CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF SELECTED EXUDATE GUMS

Miss Parichart Hongsing

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Thesis Advisor	Chanida Palanuvej, Ph.D.
Thesis Co-advisor	Associate Professor Nijsiri Ruangrungsi, Ph.D.

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……Dean of College of Public Health Sciences
(Professor Surasak Taneepanichskul, M.D.)

THESIS COMMITTEE

…… Chairman
(Professor Surasak Taneepanichskul, M.D.)

…… Thesis Advisor
(Chanida Palanuvej, Ph.D.)

…… Thesis Co-advisor
(Associate Professor Nijsiri Ruangrungsi, Ph.D.)

…… Examiner
(Tepanata Pumpaibool, Ph.D.)

…… External Examiner
(Associate Professor Penpun Wetwitayaklung, Dr. rer. nat.)

ปริชาติ หงษ์สิงห์ : องค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพของยางไม้บางชนิด (CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES FROM SELECTED EXUDATE GUMS) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : อ. ดร. ชนิดา พลานุเวช อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ. ดร. นิจศิริ เรืองรังษี, 81 หน้า.

กัมคอนดาโกกุ กัมคารายา และกัมอคาเชียเป็นยางที่ได้จากต้นสุพรรณิการ์ ต้น *Sterculia urens* Roxb.และต้น *Acacia senegal* (Linn.) Willd. ตามลำดับ มีคุณสมบัติเป็นกากใยโพลีแซกคาไรด์ชนิดละลายน้ำ ทดสอบฤทธิ์ทางชีวภาพในการกักเก็บน้ำตาลในถุงไดอะไลซิส ฤทธิ์ยับยั้งการทำงานของเอนไซม์แอลฟากลูโคซิเดส ไลเปส และไทโรซิเนส โดยมีสารควบคุมผลบวกคือ 1-ดีออกซีโนจิริมัยซิน ออริสแตส และกรดแอสคอร์บิคตามลำดับ ทดสอบฤทธิ์รบกวนการละลายของไขมันในไมเซลล์จากเกลือน้ำดีสังเคราะห์และน้ำดีจากตับหมู ทดสอบฤทธิ์ในการต้านอนุมูลอิสระเปรียบเทียบกับบิวทิเลเตดไฮดรอกซีโทลูอีน ศึกษาคุณสมบัติทางกายภาพได้แก่ การหาค่าความหนืดและน้ำหนักโมเลกุลโดยเฉลี่ย ศึกษาคุณสมบัติทางเคมีโดยวิเคราะห์ชนิดและปริมาณของน้ำตาลโมเลกุลเดี่ยวที่เป็นองค์ประกอบในกัมแต่ละชนิดด้วยวิธีแกสโครมาโทกราฟีวิเคราะห์ปริมาณโปรตีนรวมโดยวิธีลาวรี ผลการศึกษาพบว่ากัมคอนดาโกกุมีฤทธิ์ในการกักเก็บน้ำตาลในถุงไดอะไลซิสได้ร้อยละ 39 กัมทั้งสามชนิดไม่มีฤทธิ์ในการยับยั้งเอนไซม์แอลฟากลูโคซิเดส กัมคอนดาโกกุและกัมอคาเชียลดการละลายของคอเลสเทอรอลในไมเซลล์จากเกลือน้ำดีสังเคราะห์ได้ร้อยละ 16 และ 23 กัมคอนดาโกกุและกัมอคาเชียยับยั้งเอนไซม์ไลเปสได้ร้อยละ 16 และ 20 ตามลำดับ ฤทธิ์ยับยั้งไลเปสน้อยลงเมื่อความเข้มข้นของกัมสูงขึ้น กัมทั้งสามแสดงฤทธิ์ในการกระตุ้นเอนไซม์ไทโรซิเนส กัมคอนดาโกกุและกัมคารายาแสดงฤทธิ์ในการต้านอนุมูลอิสระได้ร้อยละ 10 และ 20 ตามลำดับ ศึกษาคุณสมบัติทางกายภาพพบว่ากัมคอนดาโกกุมีความหนืดมากที่สุด ค่าความหนืดของกัมทั้งสามโดยวิธีวัดการตกของลูกบอลเท่ากับ 574.1, 25.2 และ 1.0 เซนติพอยส์ ตามลำดับ น้ำหนักโมเลกุลโดยเฉลี่ยโดยวิธี SEC-HPLC พบว่ากัมที่ศึกษาทุกชนิดมีน้ำหนักมากกว่า 2,350 กิโลดาลตัน บ่งชี้ว่ากัมเหล่านี้มีโครงสร้างขนาดใหญ่ กัมคอนดาโกกุประกอบด้วยน้ำตาลกาแล็กโทส แรมโนส และกรดกาแลกตูโรนิก ร้อยละ 14.6, 17.1 และ 17.2 โดยน้ำหนัก ตามลำดับ กัมคารายาประกอบด้วยน้ำตาลกาแลกโตส แรมโนส กรดกาแลกตูโรนิก และกรดกลูคูโรนิก ร้อยละ 14.7, 8.9, 9.1 และ 36.0 โดยน้ำหนัก ตามลำดับ กัมอคาเชียประกอบด้วยน้ำตาลอะราบิโนส กาแลกโตส แรมโนส และกรดกลูคูโรนิก ร้อยละ 21.4, 42.6, 10.6 และ 22.5 โดยน้ำหนัก ตามลำดับ ปริมาณโปรตีนรวมในกัมทั้งสามเท่ากับร้อยละ 2.4, 9.5 และ 4.7 โดยน้ำหนัก ตามลำดับ

สาขาวิชา วิทยาศาสตร์สาธารณสุข ลายมือชื่อนิสิต……..

ปีการศึกษา 2554 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก……...

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม……

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KEYWORDS : CARBOHYDRATE / DIETARY FIBER / VISCOUS POLYSACCHARIDES / MONOSACCHARIDE COMPOSITION / PHYSICAL CHARACTERIZATION

PARICHART HONGSING : CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF SELECTED EXUDATE GUMS. ADVISOR : CHANIDA PALANUVEJ, Ph.D., CO-ADVISOR : ASSOC. PROF. NIJSIRI RUANGRUNGSI, Ph.D., 81 pp.

The selected exudate gums from medicinal plants, Kondagogu (*Cochlospermum gossypium* Dc.), Karaya (*Sterculia urens* Roxb.) and Acacia (*Acacia senegal* (Linn.) Willd.) were recognized in the term of polysaccharide dietary soluble fibers. The *in vitro* biological activities were performed namely glucose entrapment ability against dialysis; α- glucosidase, pancreatic lipase and tyrosinase inhibition effects using 1-Deoxynorjirimysin, Oristat and Ascorbic acid as positive control; disturbance on lipid's solubility in synthetic bile salt and pig bile micelles; and DPPH scavenging effect compared to Butylated hydroxytoluene. The physical properties for viscosity and average molecular weight were investigated. The monosaccharide composition was analyzed qualitatively and quantitatively using gas chromatographic technique. Total protein contents were quantities by Lowry method. Only Kondagogu gum showed 39 % of glucose entrapment property against dialysis tubing. The exudate gums had no effect on α-glucosidase inhibition. The effect on cholesterol solubility in bile salt micelles showed that Kondagogu and Acacia gums had slightly inhibitory effects (16 and 23%). Kondagogu and Acacia gums inhibited 16 and 20 % of lipase activity. The lipase inhibitory effect was reciprocal relationship to the concentration of gum gels. Tyrosinase enhancing activity was shown among these gums**.** Kondagogu and Karaya gums expressed 10% and 20% DPPH scavenging effect respectively, while Acacia gum had no effect. The viscosity determined by falling ball viscometer was 574.1, 25.2 and 1.0 cP for Kondagogu, Karaya and Acacia gums respectively. The determination of average molecular weight performed by size exclusive chromatography using SEC-HPLC with refractive index detector revealed the average molecular weight more than 2,350 kDa for Acacia, Kondagogu and Karaya gums, indicating their large structures. Kondagogu gum was composed of galactose, rhamnose and galacturonic acid (14.6, 17.1 and 17.2 % by weight respectively). Karaya gum was composed of galactose, rhamnose, galacturonic acid and glucuronic acid (14.7, 8.9, 9.1 and 36.0 % by weight respectively). Acacia gum contained arabinose, galactose, rhamnose and glucuronic acid (21.4, 42.6, 10. 6 and 22.5 % by weight respectively). Total protein contents among these gums were 2.4, 9.5 and 4.7 % by weight respectively.

Field of Study : Public Health Sciences ..... Student's Signature .....

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Co-advisor's Signature .....

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LIST OF ABBREVIATIONS

Ara	=	Arabinose
cP	=	Centipoise
DPPH	=	1,1-Diphenyl-2-picrylhydrazyl
g	=	Gram
Gal	=	Galactose
GalA	=	Galacturonic acid
GC	=	Gas chromatography
Glc	=	Glucose
HCl	=	Hydrochloric acid
HPLC	=	High performance liquid chromatography
IC_{50}	=	Half maximal inhibitory concentration
kDa	=	Kilodalton
L	=	Liter
M	=	Molar per liter
Man	=	Mannose
mg	=	Milligram
mM	=	Millimolar per liter
MW	=	Molecular weight
NaTDC	=	Sodium taurodeoxycholate
nm	=	Nanometer
p	=	Pyranose

PVDF	=	Polyvinylidene fluoride
Rha	=	Rhamnose
TMS	=	Trimethylsilyl
Xyl	=	Xylose
α	=	Alpha
β	=	Beta
µl	=	Microliter
µm	=	Micrometer
λmax	=	Wavelenght at maximum absorption
mPa	=	Megapascal
s	=	Second
cm	=	Centimeter

CHAPTER I

INTRODUCTION

Background and Significance of the Study

Carbohydrates are the primary constituents in plants such as fruits, vegetables and grains. The complex structure of carbohydrates or polysaccharides had been proved as a dietary fiber in 1953 by Hipsley, stating that this carbohydrate fiber contains the healthy nutrients with high benefits for cholesterol and blood glucose monitoring [1]. Over the years, the association between Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO), which is authorized by World Trade Organization (WTO) established The Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) to categorise dietary fiber into three groups "1) edible carbohydrate polymers naturally occurring in the food consumed, 2) carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological benefit to health, as demonstrated by generally accepted scientific evidence to competent authorities, 3) synthetic carbohydrate polymers that have been shown to have a physiological benefit to health, as demonstrated by generally accepted scientific evidence to competent authorities".

Dietary fibers improve weight loss by yielding a satisfied feeling after meal, especially unrefined carbohydrate fibers [2]. Dietary fibers cause a reduction of blood glucose, leading to a decrease the insulin released. Hence, this mechanism causes the less fat storage. Many researches revealed that the higher intake of dietary fiber can improve and manage the body weight. The observational research on lean and obese women and men demonstrated that lean women and men have higher intake of dietary fiber than obese women and men (22.7 g/day and 27.0 g/day *vs.* 15.0 g/day and 22.0 g/d respectively) [3]. The cohort study on over 5,000 subjects supported the previous study that high fiber intake affected to the lower of body mass index (IBM) in both women and men [4]. Another randomized crossover research stated that adding viscous dietary fibers reduced the postprandial glucose, insulin, and cholecystokinin by assisting a reverse cholesterol transport pathway [5].

Many agencies and organizations recommended dietary fiber intake for individuals, particularly people with type 2 diabetes [6]. Diabetes mellitus is a common disease, resulting from radical changes in eating patterns. This lifelong undesirable disease results in improper use of sugar (glucose) in the body [7]. The increase of obesity and physical inactivity in the general population leads non-insulin-dependent or type 2 diabetes to be the most common form of diabetes. The prognosis by The World Health Organization (WHO) and The American Diabetes Association (ADA) showed an increased number of people with diabetes in developing countries. The number of diabetic people will double over the next 25 years, eventually reaching more than a hundred million diabetics by 2030. Numerous clinical researches supported various benefits of viscous dietary fibers. The ability to reduce the absorption of micronutrients across the intestinal lumen has advantages in management and prevention of insulin resistance which is the cause of type 2 diabetes [8]. The ability to diminish serum glucose of Xanthan and Guar gum supported the idea of viscous fiber bearing with hypoglycemia in type 2 diabetic people [9, 10, 11]. In terms of glycemic index, the clinical study between healthy and type 2 diabetic participants, consuming highly viscous fiber biscuits reveled the ability to reduce blood postprandial glucose by 74% (7.4 glycemic index units/g of fiber) and 63% (6.3 glycemic index units/g of fiber) in healthy and diabetic participants respectively [12]. Abundant researches assured the association between diabetes mellitus and obesity significantly increased the risk of coronary heart disease [13, 14, 15]. Therefore, preventing and controlling obesity and type 2 diabetes are the indirect benefit for heart disease. These conditions above lead viscous dietary fiber to be the key to over come many undesirable diseases. The recommendation of The National Cholesterol Education Program (NCEP) to increase viscous dietary fiber consumption is also supported by the Food and Drug Administration (FAD), stating that taking 7 g/day of Psyllium and 3g/day of beta-glucan from barley and oats effectively reduce coronary heart disease. National Cancer Institute recommends 20 – 30 grams of high fiber foods to reduce the risk of colon cancer [16]. People without diabetes are recommended a daily fiber intake 20 -35 grams; in addition, clinical studies have shown that daily fiber intake more than 50 grams can improve blood glucose control and reduce lipid levels in people with type 2 diabetes [17,18]. Therefore, choosing

viscous carbohydrates gives various benefits for health.

Gums are the edible part of exudates from plants. The biopolymer natural gums are obtained from the breakdown of cell wall (gummosis) by stripping of the bark, yielding viscous sticky amorphous tear-like lumps [19]. The main component in gums is hydrophilic polysaccharide which is the polymer of soluble monosaccharide. The physical properties of polysaccharide gums are used as stabilizing, suspending, gelling, emulsifying, thickening, binding and coating agents. Thus, the viscous mucilage gums can reduce and retard the absorption of cholesterol, glucose, and organic compounds in gastrointestinal tract because of their water holding capacity. The fiber gums become swollen when merged with water. Even high-fiber carbohydrate cannot be digested by any enzymes in the human body; however, the bacteria in the lower gastrointestinal tract can degrade the dietary fiber [2, 20]. The indigestible process of dietary fibers is attested that high consumption of fiber carbohydrates yields small amount of calorie. Presently, gums are classified as water soluble dietary fibers because of their ability to resist the digestion and absorption of aliment in the intestinal digestive system.

Kondagogu gum from the tree *Cochlospermum gossypium* De Candole belonging to Cochospermaceae family is the newest commercially natural product for food and pharmaceutical use. Traditional medicine uses exudate gums as a laxative to soften the intestinal waste and makes it easy to go through the bowel. The exceptional properties of water soluble Kondagogu gums that the molecular mass and intrinsic viscosity of Kondagogu gum is much higher than the values reported for the other tree gums such as Karaya gum from the tree *Sterculia urens* Robx and Acacia gum from the tree *Acacia cenegal* (Linn) Willd. Therefore, the ability to swell within the water, entrap water and nutrients in the intestinal tract make it an interesting topic to study, especially to search new paths for diabetes prevention and care. Moreover, other components in the viscous fiber gums are also interesting to explore its biological activities.

Research Questions

1. What are monosaccharide constituents of selected exudate gums?
2. What are the biological activities of selected exudate gums?

Hypothesis

Selected exudate gums are heteropolysaccharide with functional food potential.

Objectives

1. To analyze monosaccharide constituents of selected exudate gums
2. To investigate biological activities of selected exudate gums

CHAPTER II

LITERATURE REVIEWS

Carbohydrates

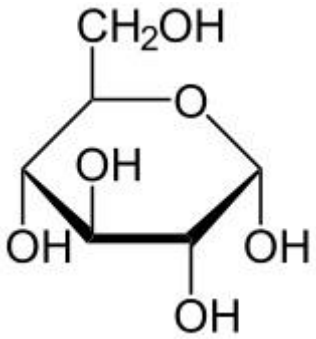
Carbohydrates (saccharide or glycan) are organic compounds. The molecular components are composed of carbon, oxygen and hydrogen atoms. Macromolecules are the common form of carbohydrates that can be found in nature; such as starches, glycogen, and cellulose. Most carbohydrates yield 4 calories per gram; however, all carbohydrates do not have the same nutrition. The numbers of single saccharide molecules in chemical structures are used to classify carbohydrates. There are three types of carbohydrates. These are monosaccharides, oligosaccharides and polysaccharides.

Monosaccharides

Monosaccharides are the main constituents of carbohydrates, yielding a sweet taste in the form of a water soluble organic compound. It is a simplest form of carbohydrate which is composed of either aldehyde or ketone group with at least two hydroxyl groups. Thus, the empirical formula for unmodified monosaccharides is $(CH_2O)_n$. Aldoses and ketoses are the general derivative types of monosaccharides. In addition, monosachariedes are named according to the number of carbon atoms they possess. The asymmetric carbon atoms in monosaccharides cause optical isomer (enantiomer) for one another. Therefore, the L-isomer is represented as the projecting of hydroxyl group to the left while the projection of hydroxyl group to the right is D-isomer. Both aldose and ketose series are shown in figures 1 and 2. However, most natural monosaccharides in food have five (furanoses) to six (pyranoses) carbon atoms in the form of a ring structure. The intramolecular reaction between aldehyde and hydroxyl groups in aldose monosaccharides is called hemiacetal reaction. In contrast, the intramolecular reaction between carbonyl and hydroxyl groups in ketose monosaccharides is called hemiketal reaction. These two reactions increase asymmetric carbon atoms which cause alpha form of anomer when the anomeric hydroxyl group points down while the anomeric hydroxyl group points up is called a

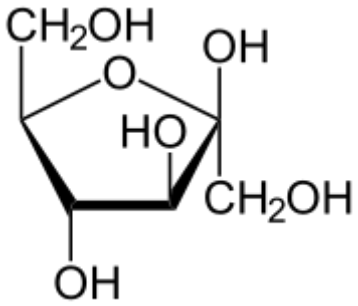
beta form of anomer. The most known monosaccharides are glucose, fructose, galactose and ribose [21].

Glucose



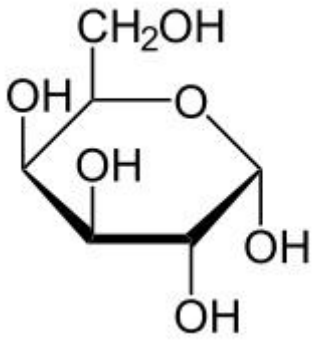
The empirical formula of glucose is $C_6H_{12}O_6$ (aldohexoses). Glucose is an aldehyde monosaccharide because the first carbon atom of glucose is an aldehyde group. Blood sugar monitoring is the common name of glucose because it is measured for glycemic index. Cells use glucose as a source of fuel, according to glycolysis metabolism pathway which uses glucose to provide ATP (Adenosine triphosphate). After digestion, glucoses are absorbed in the small intestine and travel in the blood to the liver. The amount of glucose in blood can be a health indicator. The improper use of glucose in the body is considered for diabetic people [22].

Fructose



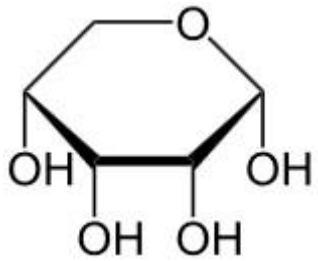
Fructose has the same empirical formula as glucose ($C_6H_{12}O_6$) but differs in structure because fructose is a ketone monosaccharide. Fruit sugar is fructose because this sugar is commonly found in fruits and vegetables. Fructose has less effect for diabetic people than glucose.

Galactose



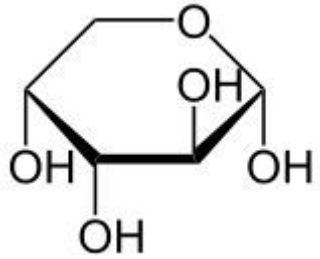
Galactose is classified as an aldohexose monosaccharide. Carbohydrate is not only found in plants but also in dairy products such as milk, which contains lactose. Lactase is a specific enzyme that could hydrolyze lactose to yield galactose, which the intestine can absorb [2].

Ribose



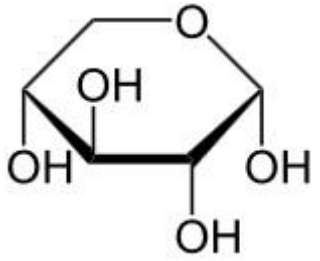
Ribose which contains five carbon atoms is an aldopentose monosaccharide ($C_5H_{10}O_5$). It is a building block of the backbone chains of genetic molecule, RNA (ribonucleic acid). RNA is the polymer of ribonucleotides. Each ribonucleotide contains 1 ribose, 1 purine or pyrimidine base and 3 phosphate groups. The other genetic molecule, DNA (deoxyribonucleic acid) contains deoxyribose instead of ribose.

Arabinose



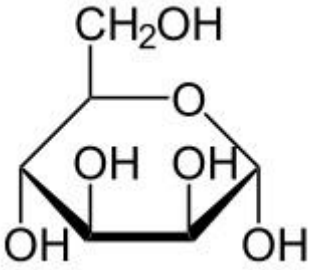
Arabinose which consists of five carbon atoms with an aldehyde group is an aldopentose monosaccharide. It was first isolated from Arabic gum. In nature, it is found as a component of polysaccharides in gums, pectin and hemicelluloses.

Xylose



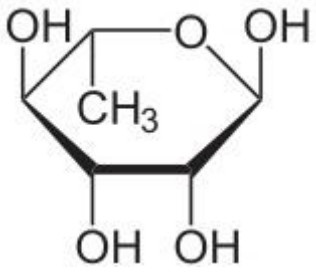
Xylose is aldopentose monosaccharide which was first isolated from woody materials and proved its properties in the time of World War I by a Finnish scientist. Human body can produce a small amount of it. When xylose is reduced, it produces xylitol, a sugar alcohol as a product [23]. Xylitol is widely used as a sweetener in reduced caloric food for medicinal purposes.

Mannose



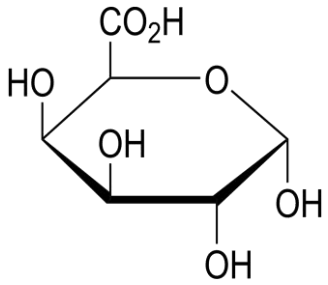
Mannose is a sugar monomer containing aldehyde group with six carbon atoms. It has an influence in human metabolism, especially in a glycosylation. In medicinal term, D-mannose is used for congenital disorders by its ability to adhere the bad bacteria in the urinary tract and forming complex molecules which are discharged by voiding [24].

Rhamnose



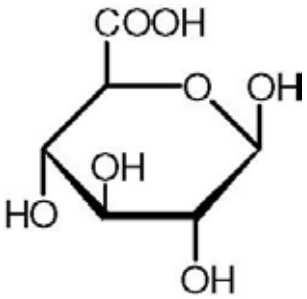
Rhamnose is a six-deoxy-hexose monosaccharide that can be found naturally in plant glycoside. This monosaccharide is the constituent in polysaccharide gums and mucilages. Rhamnose can be obtained from the extraction of *Uncaria* genus plants and some bacteria [25].

Galacturonic acid



Galacturonic acid is the derivative of glucose that can be found in plants and human body. This monobasic saccharide comes from the oxidation of the first alcohol group of D-galactose to carboxylic acid [21]. Galacturonic acid is an abundant constituent in plants such as pectin and polysaccharide gums. Moreover, D-galacturonic acid obtained from pectin can be used as a source of ascorbic acid synthesis [26].

Glucuronic acid



Glucuronic acid, a hexuronic monosaccharide is naturally occurred in mammals and plant cell wall as glucuronide conjugates and glycosaminoglucans. Glucuronic acid is a derivative of glucose. It is known as a detoxifying agent which is eliminated by kidney as aryl/alkyl D-glucosiduronic acids form in urine [27].

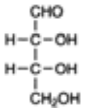
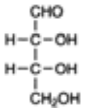
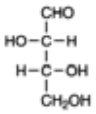
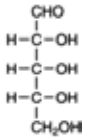
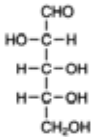
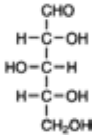
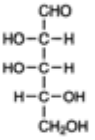
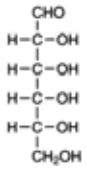
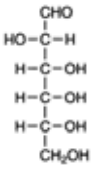
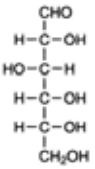
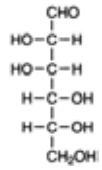
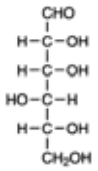
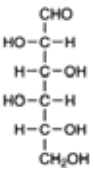
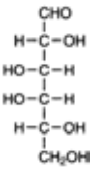
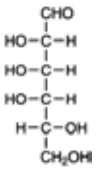
D- Aldoses							
 D-Glyceraldehyde							
 D-Erythrose				 D-Threose			
 D-Ribose		 D-Arabinose		 D-Xylose		 D-Lyxose	
 D-Allose	 D-Altrose	 D-Glucose	 D-Mannose	 D-Gulose	 D-Idose	 D-Galactose	 D-Talose

Figure 1 D-aldose monosaccharide series [21]

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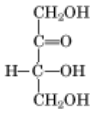
<tr><td colspan="4"> 
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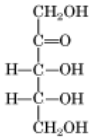
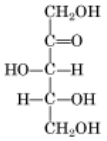
<tr><td colspan="2"> 
D-Ribulose</td><td colspan="2"> 
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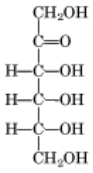
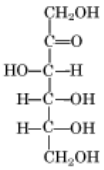
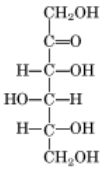
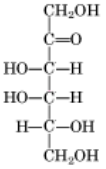
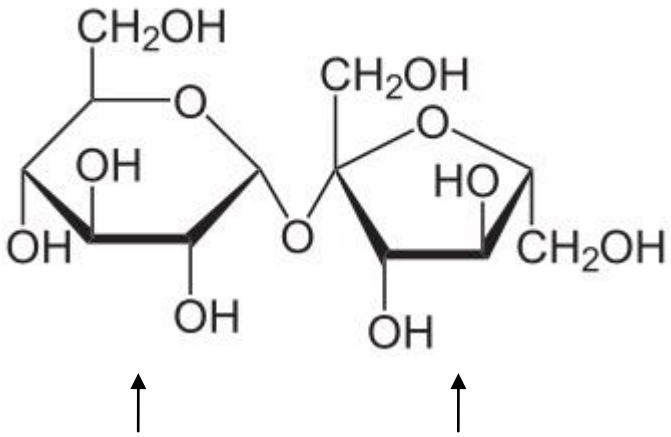
<tr><td> 
D-Psicose</td><td> 
D-Fructose</td><td> 
D-Sorbose</td><td> 
D-Tagatose</td></tr>
</table>

Figure 2 D-ketose monosaccharide series [21]

Oligosaccharides

Oligosaccharides are a short chain of polysaccharides which are made up of 2- 10 units of monosaccharides, yielding a sweet taste in form of water soluble organic compound. The most abundant form of oligosaccharides is disaccharides. Disaccharides are formed from two monosaccharides bonded together by a glycosidic linkage. Different types of monosaccharides and the glycosidic linkage configuration (alpha/beta) are used to distinguish disaccharides. Moreover, the bonding position between the anomeric carbon of the first monosaccharide with the hydroxyl group of another monosaccharide is also an indicator of the different types of disaccharides. Most disaccharides are reducing sugar because they contain a free anomeric carbon, except sucrose, which is a non-reducing sugar. The most important disaccharides are sucrose, maltose and lactose.

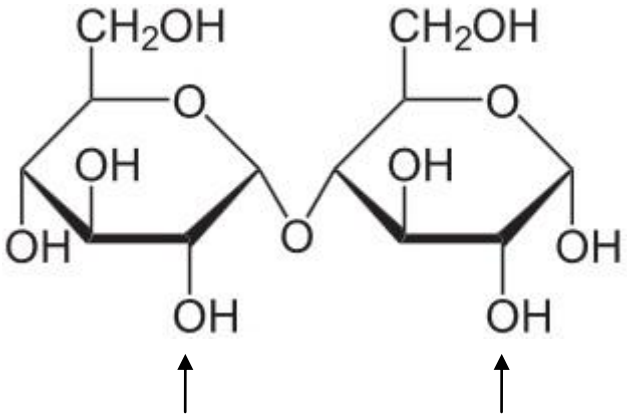
Sucrose



Glucose **Fructose**

Sucrose is also known as saccharose or table sugar. It is a combination of glucose and fructose which is linked together by glycosidic bond, yielding one molecule of sucrose ($C_{12}H_{22}O_{11}$). Unlike most disaccharides, the anomeric carbon of fructose connected with hydroxyl group of anomeric carbon of glucose, thus sucrose could not be reduced. Sucrose can be digested by enzyme sucrase giving glucose and fructose which are absorbed by intestine. Thus, taking table sugar daily is a concern for diabetics.

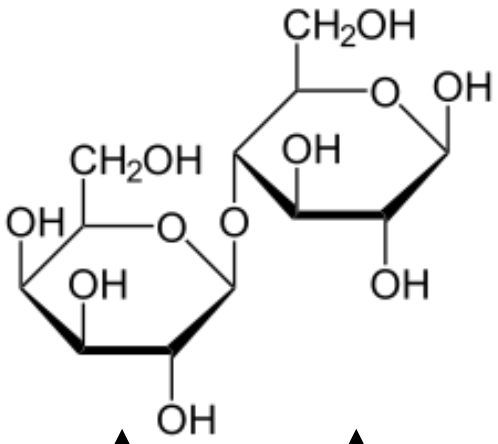
Maltose



Glucose **Glucose**

Maltose is formed by two glucose molecules linking together by α (1→4) bond. The empirical formula of maltose is ($C_{12}H_{22}O_{11}$). The breakdown of maltose occurs in the small intestinal tract by enzyme maltase, then two glucose molecules are released.

Lactose



Galactose **Glucose**

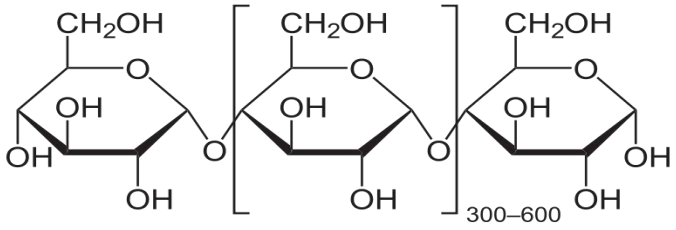
Lactose can be found in milk and dairy products. Lactose is a combination of galactose and glucose. They combine together by β (1→4) glycosidic linkage. The small intestine releases adequate amounts of enzyme lactase to digest lactose. Lactose intolerance is the condition of which the small intestine cannot produce the enzyme lactase.

Polysaccharides

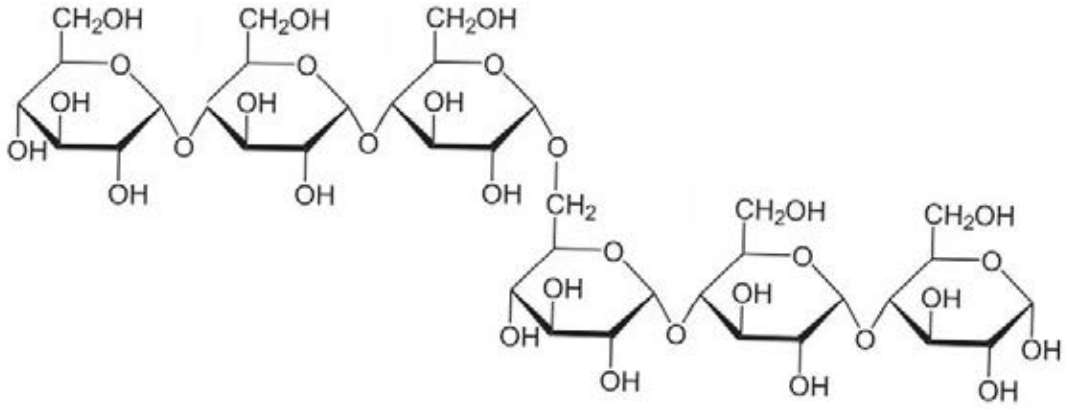
Polysaccharides are an insoluble macromolecular of carbohydrate that generally found in nature. The structure in either branched or linear polymer indicates different types of polysaccharides. Homopolysaccharides are the repeating of one type of sugar or sugar derivative unit in their structures, while heteropolysaccharides are composed of various types of sugars unit in their structures. The most important polysaccharide is starch.

Starch

Starch is a powdery substance that acts as a food storage device. It is primarily found in plants. Many foods containing high amounts of starch include rice, bread, potato, corn and beans. These foods are central to meals in many countries. All macromolecular molecules belonging to carbohydrate must be digested into monosaccharides before they can be absorbed in the intestine and transferred to the liver *via* the hepatic portal vein. The chemical structure of starch which consists of glucoses is used to classify the type of starch. Amylose is made up by many units of glucose and connected to each other by α (1→4) linkage. The structure of amylose is helical coil. Amylopectin also has a long chain of glucose but differs in branches along the chain in every 25 – 30 units of glucose. In addition, the new existing branches are bonded by α-(1→6) linkage. Starch digestion firstly begins in the oral cavity by mastication. Saliva glands produce amylase to hydrolyze starch into short chain sugars, resulting in maltose, maltotriose, α-dextrins and even some glucose. The pancreatic α-amylases are secreted into the small intestine (duodenum) to cut down disaccharides that remain from the first part. Finally, α-glucosidase, maltase-glucoamylase complex, sucrose-isomaltase complex hydrolyzed the short chain sugars to complete the digestion, yielding glucose to travel into the bloodstream *via* the small intestinal villi [2].



Amylose



Amylopectin

Figure 3 Amylose is bonded by α (1→4) linkage. Amylopectin has more branches that consists of α-(1→6) linkage [21].

Dietary fiber

Dietary fiber is an indigestible carbohydrate because it is macromolecular of many sugars joining together with a mainly β-glycosidic bond. The term of dietary fiber was introduced in 1953 by Hipsley [28]. The dietary fiber composed of edible plant cells, polysaccharides, lignin and associated substances resistant to digestion (hydrolysis) by the alimentary enzymes of human [29]. There are two types of fiber and based on the water fraction analysis method [30]. The fiber that cannot dissolve in water is called insoluble fibers. Most of insoluble fibers are plant cells, including cellulose, hemicelluloses and lignin. The dietary fiber that can dissolve and blend with water to form a gel in gastrointestinal tract is called soluble fiber. Soluble fiber included seeds, beans, mucilage plants and gums. Over the years, the association between Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO), which is authorized by World Trade Organization (WTO) established The Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) to categorise dietary fiber into three groups " 1) edible carbohydrate polymers naturally occurring in the food consumed, 2) carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological benefit to health, as demonstrated by generally accepted scientific evidence to competent authorities, 3) synthetic carbohydrate polymers that have been shown to have a physiological benefit to health, as demonstrated by generally accepted scientific evidence to competent authorities" [31].

Fiber rich food has been proven as the essential for a healthy diet. Therefore, dietary fibers play an important role in a healthy diet recommendation. The main idea of the dietary fiber recommendation is to choose the right type of carbohydrate which reduces the risk factors of undesirable diseases. The physical properties of dietary fiber have positive physiological response to human body. The ability to trap the aqueous medium (water, nutrients) and form viscous solution elevates the stool bulk in the gastrointestinal tract and makes you feel satisfied, improving weight loss. The stool bulk content causes a reduction of the alimentary enzyme mobility in hydrolyzing process (digestion), leading to decrease the rate of nutrients absorption in

the intestinal lumen. This physical characteristic of viscous fiber facilitates the defecation and reduces the risk of constipation, hemorrhoids and diverticular disease. Dietary fiber causes bacterial fermentation in the lower gastrointestinal tract which assists in bacterial degradation. The breakdown of fiber carbohydrates by bacteria forms fatty acids in the colon. The fatty acids enrich the growth of *Bifidobacterai*, leading to lower rate of colon cancer. The water-holding capacity does not only contribute to fecal bulking, but also in the retardation of cholesterol solubility in bile acids, hence lowering plasma cholesterol levels. In addition, dietary fibers that form a viscous solution such as Guar gum reduced blood sugar (glucose) by retarding the absorption of glucose in small intestine [2, 32]. The National Cancer Institute recommends a daily intake of 20 – 35 grams of dietary fiber for cleaning the potential carcinogens in gastrointestinal tract [33].

Exudative gums from plants are natural hydrocolloids, the complex hydrophilic polymer which composes of hydroxyl group (-OH) [34]. The constitutions in each exudative gum are a mixture of monosaccharide and uronic acids. Even the constitutions in gums and mucilages are similar; however, their origins are different. Mucilages are formed by normal plant metabolism, while exudative gums are the pathological products caused by the breakdown of plant's cell wall (gummosis) such as Karaya, Acacia and Kondagogu gums.

Viscous dietary fiber

Viscosity is the famous physiochemical property of many polysaccharide dietary fibers which has been credited for the physiological advantages in human. The ability to form a sticky viscous solution when merged with fluids leads soluble fiber to be a majority of viscous dietary fibers, such as glucomannan, pectin and gums. Viscosity is the intrinsic property that depended on a degree of concentration and the composition of chemical structure in each viscous dietary fiber [35]. Numerous researches revealed the physiological benefits of viscous dietary fibers against many undesirable diseases. The change of eating patterns to high consumption of refined food, particularly refined carbohydrates caused the elevation of glycemic response in human population [36]. The clinical study on various concentrations of oat gum consumption in healthy participants showed 79 – 96% significantly reduction in

plasma glucose concentration ($P<0.05$). The result evidenced that viscosity of dietary fibers has an influence to plasma glucose levels [37]. The consumption of viscous dietary fiber decreased plasma glucose concentration may be due to their water holding capacity, resulting in the formation of a gel matrix. The gel matrix caused the slow digestion and absorption, leading to delay the rise of postprandial glucose. The meta-analysis of 8 controlled trails [38] was conducted to study the effects of Psyllium consumption (10.2 g/day) against the cellulose placebo with a low-fat diet for ≥ 8 weeks. The results showed the ability of Psyllium to reduce 4% serum total cholesterol ($P<0.0001$), 7% LDL cholesterol ($P<0.001$) and 6% of serum apolipoprotein B to apolipoprotein A-1 ($P<0.05$). These results were related to placebo in participants that consumed low-fat diet. There was no adverse effect to the participants from Psyllium consumption in this study. Previous studies suggested many mechanisms to reduce the cholesterol from viscous dietary fibers. The property to trap the water and form the sticky viscous solution retarding the digestion and absorption of fat alimentary in the intestinal lumen is the general property of soluble fiber. In addition, the reduction of serum total and LDL cholesterols may be due to the ability to assist the releasing of bile acid deviating hepatic cholesterol for bile acid production [39]. The second famous property of soluble fiber is fermentation. The indigestible part of dietary fiber was fermented by intestinal bacteria, yielding short chain fatty acid that may prevent the synthesis of cholesterol in liver. These fatty acids may treat the inflammation in the intestinal lumen and prevent colon cancer [2]. The excess of glucose and fat in the blood circulation is the cause of diabetes mellitus, coronary heart disease and many complications. The therapeutic effect of Psyllium was adapted from the beneficial mechanisms of viscous dietary fiber to evaluate diabetic parameters in type 2 diabetic patients. This clinical study was conducted in Spain including type 2 diabetic 12 men and 8 women. The first week (phase 1), participants received diabetic food and drug then 14 g/day of Psyllium was added for 6 weeks later (phase 2, treatment). The last 4 weeks (phase 3) were the washout period. The study was significantly reduced 12.2% of blood glucose, while uric acid, LDL and total cholesterol were reduced to 10, 9.2, and 7.7% respectively. There is no significant change in vitamins, but only sodium increased after Psyllium consumption [40]. From this research, the therapeutic effects of Psyllium preventing diabetic and

coronary heart disease encouraged viscous dietary fiber to be the interesting topic to explore its conceal therapeutic abilities.

Carbohydrate digestion and absorption

Carbohydrate foods (polysaccharides) are macromolecules that cannot be absorbed into the body. Therefore, the digestion of carbohydrates by alimentary enzymes in gastrointestinal tract is occurred. The carbohydrate digestion begins firstly in the mouth. Parotid glands produce salivary amylase to break down α (1 → 4) glycosidic linkage of carbohydrates, yielding shorter polysaccharides and later pass through the stomach. Hydrochloric acid in the stomach inactivates salivary amylase from the mouth; however, the polysaccharides remain from stomach are totally digested by pancreatic amylase in the small intestine. At the brush border of small intestine, the enzymes, especially α-glucosidase attach to disaccharides and oligosaccharides breaking down into monosaccharides. The absorption of monosaccharides at the intestinal villi will be complete by carrier-mediated process. Active transport is significant to glucose and galactose absorption, while facilitated diffusion is significant to fructose. The picture below shows the carbohydrate digestion and absorption pathway.

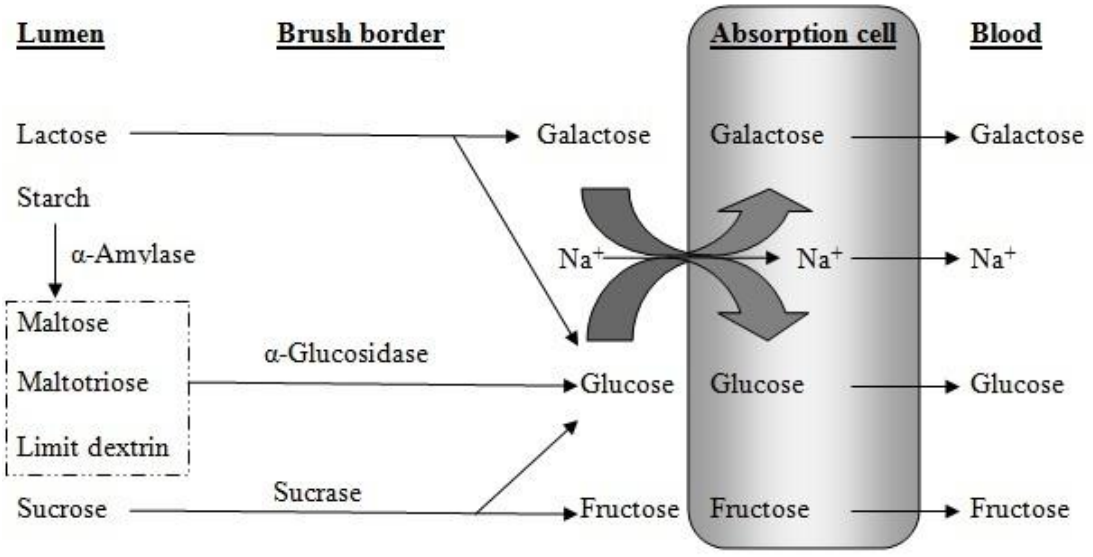


Figure 4 Dietary carbohydrate digestion and absorption [21]

Lipid digestion and absorption

Hepatocytes synthesized bile acids from cholesterol and then the bile acids are conjugated to glycine or taurine (an amino acid), secreting into gallbladder as the conjugated form. Thus, bile acids are the derivatives of cholesterol. Bile acids play an important role in emulsification of lipid aggregation. This emulsifier is the key for micelles formation by facilitate lipid solubility in aqueous matrix. Bile acids perform as the detergent on lipid particles, causing the droplets of lipid (micelles). Lipase enzymes cannot access into the micelles; however, the previous process from bile acids greatly increases surface area of lipid, causing the opportunity for lipases digestion. Therefore, the efficiency of lipid metabolism depends on its solubility. The interruption of lipid solubility reduced lipids digestion and absorption in the intestinal lumen into the blood circulation. Even the mechanism to retard the cholesterol absorption is not fully explained; however, the studies on viscous polysaccharides from plant showed the ability to reduce the absorption of cholesterol in the intestinal lumen [38, 39, 40].

Most lipids from diets are triacylglycerol or triglyceride. Complete digestion of dietary fat in small intestine needs triacylglycerol lipase or pancreatic lipase which is mainly produced in pancreas. After the emulsification of dietary fat by bile salts, the water-soluble enzyme (pancreatic lipase) works at the lipid-water interface area. Pancreas releases lipase to breaks down (hydrolyze) fat in the alimentary digestive system, resulting in monoacylglycerol and free fatty acids [41]. Lipase inhibitor is a target in drug or food supplement development for hyperlipidemia protection.

Free radicals and vascular diseases

Free radical is the atom or molecule containing unpaired electrons at the most outer shell (unstable state) causing highly reactive. Any free radicals containing oxygen are the type of reactive oxygen species (ROS). At the ground state, free radical molecules must be stable by stealing the electron from other molecules causing new free radical formations. Thus, thousands of the chain reaction events are occurred continually. Free radical is the deleterious cause of many health problems such as diabetes mellitus, coronary heart diseases and cancer by attacking healthy

cells in the body. These damages cause the abnormality of cells and organ systems [42]. The interesting of natural antioxidants from herbal medicines and plants become popular for pharmaceutical and food industrials around the world. Previous report using DPPH radical-scarvenging activity evidenced that viscous polysaccharides showed the ability to inhibit the oxidation from 2,2-diphenyl-1-picrylhydrazyl free radical [43].

Hyperpigmentation and protection

Figure 5 showed tyrosinase enzyme catalysis of the skin pigments (melanin) formation from the amino acid (tyrosine). The increasing of the enzyme ability in melanogenesis (melanin production) caused hyperpigmentation or dark skin. Currently, skin-whitening products are the most popular cosmetic for many Asians. Numerous plants have been screened for its tyrosinase inhibitory activity [44]. Plant polysaccharides were revealed for the potential on tyrosinase inhibitory activity. For example, 10 μg/ml of polysaccharide fraction from Pomegranate showed around 40 % tyrosinase inhibition [45]. Crude polysaccharide from Tea leaves at the concentration of 1 μg/ml was reported 75 % of tyrosinase inhibition [46].

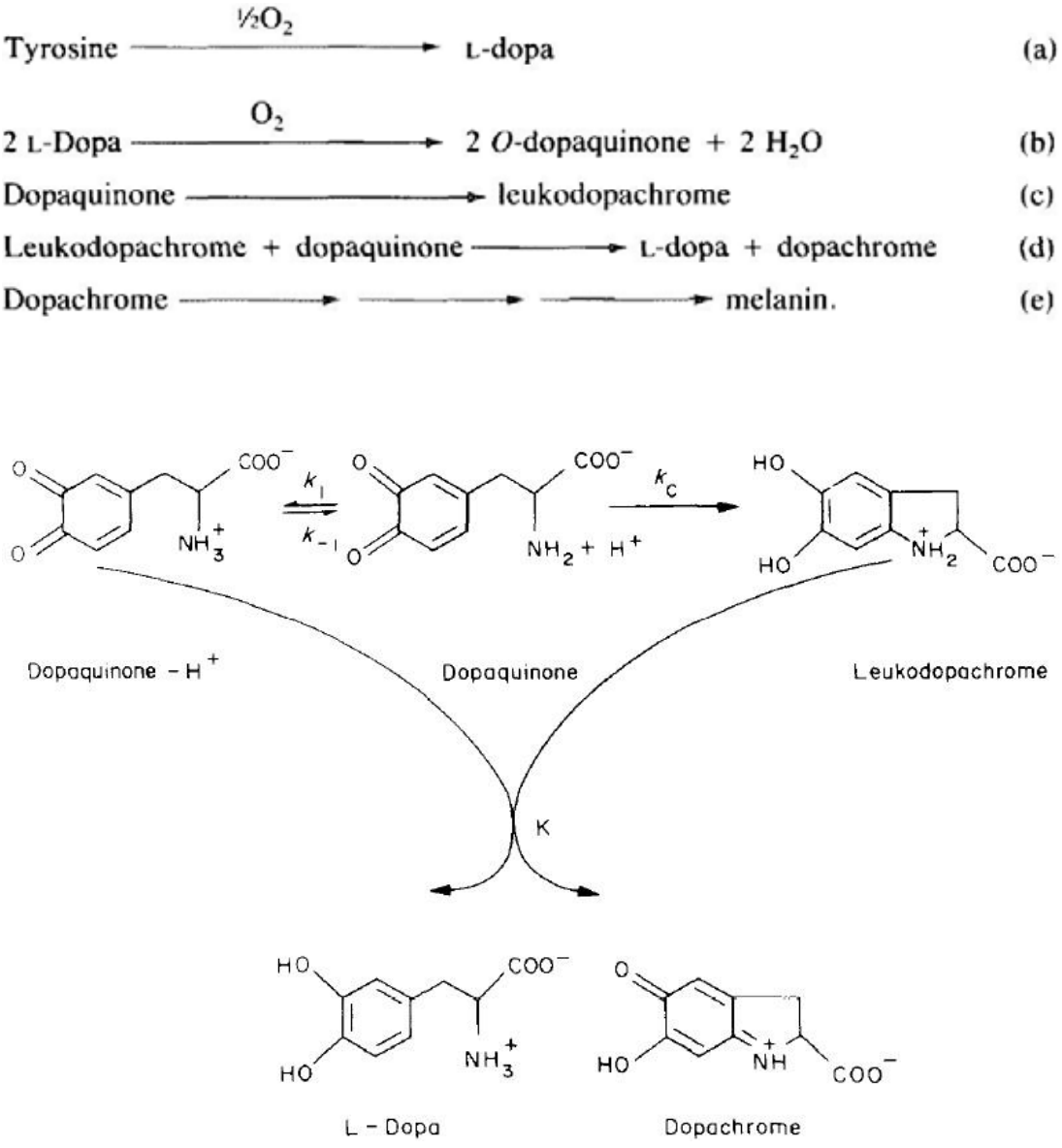


Figure 5 (above) Melanin biosynthesis: (a) and (b) reactions are tyrosinase enzymatic catalysis; (c) and (d) reactions are spontaneously chemical process; (e) reactions involved by tyrosinase and tyrosinase related protein. (below) Chemical reactions from dopaquinone to dopachrome. [47, 48]

Exudate gums

Exudate gums are pathological product from plants. The production of exudate gums occurs when the plants bark is injured; the liquid solution is exuded to seal the wound for prevention of dehydration and infection of the plants. The gum solution is dried when contact with air and sunlight, forming the hard tear like lumps. The chemical composition of exudate gums is complex. The amount of chemical constituents in exudate gums is varied, depending on the source and their age. Exudate gums are the economic natural product in third-world countries because of their applications, mainly in pharmaceutical and food industrials [49].

Kondagogu gum

Kondagogu gum exudates from *Cochlospermum gossypium* De Candole tree (family COCHLOSPERMACEAE). According to Flora of Andhra Pradesh, [50] *"Cochlospermum gossypium* tree is a small deciduous tree with short, thick, spreading branches. Bark thick, fibrous, deeply furrowed; inner substance red. Leaves near the ends of branches, palmately 5 lobed, 3-8 in. diam., lobes shortly acuminate, entire grey-tomentose beneath ; petioles 4-6 in. long. Flowers are 2 sexual 4-5 in. diam., bright golden yellow, in few-flowered terminal panicles, appearing before the leaves. Sepals 5, silky, deciduous. Petals 5, contorted in bud. Stamens indefinite, inserted on a disk without glands; anther-cells opening with a short slit at the top. Ovules numerous on 3-5 parietal placentas; style 1, filiform. Fruit an egg-shaped capsule 5 valved, valves 2-3 in. long. Seeds covered with soft silky hair. Wood extremely soft, greyish-brown ; no heartwood. It is quite useless being when dry, nothing but a loose bundle of fibers, when green, a soft spongy mass. It is a very common and conspicuous tree characteristic of the hottest, driest, stoniest slopes. The cotton from the seeds can be used for stuffing pillows, for which purpose it is even better than that of Bombax. The bark gives a cordage fiber (Gamble)".

During April to June, stripping of the bark *of Cochlospermum gossypium* tree yields the best quality of exudative gum. Kondagogu gum is classified in many grades, depends on the color and foreign matter in the gum [51]. The physico-chemical analyses revealed Kondagogu gum grade two and three have higher water-

binding capacity than that of Kondagogu gum grade one [52]. Previously study reported that the intrinsic viscosity values were in the range of 720–1160 ml g^{-1} [53]. Later study reiterated that the intrinsic viscosity value of Kondagogu gums was found as 729 ml g^{-1} [52]. Therefore, intrinsic viscosities could lead to considerable change between the properties and functionality of the gums, as intrinsic viscosity gives an indication of the differences in the molecular weight and space occupied in solution and therefore has implications for molecular shape. Toxicity study in rats shown that Kondagogu gum is non-toxic; and, an increased faecal bulk was observed in rats fed with 5% Kondagogu gum. Thus, Kondagogu gum is an effective laxative [54].

Figure 6 *Cochlospermum gossypium* tree, flowers and Kondagogu gum

Karaya gum

Karaya gum is the natural product exudates from *Sterculia urens* Roxb tree (family STERCULIACEAE). According to Agoforestry Database, [55] *"Sterculia urens* tree is a deciduous medium tree with reddish or grayish-white bark. Leaves on long petioles, crowded at the ends of branches, palmately 5-lobed, 20-30 cm diameter; tomentose beneath, glabrous above, entire, acuminate; stipules caducous. Flowers greenish yellow, small, in terminal panicles; follicles 4-6, ovoid-oblong, about 2.5 cm diameter, coriaceous, red, covered with stinging hairs. Fruit consists of 5 sessile, radiating, ovate-lanceolate hard, coriaceous carpels, 7.5 cm long, red when ripe,

covered outside with many stiff bristles. Seeds 6 mm long, oblong, dark chestnut-brown, 3-6 per carpel." Karaya gum is a pathological product from *Sterculia urens* tree. The famous processes to get the gum are blazing or stripping of the trunk. The thoroughness of the gum is a quality indicator. Therefore, most of food and drug grade gums are usually a pinkish-white powder with faded acetic odor [16, 19]. A low concentration (1%) of Karaya gum can cause a formation of viscous colloidal solution by mergence with water; moreover, at a high concentration up to 4% of Karaya gum causes gel and plate textures [19, 56]. The structure of Karaya gum consists of acetyl groups which have an influence to its swelling property. Another unique property of Karaya gum is an ability to swell within 60% alcohol, but not in other organic solvents. Karaya gum is used as a laxative drug because the ability to swell up to 60 – 100 times its original volume, so the intestine contents is increased [56, 57]. The sticky property is similar to glue, so it has been used as ostomy adhesive and dental fixture [58, 59]. When applied Karaya gum as coating agent on tooth, it reduced 98% bacteria adhesion [60]. Some preliminary researches suggest that viscous dietary fiber gums may cause a reduction of plasma lipid and blood sugar levels [61]. Laboratory experiments shown the ability of Karaya gum as a drug delivery by carry out the drugs in aqueous medium [62, 63].

Figure 7 *Sterculia urens* tree, flowers and Karaya gum

Acacia gum

Acacia gum is the exudate gum of *Acacia senegal* (Linn.) Willd tree (family FABACEAE). According to Agoforestry Database, [64] "*Acacia senegal* is a deciduous shrub, growing to 15 m tall. The bark is greyish-white. Leaves bipinnate, 3-8 pinnae (glands between uppermost and lowermost pinnae); rachis up to 2.5 cm long; pinnacles are pairs of 8-15, green; 2 stipular spines strongly recurved with a 3rd pseudo-stipular between them. Flowers yellowish-white and fragrant, in cylindrical, axillary pedunculate spikes, 5-10 cm long; calyx of each flower has 5 deep lobes, 5 petals. The pods are straight, thin, flat; seeds 3-6, smooth, flat, rather small, shiny, dark brown**".** The Egyptian materials for a glue adhesion and painting evidence a long history use of Acacia gum. A wide variety of illnesses were treated with the gum by Arabic physicians [65]. Currently, Acacia gum is used in food and pharmaceutical industry as a coating, stabilize emulsions and demulcent agents. Acacia gum consists of monosaccharides, acids, calcium, electrolytes and peroxides enzyme [19]. The main constituents are calcium salt called arabin which is the polysaccharide arabic acid [58]. A complex structure of Acacia gum has been fully explained. It is an arabinogalactan-type polysaccharide. The international standardization of Acacia gum was performed by NMR spectra [66]. The structure of Acacia gum indicates the range of its size around 200,000 to 600,000 daltons, consisting of D-glactose, D-glucuronic acid, L-rhamnose, and L-arabinose [19]. This soluble gum does not merge with alcohol but very soluble in water. Acacia gum is a part of dietary fiber; however, the study of hypercholesterolemic patients administered with Acacia gum had shown no effect on plasma lipid level [67, 68]. The *in vitro* dental study in 0.5 – 1% culture medium shown that Acacia gum inhibited the growth of *Porphyromonas gingivalis* and *Prevotella intermedia* [69]. The reduction of antibacterial effectiveness of methyl-*p*-hydroxybenzoate, preservative, against *Psudomonas aeruginosa* by Acacia was performed to protect the action of the preservative from microbial cells [70]. Acacia gum composes of pectinases, oxidases, peroxidases, and cyanogenetic glycosides. An inactive peroxidase enzyme in Acacia gum causes some phenols and amines, which is normally destroyed by heat. This enzyme increases the eradication of alkaloid products and directly combines with oxygen in some vitamins [16, 19].

Another enzyme study of trypsin inhibitor has been testified, but clinical study is not fully explained [71].

Figure 8 *Acacia senegal* tree, flowers and Acacia gum

CHAPTER III

METERIALS

Exudate Gums

1. Kondagogu gum (Panacea Biotec Ltd., India)
2. Karaya gum (Sigma–Aldrich Company Co., St. Louis, MO, USA)
3. Acacia gum (Taian Dingli Gum Industrial Co., Ltd., Shandong, China)

Chemicals and Reagents

1. Cholesterol (Merck, Darmstadt, Germany)
2. Cholesterol Liquicolor (Human Gasellsthaft, MBH, Germany)
3. D-Glucose monohydrate (Sigma–Aldrich Company Co., St. Louis, 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 Gasellsthaft, 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.,31 Thailand)
10. Lipase (EC 3.1.1.3) from porcine pancrease (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. Mushroom tyrosinase (Sigma–Aldrich Company Co., St. Louis, MO, USA)
14. *p*-Nitrophenyl- α-D-glucopyranoside (Sigma–Aldrich Company Co., St. Louis, MO, USA)
15. Phosphorus pentoxide (Sigma–Aldrich Company Co., St. Louis, USA)

16. Pullulan standard P-series (Shodex, Showa Denko KK, Japan)
17. Trimethylchlorosilane (Supelco, Bellefont, PA, USA)
18. All other chemicals were analytical grade.

Materials

1. Dialysis tubing cellulose membrane (molecular weight cut off = 12,000 Da) (Sigma–Aldrich Company Co., St. Louis, MO, USA)
2. OHpak SB-806 M HQ HPLC column (8.0 mm, i.d. x 300 mm length) (Shodex, Showa Denko KK, Japan)
3. PVDF syringe membrane filter (0.45 µm x 13 mm) (VertiPureTM, Vertical Chromatography Co., Ltd., Bangkok, Thailand)
4. PVDF Centrifugal filter (0.45 µm) (Merck Millipore Corporation, Billerica, MA, USA)
5. SGE BPX5 capillary GC column (30 m x 0.25 mm x 0.25 µm) (SGEAnalytical Science Pty Ltd., Victoria, Australia)

Instrumentation and Equipments

1. Falling ball viscometer (HAAKE Mess-Technik GmbH u. Co, Germany)
2. Gas chromatography (GC) Thermo Finnigan model Trace GC Ultra equipped with flame ionization detector (Thermo Fisher Scientific Inc.,USA)
3. High-performance liquid chromatography (HPLC) system consisted of a Hitachi L-600 pump (Hitachi Seisakucho Co., Japan), Rheodyne 7725iloop injector (USA) and an TRD-880 refractive index detector (Shimamura Instruments Co., Japan)
4. UV-1800 Spectrophotometer (Shimadzu Scientific Instruments, USA)
5. Homogenizer (Model DI 18) (Yellow Line (IKA), Germany)
6. Transferpettor (Brand GMBH and CO KG Postfach, 97877 Wertheim, Germany)

CHAPTER IV

METHODOLOGY

Exudate Gums Preparation

A high-speed mechanical blender is used to powder the exudate gums. Gelation of gums was prepared by dispersing gum powder in ultrapure water and mixing by homogenizer.

Effect on Glucose Diffusion

The study of the effect on glucose diffusion was followed by the dialysis tubing method [72]. Various concentrations of polysaccharide gels (0, 0.5, 1, 2% w/v) with 2% glucose at the final concentration were prepared. Prior to use, the dialysis tubing cellulose membrane (molecular weight cut off = 12,000 Da) was rinsed with tap water for several minutes following by pure water. The tubing was filled with 4 milliliters of each concentration and then put in the bottle which contained 60 ml of Ringers buffer for 2 h under rotational shaking at 150 rpm. The Glucose oxidase method was used to determine the released glucose by which glucose oxidase enzyme oxidized the released glucose to yield gluconic acid and hydrogen peroxide. Eventually, hydrogen peroxide further reacted with 4-aminophenazone and phenol in the presence of peroxidase to form quinoneimine chromophore with a λ_{max} at 510 nm. In this study, the procedure was performed using Glucose Liquicolor kit according to manufacturer's instructions with some modification.

Briefly, ten microliters of the sample (tested buffer and standard glucose solution) was added to 1 ml of the reagent, mixed and incubated at room temperature for 15 min. The absorbance of the standard glucose solution and the sample was measured against the reagent blank at 510 nm. The assay was done in triplicate.

Effect on α-Glucosidase Inhibition

Various concentrations of polysaccharide gels (0.06, 0.13, 0.25, 0.50, 1 %w/v) were incubated with 10 µl of α-glucosidase (1 U/ml) at 37ºC for 10 min, then 20 µl of the substrate, 1 mM p-nitrophenyl- α -D-glucopyranoside in 0.1M sodium phosphate

buffer at pH 6.9 was added to start the reaction and furture incubated at 37ºC for 20 min. The reaction was terminated by adding 50 µl of 1M sodium carbonate [73]. The appearance of p-nitrophenol from enzymatic reaction was measured at 450 nm. The positive control was 1-Deoxynojirimycin ranging from 100 - 500 µg/ml. The calculation of percent inhibition was prepared by this equation:

$$\text{Inhibition (\%)} = (1 - B/A) \times 100$$

A = the absorbance of reactive mixture without sample or positive control

B = the absorbance of reactive mixture with test sample or positive control

The half maximal inhibition concentration or IC50 was determined from the concentration inhibition curve using Sigmaplot software.

Effect on Cholesterol Solubility in Single Bile Micelles

Matsuoka's method was adjusted to this study [74]. The test-tubes contained 10 mg of solid cholesterol (excess for saturation) and 15 mg of the exudate gums (equal to 0.5% w/v) were filled with 3 ml of 25 mM sodium taurodeoxycholate (NaTDC) in 15 mM phosphate buffer saline (pH 7.5). The mixtures were stirred by magnetic stirrer for 24 h (to reach the equilibrium system). Polyethersulfone syringe membrane filter (0.22 µm x 33 mm) was used for the separation of soluble micelles afterward. The Cholesterol oxidase method was used to determine the cholesterol concentration in the filtrates. The cholesterol was oxidized 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 λ_{max} at 510 nm. The procedure was performed using Cholesterol Liquicolor kit according to manufacturer's instructions with some modification.

Briefly, ten microliters of the filtrate was added to 1 ml of the reagent, mixed and incubated at room temperature for 15 min. The Measurement of the absorbance of the standard cholesterol and the sample was performed against the reagent blank at 510 nm. The inhibitory effect on cholesterol solubility was shown as % reduction of cholesterol concentration in the filtrate with and without the exudate gum.

Effect on Cholesterol Solubility in Pig's Bile Micelles

Ten milligrams of solid cholesterol and 15 mg of the exudate gums were added into a test- tube containing 3 ml of Pig's bile and the experiments were performed as described above [74].

Effect on Lipase Activity

The study on lipolytic activity was adapted from previous reports [43, 75]. The emulsion was prepared by adding 5 mM sodium acetate buffer (pH 5.0) into 2% Triton X-100, and then added 0.08 % w/v of *p*-Nitrophenyl laurate. This *p*-Nitrophenyl laurate emulsion must be heated for dissolution by boiling in water baht (5 min), cooled down at 37 ºC and then mixed well before use. Ten milligrams of porcine pancreatic lipase was dissolved in 1 ml of water. The pancreatic lipase solution was centrifuged at 12,000 rpm for 5 min. The positive inhibitor was Orlistat in 1% DMSO. The assay mixture was composed of 50 µl of tested substance (Orlistat or polysaccharide gels at 0, 0.12, 0.25, 0.5 and 1.0 % w/v), 150 µl of pancreatic lipase and 400 µl of 0.1 M Tris buffer (pH 8.2), incubating in water bath at 37°C for 5 min, then start the reaction by adding 450 µl of p-Nitrophenyl laurate emulsion. The final mixture was incubated at 37°C for 2h and filtered through 0.45 µm PVDF filter. The activity of lypolysis yields *p*-nitrophenol that can measure at 410 nm by spectrophotometer. All test samples in this study were assayed in triplicate. The effect on lipase activity was shown as the percentage ratio of the absorbance at 410 nm between the filtrate with and without tested substance.

Tyrosinase Inhibitory Activity

The determination of tyrosinase inhibitory activity was performed using dopachrome method [76]. The kinetic assay of enzyme activity was used instead of the end-point assay. The enzyme solution was prepared by adding 0.5 mg of mushroom tyrosinase enzyme in 5 ml of 15 mM phosphate buffer (pH 7.3). The preparation of 0.85 mM L-DOPA substrate was done by adding 0.8 mg of L-DOPA in 5 ml of 15 mM phosphate buffer (pH 7.3). Twenty microliters of each polysaccharide gel (0.25, 0.5, 1% w/v) were added into 40 µl of the enzyme solution and 140 µl of 15

mM phosphate buffer (pH7.3). The reaction was started by adding 20 µl of 0.85 mM L-DOPA in the mixture and the absorbance was monitored at 405 nm for 10 min at the room temperature. The positive control was 0.1 M ascorbic acid [77]. The initial rate of absorbance change was calculated by MikroWin 2000, version 4. The percent inhibitory activity was calculated by the following equation:

$$\text{Tyrosinase inhibition (\%)} = (1 - B/A) \times 100$$

A = the absorbance change of reactive mixture without sample or positive control

B = the absorbance change of reactive mixture with test sample or positive control

DPPH Radical-Scavenging Activity

The stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) is used for a simple method that has been developed to determine the antioxidant activity of natural products including polysaccharide gels [78, 79]. Added 0.5 ml of different concentrations of exudate gums to 1.5 ml of a 0.004% methanolic solution of DPPH in cuvette. The mixtures were incubated under the room temperature without light. The absorbance at 517 nm was determined after 30 min. The percent scavenging activity calculated by the following equation:

$$\text{Scavenging effect (\%)} = (1 - B/A) \times 100$$

A = the absorbance of reactive mixture without sample or positive control

B = the absorbance of reactive mixture with test sample or positive control

The IC50 for scavenging activity was determined from the concentration inhibition curve using Sigmaplot software.

Monosaccharides Analysis

The powder of exudate gum samples (1 mg) were subjected to hydrolysis with 4 M methanolic HCl at 80°C for 24 h in acid-washed vial. The hydrolyzed samples were dried under nitrogen, then methanol was added and the samples were dried

again. This washing was repeated twice. 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. 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 initiated at 140°C, then ramped at the rate 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 [80].

Estimation of the Average Molecular Weights

Ultrapure water was used to prepare the sample solutions, making 0.1% (1 mg/ml) sample concentrations. The sample solutions were filtered through 0.45 μm PVDF centrifugal filters. Size exclusive chromatography using HPLC with refractive index detector (RID) at 20 ºC was used to estimate the average molecular weights of the soluble gum polysaccharides [64]. OHpak SB-806 M HQ HPLC column with a mobile phase of ultrapure water eluted at a flow rate of 0.5 ml/min was used to perform the separations [81]. The injection volume of the samples was 10 μl. Showa Denko's standard set of Pullulans was prepared in ultrapure water and filtered through 0.45 μm PVDF. The retention time of standard pollulans were plotted against their average molecular weights and used as a calibration curve for estimation of the average molecular weights of tested polysaccharide gels.

Total Protein Analysis

The estimation of protein content in the polysaccharide samples was determined by folin reaction, according to the Lowry assay [82]. Various concentrations of Bovine serum albumin (0-100 μg/ml) were prepared as the protein standard. The polysaccharide sample (1 mg) was prepared in 0.5 ml of ultrapure water, then sample solution was mixed to 0.7 ml of Biuret reagent, and incubated for 20 min at the room temperature. Later, 0.1 ml of folin-ciocalteu in ultrapure water (1:1.2) was added in the mixed-well sample solutions and incubated at room

temperature for 30 min. The standard and sample solutions were measured at 750 nm against the reagent blank solution.

Viscosity

The viscosity of the polysaccharide gums was measured by a falling ball viscometer at room temperature [43]. Five grams of the polysaccharide gums were prepared in 250 ml of water to make the viscous sample solutions. The sample solutions were filled into the boron silica glass inclined cylindrical measuring tube with known inner diameter (approximately 15.94 ± 0.01 mm), and then placed the standard ball with known radius and density to pass the sample solutions through the cylindrical measuring tube. A set of standard ball was screened. Ball number 2 was used for the viscosity determination of Karaya and Acacia gums, while ball number 5 was used for Kondagogu gum (Table1). The falling time of the standard ball in the defined distance was recorded to calculate the viscosity, using the following equation.

$$\text{Viscosity (in mPa.s)} = K\,(\rho 1 - \rho 2) \times t$$

K = ball constant (mPa.s.cm^3/g.s)

ρ1, ρ2 = density of the ball and sample solution (g/cm^3) respectively

t = falling time of the ball in seconds.

Table 1 A set of standard ball contains six balls.

Ball No.	**Made of**	**Density, ρ (g/cm^3)**	**Diameter of the ball (mm)**	**Constant, K (mPa.s.cm^3/g.s)**
1	boron silica glass	2.2	15.81±0.01	0.0007
2	boron silica glass	2.2	15.6±0.05	0.05
3	nickel iron alloy	8.1	15.6±0.05	0.09
4	nickel iron alloy	8.1	15.2±0.1	0.7
5	W.-No. 4034	7.7 – 8.1	14.0±0.5	4.5
6	W.-No. 4034	7.7 – 8.1	11.0±1	33

CHAPTER V

RESULTS

Effect on Glucose Diffusion

Figure 9 illustrates percentage of released glucose from the concentration of 0, 0.5, 1, 2 % w/v of polysaccharide gels. The entrapment ability of polysaccharide gel was demonstrated as decreasing in glucose diffusion from dialysis tubing into the external solution. Kondagogu gum at 2% gel showed 60.8 ± 0.2% of glucose releasing compared to 95.8 ± 0.2% and 93.5 ± 0.1% by 2% Karaya and 2% Acacia gels respectively.

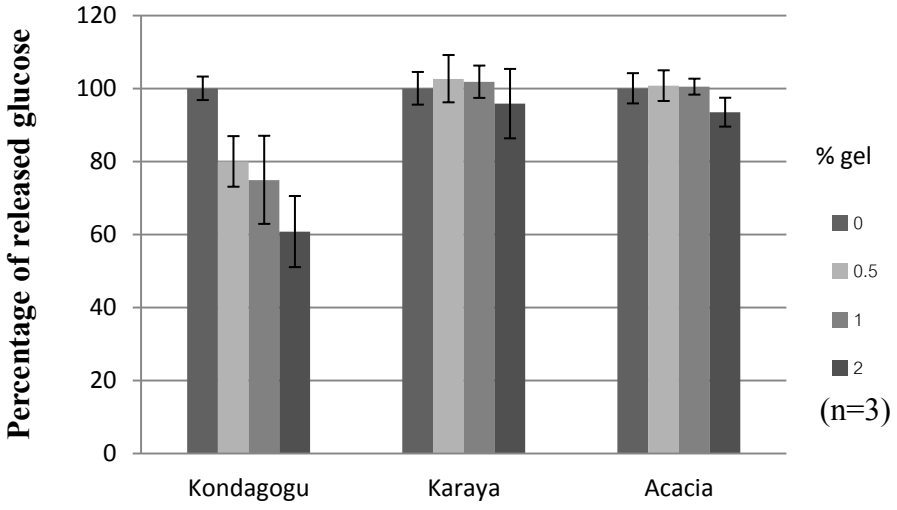


Figure 9 Percentages of glucose releasing from 0, 0.5, 1, 2% polysaccharide gels containing 2% glucose after 2 hr dialysis (n = 3).

Effect on α-Glucosidase Inhibition

The exudate gums in the form of polysaccharide gels at 0, 0.5, 1, 2% w/v had no effect (0% inhibition) on α-Glucosidase inhibition, while the half maximal inhibitory concentration (IC_{50}) of a positive control, 1-Deoxynorjirimycin was 124.3 µg/ml.

Effect on Cholesterol Solubility in Single Bile and Pig's Bile Micelles

The extent of cholesterol solubility in the bile acid micelles was determined when 0.5% of polysaccharide gums was mixed in cholesterol-bile acid system, compared to bile acids alone. In single bile micelles system, Kondagogu and Acacia gums showed the slightly inhibition (16 ± 0.04% and 23 ± 0.04%), while Karaya gum had no effect on cholesterol solubility. For pig's bile micelles system, Kondagogu and Acacia gums had no effect on cholesterol solubility, while Karaya gum showed a slightly activation (3 ± 0.06%).

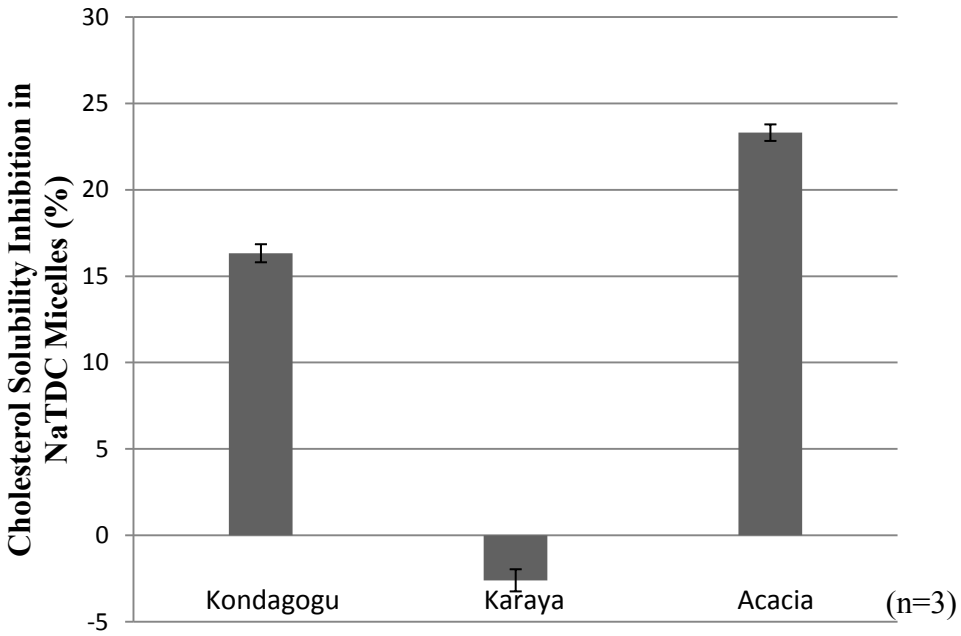


Figure 10 The inhibitory effect in percentage on micellar solubility of cholesterol using NaTDC system (n=3).

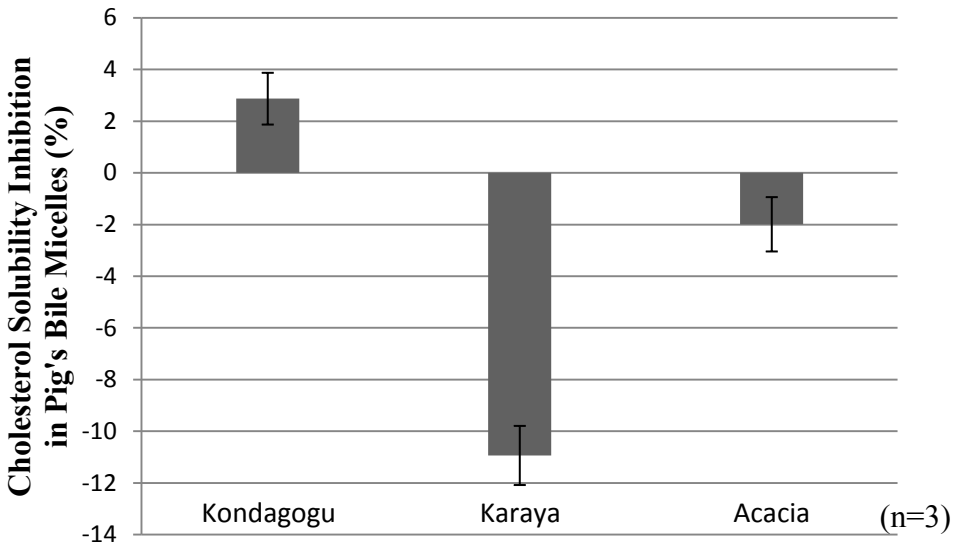


Figure 11 The inhibitory effect in percentage on micellar solubility of cholesterol using pig's bile system (n=3).

Effect on Lipase Activity

At 0.25% gel, Kondagogu and Acacia gums had 16.2 ± 4.9% and 20.4 ± 1.1% inhibitory effect on lipase activity respectively. The reciprocal relationship between the inhibitory effect and gel concentration was shown. Karaya expressed lipase activation at higher concentration. Surprisingly, at 0.13% gel, kondagogu enhanced lipase activity as well (Figure 12). Oristat was tested as a positive control and shown the IC50 at 2.4 µg/ml (0.24 % w/v).

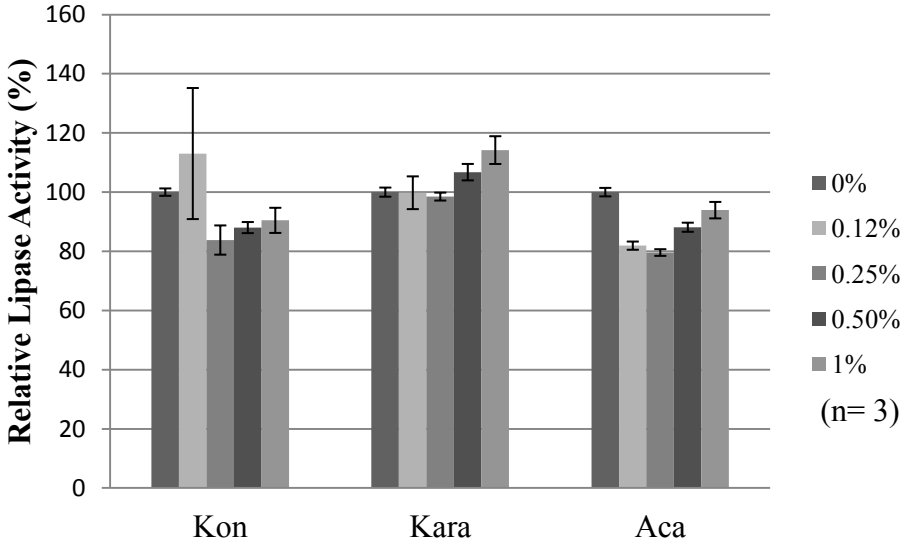


Figure 12 The lipase activity as percentage relative to gel 0

Tyrosinase Inhibitory Activity

The selected polysaccharide gels had no promising tyrosinase inhibitory activity. Kondagogu at 1 % w/v exhibited slightly (6%) inhibitory activity, on the contrary; most of them demonstrated tyrosinase enhancing activity. The positive control, 0.1M Vitamin C showed 94 % inhibition against tyrosinase.

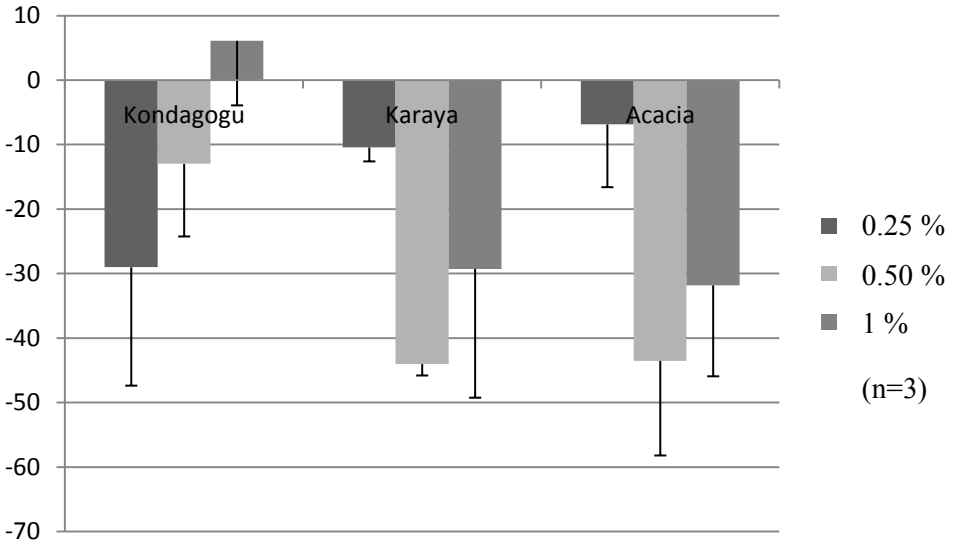


Figure 13 Percentage of tyrosinase inhibitory activity of 0.25, 0.5, 1% w/v of polysaccharide from exudate gums compared to gel 0 (n=3)

DPPH Radical-Scavenging Activity

Figure 14 showed the percentage of the scavenging effect from various concentrations (0.25, 0.50, 1 % w/v) of polysaccharide gels (n=3). Butylated hydroxytoluene was used as a positive control in this experiment. Kondagogu and Karaya gums at 1% gel (w/v) expressed 11.6 ± 5.5% and 20.2 ± 6.4% scavenging effect against DPPH respectively. Whereas 1% gel (w/v) Acacia gum had slightly effect on scavenging activity. Butylated hydroxytoluene, a positive control showed more than 80 % at the same concentration.

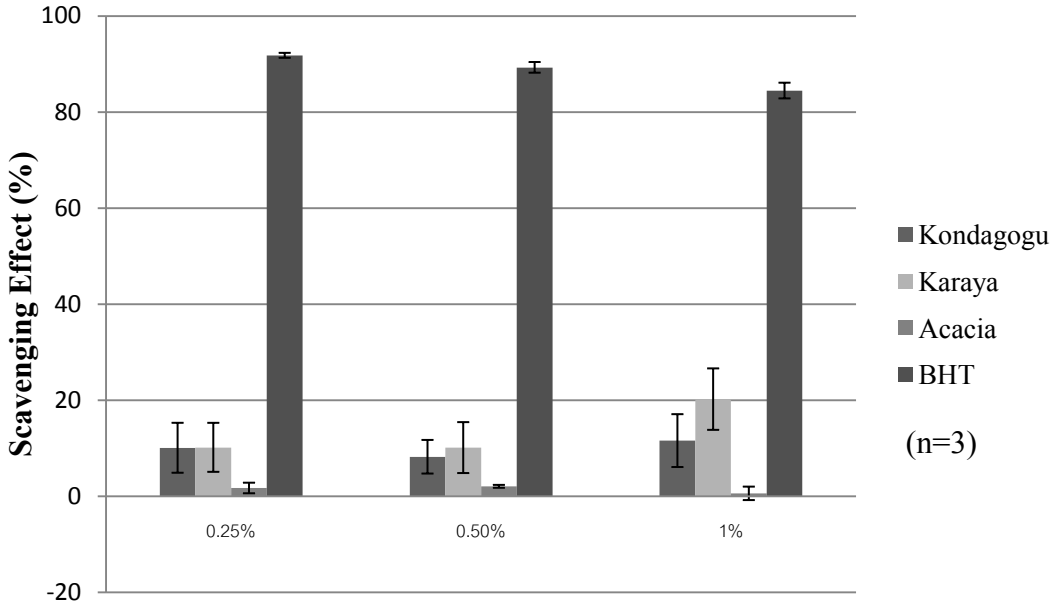


Figure 14 Percentage of scavenging effect of 0.25, 0.5, 1% gels containing 0.004% methanolic solution of DPPH after incubation for 30 min. (n=3).

Monosaccharides and Total Protein Analysis

The exudate gums were analyzed for monosaccharide constituents using the gas chromatographic technique. Qualitative and quantitative determinations were performed using external standard method. A set of monosaccharide standard included Manitol, Galacturonic acid, Glucuronic acid, Fucose, Rhamnose, Arabinose, Xylose, Mannose, Galactose, Glucose, Fructose and Ribose. Rhamnose and galactose were found in three kinds of the studied gums. Galacturonic acid was found in Kondagogu and Karaya gums, while Glucuronic acid was found in Karaya and Acacia gums. Moreover, Arabinose was found only in Acacia gum. The protein content in polysaccharide samples was determined by folin reaction of Lowry assay, comparing to various concentrations of protein standard.

Table 2 The amount of monosaccharide and total protein (TP) contents (μg/mg) in selected exudate gums.

	Rha	**Ara**	**Gal**	**GalA**	**GlcA**	**TP**
Kondagogu	171.141	-	146.378	172.353	-	2.45
Karaya	89.051	-	146.723	91.262	359.790	9.48
Acacia	106.304	214.197	425.735	-	225.398	4.68

Estimation of the Average Molecular Weights

Molecular weight (MW) plays an essential role as the important property in many polymeric materials. The estimation of average molecular weights was assayed by size exclusive chromatography using HPLC with refractive index detector. The separation was performed by OHpak SB-806 M HQ HPLC column. Showa Denko's standard set of Pullulans was prepared for a calibration curve to estimate the average molecular weights (Figure 20). Table 3 presented the molecular sizes of three selected gums.

Table 3 Average molecular weights (Da) among selected exudate gums.

Polysaccharide gels	MW (Da)*
Kondagogu gum	9,639,622
Karaya gum	11,140,380
Acacia gum	4,350,507

*Pullulans equivalent molecular weight.

Viscosity

The intrinsic viscosity of exudate gums in the form of polysaccharide gels is a natural property of many soluble dietary fiber foods. A falling ball viscometer is a simple instrument for measuring the viscosity of sticky viscous solution. The viscosity of polysaccharide gels was required the time that the standard ball passed through the sample solution in cylindrical tube with measuring scale. At the applicable concentration of polysaccharide gels (2% w/v), Kondagogu gum showed the highest viscosity (574 cP) when mixed with water, resulting in the paste texture.

Table 4 The viscosity among exudate gums in the form of polysaccharide gels

Polysaccharide gels (2% w/v)	Viscosity (cP)
Kondagogu gum	574.08 ± 0.01
Karaya gum	25.02 ± 0.01
Acacia gum	1.10 ± 0.01

CHAPTER VI

DISCUSSION

Effect on Glucose Diffusion

The ability to retard the absorption and slow the digestion (hydrolysis) of nutrients in the intestinal lumen by dietary fibers have been proved in many viscous polysaccharide plants, resulting in lowering postprandial glucose concentration in the blood circulation [37, 40]. This mechanism is a strategy to prevent many acute and chronic diseases related to hyperglycemia, such as diabetes mellitus, endothelia dysfunction, heart diseases and complications. The reciprocating dialysis tubing method is a simple technique to estimate the intrinsic latency of soluble dietary fiber for the aqueous medium entrapment ability [83], especially for glucose entrapment. The dialysis tubing method mimics the *in vivo* events that occur in the intestinal jejunal lumen [84, 85]. This model reveals the potency of viscous fiber to entrap glucose before it diffuses across the intestinal lumen into the blood steam by measuring the amount of releasing glucose in the external solution. Additionally, numerous studies of viscous dietary fibers showed the ability to reduce glucose concentration in the blood circulation [37, 40]. These reports encouraged the *in vitro* study of glucose entrapment among selected viscous polysaccharide gums. The effect on glucose diffusion of Kondagogu, Karaya and Acacia gums, resulting from dialysis tubing model showed the percentage of released glucose in the concentration dependence (0, 0.5, 1, 2 % w/v of polysaccharide gels). At the same concentration (2% w/v), Kondagogu (*Cochlospermum gossypium*) showed the highest ability to decrease glucose diffusion, comparing to Acacia (*Acacia Senegal*) and Karaya (*Sterculia urens*) from dialysis tubing into the external solution (60.8 ± 0.2, 95.8 ± 0.2, 93.5 ± 0.1% respectively). This study advantaged many health benefits by prevention and reduction the risk for excess glucose concentration in the circulation from relative diseases and its complications.

Effect on α-Glucosidase Inhibition

Alpha-Glucosidase enzyme breaks down carbohydrates (starch, disaccharides) into a single alpha-glucose molecule (monosaccharide). The name of this enzyme obtained from the hydrolyzing action upon the terminal non-reducing 1, 4 linked alpha bonds. Alpha-glucosidase inhibitor is one of various oral anti-diabetic drugs, especially for *non-insulin-dependent* diabetes mellitus or diabetes mellitus type 2. The mechanism of alpha-glucosidase inhibitor (competitive inhibitor) is preventing the digestion of carbohydrates, leading to less alpha-glucose absorption into the brush border of the small intestines. This action causes the reduction of postprandial glucose level in the circulation. Furthermore, previous studies revealed a large number of natural alpha-glucosidase inhibitor from plants [43]. Therefore, the effect on α-glucosidase inhibition is an interested topic to explore from selected exudate gums. Even there are numerous studies of viscous dietary fibers effect on reducing blood glucose level; however, the *in vitro* study of dietary fiber gums Kondagogu (*Cochlospermum gossypium*), Acacia (*Acacia Senegal*) and Karaya (*Sterculia urens*) in the form of polysaccharide gels had no effect on α-Glucosidase Inhibition, while the half maximal inhibitory concentration (IC_{50}) of a positive control, 1-Deoxynorjirimycin was 124.3 µg/ml. This study evidenced that not all soluble dietary fibers can be the α-glucosidase inhibitor.

Effect on Lipids Solubility in Single Bile and Pig's Bile Micelles

Sodium taurodeoxycholate (NaTDC) has been reported for its stability in cholesterol-bile salt micelles formation [86, 87, 88]. In this study, the ability to inhibit cholesterol absorption of selected exudate gums was performed by the competitive mechanism with cholesterol for solubilization in micelles (single bile and pig's bile micelles) [43]. After 12 h incubation period for the experiment between 0.5% w/v polysaccharide gels with single bile micelles (NaTDC), Kondagogu gum (*Cochlospermum gossypium*) and Acacia gum (*Acacia Senegal*) had the slightly inhibition (16% and 23%), while Karaya gum (*Sterculia urens*) had no effect to cholesterol solubility. The next experiment was done as the previous assay, but used pig's bile micelles instead of NaTDC to mimic the test emulsifier as the real bile in

the animal. The result showed no effect to cholesterol solubility from Karaya gum (*Sterculia urens*) and Acacia gum (*Acacia Senegal*), while Kondagogu gum (*Cochlospermum gossypium*) showed a very slightly inhibition (3%).

Effect on Lipase Activity

In this study, *p*-nitrophenyl acyl ester of lauric acid was used as a model substrate in the lipase activity, yielding *p*-nitrophenol for spectrophotometric determination. Kondagogu gum, Acacia gum expressed 16 ± 4.92% and 20 ± 1.12% of the effect on lipase activity while Karaya gum (*Sterculia urens*) had barely inhibitory effect (1.5 ± 1.34%). The half maximal inhibitory concentration (IC_{50}) of Oristat, the lipase inhibitor was 2.4 μg/ml, which has a different IC_{50} value comparing to previous report (IC_{50} = 8.4 μg/ml) [43]. The difference IC_{50} values of Oristat might cause from the different in lipases, substrates and the assay conditions. Pancreatic lipase is a class of interfacial enzymes that interact only with lipid-water interfaces such as emulsions, micelles or bilayers [ref]. Surface active property of polysaccharide gel may play a role on compartmentalization between two phases that affected lipolytic activity. [49].

Tyrosinase Inhibitory Activity

In this study, L-DOPA was used as the substrates to form dopachrome. This intermediate substance (dopachrome) in the melanin production [44] can be detected by spectrophotometer. The log period of dopachrome formation showed the slope of each test sample leading to the calculation of tyrosinase inhibitory activity. This concept can be used as the process to find out the treatment for skin diseases related to melanin disorder. Ascorbic acid (Vitamin C) was used as a positive control, showing 94% inhibition at 0.1 M [77]. Kondagogu at 1% w/v slightly inhibited activity while low concentration of Kondagogu as well as all concentration of Karaya and Acacia activated tyrosinase. Therefore, this study revealed the effect of selected polysaccharides on either inhibition or activation of tyrosinase enzyme. The results are interested for further investigation of the whitening potential of Kondagogu gum as well as the skin coloring (re-pigmentation) potential of Karaya and Acacia gums.

DPPH Radical-Scavenging Activity

In this study, DPPH radical-scavenging was performed to determine the ability of polysaccharide gums as antioxidant agents. DPPH radical-scavenging activity is the most widely reported antioxidant method. This simple method is based on the discoloration of DPPH methanolic solution by free radical scavengers at 517 nm. The mixture between 500 µl of various sample concentrations and 1.5 ml of 0.004% methanolic solution of DPPH was incubated at the room temperature for 30 min. The determination of methanolic DPPH discoloration was performed by spectrophotometer. At the same concentration 1% gel w/v, Karaya had the highest scavenging effect (20 ± 6.4%), while Kondagogu and Acacia gums had slightly effect on scavenging activity (10 ± 5.5, 1 ± 1.4% respectively). Thus, three polysaccharide gums in the form of gel mixture had slightly effect on scavenging activity, comparing to Butylated hydroxytoluene (positive control). Even the exudate gums have slightly antioxidant activity; however, the water holding property of these polysaccharide gums may trap the carcinogens in the alimentary bowel.

Monosaccharides and Total Protein Analysis

Table 5 showed the previous studies using various methods to analyze monosaccharide constituents in Kondagogu, Karaya and Acacia gums. In this assay, the exudate gums were analyzed to find monosaccharide constituents by gas chromatography technique. One milligram of the each exudate gum in the experiment revealed different types of monosaccharides. Acacia gum from *Acacia senegal* tree is composed of four different monosaccharides including rhamnose, arabinose, glucuronic acid and galactose (10.6, 21.4, 22.5, 42.6% by weight respectively). Karaya gum from *Sterculia urens* tree composed of four different monosaccharides including rhamnose, glacturonic acid, galactose and glucuronic acid (8.9, 9.1, 14.7, 36% by weight respectively). Kondagogu gum from *Cochlospermum gossypium* tree composed of three monosaccharides including galactose, rhamnose and galacturonic acid (14.6, 17.1, 17.3% by weight respectively). The differences in types and amount of monosaccharides between this study and the literature studies may due to the extraction process, the obtained origin, methodology and grades of the exudate gums.

The total protein content was analyzed from Kondagogu, Karaya and Acacia gums in this study resulting in 0.2, 0.9 and 0.5% by weight respectively.

Table 5 Monosaccharide constituents among selected exudate gums from literatures

Polysaccharide gum	**Monosaccharide constituents % (w/w)**					
	Rha	Ara	Gal	GlcA	GalA	Glu
Acacia gum (*Acacia senegal*) [89]	16	28	39	18	-	-
Karaya gum (Sterculia urens) [90]	31	0.1	48	1	20	-
Kondagogu (Cochlospermum gossypium) [52]	20	1	17	37	27	-

Estimation of the Average Molecular Weights

Molecular weight is an important physical property in many polymeric materials. The sizes of molecules are responded to their structure and composition. Many parameters such as the type of linkage, the kind of monosaccharide and the intrinsic viscosity also predictably indicated the size of molecular weights. Table 6 showed the average molecular weights estimation of Karaya (*Sterculia urens*), Acacia (*Acacia senegal*) and Kondagogu gums (*Cochlospermum gossypium*). In this study, the average molecular weights of three selected polysaccharide gums were estimated by the size exclusive chromatography method with refractive index detection. The separation process was performed on OHpak SB-806 M HQ HPLC column with some modification by using ultrapure water instead of ammonium bicarbonate. Showa Denko's standard set of Pullulans was prepared for a calibration standard to estimate the average molecular weights. The difference values of the average molecular weights from the literatures may due to the extraction process, the analysis method and the origin of exudate gums.

Table 6 The average molecular weight of selected exudate gums

Polysaccharides	Molecular weight (Da)
Karaya gum (*Sterculia urens*)	460,000 [91] 9,500,000 [92]
Acacia gum (*Acacia senegal*)	600,000 [93] 650,000 [94]
Kondagogu (*Cochlospermum gossypium*)	7,230,000 [53]

Viscosity

Viscous polysaccharides form the gel matrix and dilute the content in gastrointestinal lumen. This property advantaged many health benefits by reducing the digestion and absorption of nutrients and trapped some carcinogens inside intestinal lumen [15]. High consumption of viscous dietary fiber has been proved as beneficial diets for preventing of many undesirable diseases based on the scientific reports [35-40]. Viscosity is the physiochemical property related to soluble polysaccharide fibers. The relationship between the flow of a fluid and the direct force on a fluid was firstly established by Sir Isaac Newton [95]. In this study falling ball viscometer was used to measure the intrinsic viscosity at 2% (w/v) of Kondagogu, Karaya, Acacia gums showing 574.08, 25.02 and 1.10 cP respectively. The limitation of this study may due to the degree of gel matrix concentration, thus the highest concentration that the polysaccharide gels can be measured by viscometer is 2% (w/v).

CONCLUSION AND RECOMMENDATION

Cochlospermum gossypium De Candole, *Sterculia urens* Roxb. and *Acacia senegal* (Linn.) Willd. are the economic trees in India and some African countries. Kondagogu, Karaya and Acacia are exudate gums obtained from these plants. The physical properties of these gums are attractive to pharmaceutical and food industries as the stabilizing, suspending, gelling, emulsifying, thickening, binding and coating agents. The studied gums from this *in vitro* study showed high intrinsic viscosity which are related to their macrostructure (high molecular weight). The main constituents of exudate gums are the combination of various monosaccharides. Additionally, the exudate gums are concerned as a dietary soluble fiber because of their viscous and gelling properties. This reason makes the exudate gums as an interesting topic to find out their biological activities for therapeutic effects such as the abilities to reduce and retard the gastrointestinal digestion and absorption. The selected exudate gums are revealed their selectively marginal effects on glucose entrapment ability, cholesterol solubility, DPPH scavenging activity, α-glucosidase, pancreatic lipase and tyrosinase activities.

This study provided the basic knowledge of chemical constituents and physical characteristics as well as bioactive properties crude exudate gums. The *in vitro* models in this research are simple, economical and useful to explore viscous polysaccharide potential in herbal medicine research.

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APPENDICES

APPENDIX A

Data of *In Vitro* Biological Activities

Table 7 Concentration of glucose releasing through dialysis tubing (% v/v)

Gel concentration (% w/v)	Reseaing Glucose (%v/v)		
	Kondagogu	**Karaya**	**Acacia**
0	1.76 ± 0.05	1.67 ± 0.07	1.71 ± 0.07
0.5	1.41 ± 0.12	1.71 ± 0.10	1.72 ± 0.07
1.0	1.32 ± 0.21	1.70 ± 0.07	1.71 ± 0.03
2.0	1.07 ± 0.17	1.60 ± 0.15	1.59 ± 0.06

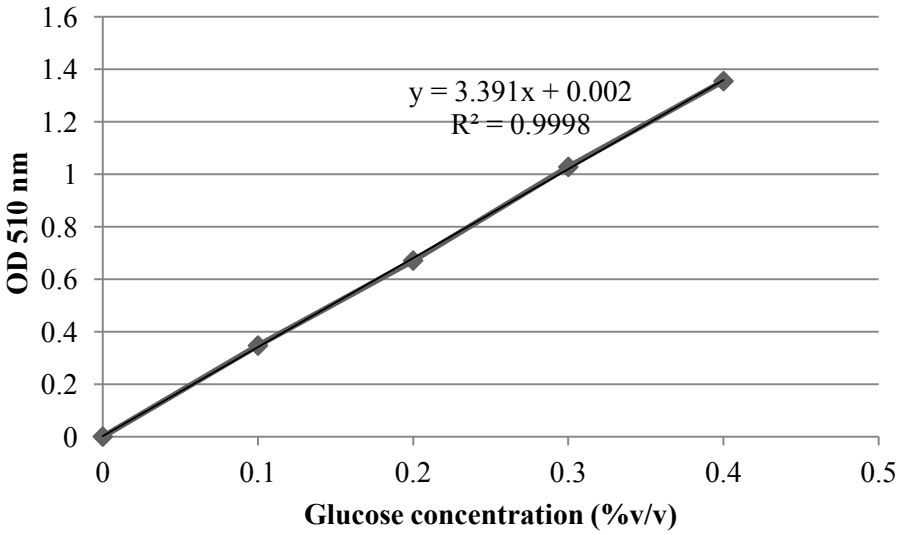

Figure 15 Calibration curve of glucose

Table 8 The absorbance at 450 nm which equivalent to *p*-nitrophenyl- α -D-glucopyranoside - α-Glucosidase catalysis under various concentration of 1-Deoxynorjirimycin

1- Deoxynorjirimycin concentration (µg/ml)	**OD 450**
0	0.57
100	0.31
500	0.14
750	0.16
1000	0.11

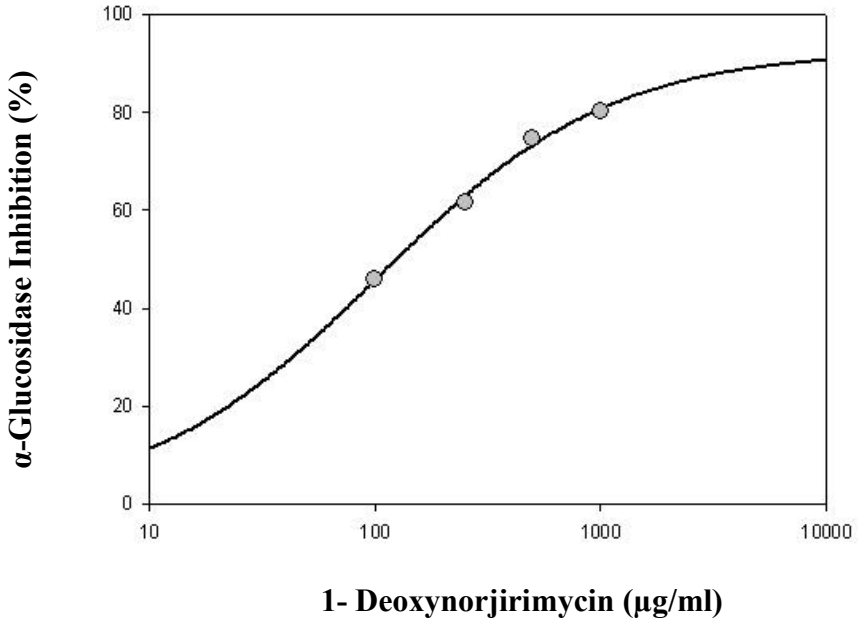

Figure 16 Percentage of α-Glucosidase Inhibition by 1- Deoxynorjirimycin, a positive control showed the half maximal inhibitory concentration (IC_{50} = 124.3 µg/ml).

Table 9 Concentration of cholesterol soluble in bile micelle (%w/v) with and without 0.5 % polysaccharide gel

Polysaccharides	Cholesterol in filtrate (% w/v)	
	NaTDC	Pig's bile
-	0.62 ± 0.01	1.03 ± 0.01
Kondakoku	0.52 ± 0.03	1.00 ± 0.04
Karaya	0.63 ± 0.01	1.14 ± 0.05
Acacia	0.47 ± 0.04	1.05 ± 0.01

Table 10 The absorbance at 410 nm which equivalent to *p*-Nitrophenyl laurate - pancreatic lipase catalysis under various concentration of each polysaccharide gel

Gel concentration (% w/v)	OD 410		
	Kondakoku	Karaya	Acacia
0	2.06 ± 0.05	2.09 ± 0.02	2.07 ± 0.01
0.12	2.33 ± 0.45	2.05 ± 0.11	1.93 ± 0.05
0.25	1.72 ± 0.10	2.03 ± 0.02	1.81 ± 0.03
0.50	1.81 ± 0.03	2.20 ± 0.05	1.64 ± 0.02
1.00	1.86 ± 0.08	2.35 ± 0.09	1.68 ± 0.02

Table 11 The absorbance at 410 nm which equivalent to *p*-Nitrophenyl laurate - pancreatic lipase catalysis under various concentration of Orlistat

Orlistat concentration (µg/ml)	OD 410
0	0.97
1.0	0.55
10.0	0.41
50.0	0.37
100.0	0.36

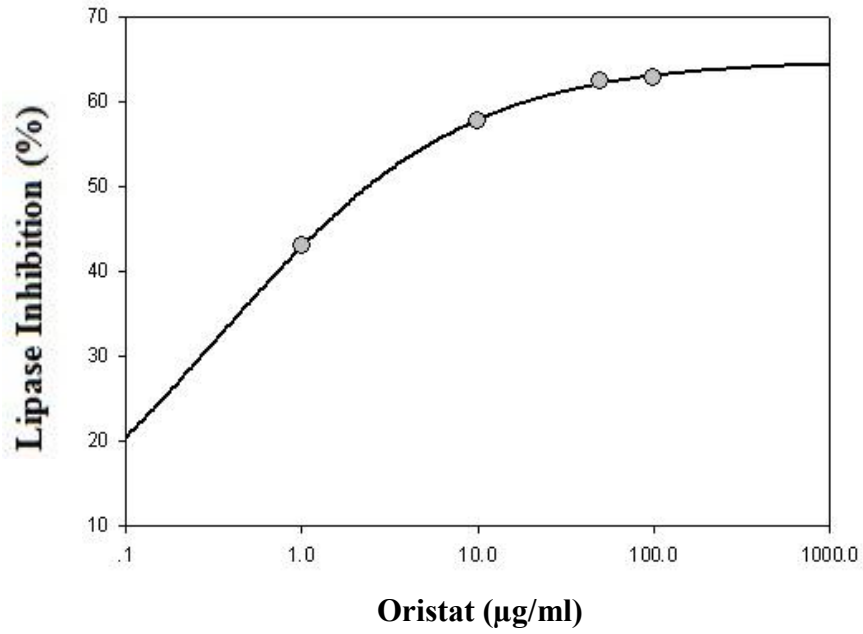

Figure 17 Percentages of lipase inhibition with orlistat, a positive control showed the half maximal inhibitory concentration (IC_{50} = 2.4 µg/ml)

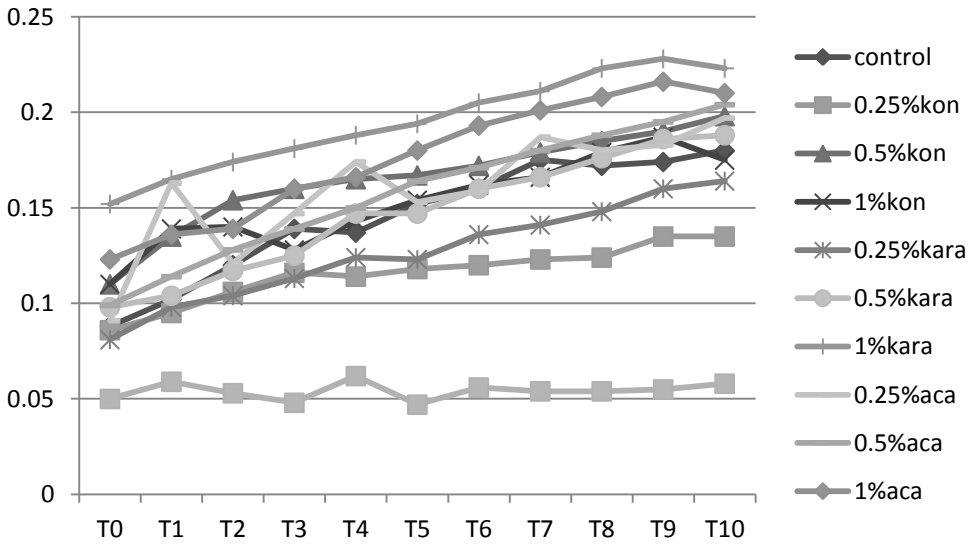

Figure 18 The kinetic activity of tyrosinase enzyme with 1% w/v of polysaccharide gels and 0.1 M vitamin C (Ascorbic acid), a positive control compared to gel 0 (n=3).

Table 12 The absorbance change (slope) at 405 nm which equivalent to L-Dopa - tyrosinase catalysis under various concentration of each polysaccharide gel

Gel concentration (% w/v)	**Δ OD 405**		
	Kondakoku	**Karaya**	**Acacia**
0	0.00655 ± 0.00147[a] (r^2 0.9092)[b]		
0.25	0.00845 ± 0.00173 (r^2 0.9493)	0.00723 ± 0.00074 (r^2 0.9729)	0.00700 ± 0.00066 (r^2 0.8451)
0.5	0.00740 ± 0.00014 (r^2 0.9478)	0.00943 ± 0.00012 (r^2 0.9880)	0.00940 ± 0.00131 (r^2 0.9823)
1.0	0.00507 ± 0.00193 (r^2 0.9008)	0.00847 ± 0.00096 (r^2 0.9658)	0.00863 ± 0.00092 (r^2 0.9750)
0.1 M Vitamin C	0.00040 ± 0.00010 (r^2 0.1698)		

[a] Mean ± SD of the slope of absorbance change per minute, [b] Correlation coefficient

Table 13 The percentage of tyrosinase inhibitory activity among various concentration of each polysaccharide gel

Gel concentration (% w/v)	% Tyrosinase inhibitory activity		
	Kondakoku	Karaya	Acacia
0.25	-29.01 ± 18.35	-10.43 ± 11.25	-6.87 ± 10.01
0.5	-12.98 ± 2.16	-44.02 ± 1.76	-43.51 ± 19.96
1.0	6.11 ± 9.72	-29.26 ± 14.67	-31.81 ± 14.10
0.1 M Vitamin C	93.89 ± 1.53		

[a] Mean ± SD of the slope of absorbance change per minute, [b] Correlation coefficient

Table 14 The absorbance at 517 nm which equivalent to $DPPH^+$ under various concentration of each polysaccharide gel and BHT

Gel concentration (% w/v)	OD 517			
	Kondakoku	Karaya	Acacia	BHT (0.001 M)
0	0.98 ± 0.01	0.94 ± 0.01	0.90 ± 0.02	0.08 ± 0.01
0.25	0.88 ± 0.12	0.85 ± 0.03	0.89 ± 0.01	
0.50	0.89 ± 0.02	0.85 ± 0.08	0.88 ± 0.02	
1.00	0.86 ± 0.01	0.75 ± 0.10	0.90 ± 0.01	

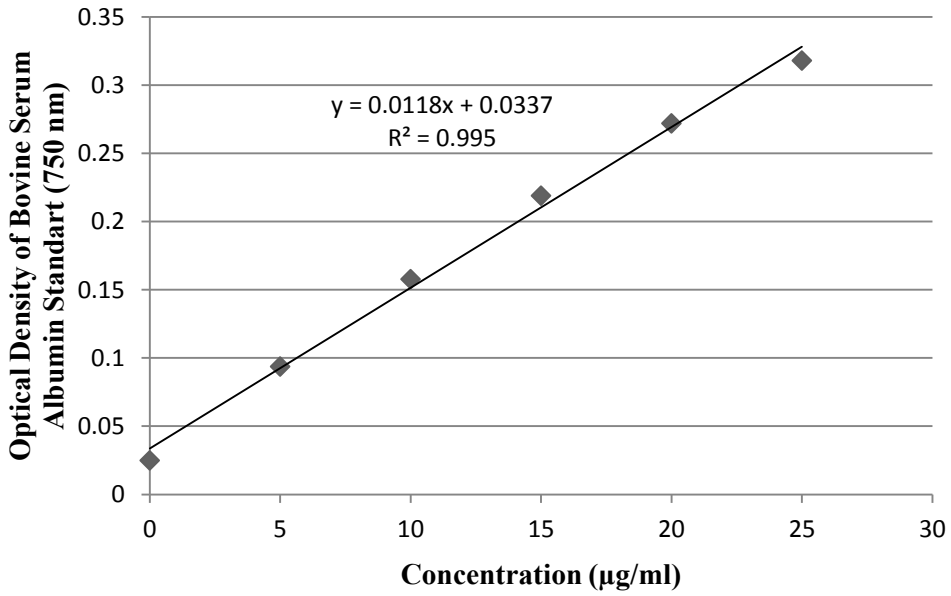

Figure 19 Calibration curve of Bovine serum albumin

Table 15 Falling time of the ball (second) against each polysaccharide gel

Ball No.	Polysaccharide	Density, ρ (g/cm^3)	Falling time, t (sec)
5	Kondagogu	0.98	19
2	Karaya	1.00	420
2	Acacia	1.02	18

APPENDIX B

Data of Physicochemical Determination

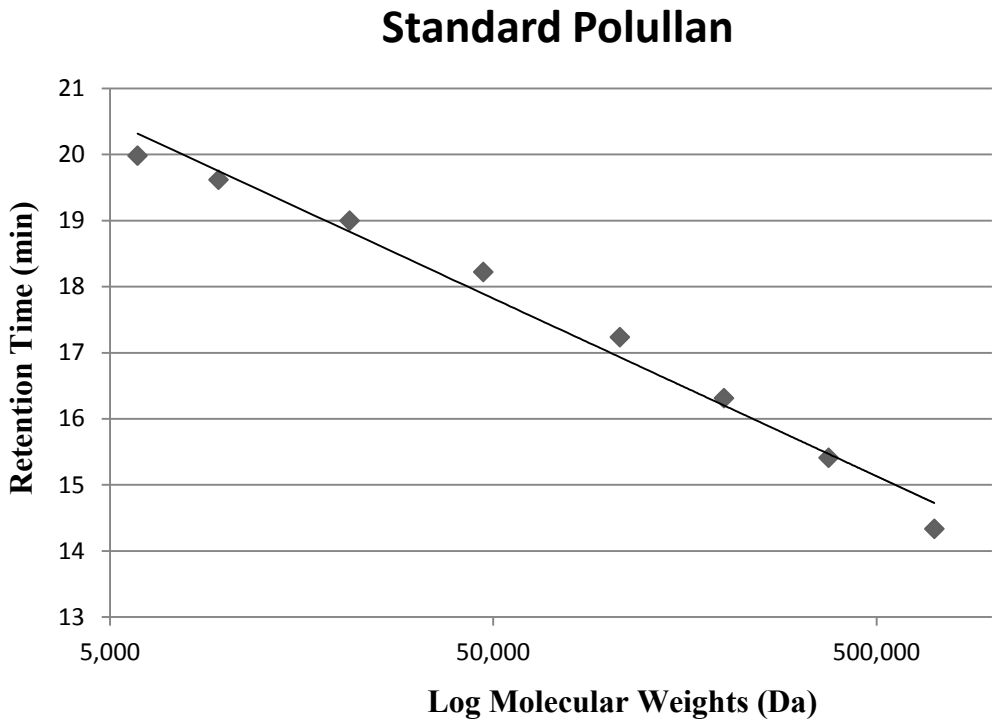

Figure 20 Standard Pullulans as a calibration standard to estimate average molecular weight among selected exudate gums.

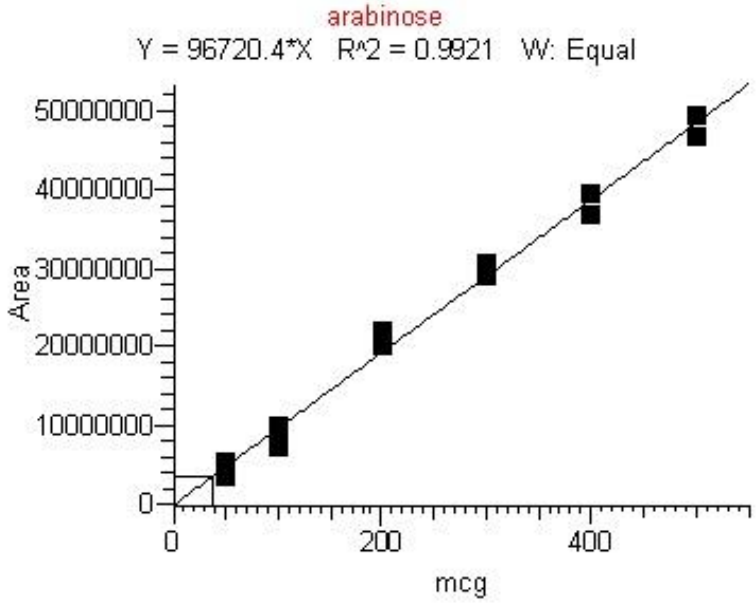

Figure 21 Calibration curve for arabinose determination by GC

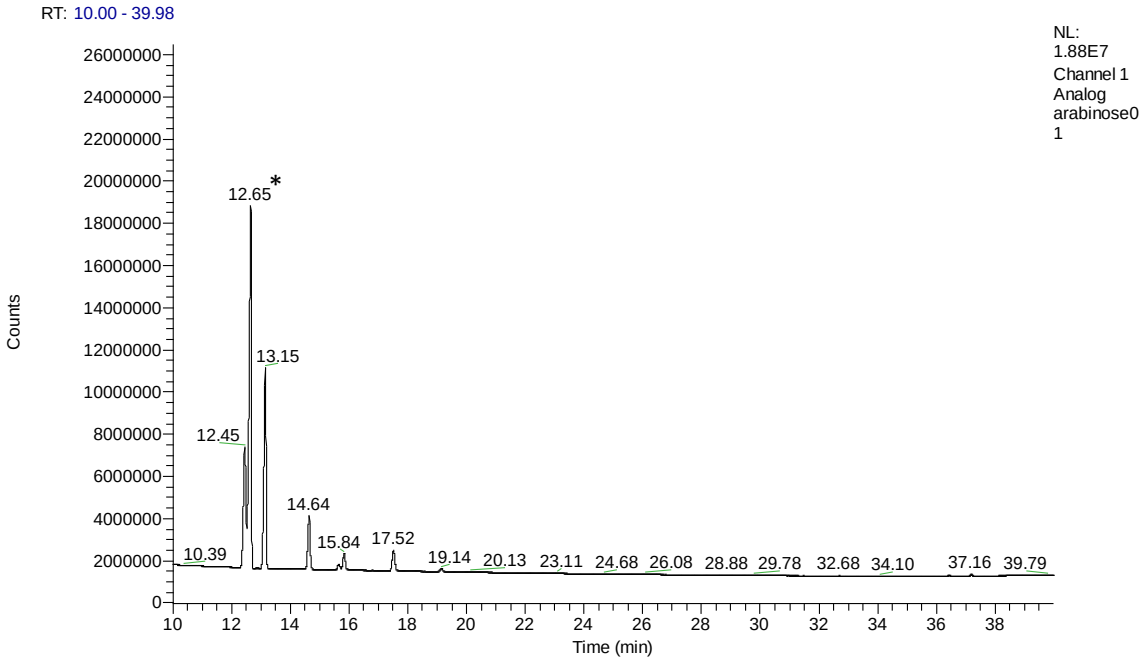

Figure 22 GC chromatogram of arabinose

* The peak used for calibration curve of each monosaccharide.

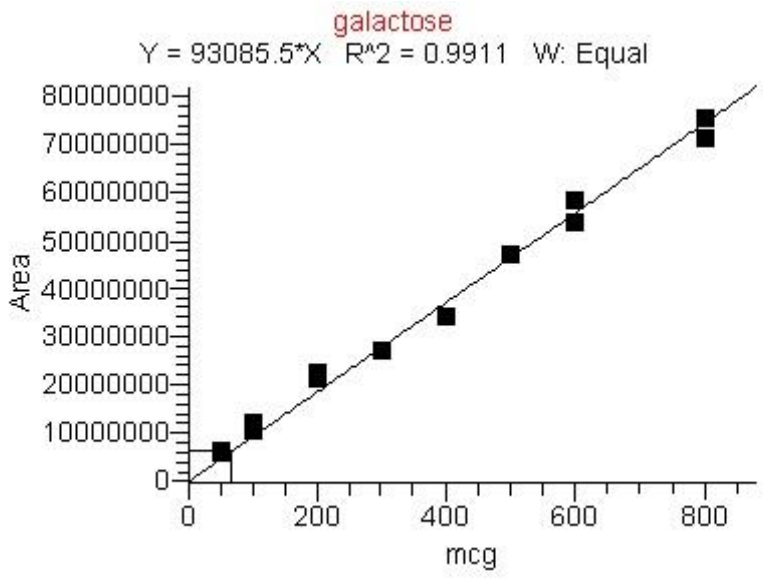

Figure 23 Calibration curve for galactose determination by GC

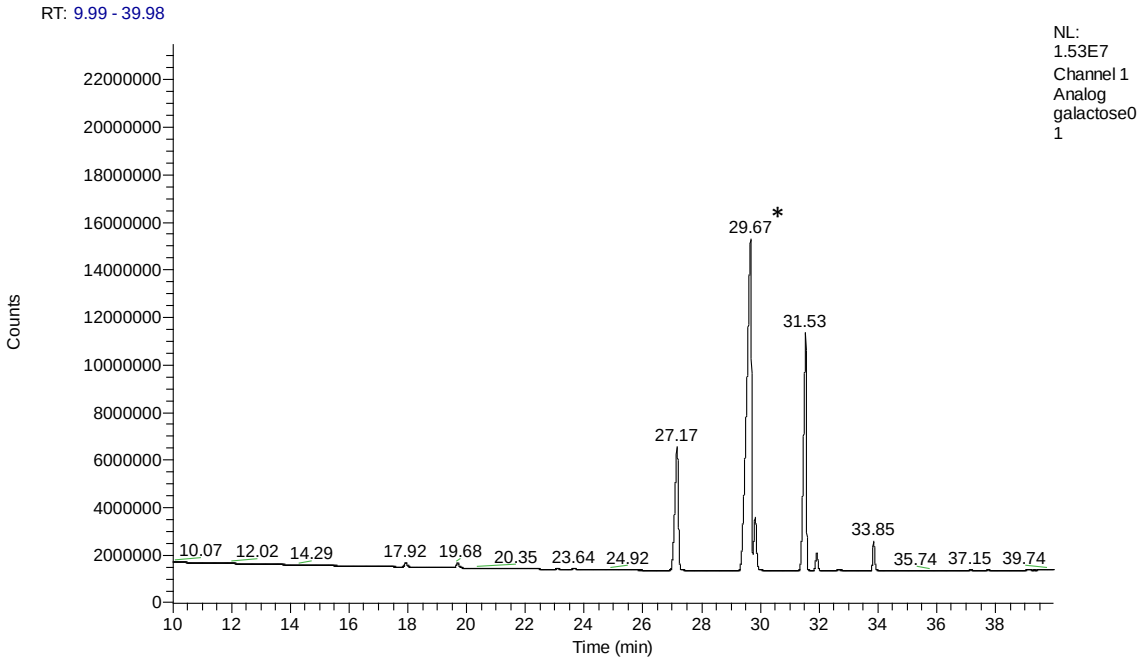

Figure 24 GC chromatogram of galactose

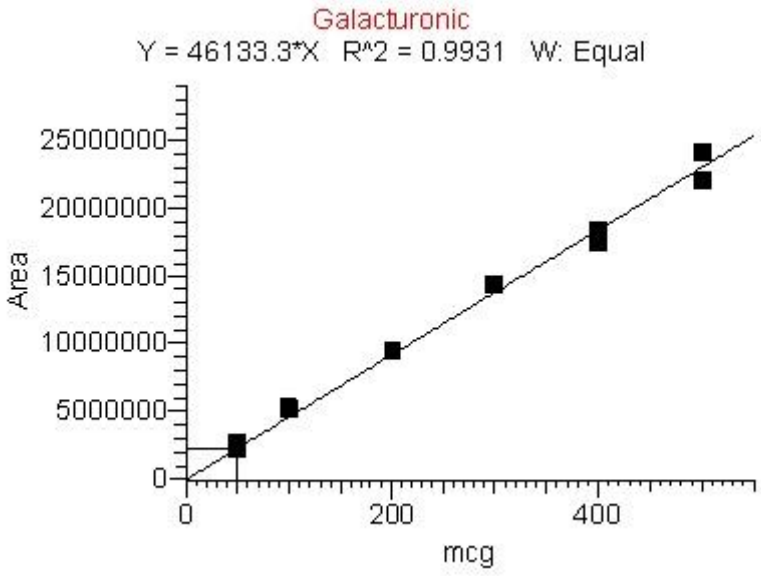

Figure 25 Calibration curve for galacturonic acid determination by GC

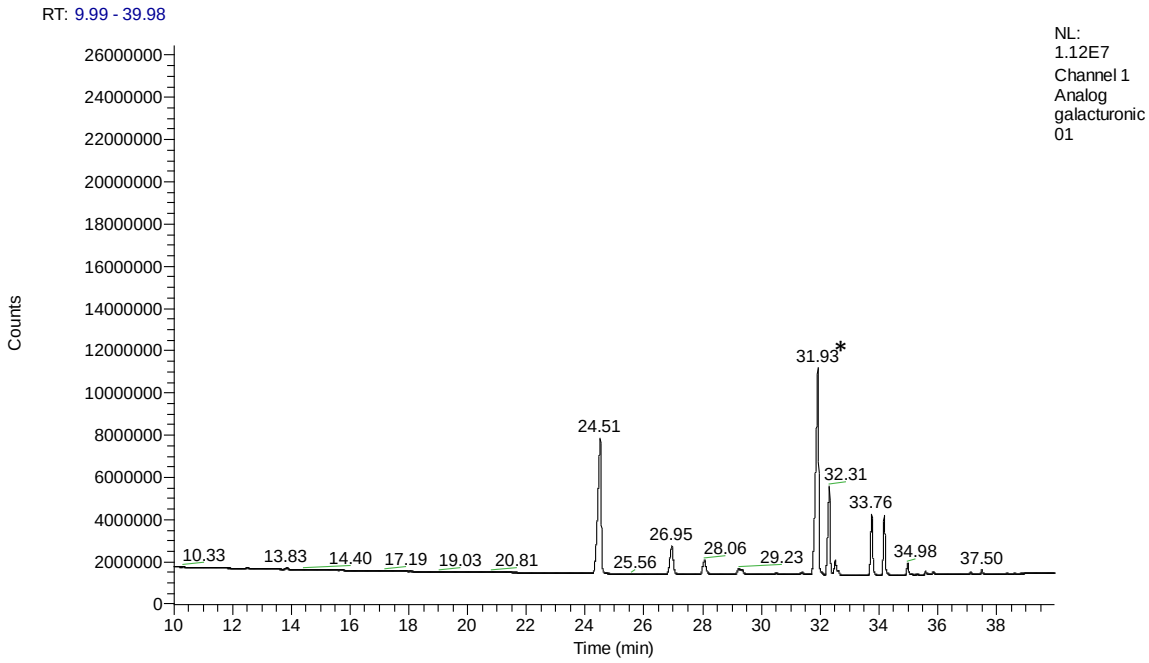

Figure 26 GC chromatogram of galacturonic acid

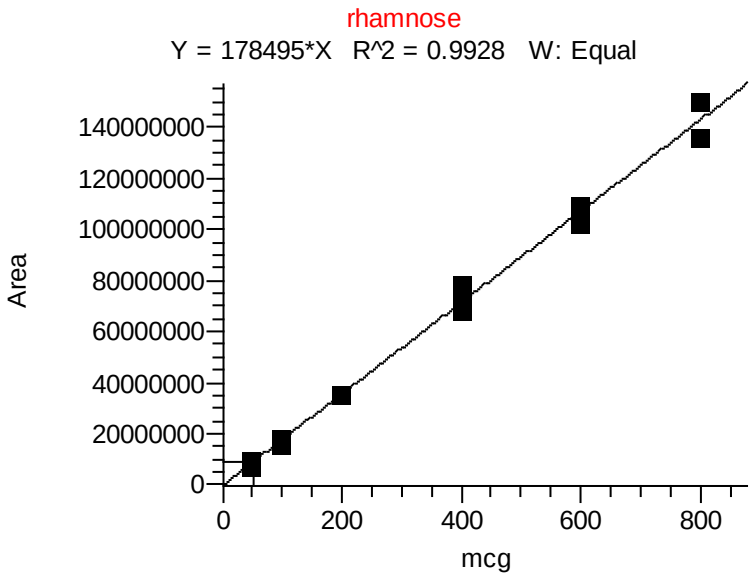

Figure 27 Calibration curve for rhamnose determination by GC

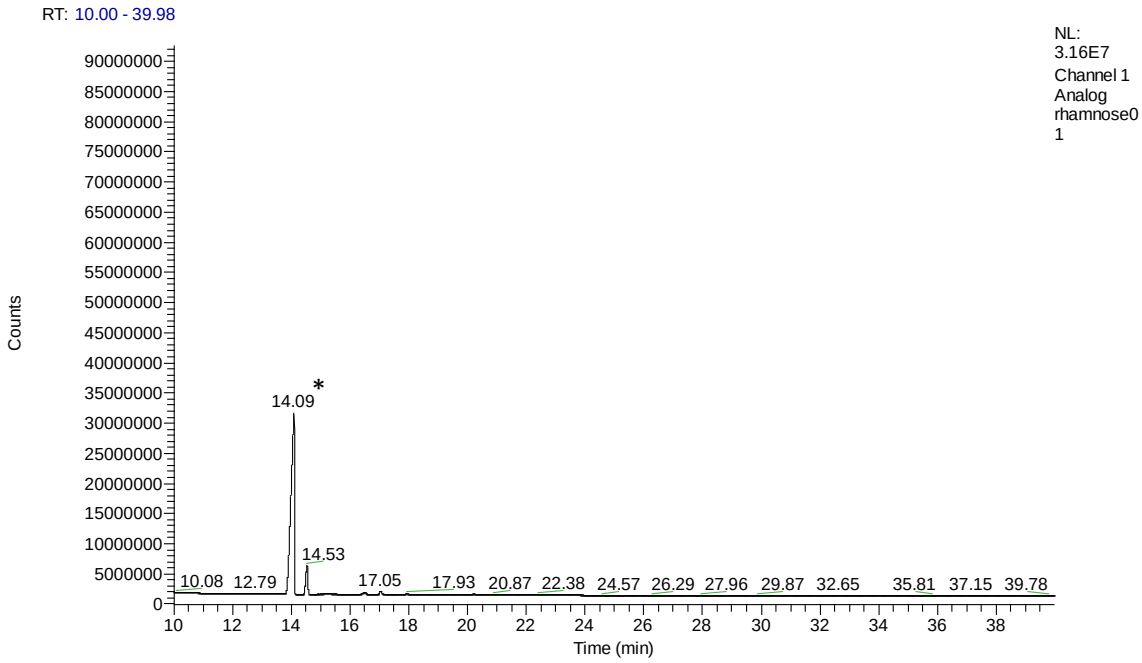

Figure 28 GC chromatogram of rhamnose

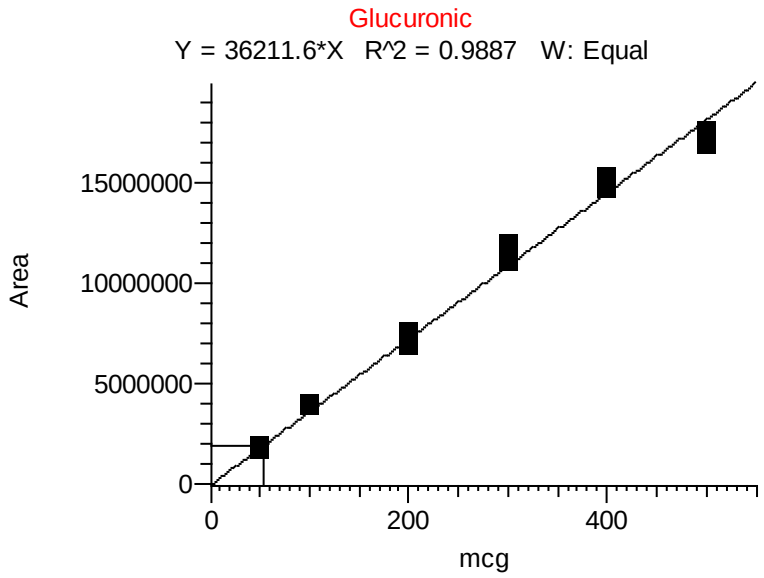

Figure 29 Calibration curve for glucuronic acid determination by GC

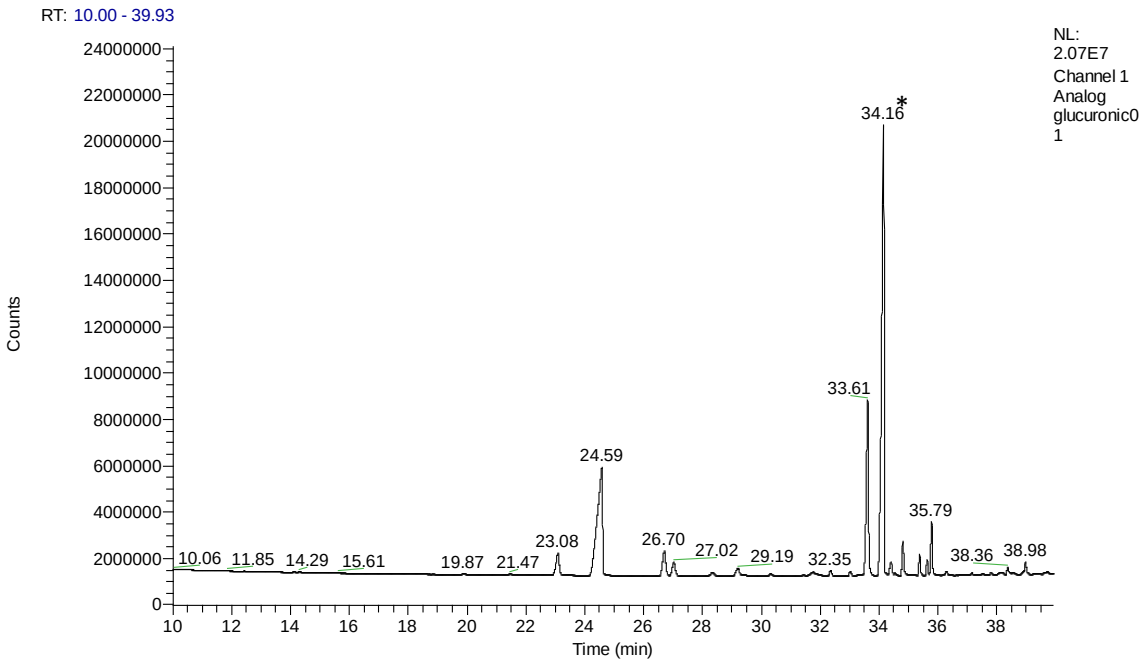

Figure 30 GC chromatogram of glucuronic acid

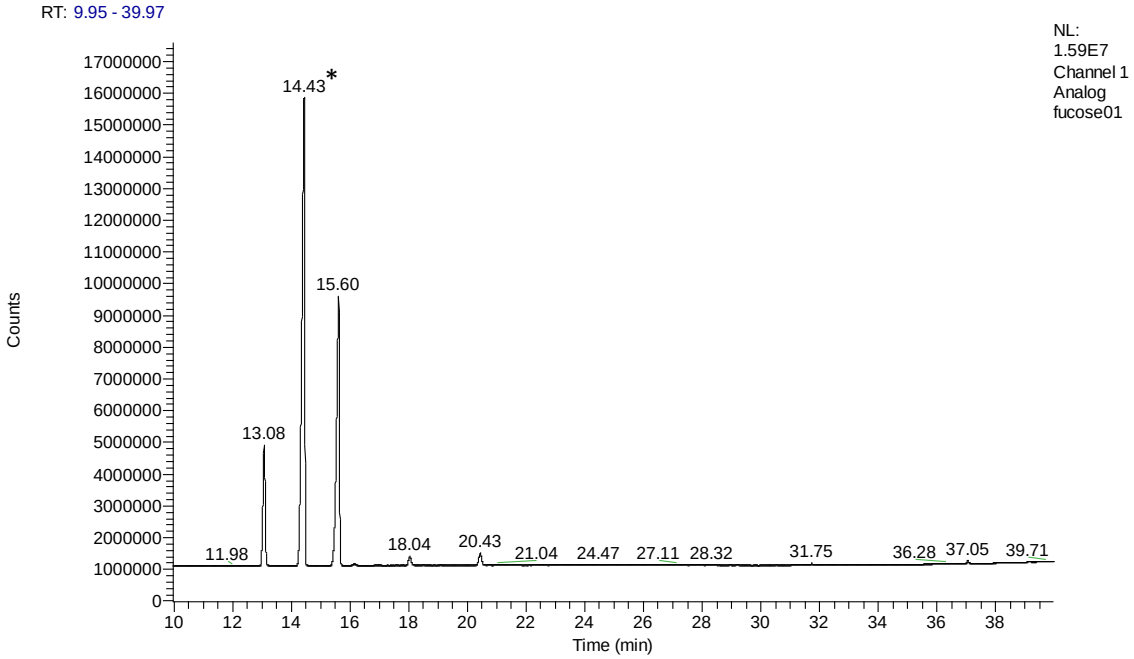

Figure 31 GC chromatogram of fucose

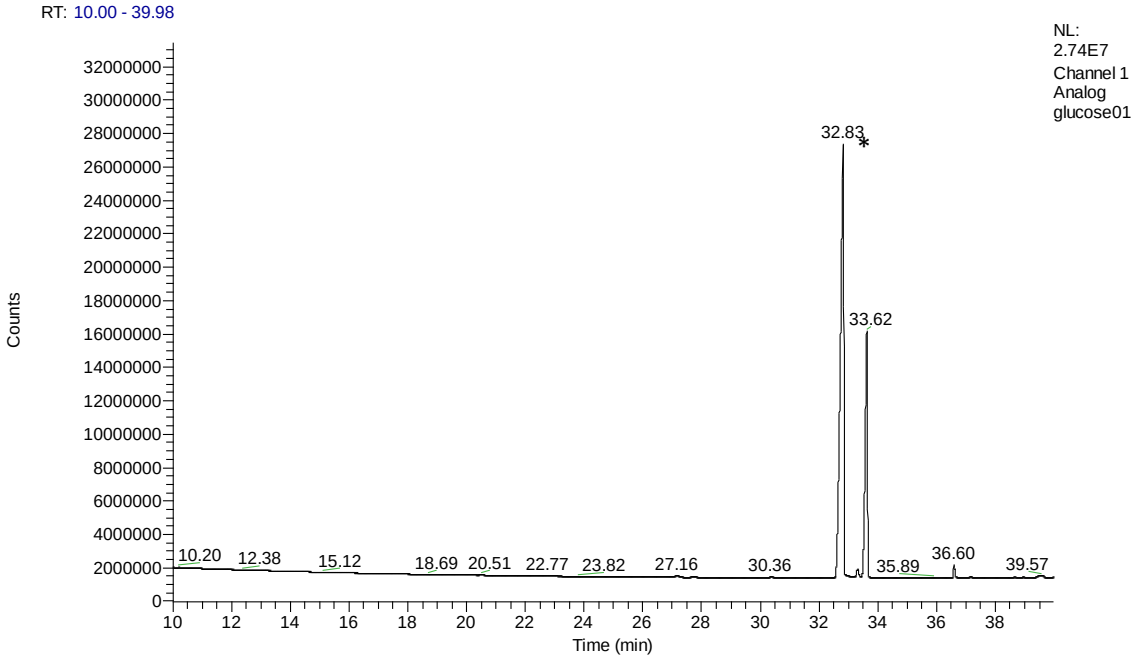

Figure 32 GC chromatogram of glucose

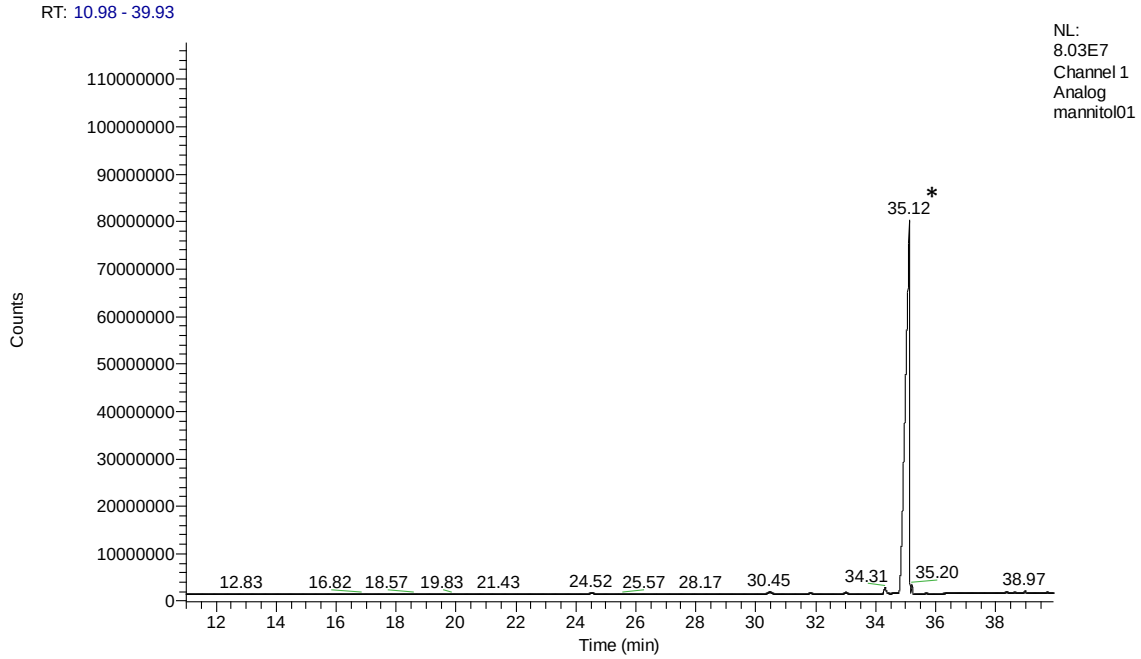

Figure 33 GC chromatogram of mannitol

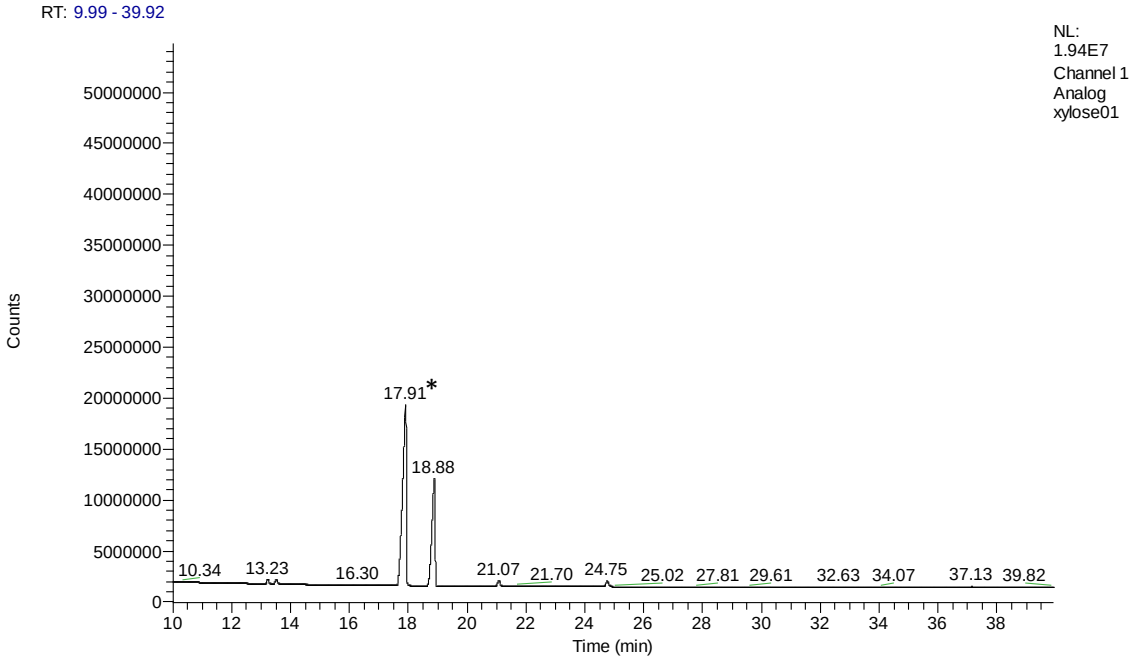

Figure 34 GC chromatogram of xylose

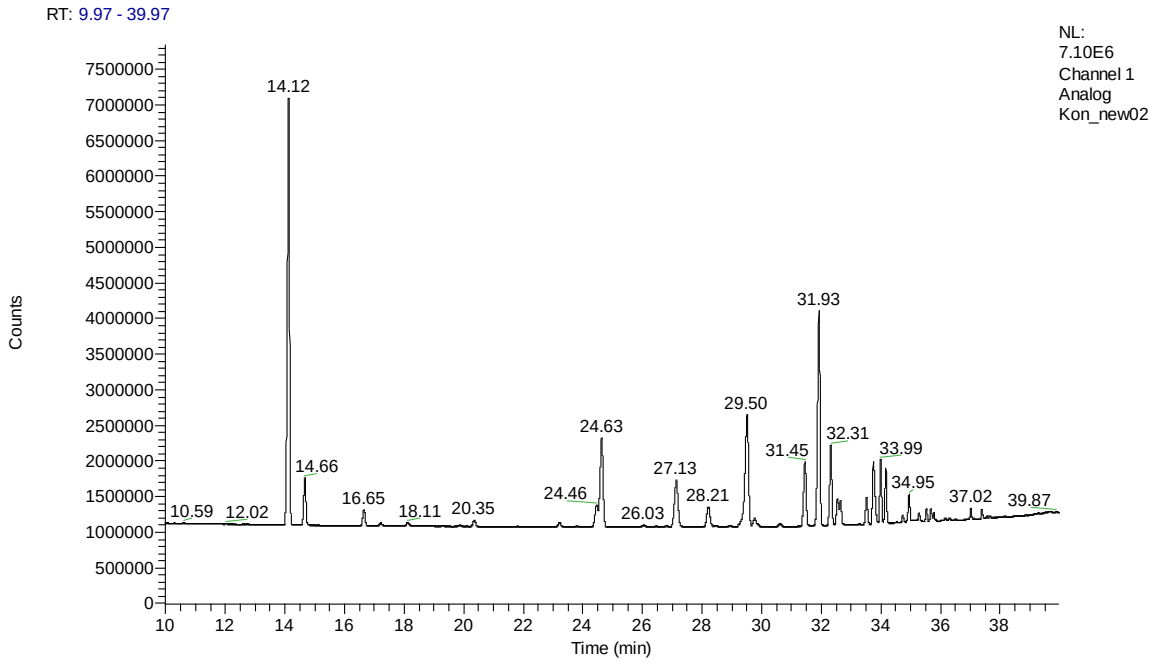

Figure 35 GC chromatogram of Kondagogu gum

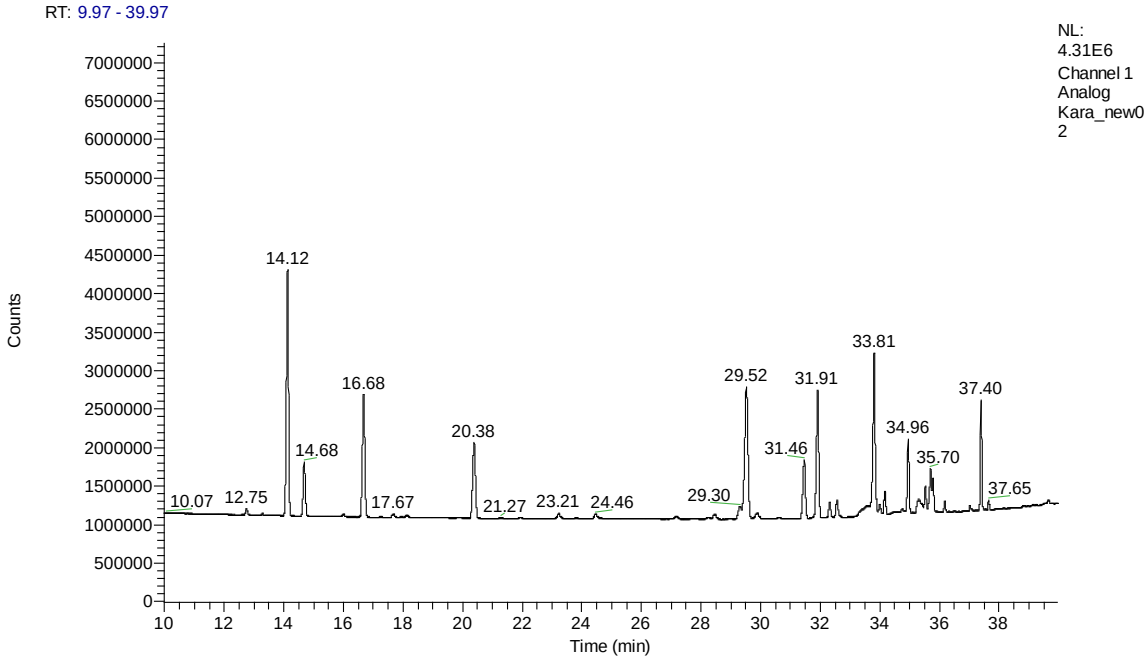

Figure 36 GC chromatogram of Karaya gum

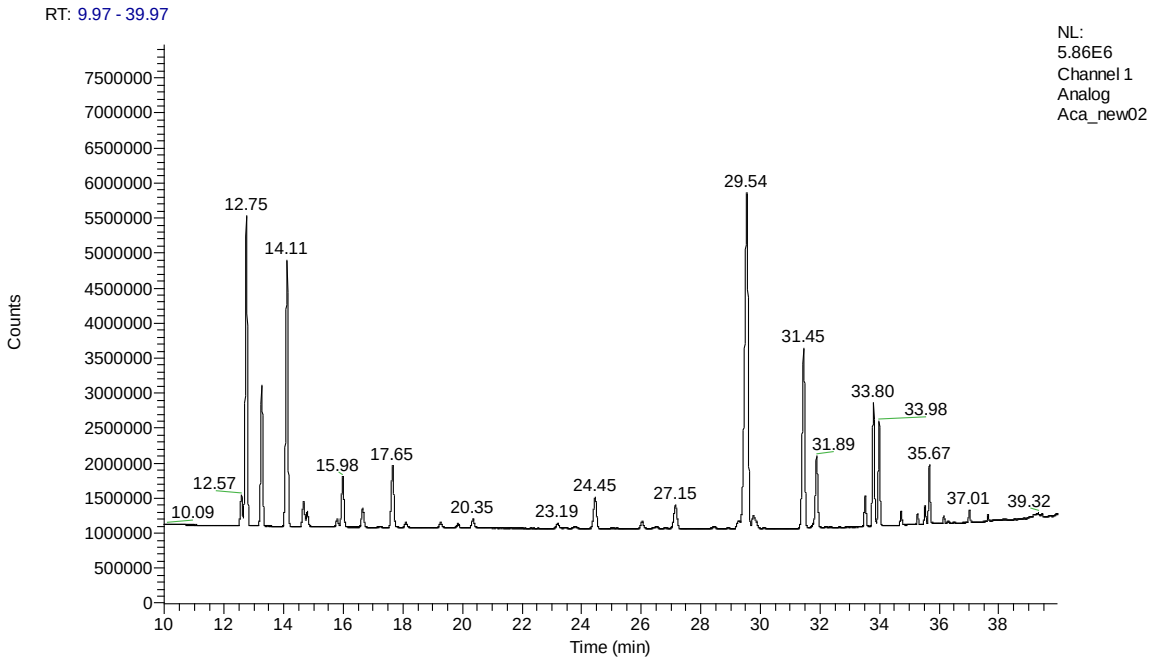

Figure 37 GC chromatogram of Acacia gum

VITAE

Miss Parichart Hongsing was born on June 29, 1987 in Roiet, Thailand. She received her Bachelor's degree of Applied Thai Traditional Medicine (Applied Thai Traditional Medicine) with second class honor in 2010 from Mae Fah Luang University.

Publication

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