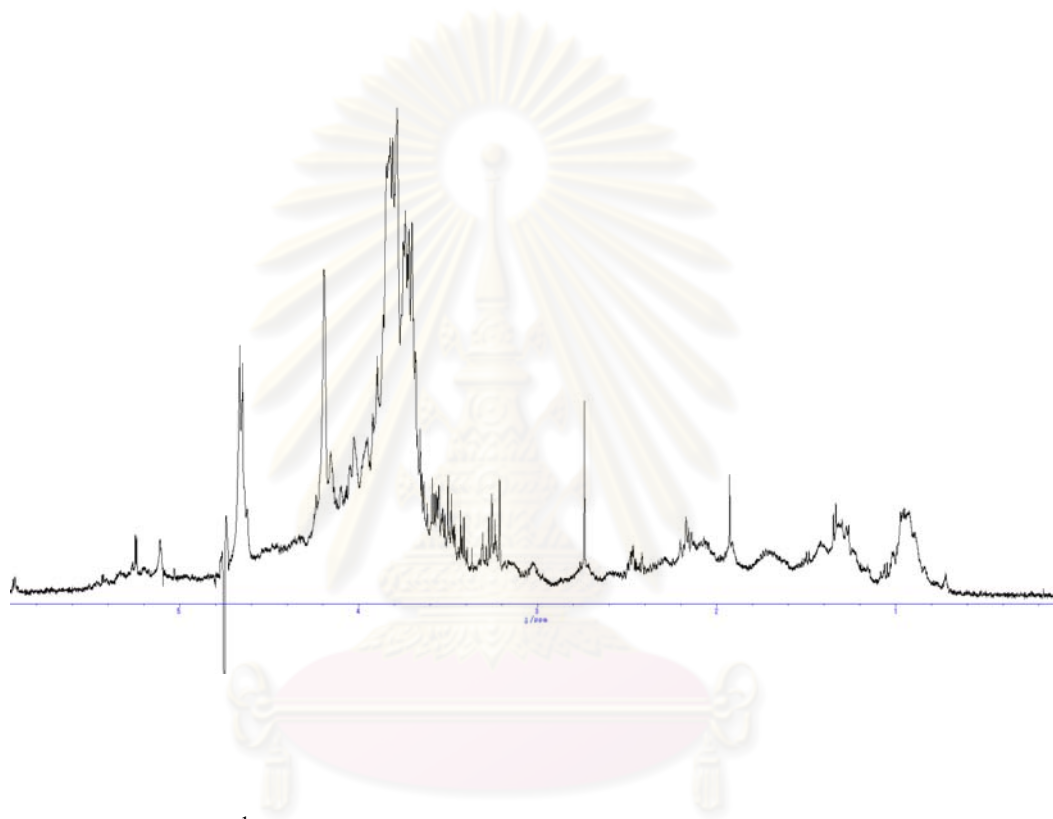
Figure 24 ^{1}H NMR spectra of *B. alba* mucilaginous polysaccharide

CHAPTER VI

DISCUSSION

Effect on Glucose Diffusion

Dialysis tubing technique is a simple model to evaluate the potential of soluble dietary fibers to additionally retard the diffusion of glucose in the intestinal tract. Although movement of glucose in the gut is not affected by the true diffusion but by the convective activity of intestinal contractions *In vitro* dialysis with shaking or stirring could mimic events occurring *in vivo* [139, 177]. The entrapment ability of mucilage gel resulted in decreasing of glucose diffusion into the external solution. The retardation of the nutrient flow into the external medium is an indication of the modulating effect of that fiber on glucose absorption in the jejunum [177]. From the studied model, all mucilages showed concentration dependence (0.5, 1.0 and 2.0%w/v) on glucose entrapment activity. The percentage of glucose releasing from 2% mucilages were 61.6, 70.8, 71.7, 80.6, 83.4, 85.8 and 92.8 % for mucilages from *O. canum, P. ovata, T. foenum-graecum, L. glutinosa, H. esculentus, B. alba* and *S. scaphigerum* respectively, while 65.4% of glucose release was obtained from the glucomannan at the same concentration. Glucomannan seems to be the most impressive natural fiber with increasing importance in the biomedical and pharmaceutical fields. It has been found to decrease the serum glucose levels and also the postprandial insulin flow which aiding diabetic control [36]. Psyllium seeds from *P. ovata* and fenugreek seeds from *T. foenum-graecum* are also reported as an adjunct to dietary therapy in patients with type II diabetes, to reduce glucose and glycosylated hemoglobin [12, 103]. Figure 1 showed that the retardation effect on glucose movement of the mucilages from *P. ovata* and *T. foenum-graecum* were lower degree than glucomannan. There have been previous studies of hypoglycemic effect of *O. canum* aqueous extract from leaves [178]. This study showed that *O. canum* mucilage of all concentration superiorly entrapped glucose compared to glucomannan.

Effect on *α*-Glucosidase Inhibition

$α$-Glucosidase inhibitor is a class of oral medications for type 2 diabetes. Its action affects on decreasing the absorption of dietary starches from the intestinal lumen so lowering the level of postprandial glucose and consequently reducing glucotoxicity on the endothelial vessels [118]. The effect of selected mucilages against α-glucosidase activity was evaluated. At the concentration of 0.5%, mucilages from *S. scaphigerum*, *L. glutinosa*, *H. esculentus*, *O. canum*, *T. foenum-graecum*, *P. ovata*, *B. alba* and glucomannan showed the inhibitory percentage of 82.6, 41.0, 37.6, 32.8, 30.6, 27.0, 25.0 and 19.7 % respectively. A positive control (1-Deoxynorjirimycin) at the same concentration showed the inhibition of 47.6%. *S. scaphigerum* mucilage was further investigated and found that the concentration for 50% inhibition of α-glucosidase activity (IC$_{50}$) was 0.17% or 1.7 mg/ml. *S. scaphigerum* or Malva nut tree is mostly found in the East of Thailand. The gel made from malva nuts is used as ingredients in dishes and beverages. Malva nut drink is traditionally used to relief coughing and sore throats. The previous study in type 2 diabetic patients reported that after the intake of malva nut drink, fasting plasma glucose and HbA1c decreased significantly [179]. The present study showed a possible mechanism in $α$-glucosidase inhibition.

Effects on Lipids Solubilities in Bile Micelles

Bile acids play an important role to increase lipid solubility in aqueous matrix by micelles formation. Lipid metabolism depends on its solubility which leads to permeability from intestinal lumen to circulation. Interfering of lipid-bile micelles characters can affect lipid solubility which influences on lipid absorption, distribution and metabolism. The bile acids are synthesized from cholesterol and conjugated to glycine or taurine with the ratio of glycine to taurine 2:1 in the liver and secreted into the gallbladder. Typical biosynthesized bile acids are classified into cholic acid (CA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) by the ratio of 12 (CA) : 7 (DCA) : 13 (CDCA) : 1 (UDCA) in human gallbladder [180]. Deoxycholic acid is a secondary bile acid converted from cholic acid by intestinal bacterial enzyme. This secondary bile acid is more hydrophobic and more capable to solubilize cholesterol and fatty acids in the intestinal lumen [181]. NaTDC has been previously reported for its stability in cholesterol-bile salt micelles formation [166, 182, 183].

Absorption of cholesterol and fatty acids depends on their ability to form micelles within the intestinal lumen. Several dietary factors have been reported to reduce cholesterol absorption. The exact mechanism to inhibit cholesterol absorption is not fully understood. Several mechanisms have been proposed, including competition with cholesterol for solubilization in micelles within the intestinal lumen, co-crystallization with cholesterol to form insoluble crystals, interaction with digestive enzymes and regulation of intestinal transporters of cholesterol [181]. There have been experimental evidences for competition solubilization between cholesterol and phytosterols [156, 157]. When equimolar amounts of cholesterol and sitosterol were sonicated together, the extent of cholesterol solubility in the micelles was significantly reduced (0.131 ± 0.010 to 0.043 ± 0.009) [159]. The cholesterol-lowering effect was similar for plant sterols and plant stanols [158]. It was found that pectin and acacia (0.5%) significantly increased the aqueous solubility of cholesterol while slight increases were observed for the solubility of cholesterol in both high and low molecular weight dextran solutions [184, 185]. Carageenans (0.1%) significantly decreased cholesterol solubility [185].

At 25 mM of NaTDC (above the critical micelles concentration [166]), cholesterol and stearic acid (saturated fatty acid, C 18:0) were saturated. Oleic acid (unsaturated fatty acid, C 18:1) has lower critical micelles concentration than stearic acid and cholesterol. So the concentration of NaTDC used for oleic acid experiment was adjusted to 80-fold lower than for stearic acid and cholesterol. Bile acids are naturally formed mixed micelles with phospholipids, fatty acids and monoglycerides. Many studies have reported enhanced bioavailability of drugs when administered in combination with mixed micellar solutions [186]. Oleic acid was found to up-regulate specific cholesterol uptake in the rat intestinal cell line [187] and enhance the affinity of drugs to the micellar phase [188]. From this study, cholesterol solubility was increased 2-fold in oleic-NaTDC mixed micelles.

The mucilages from *H. esculentus, L. glutinosa, P. ovata and S. scaphigerum* at the concentration of 0.5 % had no effect on cholesterol solubility in both simple and mixed micelles. Mucilages from *O. canum* and *T. foenum-graecum* showed slightly inhibition as same as glucomannan. Their inhibitory effects were not different between simple and mixed micelles. Even though all studied polysaccharides especially glucomannan strongly inhibit oleic acid solubility in micelles. Furthermore

B. alba which had the least effect on oleic acid solubility showed the extent of cholesterol solubilization inhibition in only mixed micelle. The mechanisms of interference on cholesterol micellar solubilization among mucilages from *B. alba, O. canum, T. foenum-graecum* and glucomannan might be different. The experiments on stearic acid showed that most mucilages and glucomannan, except mucilages from *B. alba* and *S. scaphigerum*, increased stearic solubilization in bile salt micelle. The previous clinical studies in healthy males and females found that high stearic acid diet resulted in less postprandial plasma triglyceride concentration than other saturated and unsaturated fatty acids [189, 190]. The studies in rats and hamsters showed that high dietary stearic acid reduced cholesterol absorption and increased excretion of endogenous cholesterol [191, 192]. One postulation was that the stability of micellar phospholipids containing stearic acid was impaired compared to unsaturated fatty acids. Otherwise, stearic acid might inhibit cholesterol absorption through systemic mechanisms within the intestinal lumen [193]. This study demonstrated the effects of the mucilaginous polysaccharides on decreasing oleic acid and increasing stearic acid in micelle formation which might be relevant to their cholesterol lowering potentials.

Effect on Lipolytic Activity

Pancreatic lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) is a key factor of postprandial lipid digestion and absorption. It catalyzes the hydrolysis of triglycerides to monoglycerides and free fatty acids. This enzyme is a class of interfacial enzymes that interact only with lipid-water interfaces such as emulsions, micelles or bilayers [194, 195]. The assay of lipolysis activated by lipase in this study was spectrophotometric determination using *p*-nitrophenyl acyl ester of lauric acid (C 12:0 fatty acid) as model substrate. The hydrolysis of *p*-nitrophenyl acyl esters yielded fatty acid and *p*-nitrophenol, a chromophore in slightly alkaline media [196]. Without enzyme and mucilage in the media, no lipolysis occurred. Enzymatic lipolysis in 37°C for 2 h was set as 100% of activity. The reaction showed dose dependent effect by lipase inhibitor, orlistat. The IC_{50} of orlistat in this study (8.4 µg/ml) was higher than the previous reports (28 ng/ml [197] and 0.22 µg/ml [198]). This was due to the difference in substrates, lipases and the assay conditions. The effect of the mucilages on enzymatic lipolysis was studied and found that the mucilages from *B. alba, H. esculentus, L. glutinosa, O. canum, P. ovata, S. scaphigerum, T foenum-graecum* as well as glucomannan did not inhibit lipase activity. On the other hand, these

mucilages showed the catalytic activity effect on non-enzymatic lipolysis of the model substrate. Micellar catalyzed chemically hydrolysis of *p*-nitrophenyl acyl esters were previously reported [199, 200]. Under alkaline condition (1 mole of NaOH per mole of ester), hydrolysis of an ester in microemulsion could be occured. The reaction in microemulsion type medium would be accelerated by compartmentalization, concentration of reactants and specific attraction between the reactant and the surfactant palisade layer [200]. The studied mucilages were found to be surface active on lipid-water interface but not on lipase enzyme. There was different interfacial activation among these mucilaginous polysaccharides.

DPPH Radical-Scavenging Activity

Endothelial dysfunction is a key factor in all stages of atherosclerosis development. This term refers to an imbalance in the production of vasodilators at the circumstance of high concentrations of reactive oxygen species or oxidative stress [118]. Scavenging of the stable free radical diphenylpicrylhydrazyl (DPPH) is the basis of a common antioxidant assay. There have been widely different protocols which differed in the conditions (i.e. pH, solvents, wavelength of measurement), yielding different results. In the present study, the polysaccharide samples were incubated with 100 μM DPPH in methanol for 30 min using ascorbic acid as standard antioxidant. The number of DPPH reduced by one molecule of ascorbic acid (antioxidant stoichiometry) was 2.5 which was higher than the theoretic value of 2.0 but close to the previous experiments [201, 202]. The study demonstrated the scavenging activity of the polysaccharide samples on the DPPH radical. Except for mucilages from *O. canum* and *P. ovat*a, other mucilages showed scavenging activities higher than glucomannan. Especially the mucilages from *L. glutinosa, S. scaphigerum* and *H. esculentus* had IC_{50} less than 1 mg/ml. Although the abilities were lower than that of ascorbic acid, these mucilages counteracted with DPPH as strongly as *Ganoderma* polysaccharides (IC_{50} between 3-13 mg/ml) [203].

Swelling Properties and Viscosity

There are a series of physical interactions in the gastrointestinal tract which affect absorption, as follow: diffusion of nutrients from the bulk solution to the intestinal epithelia, the rate of removal of waters of hydration from a complex nutrient solvent system, counter diffusion of nutrients away from the intestinal surface and

diffusion of nutrients along the epithelial surface to an appropriate absorptive site [204]. Water can be held within the polysaccharide matrix causing considerable swelling and viscous solution or gelation. Viscous polysaccharide gels may slow absorption by trapping nutrients, digestive enzymes or bile acids in the matrix and by slowing mixing and diffusion in the intestine [205]. These physical properties of selected mucilages were studied. *O. canum* and *S. scaphigerum* mucilages showed highest values of swelling volume and water absorption index followed by *P. ovata* and *T. foenum-graecum* mucilages respectively. Other mucilages showed the same values of swelling volume and water absorption index as glucomannan. Viscosity was determined using falling ball viscosity method. *O. canum* mucilage had highest viscosity value even at low concentration (0.5%). Viscous character seemed to be a prominent factor affected the hypoglycemic potential of *O. canum* but not *S. scaphigerum* mucilage.

Estimation of the Average Molecular Weights

The weight average MW of the mucilaginous polysaccharides in this study were relative MW based on the standard linear polymaltotriose polysaccharides - pullulans. The MW of glucomannan, around 800 kDa was in the range of previously reported (1000 kDa) [38]. Average molecular weights of polysaccharides isolated from *H. esculentus* and *S. scaphigerum* were ~ 2,000 kDa compared to 1,700 kDa of water extracted okra pod analyzed by Tomada *et al.* using gel chromatography [63] and 2,270 kDa of alkaline extracted malva nut gum reported by Somboonpanyakul and the colleagues using HPSEC [98]. The previous study of *L. glutinosa* mucilage of the bark revealed highly viscous polysaccharide product [71]. This study found that *L. glutinosa* mucilage of the leaves had MW of ~ 1,500 kDa. *P. ovata* mucilage extracted with water in this study showed the same range of MW (~ 400 kDa) as one extracted with alkali solution [206]. *T. foenum-graecum* mucilage by this method (> 2,350 kDa) showed higher MW than the previous reports (1,418 – 2,350 kDa) [100, 101]. *O. canum* seeds mucilage has been reported for MW of 3,030 kDa [74] which corresponded to the highest MW of this mucilage among selected polysaccharides. *B. alba* mucilage was revealed in this study for its MW of 140 kDa. It should be noted that this MW estimation had the limitation due to some factors. The first one was that the MW of the studied polysaccharides was determined from the elution time of each

polysaccharide across the gel permeation chromatography column compared to the elution characteristics of pullulan. The MW corresponded to the size of the polysaccharides. The differences in structure and conformation among the tested polysaccharides and pullulan standard affected the uncertainty of the results [207]. The second limitation was due to the lack of information about the MW distribution among these studied polysaccharides.

Monosaccharides and Total Protein Analysis

The selected mucilages as well as glucomannan were analyzed for the primary structures of their monosaccharide compositions. Konjac glucomannan in this study showed the mannose : glucose molar ratio of around 1.6 : 1 which was in the range reported elsewhere. Galacturonic acid was found in 3 from 7 mucilages namely *B. alba*, *P. ovata* and *S. scaphigerum*. Whereas rhamnose was common sugar found in all studied mucilages. Chemical analyzes of the mucilages in this study showed some discrepancies from the previous reports. This was due to the differences in either plant origin or methodology of extraction and analysis. Literature reviews of monosaccharide composition among these mucilages were summarized in Table 8. *H. esculentus* mucilage from this study had highest glucose (79%) composition compared to 44% and 5% from the literatures. *L. glutinosa* mucilage from the leaves in this study had xylose/ arabinose ratio differed from the reported barks. *O. canum* mucilage showed higher propotion of galactose and xylose than previous studies. *P. ovata* and *T. foenum-graecum* mucilages displayed the typical characters of arabinoxylan and galactomannan respectively. The water extracts of *S. scaphigerum* mucilages in this report had similar ratio of monosaccharide with the alkaline extracts reported elsewhere. *B. alba* mucilage was firstly revealed the composition of arabinose, rhamnose, galactose, galacturonic acid and glucose (24:5:41:13:16). Total protein contents in these crude polysaccharides ranged from 2% in *P. ovata* to 38% in *L. glutinosa*.

Table 8 Monosaccharides composition among mucilaginous polysaccharides from literatures

glucomanan	Man : Glc 1.6 : 1	[35]
H. esculentus	Rha : Gal : GalA 21.8 : 25.2 : 27.3	[63]
	Rha : Gal : GalA: Glc : GlcA 1 : 2.5 : 1.8 : 0.3 : 0.2	[64]
	Ara : Rha : Xyl : Man : Gal : GalA : Glc : GlcA 5 : 3 : 5 : 3 : 17 : 16 : 44 : 7	[65]
L. glutinosa	Ara : Xyl 3.4 : 1.0 (barks)	[71]
O. canum	Ara : Rha : Xyl : Man : Gal : Glc 1 : 2 : 1 : 2 : 5 : 8 (uronic acids 8.15%)	[73]
	Ara : Rha : Xyl : Gal 6.0 : 7.7 : 9.7 : 1.1	[74]
P. ovata	Ara : Rha : Xyl : Gal 23.3 : 0.8 : 75.1 : 1.2	[206]
	Ara : Rha : Xyl : Man : Gal : Glc 20.7 : 1.1 : 50.3 : 1.1 : 4.8 : 2.0	[208]
	Ara : Rha : Xyl : Man : Gal : Glc 22.0 : 1.5 : 56.7 : 0.4 : 3.8 : 0.6	[209]
S. scaphigerum	Ara :Rha : Gal 1.1 : 1.0 : 1.0	[98]
T. foenum-graecum	Ara: Rha : Man : Gal : Glc 0.5 : 0.2 : 31.4 : 26.2 : 0.6	[101]
	Gal : Man 1.00 : 1.02 to 1.00 : 1.14	[100]

NMR spectroscopy of *B. alba* mucilage

      B. alba mucilaginous polysaccharide was further investigated for more details using its proton NMR spectrum. The strong doublet signals at 4.65 and 4.66 with the coupling constant of 7.8 Hz were assigned to the anomeric proton (H-1) of β-D-galactopyranosyl residue. The signal at 4.20 was ascribed to the H-4 of $1 \rightarrow 4$ linked galactose residue [210-212]. The weak doublet signals at 5.10-5.11 and 5.24-5.25 ppm with the coupling constant of 4.0 Hz might be assigned to α-arabinofurasyl residue [212, 213]. The NMR spectrum corresponded with the monosaccharide composition analyzed by GC which galactose was shown as major component followed by arabinose. The sharp signals at 1.93 and 2.74 ppm were not assigned. The preliminary investigation of *B. alba* mucilage postulated the backbone structure of β-galactan.

CONCLUSION AND RECOMMENDATION

The plants bearing mucilages in this study have been well known in Thailand as both edible and medicinal plants. Mucilaginous typed polysaccharides from these plants were investigated for the biological activities especially antidiabetic potential. The *in vitro* models were performed and there seemed to be various mechanisms possibly involved by the mucilages due to their physico-chemical characteristics. Konjac glucomannan was comparatively studied. *O. canum* mucilage from the seeds exhibited potential in glucose entrapment and cholesterol micellar solubilization inhibition. The glucose entrapment potential was attributable to its very high viscosity and MW. The other mucilages as well as glucomannan performed dose related viscosity and glucose entrapment effect. *S. scaphigerum* mucilage was revealed for its other potential for antidiabetes, the α-glucosidase inhibitor with IC_{50} of 0.17%. In addition to *O. canum* mucilage, *T. foenum-graecum* and *B. alba* mucilages and glucomannan could inhibit cholesterol solubilization in bile acid micelles. All polysaccharides investigated affected fatty acids solubility in bile acid micelles as well as non-enzymatic micellar catalysis of lipolysis. The mucilages from *L. glutinosa*, *S. scaphigerum* and *H. esculentus* also possessed anti- free radical characteristics with IC_{50} of less than 1 mg/ml. The monosaccharides composition among these mucilaginous polysaccharides were demonstrated and compared to another reports from distinguished techniques. *B. alba* mucilaginous polysaccharide was first reported for its composition and postulated its *β*-galactan backbone.

The *in vitro* models are beneficial tools for primary investigation of bioactive nutrients. They provide analytical supports to refine the possible and capable research designs prior to further *ex vivo* or *in vivo* studies which help in the reduction of the use of laboratory animals as well as clinical trial participants. Further researches are needed to establish the bioactivity, bioaccessibility and bioavailability of each mucilaginous polysaccharide during digestion and absorption. There also are many species of natural products bearing mucilages in Thailand. The *in vitro* study designs which mimic the physiological conditions in human gastrointestinal tract are needed to be developed for valid screening information.

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APPENDICES

APPENDIX A

Data of *In Vitro* Biological Activities Determination

Table 9 Percentages of glucose releasing from 0.5, 1, 2% mucilages containing 2% glucose after 2 hr dialysis (n=4)

Mucilages	Percentages of releasing glucose		
	0.5%	1%	2%
Glucomannan	$85.5 \pm 1.0^{*}$	$78.5 \pm 1.9^{*}$	$65.4 \pm 4.0^{*}$
B. alba	96.1 ± 4.4	93.8 ± 2.7	$85.8 \pm 3.2^{*}$
H. esculentus	97.5 ± 4.1	93.8 ± 4.3	$83.4 \pm 5.6^{*}$
L. glutinosa	$91.1 \pm 3.2^{*}$	$87.3 \pm 2.2^{*}$	$80.6 \pm 2.1^{*}$
O. canum	$70.8 \pm 1.2^{*}$	$63.5 \pm 3.1^{*}$	$61.6 \pm 2.7^{*}$
P. ovata	94.7 ± 2.4	$83.0 \pm 3.2^{*}$	$70.8 \pm 4.2^{*}$
S. scaphigerum	98.4 ± 1.9	95.6 ± 2.2	$92.8 \pm 1.1^{**}$
T. foenum-graecum	$90.5 \pm 4.4^{*}$	$88.1 \pm 4.6^{*}$	$71.7 \pm 4.4^{*}$

The significance of differences from the control (0% mucilages, $99.8 \pm 0.9\%$) was determined by ANOVA followed by Dunnett's test ($^{*}p<0.01$, $^{**}p<0.05$).
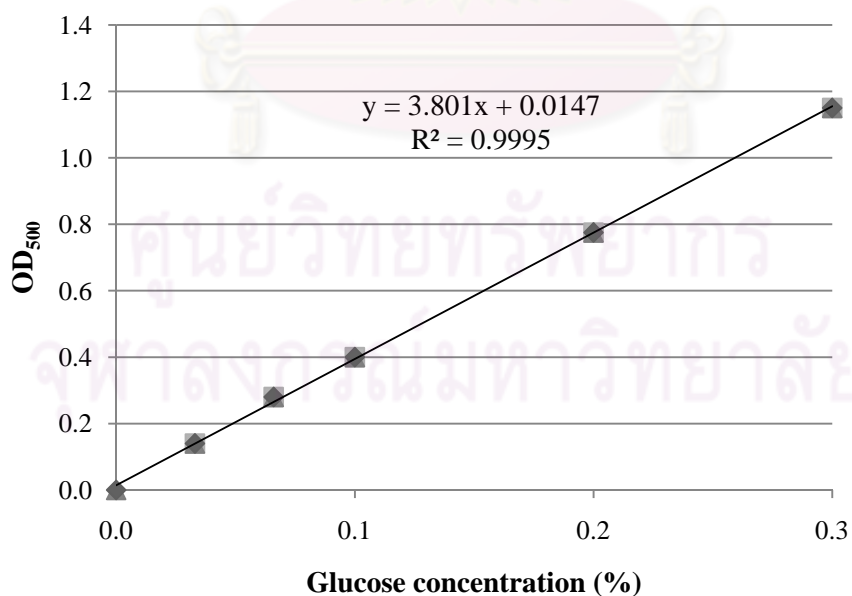

Figure 25 Calibration curve for glucose determination by glucose oxidase method

Table 10 Percentage of α-glucosidase inhibition of 0.5% mucilages (n = 3)

Mucilages	% Inhibition
Glucomannan	$19.7 \pm 5.9^{*}$
B. alba	$25.0 \pm 7.0^{*}$
H. esculentus	$37.6 \pm 8.4^{\#}$
L. glutinosa	$41.1 \pm 1.4^{\#}$
O. canum	$32.8 \pm 2.8^{*}$
P. ovata	$27.0 \pm 3.6^{*}$
S. scaphigerum	$82.6 \pm 1.5^{**}$
T. foenum-graecum	$30.6 \pm 4.7^{*}$
Deoxynojirimycin (positive control)	47.6 ± 11.4

The significance of differences from the control (Deoxynojirimycin) was determined by ANOVA followed by Dunnett's test. *% Inhibition was significantly lower than control ($p<0.05$), $^{\#}$% inhibition was not significantly different from control ($p>0.05$), **% inhibition was significantly higher than control ($p<0.05$).

Table 11 The concentration (mg/ml) of cholesterol solubilized in bile micelles with and without 0.5 % mucilages (n = 3)

Mucilages	Cholesterol[a] in NaTDC	Cholesterol[b] in NaTDC + Oleic acid
Gel 0	0.602 ± 0.015	1.296 ± 0.080
Glucomannan	$0.506 \pm 0.025^*$	$1.097 \pm 0.007^*$
B. alba	0.576 ± 0.007	$0.908 \pm 0.017^*$
H. esculentus	0.577 ± 0.013	1.213 ± 0.060
L. glutinosa	0.553 ± 0.029	1.226 ± 0.040
O. canum	$0.456 \pm 0.060^*$	$1.084 \pm 0.174^*$
P. ovata	0.591 ± 0.039	1.253 ± 0.018
S. scaphigerum	0.563 ± 0.029	1.267 ± 0.137
T. foenum-graecum	$0.473 \pm 0.013^*$	$1.105 \pm 0.067^*$

[a]The concentration of cholesterol (mg/ml) in 3 ml of 25 mM NaTDC, [b]The concentration of cholesterol (mg/ml) in 3 ml of 25 mM NaTDC containing 10 mg of oleic acid.

The significance of differences from the control (0% mucilages) was determined by ANOVA followed by Dunnett's test. *The concentration was significantly lower than control (p<0.01).

Table 12 The concentration (mg/ml) of fatty acids solubilized in bile micelles with and without 0.5 % mucilages (n = 3)

Mucilages	Stearic acid[a]	Oleic acid[b]
Gel 0	0.536 ± 0.047	0.512 ± 0.025
Glucomannan	$0.757 \pm 0.056^{\#}$	$0.011 \pm 0.020^{*}$
B. alba	$0.480 \pm 0.109^{**}$	$0.156 \pm 0.051^{*}$
H. esculentus	$0.819 \pm 0.043^{\#}$	$0.090 \pm 0.031^{*}$
L. glutinosa	$0.711 \pm 0.152^{\#\#}$	$0.089 \pm 0.020^{*}$
O. canum	$0.740 \pm 0.074^{\#}$	$0.059 \pm 0.069^{*}$
P. ovata	$0.759 \pm 0.160^{\#}$	$0.056 \pm 0.023^{*}$
S. scaphigerum	0.554 ± 0.058	$0.034 \pm 0.031^{*}$
T. foenum-graecum	$0.701 \pm 0.086^{\#\#}$	$0.052 \pm 0.008^{*}$

[a]The concentration of stearic acid (mg/ml) in 3 ml of 25 mM NaTDC, [b]The concentration of oleic acid (mg/ml) in 3 ml of 0.3125 mM NaTDC.

The significance of differences from the control (0% mucilages) was determined by ANOVA followed by Dunnett's test. **The concentration was significantly lower than control ($p < 0.01$), *The concentration was significantly lower than control ($p < 0.05$), #The concentration was significantly higher than control ($p < 0.01$), ##The concentration was significantly higher than control ($p < 0.05$).
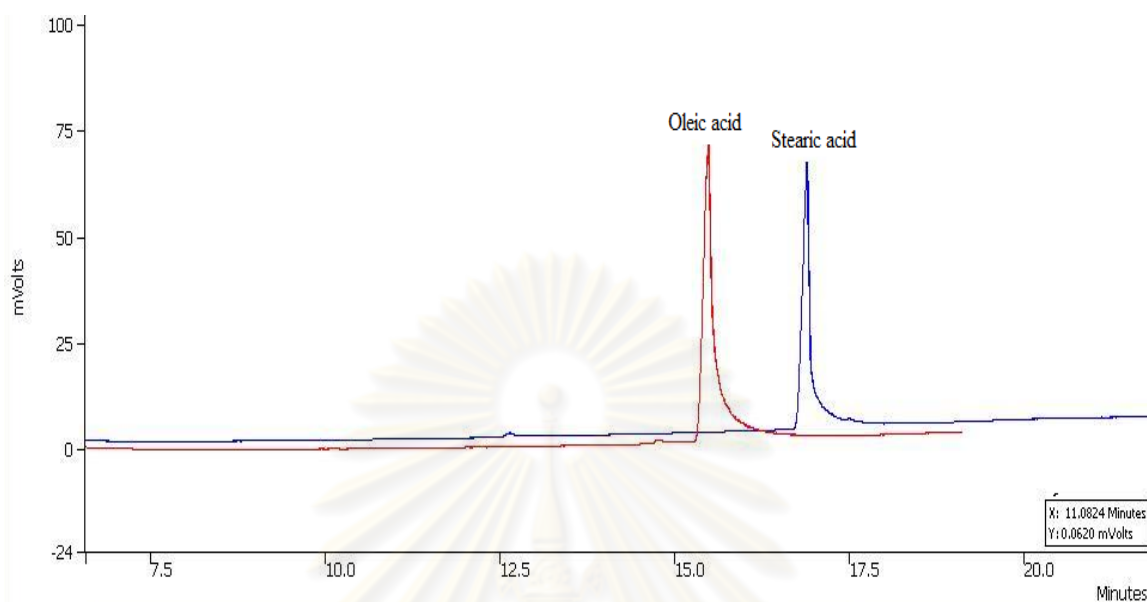
Figure 26 Chromatogram of stearic acid and oleic acid on FFAP column (30m x 0.25 mm x 0.25 μm) using flame ionization detector. The oven temperature was 200°C, raised to 250°C at the rate 2.5°C/min, the injector and the detector temperatures were set at 200 and 250°C, respectively. The helium carrier gas was controlled at 1 ml/min.
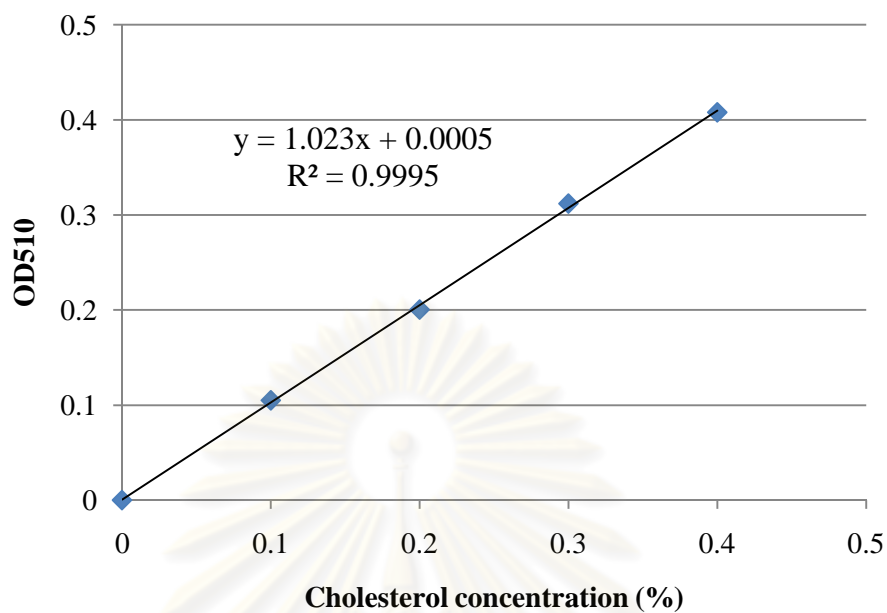
Figure 27 Calibration curve for cholesterol determination by cholesterol oxidase method
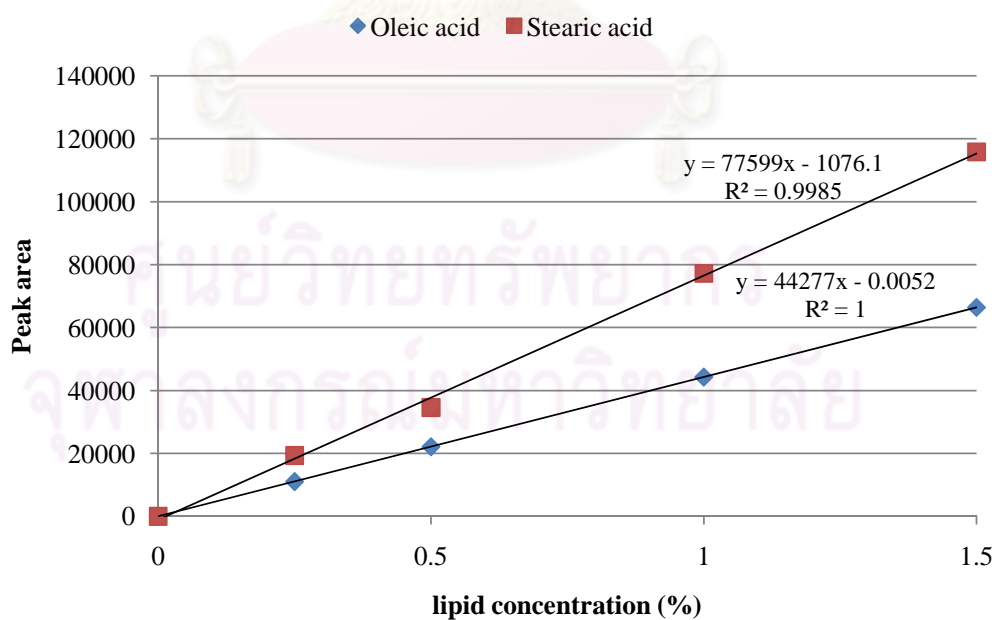
Figure 28 Calibration curve for stearic acid and oleic acid determination by GC

Table 13 Percentage of lipolytic activity of 1.0% mucilages on *p*-nitrophenyl laurate (n = 3)

Mucilages	With lipase	Without lipase
Gel 0	100.0	0
Glucomannan	104.2 ± 2.1	16.8 ± 2.0
B. alba	101.2 ± 1.8	27.8 ± 9.3
H. esculentus	98.0 ± 6.7	66.6 ± 2.6
L. glutinosa	103.3 ± 0.8	67.1 ± 0.5
O. canum	98.6 ± 0.4	92.9 ± 2.1
P. ovata	98.2 ± 6.1	34.4 ± 6.0
S. scaphigerum	99.9 ± 1.8	40.3 ± 0.9
T. foenum-graecum	93.7 ± 8.0	70.9 ± 13.9
Orlistat (µg/ml)		
1	87.5 ± 2.9	
10	46.5 ± 7.9	
50	28.7 ± 2.5	
100	24.6 ± 3.4	

Table 14 DPPH scavenging activity (%) among mucilaginous polysaccharides from selected plants

Mucilages	mg/ml	% Scavenging	Mucilages	mg/ml	% Scavenging
Glucomannan	0.5	13.4 ± 4.8	*O. canum*	5.0	
	1.0	18.5 ± 3.7		10.0	
	2.0	27.2 ± 5.1	*P. ovata*	5.0	
	3.0	37.9 ± 3.7		10.0	
	4.0	46.3 ± 0.6	*S. scaphigerum*	0.25	24.9 ± 3.9
	5.0	59.2 ± 0.4		0.5	39.1 ± 0.9
	10.0	74.2 ± 1.0		0.625	52.2 ± 0.8
B. alba	0.5	13.7 ± 1.8		1.25	67.7 ± 1.6
	1.0	23.6 ± 4.0		2.5	68.0 ± 1.2
	2.0	51.7 ± 4.1		5.0	64.8 ± 1.4
	3.0	65.3 ±2.5	*T. foenum-graecum*	0.5	17.1 ± 0.9
	4.0	66.6 ± 1.5		1.0	31.1 ± 1.4
	5.0	65.3 ± 1.4		2.0	62.1 ± 1.5
H. esculentus	0.5	36.6 ± 1.7		3.0	66.9 ± 2.2
	1.0	61.2 ± 1.0		4.0	69.7 ± 1.6
	2.0	72.8 ± 0.6		5.0	69.8 ± 0.3
	3.0	74.8 ± 0.2	Ascorbic acid[*]	0.004	5.8 ± 0.3
	4.0	76.1 ± 0.6		0.008	17.0 ± 3.9
	5.0	75.7 ± 0.3		0.012	25.7 ± 0.8
L. glutinosa	0.1	14.6 ± 4.2		0.016	36.5 ± 1.0
	0.2	20.7 ± 1.4		0.020	47.6 ± 0.5
	0.3	27.3 ± 1.3		0.050	82.1 ± 0.5
	0.4	38.1 ± 4.3			
	0.5	51.2 ± 1.1			
	1.0	73.3 ± 0.9			
	2.0	70.8 ± 1.3			
	3.0	69.5 ± 0.8			
	4.0	66.0 ± 0.7			
	5.0	66.1 ± 0.6			

[*]Positive control

APPENDIX B

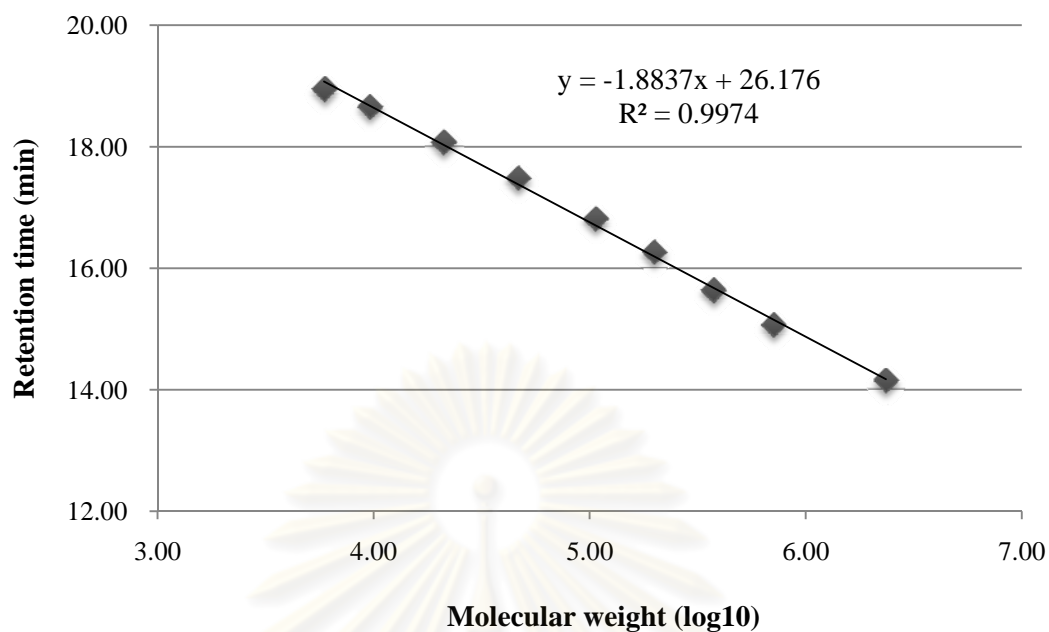Data of Physicochemical Determination

Figure 29 Calibration curve for average molecular weight determination by HPSEC
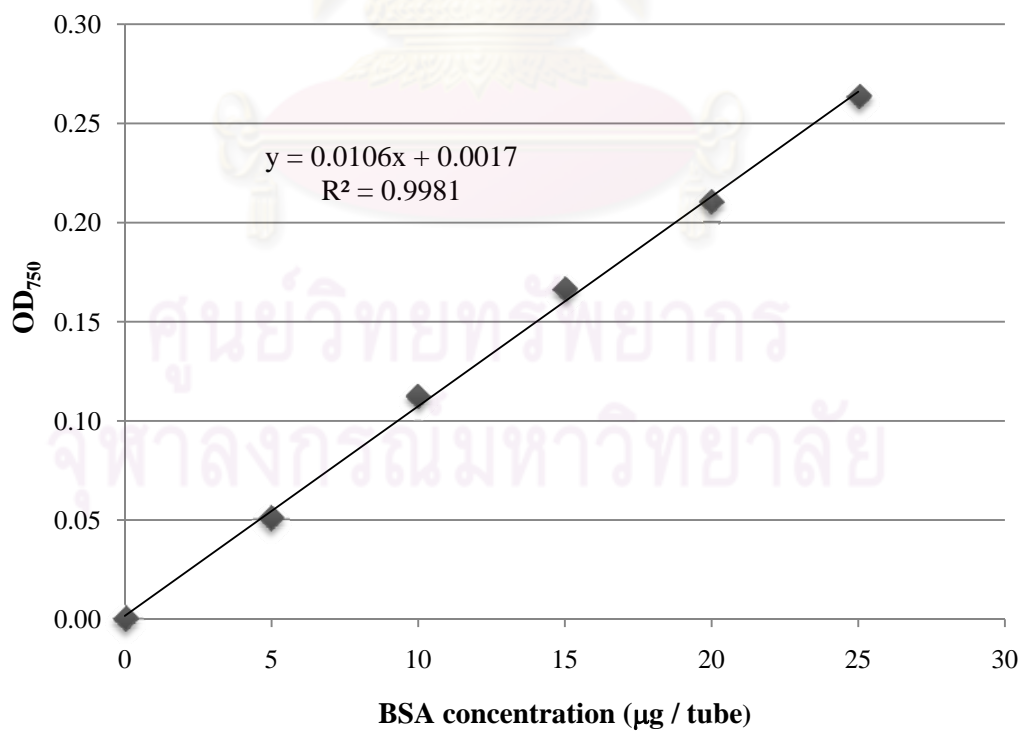
Figure 30 Calibration curve for protein determination by Lowry method
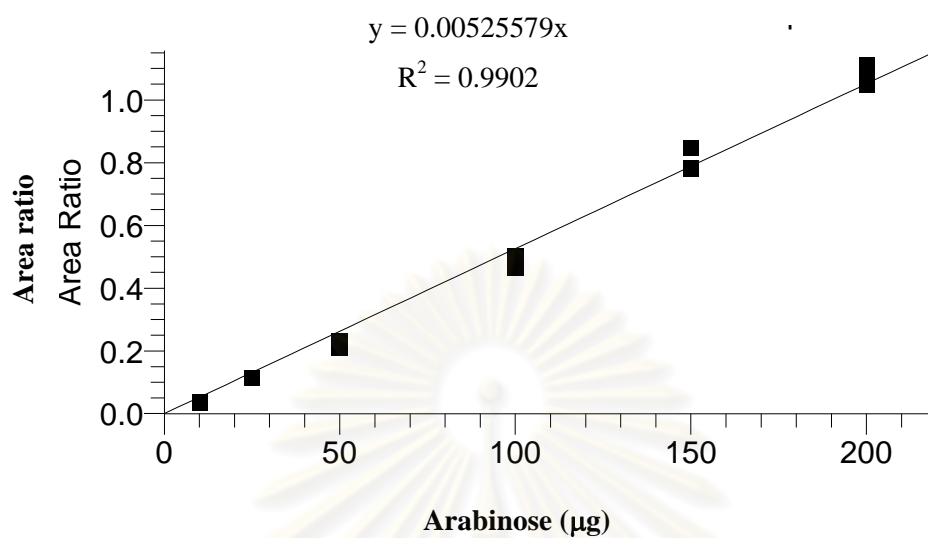
$$y = 0.00525579x$$
$$R^2 = 0.9902$$

Figure 31 Calibration curve for arabinose determination by GC
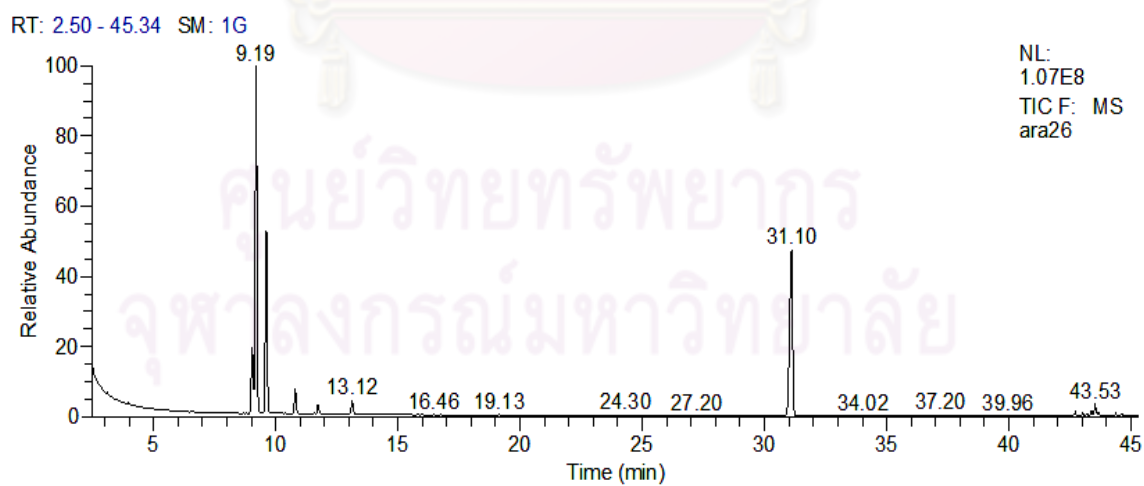
Figure 32 GC chromatogram of arabinose

$$y = -0.00404996 + 0.0049603x$$

$$R^2 = 0.9792$$
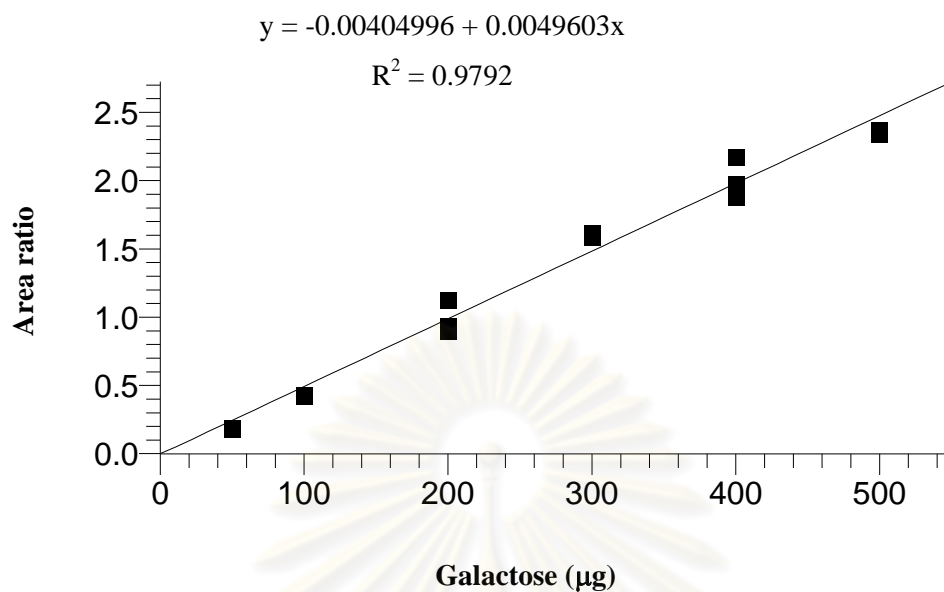
Figure 33 Calibration curve for galactose determination by GC
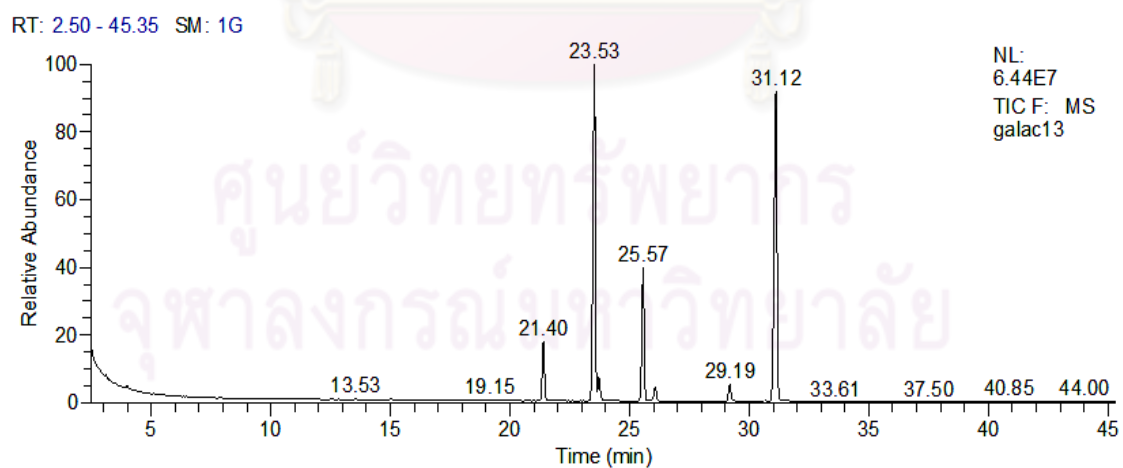
Figure 34 GC chromatogram of galactose

$$y = 0.00252781x$$
$$R^2 = 0.9803$$
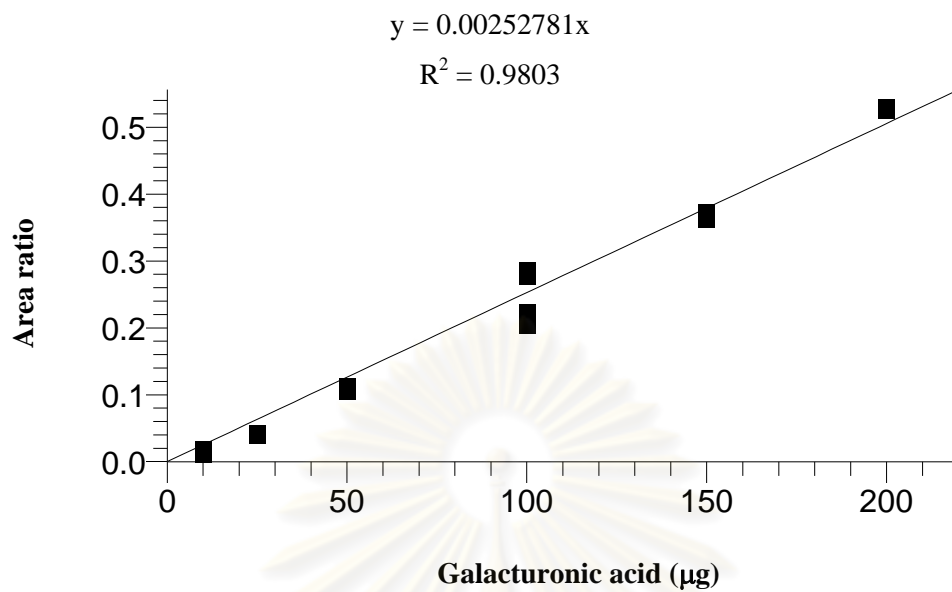
Figure 35 Calibration curve for galacturonic acid determination by GC
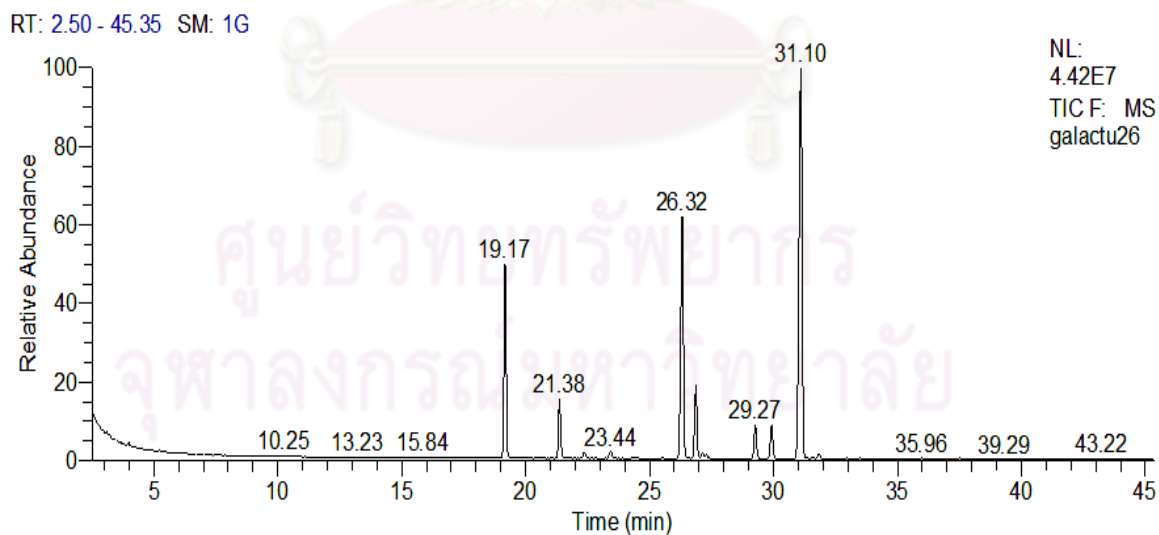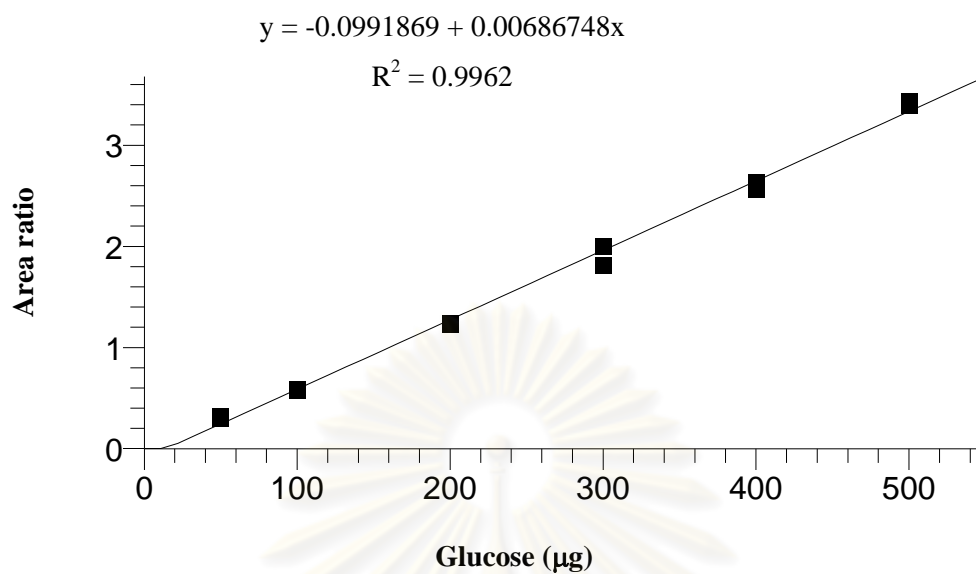

Figure 36 GC chromatogram of galacturonic acid

$$y = -0.0991869 + 0.00686748x$$

$$R^2 = 0.9962$$

Figure 37 Calibration curve for glucose determination by GC
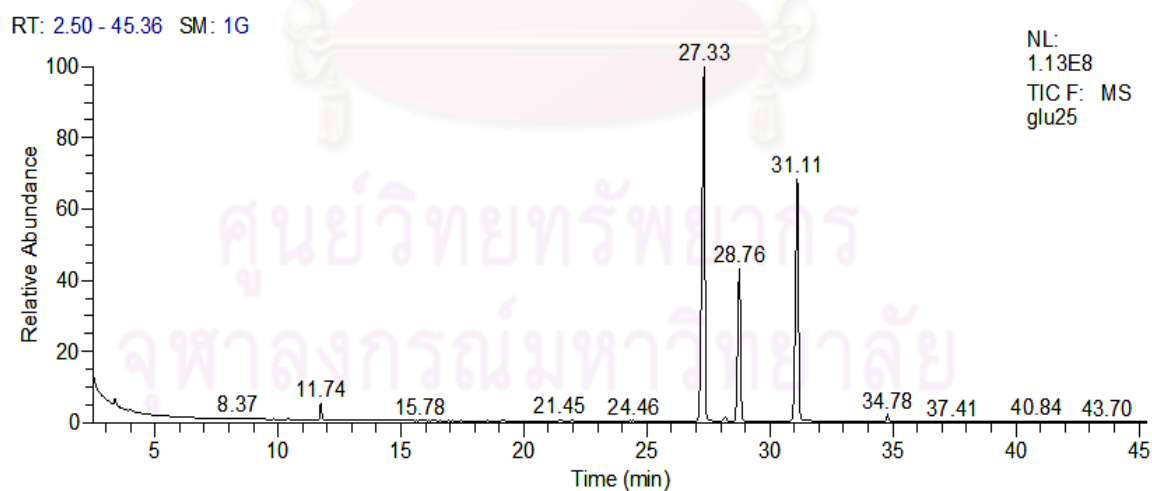
Figure 38 GC chromatogram of glucose

$$y = -0.0504843 + 0.007958x$$

$$R^2 = 0.9865$$

Figure 39 Calibration curve for mannose determination by GC
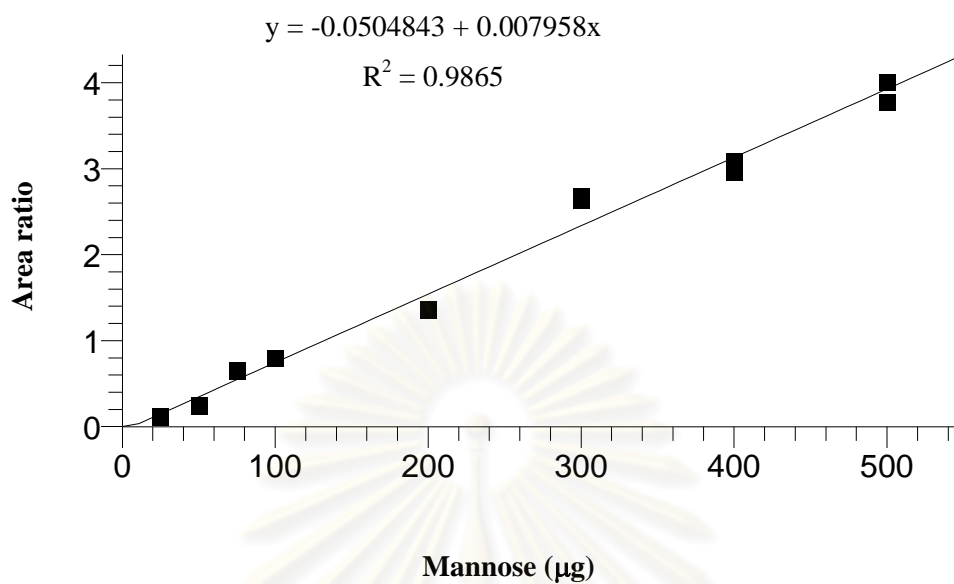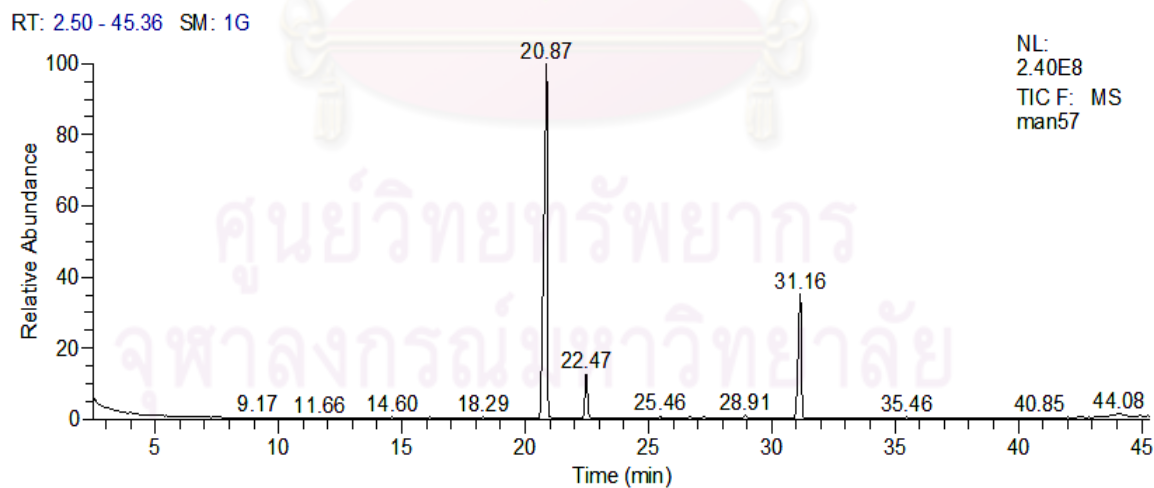

Figure 40 GC chromatogram of mannose
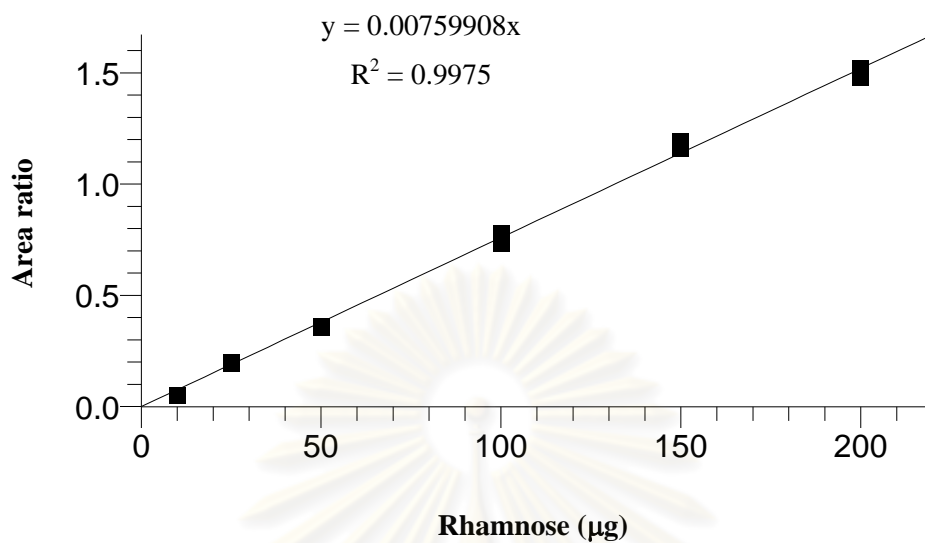
Figure 41 Calibration curve for rhamnose determination by GC
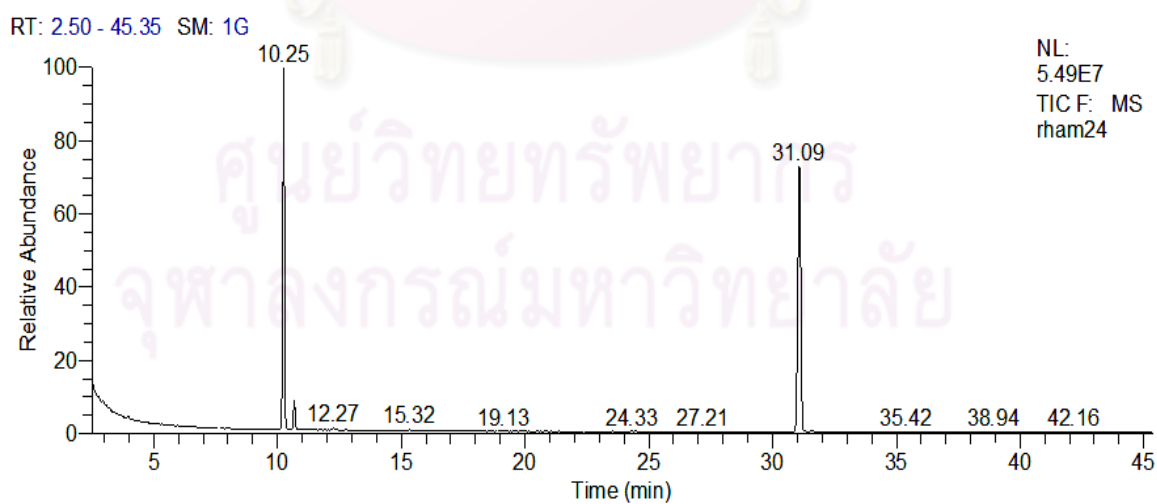
Figure 42 GC chromatogram of rhamnose

$$y = -0.0580002 + 0.00506746x$$

$$R^2 = 0.9857$$
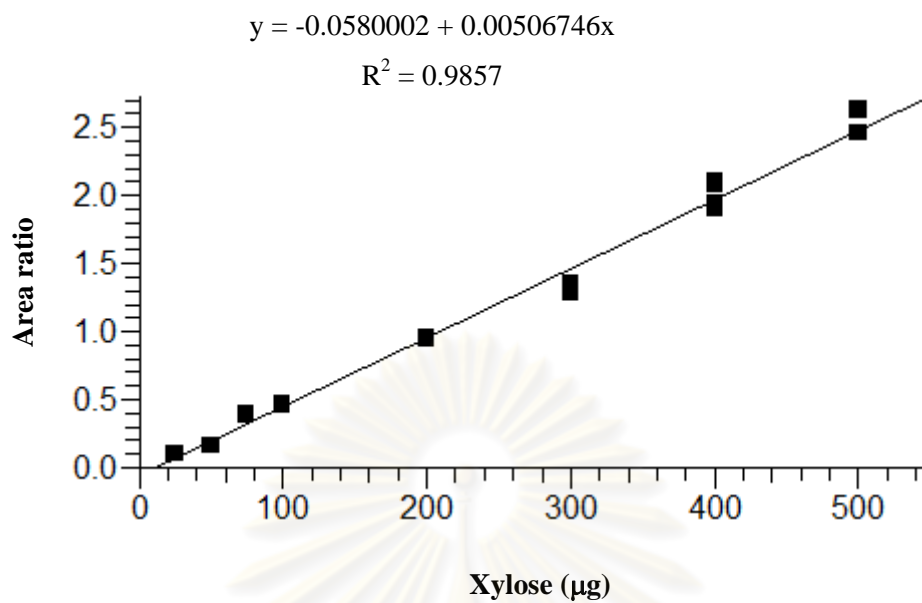
Figure 43 Calibration curve for xylose determination by GC
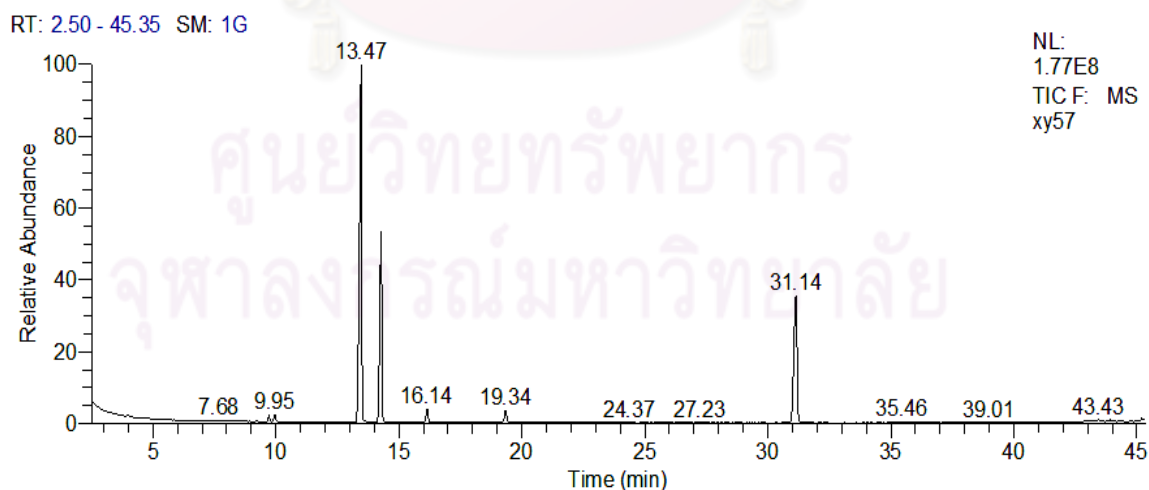
Figure 44 GC chromatogram of xylose
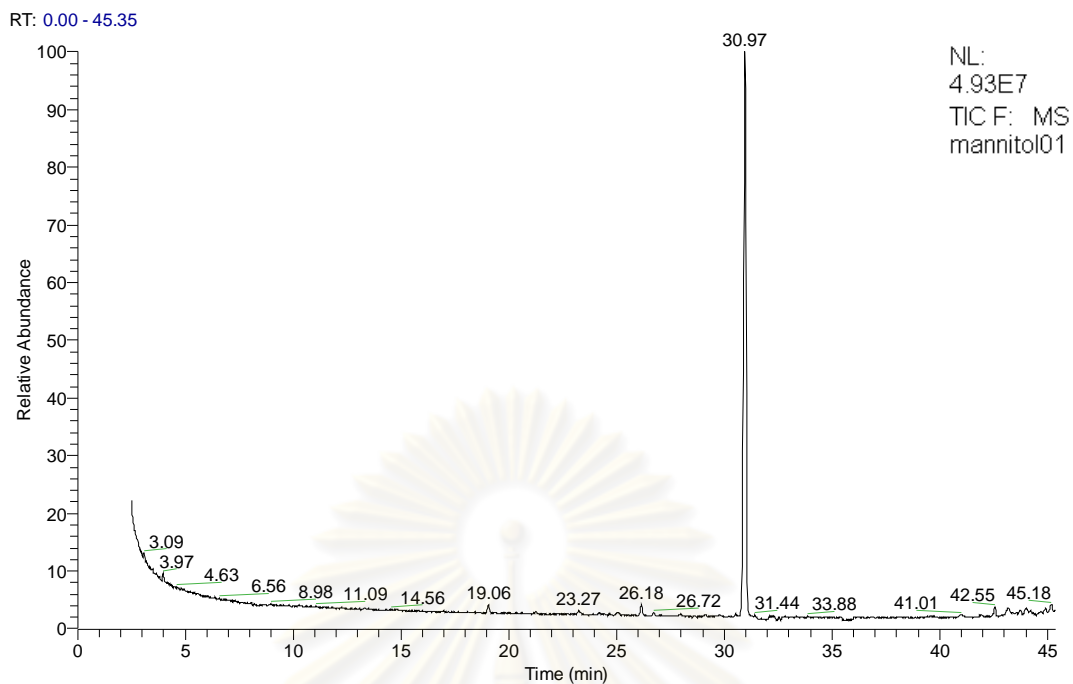
Figure 45 GC chromatogram of mannitol
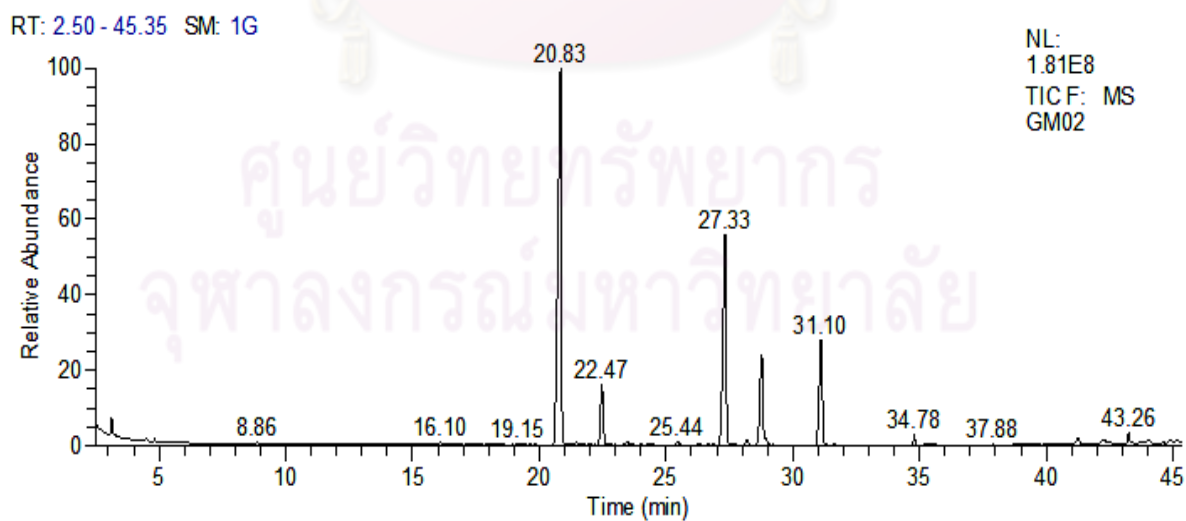
Figure 46 GC chromatogram of Konjac glucomannan
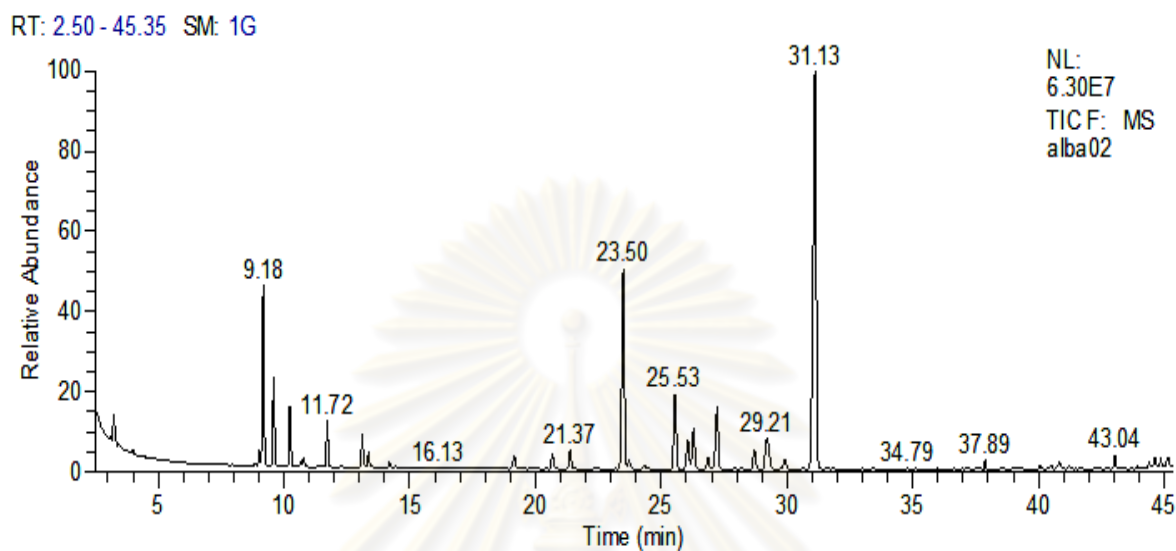
Figure 47 GC chromatogram of *B. alba* mucilage
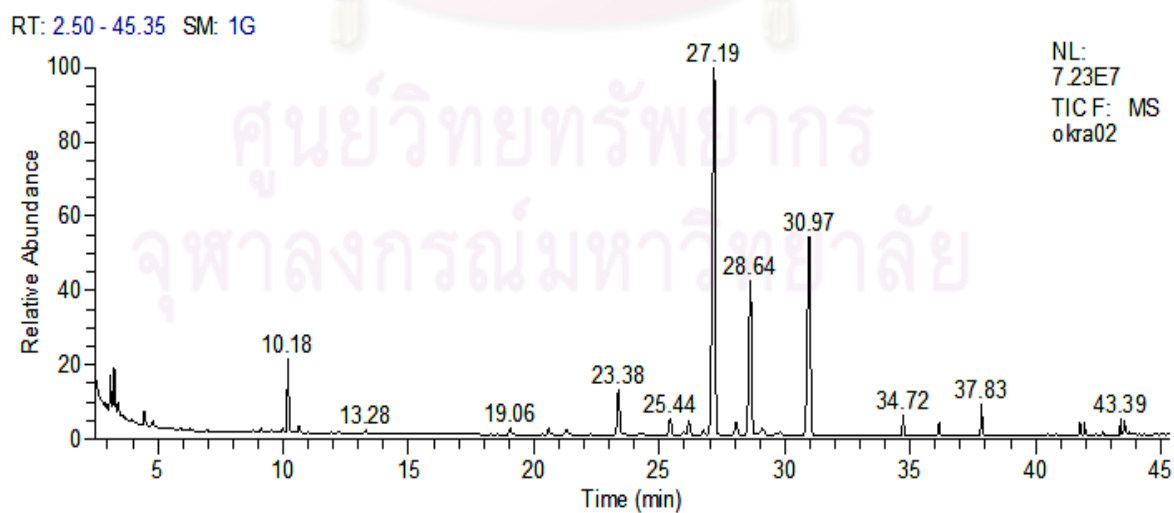
Figure 48 GC chromatogram of *H. esculentus* mucilage
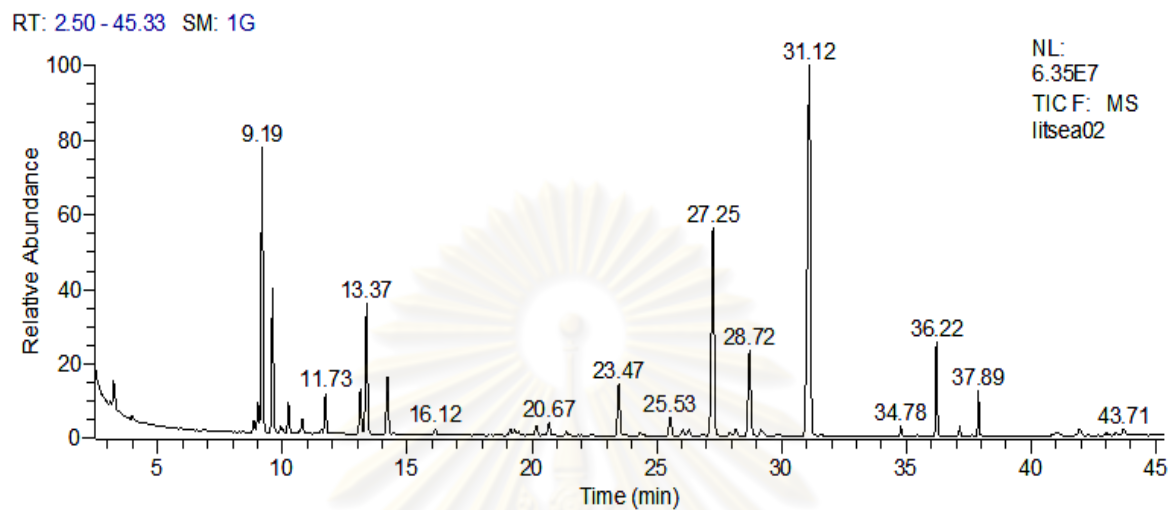
Figure 49 GC chromatogram of *L. glutinosa* mucilage
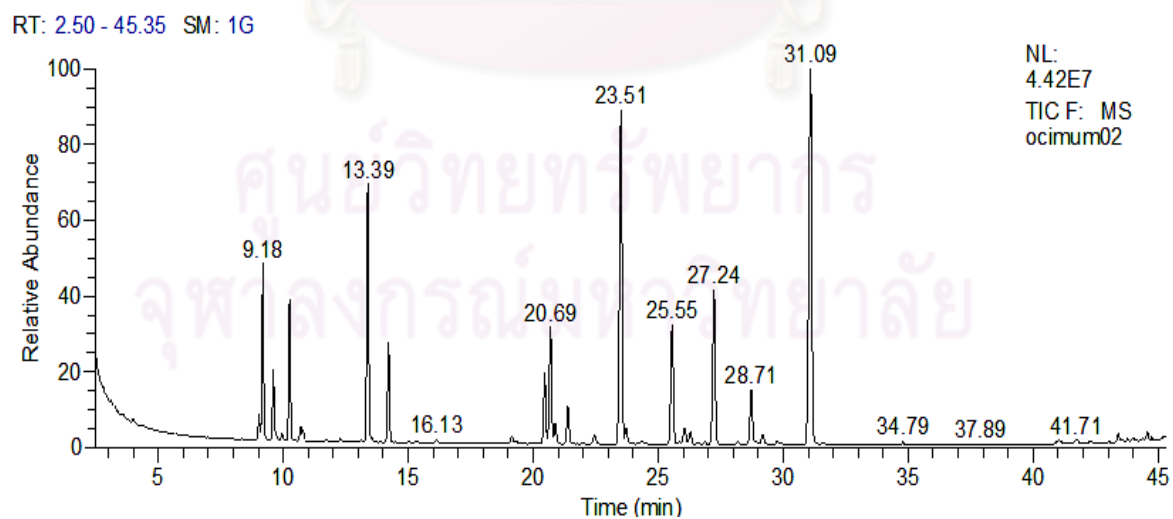
Figure 50 GC chromatogram of *O. canum* mucilage
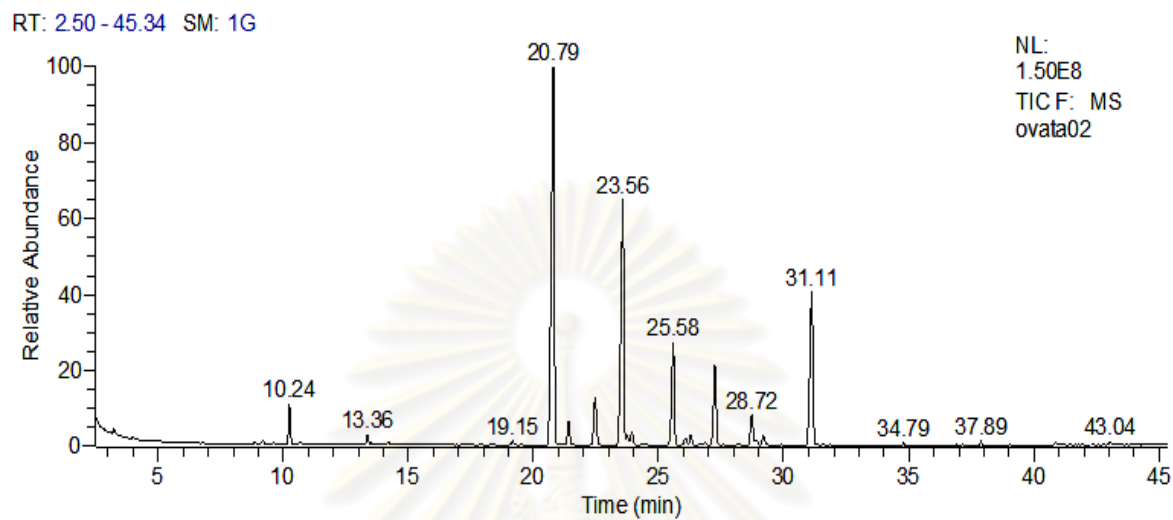
Figure 51 GC chromatogram of *P. ovata* mucilage
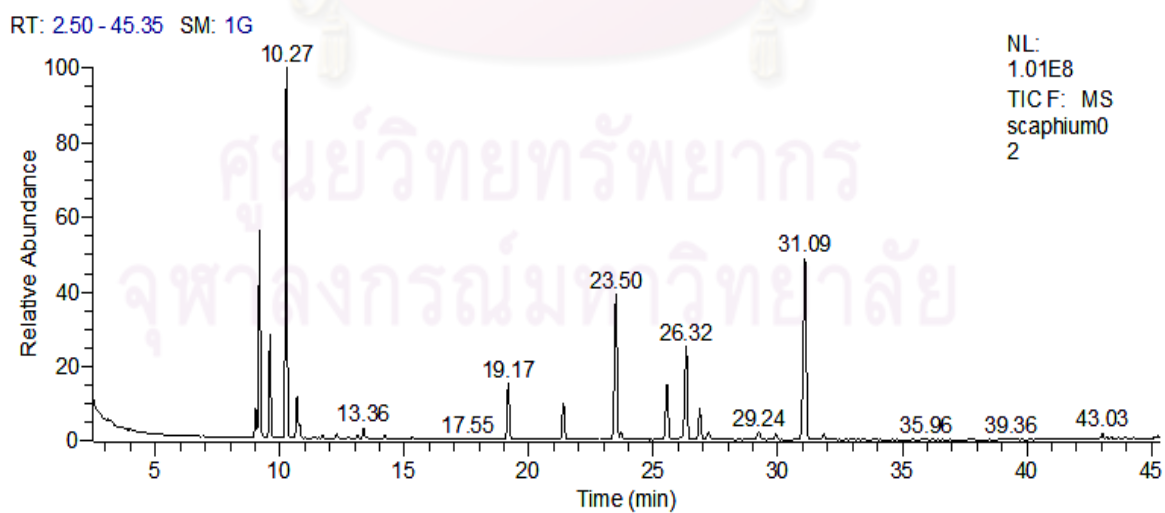
Figure 52 GC chromatogram of *S. scaphigerum* mucilage

Figure 53 GC chromatogram of *T. foenum-graecum* mucilage
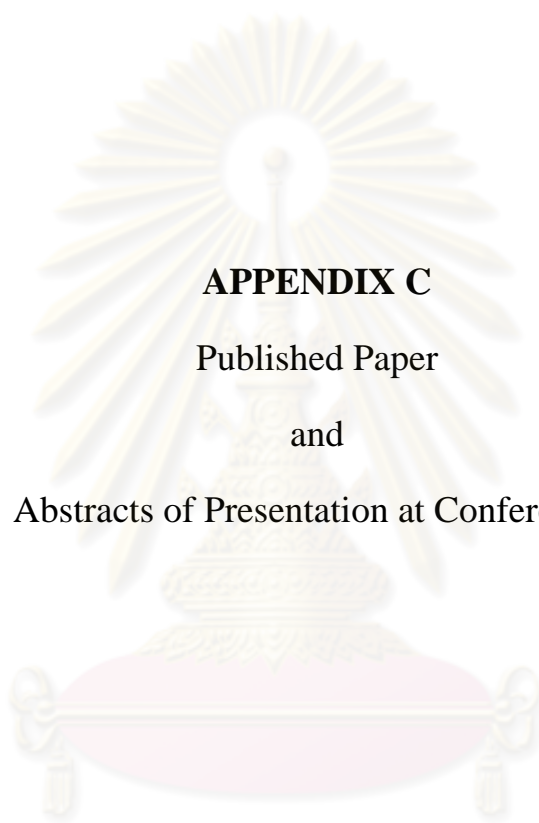
APPENDIX C

Published Paper

and

Abstracts of Presentation at Conferences

Scientia Pharmaceutica

Research article

In Vitro Glucose Entrapment and Alpha-Glucosidase Inhibition of Mucilaginous Substances from Selected Thai Medicinal Plants

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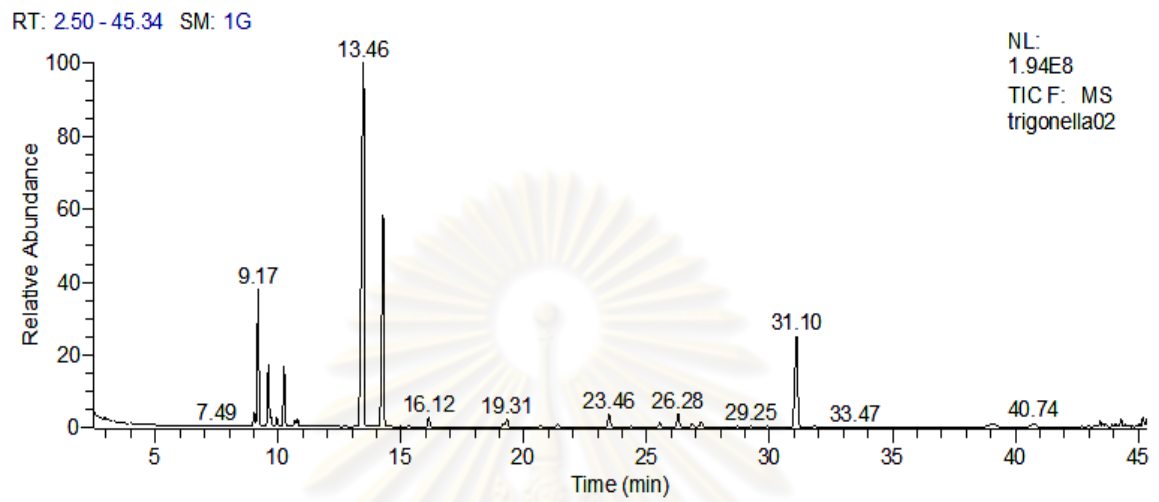[2] Research and Development Institute, Government Pharmaceutical Organization, Bangkok 10400, Thailand
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Abstract

As a continuous searching for anti-diabetic(type II) substances, seven mucilage polysaccharides from selected plants were studied as follow: aerial parts of *Basella alba* Linn., fruits of *Hibiscus esculentus* Linn., leaves of *Litsea glutinosa* (Lour.) C.B. Robinson, seeds of *Ocimum canum* Sims., seeds of *Plantago ovata* Forssk., fruits of *Scaphium scaphigerum* G. Don. and seeds of *Trigonella foenum-graecum* Linn. The bioactive properties for entrapping glucose, inhibiting enzyme alpha-glucosidase and free radical scavenger were *in vitro* studied compared to glucomannan. The physical characteristics for water holding capacity and viscosity were determined. The chemical characteristics were assayed for monosaccharide composition using methanolysis, TMS-derivatization and gas chromatography. *O. canum* mucilage superiorly entrapped glucose compared to glucomannan. This activity was relevant to its highly viscous gelation. *S. scaphigerum* showed another property of alpha-glucosidase inhibition. *S. scaphigerum* mucilage (0.5%) inhibited the enzyme activity by 82.6%, compared to 1-Deoxynorjirimycin (by 47.6%). Most mucilages, except *O. canum* and *P. ovata*, showed DPPH scavenging activity higher than glucomannan. Galacturonic acid was found in 3 from 7 mucilages namely *B. alba*, *P. ovata* and *S. scaphigerum*. Whereas rhamnose was common sugar found in all seven mucilages. Monosaccharide components of these mucilages were compared to the results from the previous reports.

Keywords

Introduction

Type 2 diabetes comprises 90% of people with diabetes around the world and is one of the major public health challenges of the 21st century. The number of cases worldwide in 2000 is estimated to be about 171 million and is projected to rise to 366 million in 2030. The World Health Organization (WHO) projects that without urgent action, diabetes-related deaths will increase by more than 50% in the next 10 years. Especially in upper-middle income countries, diabetes deaths are projected to increase by over 80% between 2006 and 2015 [1]. This circumstance results that the demand for medical care in type 2 diabetes will continue to increase. The substantial care and cost are due to the management of complications of the disease at both the starting point and the degree of deterioration over time. Macrovascular complications (ischemic heart disease, peripheral vascular disease, and cerebrovascular disease) has been estimated to be the largest cost component followed by microvascular complications (nephropathy, neuropathy and retinopathy) [2]. Endothelial dysfunction is considered to be an integral component of vascular diseases. Impaired endothelial function induces vasoconstriction, inflammatory and proliferative changes in the arterial wall and promotes atherosclerotic lesion growth. Prevention or normalization of endothelial function, contributes to the prevention of vascular lesion progression or destabilization [3]. Hyperglycemia has been proposed to be a crucial factor inducing endothelial dysfunction. High concentration of blood glucose as well as high glucose fluctuation during postprandial period correlates with the increase in reactive oxygen species or oxidative stress. Reactive oxygen species mediates the activation of the imbalance in vasoregulating factors (vasodilators and vasoconstrictors) then affects endothelial homeostasis and triggers atherogenic changes, including increases in low-density lipoprotein oxidation, sympathetic tone, vasoconstriction, and thrombogenicity [4]. Accordingly, glucose control is an important goal to diminish the risk of long term health complications of type 2 diabetes. In addition to glycated haemoglobin and fasting plasma glucose, postprandial glucose is recently recommended as essential target for diabetes management [5]. Alpha-glucosidase inhibition is one of the powerful interventions. Alpha-glucosidase is intestinal enzyme which catalyzes the degradation of diet polysaccharides to absorbable monosaccharide. Natural or synthetic glucosidase inhibitors are of therapeutic interest to delay postprandial hyperglycemia in type 2 diabetes. Amongst these, saccharide derivatives, for example Acarbose and Miglitol, have been approvable for anti-diabetic drugs [6]. Non-starch polysaccharides designated as soluble dietary fibers are also useful functional foods according to their association with the reduced risks of diabetes and cardiovascular diseases [7]. The viscous characteristics due to excellent water-holding and gel-forming capabilities have been proposed as an important mechanistic factor to delay gastric emptying and delay absorption of glucose in gastrointestinal tract [8]. Plant mucilage has been credited as one of plant chemical constituents showing hypoglycemic activity [9, 10]. This study investigated *in vitro* properties for anti type 2 diabetic potential among selected mucilaginous plants compared to a well known soluble dietary fiber, glucomannan. Some physico-chemical characteristics of these mucilages were also characterized.

Results and Discussion

Mucilage extraction

Seven mucilaginous plants were studied as follow: aerial parts of *Basella alba* Linn. (Basellaceae), fruits of *Hibiscus esculentus* Linn. (Malvaceae), leaves of *Litsea glutinosa* (Lour.) C.B. Robinson (Lauraceae), seeds of *Ocimum canum* Sims. (Labiatae), seeds of *Plantago ovata* Forssk. (Plantaginaceae), fruits of *Scaphium scaphigerum* G. Don. (Sterculiaceae) and seeds of *Trigonella foenum-graecum* Linn. (Papilionaceae). The mucilages from selected plants yielded range from 3.5% to 23.0% as shown in table 1.

Tab. 1. Mucilage polysaccharides from selected plants

Plants	Used parts	% Yield
Basella alba Linn.	aerial parts	3.5
Hibiscus esculentus L.	fruits	5.6
Litsea glutinosa Lour.	leaves	12.0
Ocimum canum Sims.	seeds	17.6
Plantago ovata Forssk.	seeds	19.0
Scaphium scaphigerum G. Don.	fruits	23.0
Trigonella foenum-graecum L.	seeds	15.0

In vitro property of entrapping glucose

Dialysis tubing technique is a simple model to evaluate the potential of soluble dietary fibers to additionally retard the diffusion and movement of glucose in the intestinal tract [11]. Movement in this system is not by the true diffusion but is assisted by the convective activity of intestinal contractions [12]. The entrapment ability of mucilage gel resulted in decreasing of glucose diffusion into the external solution. The retardation of the nutrient flow into the external medium is an indication of the modulating effect of that fiber on glucose absorption in the jejunum [11]. From the studied model, all mucilages showed concentration response (0.5, 1.0 and 2.0%w/v) on glucose entrapment activity. The percentage of glucose releasing from 2% mucilage suspension were 61.6, 70.8, 71.7, 80.6, 83.4, 85.8 and 92.8 % for *O. canum, P. ovata, T. foenum-graecum, L. glutinosa, H. esculentus, B. alba* and *S. scaphigerum* respectively. Glucomannan showed 65.4% of glucose releasing at the same concentration (Figure 1). Glucomannan seems to be the most impressive natural fiber with increasing importance in the biomedical and pharmaceutical fields. It has been found to decrease the serum glucose levels and also the postprandial insulin flow which aiding diabetic control [13]. Psyllium seeds from *P. ovata* and fenugreek seeds from *T. foenum-graecum* are also reported as an adjunct to dietary therapy in patients with type II diabetes, to reduce glucose and glycosylated hemoglobin [14, 15]. Figure 1 showed that the retardation effect on glucose movement of the mucilages from *P. ovata* and *T. foenum-graecum* were lower degree than glucomannan. Whilst *O. canum* mucilage of all concentration superiorly entrapped glucose compared to glucomannan. There have been previous studies of hypoglycemic effect of *O. canum* but from leaves extract [16].
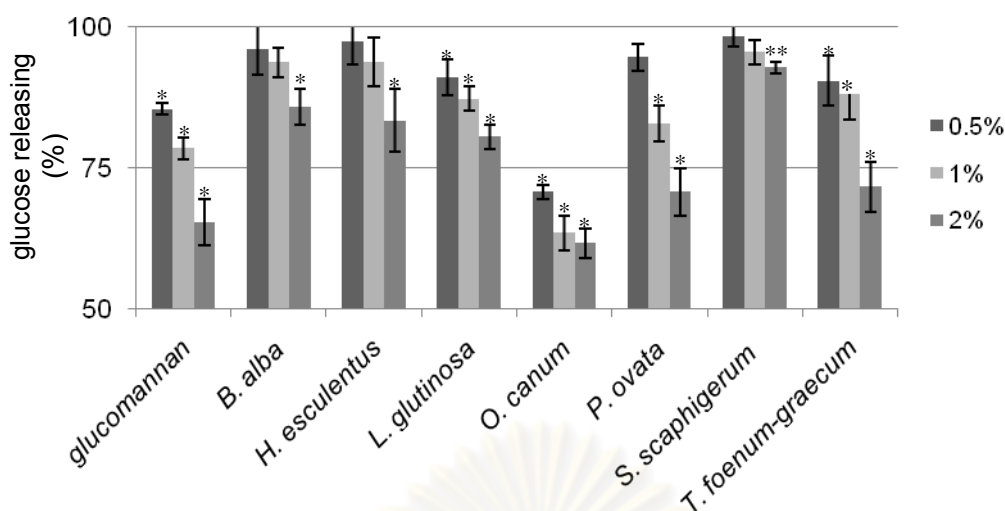
Fig. 1. Percentages of glucose releasing from 0.5, 1, 2% mucilage containing 2% glucose after 2 hr dialysis (n=4). The significance of differences from the control (0% mucilage, 99.8±0.9%) was determined by ANOVA followed by Dunnett's test (*$p<0.01$, **$p<0.05$).

In vitro property of alpha-glucosidase inhibition

The effect of selected mucilages against α-glucosidase was evaluated. At the concentration of 0.5% mucilage, *S. scaphigerum*, *L. glutinosa*, *H. esculentus*, *O. canum*, *T. foenum-graecum*, *P. ovata*, *B. alba* and glucomannan showed the inhibitory percentage of 82.6, 41.0, 37.6, 32.8, 30.6, 27.0, 25.0 and 19.7 % respectively. Whereas, 1-Deoxy-norjirimycin at the same concentration showed the inhibition of 47.6% (Figure 2). *S. scaphigerum* mucilage was further investigated and found that the concentration for 50% inhibition of α-glucosidase activity (IC_{50}) was 0.17% (Figure 3). *S. scaphigerum* or Malva nut tree is mostly found in the East of Thailand. The gel made from malva nuts is used as ingredients in dishes and beverages. Malva nut drink is traditionally used to relief coughing and sore throats. The previous study in type 2 diabetic patients reported that after the intake of malva nut drink, fasting plasma glucose and glycosylated hemoglobin decreased significantly [17]. The present study showed a possible mechanism in alpha-glucosidase inhibition.

DPPH radical-scavenging activity

Tab. 2. DPPH scavenging activity as IC_{50} among mucilage polysaccharides from selected plants

Mucilages	IC_{50} (mg/ml)	Mucilages	IC_{50} (mg/ml)
B. alba	2.00	*S. scaphigerum*	0.61
H. esculentus	0.70	*T. foenum-graecum*	1.52
L. glutinosa	0.49		
O. canum	>10	Glucomannan	4.15
P. ovata	>10	Ascorbic acid	0.02

[a] mean of 3 runs
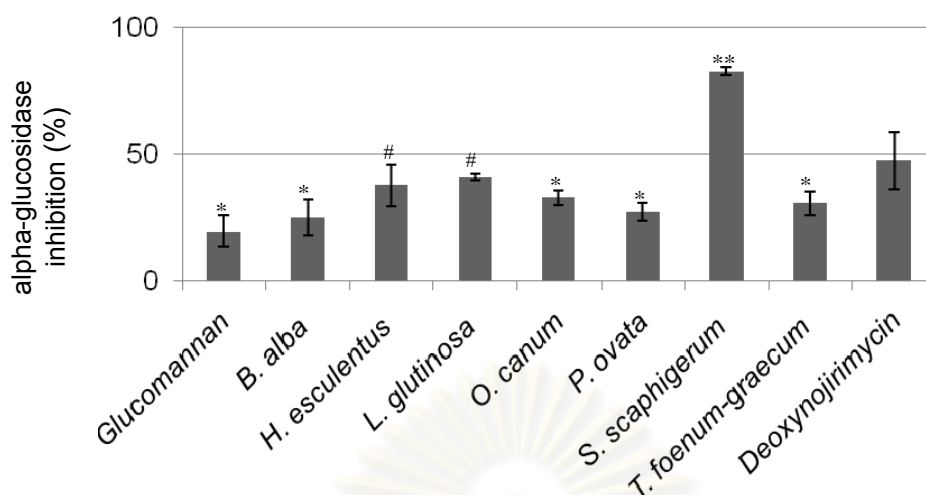
Fig. 2. Percentage of alpha-glucosidase inhibition by 0.5% mucilage (n = 3).
%inhibition was not significantly different from control (Deoxynorjirimycin)
($p>0.05$); * %inhibition was significantly higher from control ($p<0.05$);
* %inhibition was significantly lower than control ($p<0.05$) (determined by
ANOVA followed by Dunnett's test).
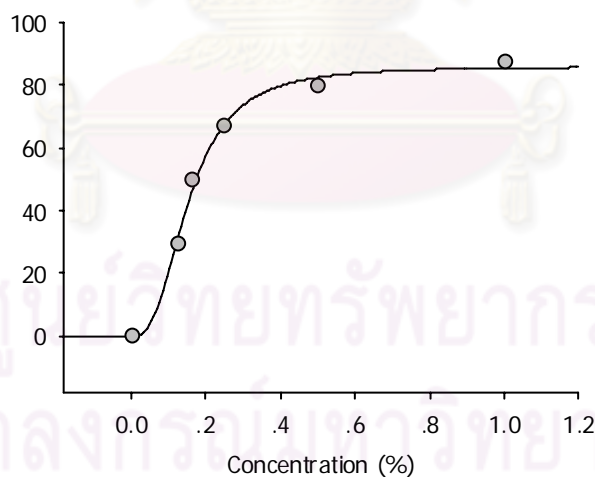
Fig. 3. Percentage of alpha-glucosidase inhibition from various concentration of *S. scaphigerum* mucilage

Endothelial dysfunction is a key factor in all stages of atherosclerosis development. This term refers to an imbalance in the production of vasodilators at the circumstance of high concentrations of reactive oxygen species or oxidative stress [4]. Scavenging of the stable free radical diphenylpicrylhydrazyl (DPPH) is the basis of a common antioxidant assay. There have been widely different protocols which differed in the conditions (i.e. pH,

solvents, wavelength of measurement), yielding different results. In the present study, the polysaccharide samples were incubated with 100 µM DPPH in methanol for 30 min using ascorbic acid as standard antioxidant. The number of DPPH reduced by one molecule of ascorbic acid (antioxidant stoichiometry) was 2.5 which was higher than the theoretic value of 2.0 but close to the previous experiments [18, 19]. Table 2 demonstrated the scavenging activity of the polysaccharide samples on the DPPH radical. Except for *O. canum* and *P. ovata*, other mucilages showed scavenging activities higher than glucomannan. Especially the mucilages of *L. glutinosa, S. scaphigerum* and *H. esculentus* had IC_{50} less than 1 mg/ml. Although the abilities were lower than that of ascorbic acid, these mucilages counteracted with DPPH as strongly as *Ganoderma* polysaccharides (IC_{50} between 3–13 mg/ml) [20].

Swelling measurements and viscosity

There are a series of physical interactions in the gastrointestinal tract which affect absorption, as follow: diffusion of nutrients from the bulk solution to the intestinal epithelia, the rate of removal of waters of hydration from a complex nutrient solvent system, counter diffusion of nutrients away from the intestinal surface and diffusion of nutrients along the epithelial surface to an appropriate absorptive site [21]. Water can be held within the polysaccharide matrix causing considerable swelling and viscous solution or gelation. Viscous polysaccharide gels may slow absorption by trapping nutrients, digestive enzymes or bile acids in the matrix and by slowing mixing and diffusion in the intestine [22]. These physical properties of selected mucilages were studied (Table 3). *O. canum* and *S. scaphigerum* mucilages showed highest values of swelling volume (SV) and water absorption index (WAI) followed by *P. ovata* and *T. foenum-graecum* respectively. Other mucilages showed the same values of SV and WAI as glucomannan. Viscosity was determined using falling ball viscosity method. *O. canum* mucilage had highest viscosity value even at low concentration (0.5%). Viscous character seemed to be a prominent factor affected the hypoglycemic potential of *O. canum* but not *S. scaphigerum* mucilage.

Tab. 3. Swelling volume (SV), water absorption index (WAI) and viscosity among mucilage polysaccharides from selected plants

Mucilages	SV (ml/g)	WAI (g/g)	Viscosity (mPa s)		
			0.5%	1.0%	2.0%
glucomannan	23.9 ± 1.9	22.1 ± 1.9	21.1 ± 0.3	143.2 ± 8.9	4582.8 ± 60.3
B. alba	25.7 ± 6.3	15.8 ± 3.0	2.2 ± 0.3	3.5 ± 0.4	7.0 ± 0.4
H. esculentus	22.2 ± 4.6	20.7 ± 4.3	7.7 ± 0.7	17.1 ± 0.9	45.1 ± 2.0
L. glutinosa	27.5 ± 8.5	20.6 ± 4.6	1.8 ± 0.2	5.9 ± 0.6	19.1 ± 2.6
O. canum	115.9 ± 17.3*	111.1 ± 17.1*	581.3 ± 59.1	>5000	>5000
P. ovata	60.4 ± 7.7*	48.3 ± 3.2*	6.2 ± 0.3	18.5 ± 1.1	1575.3 ± 57.5
S. scaphigerum	210.5 ± 4.3*	102.8 ± 1.6*	0	0	0
T. foenum-graecum	38.9 ± 1.0	19.1 ± 1.4	6.7 ± 0.4	29.6 ± 1.9	213.0 ± 17.9

[a] mean of 3 runs; * the significance of differences of SV and WAI from the control (glucomannan) was determined by ANOVA followed by Dunnett's test ($p<0.01$).

Monosaccharide analysis and protein content

The selected mucilages as well as glucomannan were analyzed for the primary structures of their monosaccharide compositions as shown in Table 4 and 5. Konjac glucomannan in this study showed the mannose : glucose molar ratio of around 1.6 : 1 which was in the range reported elsewhere. Galacturonic acid was found in 3 from 7 mucilages namely *B. alba*, *P. ovata* and *S. scaphigerum*. Whereas rhamnose was common sugar found in all studied mucilages. Chemical analyses of the mucilages in this study showed some differing results from the previous reports. This was due to the differences in either plant origin or methodology of extraction and analysis. Literature reviews of monosaccharide composition among these mucilages were summarized in Table 6. *H. esculentus* mucilage from this study had highest glucose (79%) composition compared to 44% and 5% from the literatures. *L. glutinosa* mucilage from the leaves in this study had xylose/ arabinose ratio differed from the reported barks. *P. ovata* and *T. foenum-graecum* mucilages displayed the typical characters of arabinoxylan and galactomannan respectively. The water extracts of *S. scaphigerum* mucilages in this report had similar ratio of monosaccharide with the alkaline extracts reported elsewhere. *B. alba* mucilage was firstly revealed the composition of arabinose, rhamnose, galactose, galacturonic acid and glucose (24:5:41:13:16). Total protein contents in these crude polysaccharides ranged from 2% in *P. ovata* to 38% in *L. glutinosa* (Table 4).

Tab. 4. Monosaccharide composition and total protein content (µg/mg) among mucilage polysaccharides from selected plants

	Ara[b]	Rham	Xy	Man
glucomannan				493.8 ± 11.6
B. alba	43.5 ± 0.8	10.7 ± 0.1		
H. esculentus		27.8 ± 0.6		
L. glutinosa	84.1 ± 1.2	6.0 ± 0.2	56.4 ± 0.5	9.7 ± 0.1
O. canum	47.8 ± 1.1	28.0 ± 0.7	98.43 ± 2.5	37.5 ± 1.5
P. ovata	165.2 ± 13.5	52.0 ± 3.4	697.5 ± 1.3	
S. scaphigerum	121.7 ± 3.4	155.2 ± 4.8	20.8 ± 0.7	
T. foenum-graecum		18.8 ± 0.4	20.4 ± 0.2	310.4 ± 1.2
	Gal	**Galn**	**Glu**	**TP[c]**
glucomannan			309.6 ± 3.6	107.8 ± 0.04
B. alba	88.7 ± 1.0	33.7 ± 1.3	34.6 ± 0.8	235.5 ± 0.04
H. esculentus	46.5 ± 1.4		285.7 ± 1.4	152.7 ± 0.04
L. glutinosa	29.7 ± 0.6		88.9 ± 0.5	375.1 ± 0.01
O. canum	156.2 ± 0.8		66.30 ± 0.2	81.8 ± 0.02
P. ovata		51.7 ± 3.0		20.3 ± 0.02
S. scaphigerum	144.6 ± 0.3	173.4 ± 2.9	21.1 ± 0.2	195.3 ± 0.03
T. foenum-graecum	302.2 ± 1.2		82.2 ± 0.5	209.5 ± 0.01

[a] mean of 3 runs; [b] monosaccharides found in these mucilages included arabinose (ara), rhamnose (rham), xylose (xy), mannose (man), galactose (gal), galacturonic acid (galn) and glucose (glu). Fucose (fu) and glucuronic acid (glun) were absent; [c] Total protein.

Tab. 5. Monosaccharide composition (% mole ratio) among mucilage polysaccharides from selected plants

	Ara	Rham	Xy	Man	Gal	Galn	Glu
glucomannan				61			39
B. alba	24	5			41	13	16
H. esculentus		8			13		79
L. glutinosa	33	2	22	3	10		29
O. canum	12	6	25	8	34		14
P. ovata	18	5	74			4	
S. scaphigerum	23	24	4		23	23	3
T. foenum-graecum		3	3	42	41		11

Tab. 6. Monosaccharide composition among mucilage polysaccharides from literatures

glucomannan	man : gluc 1.6 : 1	[23]
H. esculentus	rham : gal : galn: glu : glun 1 : 2.5 : 1.8 : 0.3 : 0.2	[24]
	ara : rham : xyl : man : gal : galn : glu : glun 5 : 3 : 5 : 3 : 17 : 16 : 44 : 7	[25]
L. glutinosa	ara : xy 3.4 : 1.0 (barks)	[26]
O. canum	ara : rham : xy : man : gal : glu 1 : 2 : 1 : 2 : 5 : 8 (uronic acids 8.15%)	[27]
P. ovata	ara : rham : xy : man : gal : glu 20.7 : 1.1 : 50.3 : 1.1 : 4.8 : 2.0	[28]
S. scaphigerum	ara :rham : gal 1.1 : 1.0 : 1.0 (%w/w)	[29]
T. foenum-graecum	ara: rham : man : gal : glu 0.5 : 0.2 : 31.4 : 26.2 : 0.6	[30]
	gal : man 1.00 : 1.02 to 1.00 : 1.14	[31]

Conclusion

The plants bearing mucilage in this study have been well known in Thailand as both edible and medicinal plants. Mucilagenous typed polysaccharides from these plants were investigated for the biological activities especially antidiabetic potential. Despite the limitations of this *in vitro* study, there seemed to be various mechanisms possibly involved by mucilages due to their physico-chemical characteristics. The *in vitro* models could be beneficial tools for the survey of other potential plant mucilages. Moreover they could refine the possible and capable research designs prior to the expensive further studies of either the animal models or the clinical trials.

Experimental

Seven mucilaginous plants were studied as follow: aerial parts of *Basella alba* Linn. (Basellaceae), fruits of *Hibiscus esculentus* Linn. (Malvaceae), leaves of *Litsea glutinosa* (Lour.) C.B. Robinson (Lauraceae), seeds of *Ocimum canum* Sims. (Labiatae), seeds of *Plantago ovata* Forssk. (Plantaginaceae), fruits of *Scaphium scaphigerum* G. Don. (Sterculiaceae) and seeds of *Trigonella foenum-graecum* Linn. (Papilionaceae). Glucomannan flour (the Siam Konjac Co., Ltd.) was used for comparison. Chemicals and

reagents included *p*-nitrophenyl-α-D-glucopyranoside and α-glucosidase (EC 3.2.1.20) from *Saccharomyces cerevisiae*, 1-Deoxynojirimycin, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), standard monosaccharides (Sigma Chemical Co. Ltd, St. Louis, MO.); methanolic HCl, trimethylchlorosilane, hexamethyldisilazane (Supelco, Bellefont, PA); Glucose Liquicolor kit (Human Gesellschaft für Biochemica und Diagnostica mbH, Germany); Lactated Ringers Buffer pH 7 (General Hospital Product Public Co., LTD., Thailand), All other chemicals were analytical grade. Dialysis tubing cellulose membrane (molecular weight cut off = 12,000 Da) was from Sigma Chemical Co. Ltd, St. Louis, MO. Dialysis tubing cellulose membrane (molecular weight cut off = 3,500 Da) was from Spectrum Medical Industries, Inc., Los Angeles, CA.

Mucilage extraction

The mucilage were extracted from the specified plant parts with warm water and concentrated by lyophilization. The lyophilized samples were re-dissolved in water, precipitated twice with 2 volume of 80% ethanol and dialyzed against distilled water in a dialysis tubing cellulose membrane (molecular weight cut off = 3,500 Da). The samples were lyophilized, ground and kept in refrigerator for further studies.

In vitro property of entrapping glucose

The mucilage and glucomannan were dissolved in Ringers buffer. Glucose was added to make the final concentration of 2% glucose and 0, 0.5, 1 and 2 % w/v polysaccharide gel. Four milliliter of each concentration was dialysed against 60 ml of Ringers buffer in a dialysis tubing cellulose membrane (molecular weight cut off = 12,000 Da) for 2 hours under rotationally shaking at 150 rpm. The released glucose was determined by glucose oxidase - phenyl ampyrone (GOD-PAP) colorimetric method using Glucose Liquicolor kit according to manufacturer's instructions.

In vitro property of alpha-glucosidase inhibition

Alpha-glucosidase activity was assayed using 0.1M sodium phosphate buffer at pH 6.9, and 1 mM p-nitrophenyl-α-D-glucopyranoside was used as a substrate [32]. The concentration of α-glucosidase was 1 U/mL in each experiment. The enzyme (4 µl) was incubated in the absence or presence of various concentrations of tested polysaccharides at 37 °C. The preincubation time was specified at 10 min and the substrate (95 µL) was added to the mixture. The reaction was carried out at 37 °C for 20 min, and then 100 µL of 1M Na_2CO_3 was added to terminate the reaction. Enzymatic activity was quantified by measuring the absorbance of *p*-nitrophenol at 405 nm. One unit of α-glucosidase is defined as the amount of enzyme liberating 1.0 µmol of *p*-nitrophenol per minute under the conditions specified. 1-Deoxynojirimycin was used as the positive control.

DPPH radical-scavenging activity

The potential antioxidant activity of polysaccharide samples was determined on the basis of the scavenging activity of the stable DPPH free radical [33]. Various concentrations of polysaccharides samples (0.5 ml) were added to 1.5 mL of a 0.004% methanolic solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percent scavenging activity was calculated by the following formula:

Scavenging effect (%) = $(1 - A_{sample}/A_{control}) \times 100$

Where $A_{control}$ is the absorbance of control (DPPH solution plus water), A_{sample} is the test sample (DPPH solution plus test sample or positive control) and the percent inhibition activity was calculated. IC_{50} values denote the concentration of sample required to scavenge 50% DPPH free radicals.

Swelling measurements and viscosity

Swelling volume (SV) and water absorption index (WAI) were determined from the ratio of the volume and weight of swollen gel to the dry weight of sample respectively [34,35]. A 0.050 g ground sample (<60 mesh) was suspended in 25 ml of water in a 25-ml graduated cylinder for 2 hours. The volume of swollen gel was measured. The supernatant liquid was removed, the swollen gel was weighed and SV and WAI were calculated. Viscosities at various concentrations were measured with a falling ball viscometer (HAAKE Mess-Technik GmbH u. Co, Germany) at 20 °C.

Monosaccharide analysis and protein content

The polysaccharide samples (1 mg) were subjected to methanolysis with 4 M methanolic HCl at 80°C for 24 h. Mannitol was added as an internal standard. The samples were dried with nitrogen, methanol was added and the samples were dried again. This washing was repeated twice [36]. Prior to gas chromatographic analysis, the samples were trimethylsilylated using trimethylchlorosilane : hexamethyldisilazane : Pyridine 1:2:5 (0.4 ml) at room temperature for 30 min. Instrumentation was performed on a Finnigan Trace GC Ultra with DSQ MS detector and a split–splitless injector. The column was a ZB-5 fused silica capillary column (30 m × 0.25 mm i.d.) with film thickness 0.25 µm. Helium was used as carrier gas at a flow rate of 1.0 ml/min. The injector and detector temperature were 260 and 300°C respectively. The column temperature was initially 140°C, then an increase of 1°C/min to 170°C and followed by 6°C/min to 250°C. The protein contents of the samples were determined by Lowry method using bovine serum albumin as protein standard.

Statistical analysis

The significance of differences between the mean values was determined by analysis of variance (ANOVA), followed by Dunnett's test, and a *p* value of less than 0.05 was considered statistically significant.

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Authors' Statement

Competing Interests

The authors declare no conflict of interest.

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Abstracts

บทคัดย่อ

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การกักเก็บน้ำตาลกลูโคสในหลอดทดลองของสารเมือกจากพืชสมุนไพรชนิดต่างๆ
In Vitro Glucose Entrapment of Mucilaginous Substances from Various Medicinal Plants

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บทคัดย่อ: ศึกษาการกักเก็บน้ำตาลกลูโคสในหลอดทดลองของสารเมือกจากพืชสมุนไพร 5 ชนิด เปรียบเทียบกับผงบุก ได้แก่ ผลกระเจี๊ยบเขียว (*Hibiscus esculentus* Linn.) เมล็ดแมงลัก (*Ocimum canum* Sims.) เมล็ดเทียนเกล็ดหอย (*Plantago ovata* Forssk.) ผลสำรอง (*Scaphium scaphigerum* G. Don.) และส่วนเหนือดินของผักปลังขาว (*Basella alba* Linn.) สกัดสารเมือกด้วยน้ำและระเหยแห้งภายใต้สุญญากาศ เตรียมสารละลายของสารเมือกทั้งห้าและผง บุก ความเข้มข้นร้อยละ 0, 0.5, 1 และ 2 ในริงเกอร์บัฟเฟอร์ที่มีน้ำตาลกลูโคสความเข้มข้นร้อยละ 2 บรรจุในถุงเยื่อไดอะไลซีสชนิดเซลลูโลสที่แช่อยู่ในริงเกอร์บัฟเฟอร์เป็นเวลา 2 ชั่วโมง วิเคราะห์ ปริมาณน้ำตาลกลูโคสที่ซึมผ่านถุงเยื่อออกมาโดยวิธีกลูโคสออกซิเดส สารละลายของสารเมือกที่ ศึกษายกเว้นจากสำรองแสดงคุณสมบัติเป็นเจล และการกักเก็บน้ำตาลกลูโคสสัมพันธ์กับความ เข้มข้นของสารเมือก ลำดับความสามารถในการกักเก็บน้ำตาลกลูโคสของสารเมือกทั้งห้าและผง บุกคือ แมงลัก > บุก > เทียนเกล็ดหอย > กระเจี๊ยบเขียว > ผักปลังขาว > สำรอง

Abstract: Mucilaginous substances from 5 medicinal plants were studied for *in vitro* glucose entrapment compared to glucomannan powdered. The mucilage from fruits of *Hibiscus esculentus* Linn., seeds of *Ocimum canum* Sims., seeds of *Plantago ovata* Forssk., fruits of *Scaphium scaphigerum* G. Don. and aerial parts of *Basella alba* Linn. were extracted with water and lyophilized. The mucilage solutions from 5 plants and glucomannan were prepared at concentration of 0, 0.5, 1 and 2% in Ringer buffer containing 2% glucose. The mucilage-glucose solution was dialyzed through membraneous cellulose with Ringer buffer for 2 hours. The released glucose was assayed by glucose oxidase (GOD-PAP) method. All mucilage solutions (except *S. scaphigerum*) showed gel-forming characteristics and concentration response on glucose entrapment activity. Glucose entrapment ability of the studied mucilages were ranked as follow: *O. canum* > *glucomannan* > *P. ovata* > *H. esculentus* > *B. alba* > *S. scaphigerum.*

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IN VITRO GLUCOSE ENTRAPMENT AND α-GLUCOSIDASE INHIBITION OF MUCILAGINOUS SUBSTANCES FROM SELECTED THAI MEDICINAL PLANTS

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As a continuous searching for anti-diabetic(type II) substances, seven mucilaginous plants were studied as follow: seeds of *Ocimum canum* Sims., seeds of *Plantago ovata* Forssk., seeds of *Trigonella foenum-graecum* Linn., leaves of *Litsea glutinosa* (Lour.) C.B. Robinson, fruits of *Hibiscus esculentus* Linn., aerial parts of *Basella alba* Linn. and fruits of *Scaphium scaphigerum* G. Don. The mucilage were extracted with water and lyophilized to dryness. The mucilage properties for entrapping glucose and inhibiting enzyme α-glucosidase were studied *in vitro* prior to in animal models. For glucose entrapment assay, the suspension of 0.5, 1 and 2 % of the mucilage with 2% of glucose were dialyzed through membranous cellulose with Ringer buffer for 2 hours. The released glucose was assayed by glucose oxidase (GOD-PAP) method. All mucilage showed concentration response on glucose entrapment activity. Percentage of glucose released from 2% mucilage suspension were 61.6, 70.8, 71.7, 80.6, 83.4, 85.8 and 92.8 % for *O. canum*, *P. ovata*, *T. foenum-graecum*, *L. glutinosa*, *H. esculentus*, *B. alba* and *S. scaphigerum* respectively. Glucomannan powder was also studied and showed 65.4% of glucose releasing at the same concentration. For enzyme inhibition assay, α-glucosidase activity was measured by spectrophotometry using *p*-nitrophenyl-α-D-glucopyranoside (PNP-G) as a substrate and released *p*-nitrophenol (PNP) was read at 405 nm. Five mg/ml of the mucilage and deoxynojirimycin (positive control) in DMSO were tested. *S. scaphigerum* was shown as the best inhibitor with 82.6 % inhibition whilst deoxynojirimycin, *L. glutinosa*, *H. esculentus*, *O. canum*, *T. foenum-graecum*, *P. ovata*, *B. alba* and glucomannan were 47.6, 41.0, 37.6, 32.8, 30.6, 27.0, 25.0 and 19.7 % respectively.

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THE EFFECT OF PLANT MUCILAGES ON LIPID SOLUBILITY IN BILE SALT

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KEYWORDS: Plant Mucilage, Lipid Solubility, Bile Salt

INTRODUCTION

Bile plays an important role to increase lipid solubility in aqueous matrix by micelle formation. Lipid metabolism depends on its solubility which leads to permeability through intestinal cells. Interfering of lipid-bile micelle characters can affect lipid solubility which influences on lipid absorption, distribution and metabolism. In this study, the solubility of cholesterol and fatty acids were investigated using *in vitro* model bile salt with and without plant mucilages in the system.

MATERIALS AND METHODS

Seven mucilaginous plants were studied as follow: seeds of *Ocimum canum* Sims. (Labiatae), seeds of *Plantago ovata* Forssk. (Plantaginaceae), seeds of *Trigonella foenum-graecum* Linn. (Papilionaceae), leaves of *Litsea glutinosa* (Lour.) C.B. Robinson (Lauraceae), fruits of *Hibiscus esculentus* Linn. (Malvaceae), aerial parts of *Basella alba* Linn. (Basellaceae) and fruits of *Scaphium scaphigerum* G. Don. (Sterculiaceae). The mucilages were extracted with warm water and lyophilized to dryness. Commercial glucomannan powder was also studied. Solid cholesterol, stearic acid and oleic acid sufficient to produce the saturated solution (*ca.* 10 mg each) was stirred in 3 ml NaTDC at room temperature for 24 hours. Soluble lipid micelles were separated by filtration through 0.2 µm membrane filter. Cholesterol was determined by enzymatic colorimetric assay. Stearic acid and oleic acid were determined by gas chromatography. The same experiments with 15 mg of each mucilage or glucomannan were performed. The soluble lipids in bile salt with 0.5% mucilages were presented in percentage of the soluble lipids in bile salt alone.

RESULTS

At 25 mM of NaTDC, the soluble cholesterol concentration was 1.56 ± 0.04 mM. With selected mucilage gels in the system, the soluble cholesterol concentration ranged from 1.53 to 1.18 mM. *O. canum, T. foenum-graecum* and glucomannan showed inhibition on cholesterol solubility (24.3, 21.3 and 16.0% inhibition respectively). *L. glutinosa, S. scaphigerum, B. alba, H. esculentus* and *P. ovata* had marginal effect on cholesterol solubility (8.0, 6.4, 4.3, 4.2 and 1.8% inhibition respectively). For stearic acid solubilization, *B. alba* showed slightly inhibition (10.5% inhibition) while another mucilages as well as glucomannan increased stearic acid solubilization. Oleic acid has lower critical micelle concentration than stearic acid and cholesterol. The concentration of NaTDC used for oleic acid saturation was 0.3125 mM. At this concentration, 0.5% mucilages and glucomannan showed strongly inhibition of oleic acid solubility (97.5-65.9% inhibition).

DISCUSSION

Deoxycholic acid, a secondary bile acid, is more hydrophobic and more capable to solubilize cholesterol and fatty acids in the intestinal lumen. NaTDC has been previously reported for its stability in cholesterol-bile salt micelle formation[1]. Soluble dietary fibers are nominated for their *in vivo* hypocholesterolemic effects. There are several mechanisms postulated to be involved. The results of this study indicated that plant mucilages may play a role in interference with lipid micelle formation and lipid solubility. In addition, the effect on solubilization varied among each type of lipids as well as each type of mucilages. The mucilages and glucomannan in this study decreased cholesterol solubility whilst increased the solubility of stearic acid. The previous studies found that pectin and acacia (0.5%) significantly

increased the aqueous solubility of cholesterol while slight increases were observed for the solubility of cholesterol in both high and low molecular weight dextran solutions[2,3]. Carageenans (0.1%) significantly decreased cholesterol solubility[3].

CONCLUSION

In vitro experiments on cholesterol and fatty acid solubilization in bile micelle showed that the selected plant mucilages and glucomannan had some effects on micellar characteristics and interference with the solubility of lipids into micelle.

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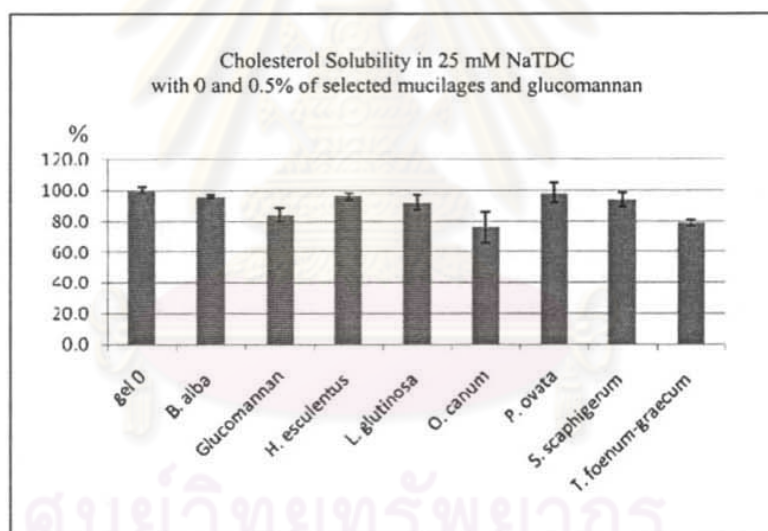
การประชุมวิชาการประจำปี

การแพทย์แผนไทย การแพทย์พื้นบ้าน การแพทย์ทางเลือกแห่งชาติ ครั้งที่ ๖

และ

การประชุมสมัชชาสุขภาพเฉพาะประเด็น ภูมิปัญญาท้องถิ่นด้านสุขภาพ
การแพทย์แผนไทย การแพทย์พื้นบ้าน และการแพทย์ทางเลือก
ในงานมหกรรมสมุนไพรแห่งชาติ ครั้งที่ ๖

ณ ห้องประชุมฟีนิกซ์ ๑ - ๖, ห้องประชุมจูปีเตอร์ ๑๑-๑๖
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ศูนย์แสดงสินค้าและการประชุมอิมแพ็ค เมืองทองธานี
อำเภอปากเกร็ด จังหวัดนนทบุรี

เพื่อเผยแพร่ผลงานวิชาการและงานวิจัยด้านการแพทย์แผนไทย การแพทย์พื้นบ้าน
และการแพทย์ทางเลือก สู่ชุมชนทางวิชาการ และสาธารณชน
และเป็นเวทีความร่วมมือเพื่อแลกเปลี่ยนองค์ความรู้ ความคิดเห็น และประสบการณ์
ด้านการแพทย์แผนไทย การแพทย์พื้นบ้าน และการแพทย์ทางเลือก

จัดโดย

กรมพัฒนาการแพทย์แผนไทยและการแพทย์ทางเลือก และเครือข่ายภาคีด้านสุขภาพ

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Bioactive Polysaccharides from Selected Thai Mucilaginous Plants

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Rationale: Plant mucilages have been used in traditional or folk medicines for treatment of many diseases. The mucilages are heterogeneous polysaccharides which exhibit hydrocolloid property. The utilizations of nutritional hydrocolloids are increasing due to their functional properties e.g. water binding, viscosity and gelation, as well as to their bio-active roles in prevention and / or treatment of certain diseases.

Objective: To investigate the biological activities of selected mucilage polysaccharides.

Methodology: Seven mucilaginous plants were studied as follow: seeds of *Ocimum canum* Sims. (Labiatae), seeds of *Plantago ovata* Forssk. (Plantaginaceae), seeds of *Trigonella foenum-graecum* Linn. (Papilionaceae), leaves of *Litsea glutinosa* (Lour.) C.B. Robinson (Lauraceae), fruits of *Hibiscus esculentus* Linn. (Malvaceae), aerial parts of *Basella alba* Linn. (Basellaceae) and fruits of *Scaphium scaphigerum* G. Don. (Sterculiaceae). The mucilages were extracted with warm water and lyophilized to dryness. Glucomannan was used as reference hydrocolloid polysaccharide. The biological activities were studied *in vitro* prior to animal models. The activity for entrapping glucose was studied by dialysis. The releasing glucose was assayed by glucose oxidase method. Alpha-glucosidase inhibitory activity which delayed carbohydrate digestion was spectrophotometrically assayed using p-nitrophenyl-α-D-glucopyranoside as a substrate. The effect on cholesterol solubility was performed using bile salt micelle model (sodium taurodeoxycholate). The soluble cholesterol was determined by enzymatic colorimetric assay. The potential antioxidant activity was tested on the basis of the scavenging activity of the stable diphenylpicrylhydrazyl (DPPH) free radical.

Results: All mucilage showed concentration response on glucose entrapment activity. Percentage of glucose released from 2 % mucilage suspension were 61.6, 70.8, 71.7, 80.6, 83.4, 85.8 and 92.8 % for *O. canum*, *P. ovata*, *T. foenum-graecum*, *L. glutinosa*, *H. esculentus*, *B. alba* and *S. scaphigerum* respectively. Glucomannan powder showed 65.4 % of glucose releasing at the same concentration. For enzyme alpha-glucosidase inhibition assay, 5 mg/ml of *S. scaphigerum* was shown as the best inhibitor with 82.6 % inhibition whilst deoxynojirimycin (standard inhibitor), *L. glutinosa*, *H. esculentus*, *O. canum*, *T. foenum-graecum*, *P. ovata*, *B. alba* and glucomannan were shown as 47.6, 41.0, 37.6, 32.8, 30.6, 27.0, 25.0 and 19.7 % inhibition respectively. At 25 mM of sodium taurodeoxycholate, the soluble cholesterol concentration was 1.56 ± 0.04 mM. With hydrocolloid polysaccharides in the system, the soluble cholesterol concentration ranged from 1.53 to 1.18 mM. *O. canum*, *T. foenum-graecum* and glucomannan showed inhibition on cholesterol solubility (24.3, 21.3 and 16.0 % inhibition respectively). *L. glutinosa*, *S. scaphigerum*, *B. alba*, *H. esculentus* and *P. ovata* had marginal effect on cholesterol solubility (8.0, 6.4, 4.3, 4.2 and 1.8 % inhibition respectively). The IC_{50} values in DPPH scavenger were 0.49, 0.61, 0.70, 1.52, 2.00 and 4.15 mg / ml for *L. glutinosa*, *S. scaphigerum*, *H. esculentus*, *T. foenum-graecum*, *B. alba* and glucomannan respectively. *O. canum* and *P. ovata* showed the least potential of DPPH scavenger ($IC_{50} > 10$ mg / ml).

Conclusion: The results revealed some biological activities among selected plant mucilaginous polysaccharides. The outcome is the scientific evidence of the potential to develop nutritional hydrocolloids from plant mucilages.

VITA

Miss Chanida Palanuvej was born on January 30, 1960 in Bangkok, Thailand. She got a Bachelor's degree of Science in biochemistry with second class honor in 1981 and got a Master of Science in Biochemistry in 1985 from Chulalongkorn University. She was granted a Royal Golden Jubilee Ph.D. Scholarship from the Thailand Research Fund (TRF) in the year 2005.