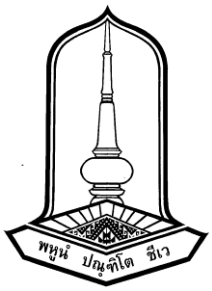

ANTIDIABETIC AND ANTIOXIDANT ACTIVITIES OF FRUIT PULP EXTRACTS FROM *CUCURBITA MOSCHATA* DUCH. AND *CUCURBITA MAXIMA* DUCH.

APINYA SUWANNAPONG

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biology at Mahasarakham University

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TITLE Antidiabetic and antioxidant activities of fruit pulp extracts from *Cucurbita moschata* Duch. and *Cucurbita maxima* Duch.

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ABSTRACT

Cucurbita moschata Duch. and *Cucurbita maxima* Duch. are commonly known as pumpkin, one of the most popular vegetables. Pumpkin has been regarded as a folk medicine using for the prevention of various human diseases and it has been reportly to have hypolipidemic, hypoglycemic, and antioxidant activities. The present study was aimed to investigate the antidiabetic and antioxidant activities of fruit pulp extracts from *Cucurbita moschata* (PCMOS) and *Cucurbita maxima* (PCMAX) to confirm the pharmacological activities of the pumpkin. The antidiabetic activity of PCMOS and PCMAX was investigated in streptozotocin (STZ)-induced diabetic rats. The rats were divided into 5 groups with 6 rats in each; group I: normal control rats treated with 0.5% Tween 80, group II: diabetic control rats treated with 0.5% Tween 80, group III: diabetic rats treated with PCMOS (500, mg/kg b.w.), group IV: diabetic rats treated with PCMAX (500, mg/kg b.w.), and group V: diabetic rats treated with glibencamide (0.5, mg/kg b.w.). The study was carried out for 6 weeks. The antioxidant activity study employing the 1,1-dipheny l-2-picrylhydrazyl free radical (DPPH) assay and ferric reducing antioxidant power (FRAP) assay was also investigated. The results showed that PCMOS and PCMAX significantly ($p<0.05$) decreased the blood glucose level in the diabetic treated rats compared to diabetic controls. In contrast, PCMOS and PCMAX significantly ($p<0.05$) increased the body weight and serum insulin in the diabetic treated rats compared to diabetic controls. Moreover, PCMOS and PCMAX slightly decreased cholesterol (CHO) and low-density lipoprotein cholesterol (LDLc), but slightly increased high-density lipoprotein cholesterol (HDLc) in the diabetic treated rats. Furthermore, PCMOS and PCMAX significantly ($p<0.05$) decreased ALP but not

AST and ALT. In addition, PCMOS and PCMAX did not alter hematological values including WBC, RBC, Hb, Hct, Neu, Lym, and Mono but increased the Plt of diabetic treated rats. Histological study in the pancreatic tissues showed the beta cell proliferation in the pancreas of STZ-induced diabetic rats treated with PCMOS and PCMAX.

The antioxidant activity using DPPH assay revealed that PCMAX exhibited potent antioxidant activity higher than PCMOS with EC_{50} of 8.0396 vs. 13.6973 µg/ml but less potent than Ascorbic acid (0.0010 µg/ml). FRAP assay revealed that PCMAX also exhibited potent antioxidant activity higher than PCMOS with the values of 42.66 vs. 31.28 mM Fe(II)/g DW. PCMAX provided the total phenolic content and total flavonoid content more than PCMOS with the amount of 11.23 mg GAE/g DW and 55.04 mg CE/g DW vs. 5.28 mg GAE/g DW and 20.85 mg CE/g DW.

The results from this study indicate that the fruit pulp extracts from *C. moschata* and *C. maxima* possess the antidiabetic and antioxidant activities, and are the good resource for the treatment of diabetes. The extracts lower the blood glucose level partially by stimulating beta cell proliferation and leading to increase serum insulin. Phenol and flavonoid content involve their antioxidant activity.

Keywords: *Cucurbita moschata* Duch., *Cucurbita maxima* Duch., antidiabetic, antioxidant, fruit pulp extract

ชื่อเรื่อง ฤทธิ์ลดระดับน้ำตาล และฤทธิ์ต้านอนุมูลอิสระ ของสารสกัดจากเนื้อฟักทอง *Cucurbita moschata* Duch. และ *Cucurbita maxima* Duch.
ผู้วิจัย นางสาวอภิญญา สุวรรณพงศ์
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บทคัดย่อ

Cucurbita moschata Duch และ *Cucurbita maxima* Duch เป็นที่รู้จักกันทั่วไปว่าเป็นฟักทอง และเป็นผักชนิดหนึ่งที่นิยมนำมารับประทาน ฟักทอง เป็นพืชสมุนไพรที่มีสรรพคุณทางยามากมาย ช่วยรักษาโรคต่าง ๆ เช่น ต้านมะเร็ง ลดอาการปวด ความดันโลหิตสูง ลดระดับไขมัน ภาวะน้ำตาลในเลือดต่ำ และต้านอนุมูลอิสระ ดังนั้นการศึกษาในครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษา ฤทธิ์ลดระดับน้ำตาลในเลือด ฤทธิ์ลดระดับไขมันในเลือด ฤทธิ์ต่อระดับอินซูลินในซีรั่ม ฤทธิ์ต้านอนุมูลอิสระ และลักษณะทางจุลกายวิภาคของเนื้อเยื่อตับอ่อนของหนูเบาหวานที่ได้รับสารสกัดจากเนื้อฟักทอง *C. moschata* (PCMOS) และ *C. maxima* (PCMAX) การศึกษาฤทธิ์ลดระดับน้ำตาลในเลือดในหนูที่ถูกเหนี่ยวนำให้เป็นเบาหวานด้วย streptozotocin (STZ) แบ่งหนูทดลองออกเป็น 5 กลุ่ม กลุ่มละ 6 ตัว ได้แก่ กลุ่มที่ 1: หนูปกติควบคุมโรคที่ได้รับ 0.5% Tween 80 กลุ่มที่ 2: หนูเบาหวานควบคุมที่ได้รับ 0.5% Tween 80 กลุ่มที่ 3: หนูเบาหวานที่ได้รับ PCMOS ขนาด 500 มิลลิกรัม/กิโลกรัม น้ำหนักตัว กลุ่มที่ 4: หนูเบาหวานที่ได้รับ PCMAX ขนาด 500 มิลลิกรัม/กิโลกรัม น้ำหนักตัว และกลุ่มที่ 5: หนูเบาหวานที่ได้รับยา glibencamide ขนาด 0.5 มิลลิกรัม/กิโลกรัม น้ำหนักตัว เป็นเวลา 6 สัปดาห์ การศึกษาฤทธิ์ต้านอนุมูลอิสระด้วยวิธี 2-2-picrylhydrazyl-1-dipheny (DPPH) free radical scavenging assay และการตรวจวัดความสามารถในการรีดิวซ์เฟอร์ริกของสารต้านอนุมูลอิสระด้วยวิธี ferric reducing antioxidant power (FRAP) assay ผลการศึกษาพบว่า หนูเบาหวานที่ได้รับ PCMOS และ PCMAX สามารถลดระดับน้ำตาลในเลือดของหนูที่เป็นเบาหวาน อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) เมื่อเปรียบเทียบกับหนูกลุ่มเบาหวานควบคุม นอกจากนี้ หนูเบาหวานที่ได้รับ PCMOS และ PCMAX ทำให้น้ำหนักตัวเพิ่มขึ้น และเพิ่มระดับอินซูลิน เมื่อเทียบกับหนูกลุ่มเบาหวานควบคุม สารสกัดจาก PCMOS และ PCMAX สามารถลดระดับคอเลสเตอรอล (CHO) และระดับไขมัน low-density lipoprotein cholesterol (LDLc) ได้เพียงเล็กน้อย อีกทั้งพบว่า หนูเบาหวานที่ได้รับ PCMOS และ PCMAX เพิ่มระดับ lipoprotein cholesterol (HDLc) ได้เพียงเล็กน้อย ในหนูกลุ่มเบาหวาน และที่น่าสนใจจากการศึกษาพบว่า หนูเบาหวานที่ได้รับ PCMOS และ PCMAX ทำให้ระดับเอนไซม์ ALP ลดลง อย่างมีนัยสำคัญ ($p < 0.05$) เมื่อเทียบกับหนูกลุ่มเบาหวานควบคุม แต่ไม่มีผลต่อระดับเอนไซม์ AST และ ALT นอกจากนี้ หนูเบาหวานที่ได้รับ PCMOS และ PCMAX ไม่มีผลทำให้ค่าโลหิตวิทยาเปลี่ยนแปลง ได้แก่ White Red blood cell (RBC), blood cells (WBC), Hemoglobin (Hb), Hematocrit (Hct), Neutrophil (Neu), Lymphocyte (Lym), Monocyte (Mono) แต่มีผลทำให้ค่า Plt ของหนูเบาหวานที่ได้รับ PCMAX เพิ่มขึ้น และจากการศึกษาลักษณะทางพยาธิสภาพของ

เนื้อเยื่อตับอ่อนในหนูเบาหวาน พบว่า สารสกัดจาก PCMOS และ PCMAX สามารถฟื้นฟูเบต้าเซลล์ของตับอ่อนได้

การศึกษาฤทธิ์ต้านอนุมูลอิสระโดยใช้วิธี DPPH assay พบว่า สารสกัดจาก PCMAX มีฤทธิ์ต้านอนุมูลอิสระสูงกว่า PCMOS โดยมีค่า EC_{50} เท่ากับ 8.0396 และ 13.6973 ไมโครกรัม/มิลลิลิตร แต่มีฤทธิ์ต้านอนุมูลอิสระน้อยกว่า ascorbic acid (0.0010 ไมโครกรัม/มิลลิลิตร) การศึกษาฤทธิ์ต้านอนุมูลอิสระโดยใช้วิธี FRAP assay พบว่า สารสกัดจาก PCMAX มีความสามารถในการรีดิวซ์เฟอร์ริกสูงกว่า PCMOS โดยมีค่าเท่ากับ 42.66 และ 31.28 ไมโครกรัม/น้ำหนักแห้ง การศึกษาปริมาณฟีนอลิกรวม และปริมาณฟลาโวนอยด์รวม พบว่า สารสกัดจาก PCMAX มีปริมาณฟีนอลิกรวม และปริมาณฟลาโวนอยด์รวมสูงกว่า PCMOS โดยมีค่าเท่ากับ 11.23 vs. 55.04 มิลลิกรัม/กรัม และ 5.28 vs. 20.85 มิลลิกรัม/กรัม

ผลการศึกษาพบว่า สารสกัดจากเนื้อฟักทอง PCMOS และ PCMAX มีฤทธิ์ต้านโรคเบาหวาน มีฤทธิ์ต้านอนุมูลอิสระ และเป็นแหล่งความรู้ที่เป็นประโยชน์อย่างยิ่งในการรักษาโรคเบาหวาน สารสกัดช่วยลดระดับน้ำตาลในเลือดได้โดยการกระตุ้นการเพิ่มจำนวนเบต้าเซลล์ของตับอ่อน และทำให้ระดับอินซูลินเพิ่มขึ้น ด้วยคุณสมบัติของสารสกัดที่มีสารประกอบ Phenol และ flavonoid ส่งผลให้มีฤทธิ์ต้านอุมูลอิสระ

คำสำคัญ: *Cucurbita moschata* Duch., *Cucurbita maxima* Duch., ยารักษาโรคเบาหวาน, สารต้านอนุมูลอิสระ, สารสกัดจากเนื้อฟักทอง

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CHAPTER I

INTRODUCTION

1.1 Background

Diabetes mellitus (DM) describes a metabolic disorder of multiple a etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (America Diabetes Association, 2010). The two most common types of diabetes: are type 1 and type 2 diabetes. Type 1 diabetes occurs due to absolute deficiency of insulin. Type 2 diabetes occurs mostly due to a combination of insulin resistance and an inadequate compensatory insulin secretory response (American Diabetes Association, 2008). Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. It is widely recognised that the prevalence of diabetes is rising rapidly. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Chronic hyperglycemia causes damage to the eyes, heart, kidneys, nerves, and blood vessels (Lebovitz, 2001).

Toxic pollution is a problem that affect the body's biochemical balance, caused changes in chemical reactions and processes to disorders, radical or free radical increase in the body. Free radical is released when cell or the body are in oxidative stress, causing damage to living systems. However, this damage pattern has relationship with diseases and disorders such as cancer, multiple types of associated with cardiovascular diseases, action of immune diseases, and aging (Wu and Hansen, 2008). It is well known that oxidative stress can be treated by substance called antioxidant. Antioxidants are important for bodily protection against oxidative stress (Zhang *et al*., 2010). The major exogenous nutritional antioxidants, vitamin E, vitamin C and β-carotene, may be beneficial to prevent several chronic disorders (Diplock, 1998). Several studies have shown that plants have antioxidant activities which could be therapeutically benefit. From the past, there are strong evidences of medicinal plants being used for treatment of diseases and revitalizing body system worldwide, especially in ancient civilizations. Medicinal plants are an important part of traditional medicine

(Atanassova *et al*., 2011). Generally, plants produce various secondary metabolites including phenols, flavonoids, quinines, tannins, alkaloids, saponins and sterols (Alghazeer *et al*., 2012). These of metabolites are being used as pharmaceutical drugs (Yazaki *et al*., 2008). Over past decades, synthetic substances were increasingly denied; therefore, plant metabolites have been explored and applied (Suhaj, 2006). In developing countries all over the world, 80% of population continues to use traditional medicine in primary medical problems (Grover, 2004). Pumpkin is one such plant that has been frequently used as functional food or medicine. And also reported that potein-bound polysaccharides from the fruit of pumpkin could obviously increase the levels of serum insulin, reduce the blood glucose levels and improve tolerance of glucose (Li *et al*., 2005). Polysaccharide, polymer of more than 20 monosaccharides, includes plant polysaccharide, animal polysaccharide and microbe polysaccharide (Zhang *et al*., 1995). Now, more than 300 types polysaccharides have been isolated from nature material. Polysaccharide compounds contained bioactivity such as antitumor, anticaducity, antiinfection, hypoglycemia and hypolipidemic (Tian and Feng, 1995). Therefore, alternative approaches like herbal remedies are increasing (Shim *et al*., 2003). And also, these plants have excellent antioxidant properties.

Pumpkin belongs to the family Cucurbitaceae, genus *Cucurbita* and common its name are Pumpkin, Squash and Cushaw (Nee, 1990; Smith, 1997). Pumpkin is native to the South America, Mexico, Peru, Bolivia and Argentina. It is then distributed to Europe and around the world. Pumpkin is cultivated worldwide. Pumpkin is one of the important plants consists of about 25-27 species. In general, 4 species of pumpkin have been commonly cultivated including *C. moschata, C. pepo, C. mixta* and *C. maxima* represent economically important species cultivated worldwide and have high production (Taylor and Brant, 2002). Pumpkin is a dicotyledonous seed vegetable and consists of a flexible succulent stem. It is a climbing vine that is an annual, shape stem, leaves, flowers and fruit varies by species (Chaimongkol, 2003). *Cucurbita moschata* Duch. is xerophytic species and can be grown in arid lands. Flowers are yellow and trumpet-shaped, pedicels are hard, fruits are oval to spherical green in color. The flesh of a yellow or dark yellow. The seeds are large can be white or dark brown. *Cucurbita maxima* Duch. has a more southern origin than other cultivated species of *Cucurbita*.

Fruits are oval to spherical orange in color, has a soft and hard shell. Flowers are yellow and lightly pubescent pedicels. The flesh of a dark are yellow. The seeds are smooth, white or brown (Bailey, 1964; Purseglove, 1968).

Popularity of pumpkin in various systems of traditional medicine for several ailments, antidiabetic, antihypercholesterolemia (Sedigheh *et al*., 2011), antioxidant (Prasad, 2014), immunomodulation (Xia *et al*., 2003) antihypertensive, antitumor, antibacterial (Fu *et al*., 2006). The phytochemical constituents of the plant, especially the carotenoid, γ-aminobutyric acid, polysaccharides, sterols, proteins, triterpens, saponins, fibers, and minerals (iron, zinc, manganese, and copper) (Fu *et al.*, 2006; Murkovic *et al.*, 2002). Li *et al*. (2005) studied the effects of protein-bound polysaccharide isolated from Pumpkin on insulin in diabetic rats. The results indicated that protein-bound polysaccharide from Pumpkin (PBPP) can obviously increase the levels of serum insulin. The hypoglycemic effect of big dose PBPP group (1000 mg/kg body weight) excelled that of small dose PBPP group (500 mg/kg body weight) and antidiabetic agent group. Sharmin *et al*., (2013) studied the effects hypoglycemic and hypolipidemic of Cucumber, white pumpkin and ridge gourd in alloxan induced diabetic rats. Screening results suggested that among the tested fruits the hypoglycemic potency follows: cucumber > white pumpkin > ridge gourd (67, 65 and 51%, respectively at 12 hours after a single intraperitoneal injection, while reduced the low density lipoprotein (LDL) level (13, 28 and 86%, respectively) and reduced total cholesterol level to 29, 15 and 38%, respectively comparing with the diabetic control group. Zhang *et al*., (2013) studied the effects of polysaccharide from pumpkin on biochemical indicator and pancreatic tissue of the diabetic rabbits. A water-soluble polysaccharide (PCE-CC) was obtained from pumpkin. The data of blood glucose (BG), total cholesterol (TC), total triglyceride (TG) and glycosylated hemoglobin (HbA1c) indicated that PCE-CC had beneficial effects on the improvement in the control of blood glucose, serum lipid and glycosylated hemoglobin levels. Observing the pancreatic tissue of the diabetic rabbits revealed that PCE-CC could promote the regeneration of damaged pancreatic islets by stimulating cell proliferation, which was accompanied by a decrease in plasma glucose levels. PCE-CC was further separated and purified to obtain PCE-CCH by ion exchange and gel chromatography.

PCE-CCH was a heteropolysaccharide and consisted of glucose, galactose, arabinose, rhamnose and little amount of hexuronic acid, with a molecular weight of 1.15 × 105 Da. Including reported by Sedigheh *et al*. (2011) showed antidiabetic activity significantly ($p<0.05$) reducing the level of glucose, cholesterol and triglyceride. Measurement of phytochemical the amount of polysaccharides and total phenolic (43.63, 12 mg/g respectively) was elements. Song *et al*. (2013) reported on the effect of antioxidant activity of polysaccharides isolated from pumpkin (*Cucurbita pepo*). The antioxidant activity study employing the DPPH radical, superoxide anion radical scavenging activity and reducing power assay. The results indicated that polysaccharides isolated from pumpkin possesses antioxidant activities. And reported by Xu, (2000) found that polysaccharides can obviously increase the superoxide dismutase and glutathione peroxidase possesses an important of antioxidant activities.

From related studies in research the two species of pumpkins including *C. moschata* Duch and *C. maxima* Duch. Pharmacological activities of *C. moschata* Duch. and *C maxima* Duch. have not yet been reported in the compare of the two species of pumpkins. Including, the mechanisms of action of polysaccharide from pumpkin in Thailand. The present study is therefore aimed to investigate the antidiabetic, lipid profile, serum insulin levels, antioxidant activities and histological of pancreatic tissue of polysaccharides from *C. moschata* and *C. maxima* in streptozotocin induced diabetic rats.

1.2 Objectives of the research

The present study was designed to determine and compare

1.2.1 Antidiabetic activity of fruit pulp extracts from *C. moschata* and *C. maxima*

1.2.1.1 Body weight

1.2.1.2 Blood glucose levels

1.2.1.3 Serum insulin levels

1.2.1.4 Lipid profiles

1.2.1.5 Biochemical values for renal function

1.2.1.6 Biochemical values for hepatic function

1.2.1.7 Hematological values

1.2.1.8 Histological features of pancreatic tissues

1.2.2 Antioxidant activity of fruit pulp extracts from *C. moschata* and *C. maxima*

. 1.2.3 Phytochemical components of fruit pulp extracts from C. *moschata* and *C. maxima.*

1.3 Scope of the research

1.3.1 Plant materials

Fruit pulp extracts from *Cucurbita moschata* and *Cucurbita maxima*

1.3.2 Measurement of polysaccharide content by using phenol - sulfuric acid method

1.3.3 Study on antidiabetic activity

1.3.4 Study on antioxidant activity

1.3.3.1 DPPH free radical scavenging assay

1.3.3.2 Ferric reducing/antioxidant power (FRAP) assay

1.3.4 Determination of phytochemical components

1.3.4.1 Total phenolic content

1.3.4.2 Total flavonoid content

CHAPTER II

LITERATURE REVIEW

2.1 Pumpkin

2.1.1 Botanical characteristics

Cucurbitaceae is a plant family generally considered to consist of melons, cucurbits and pumpkins. The pulp of pumpkin, a cucurbita species grown throughout the world in many regions, is a plant recommended for its beneficial and therapeutic properties (Zhang *et al*., 2013). Pumpkin is from genus Cucurbita belong to the family of Cucurbitaceae. It includes squash and cucumbers which are grown throughout the tropical and sub-tropical countries. There are three common types of pumpkin worldwide, namely *Curcurbita pepo*, *Curcurbita maxima* and *Curcurbita moschata ssp.* (Lee, *et al*., 2003). Pumpkin can be found in many shapes, sizes and colours. Agriculture, food-processing, pharmaceutical as well as feed industry have all taken growing interest in pumpkin fruit and pumpkin-derived products in the past few years because of the nutritional and health protective value of the proteins and oil from the seeds as well as the polysaccharides from the fruit (Sojak M. *et al*., 2010). Pumpkin is a dicotyledonous seed vegetable and consists of a flexible succulent stem. It is a climbing vine that is an annual, shape stem, leaves, flowers and fruit varies by species (Nitipon Chaimongkol, 2546).

Cucurbita moschata Duch. is xerophytic species and can be grown in arid lands. Flowers are yellow and trumpet-shaped. Pedicels are hard. Fruits are oval to spherical green in color. The flesh of a yellow or dark yellow. The seeds are large can be white or dark brown. *Cucurbita maxima* Duch. has a more southern origin than other cultivated species of *Cucurbita*. Fruits are oval to spherical orange in color, has a soft and hard shell. Flowers are yellow and lightly pubescent pedicels. The flesh of a dark yellow. The seeds are smooth, white or brown (Bailey, 1964; Purseglove, 1968)

2.1.2 Phytochemicals

Pumpkin contains chemicals, including polysaccharides, sterols, proteins, triterpens, saponins, trace elements, vitamins, starch, carotenoid, γ-aminobutyric acid, fibers, and minerals (iron, zinc, manganese, copper). Pumpkins have been accepted as a dietary constituent in China and received considerable attention in recent years because of the nutritional and health protective value of the proteins and oil, from the seeds as well as the polysaccharides from the fruits (Fu C. *et al.,* 2006; Murkovic *et al.,* 2002).

2.2 Medicinal properties of pumpkin

Li *et al.* (2005) studied the effects of protein-bound polysaccharide isolated from Pumpkin (PBPP) on insulin in diabetic rats. A total of 60 rats were randomized into 5 groups of 12 each as follows: Group 1: control group; Group 2: diabetic untreated rats; Group 3: diabetic rats treated with 1,000 mg/kg of PBPP; Group 4: diabetic rats treated with 500 mg/kg of PBPP; and Group 5: diabetic rats treated with 20 mg/kg of glibenclamide. The rats were made diabetic by alloxan injection and were treated for 10 days on a daily basis. The results indicated that PBPP can obviously increase the levels of serum insulin. The hypoglycemic effect of big dose PBPP group (1000 mg/kg body weight) excelled that of small dose PBPP group (500 mg/kg body weight) and antidiabetic agent group. The results suggest that the hypoglycemic effect of PBPP depends on the dose and PBPP possesses the possibility of being developed from a new antidiabetic agent.

Caili *et al.* (2005) To determine some properties of an acidic polysaccharide from the fruit of pumpkin (APBPP), such as weight-average molecular weight, amino acid and monosaccharide composition, analysis by HPLC showed the presence of mannose and arabinose in molar ratios of 1:2. Eighteen amino acids were identified to be components of the polymer. Alanine was the main amino acid (0.13%), followed by glutamic acid (0.113%) and serine (0.088%). But the relationship between the contents of amino acids and hypoglycemic activity of APBPP is not clear.

Caili *et al.* (2006) Popularity of pumpkin in various systems of traditional medicine for several ailments (antidiabetic, antihypertensive, antitumor, immunomodulation, antibacterial, antihypercholesterolemia, antiinflammation and antioxidant) focused the investigators' attention on this plant. Considerable evidence from several epidemiological studies concerning bioactivities leads have stimulated a number of animal model, cell culture studies and clinical trials designed to test this pharmacological actions This review will focus on the main medicinal properties and technologies of pumpkin, and point out areas for future research to further elucidate mechanisms whereby this compound may reduce disease risk.

Sedigheh *et al*. (2011) studied the hypoglycaemic and hypolipidemic effects of different doses of pumpkin (*Cucurbita pepo* L.) powder in male diabetic rats. A total of 35 rats were randomized into 5 groups of 7 each as follows: Group 1: Normal control; Group 2: Diabetic control; Group 3: Diabetics administered with low doses of pumpkin powder (1 g/kg); Group 4: Diabetics administered with high doses of pumpkin powder (2 g/kg), and Group 5: Diabetics administered with glibenclamide (0.6 mg/kg), as positive control. The rats were made diabetic by alloxan (120 mg/kg body weight (BW)) injection and were treated for 4 weeks on a daily basis. Blood samples were collected following the experiment. Pancreatic specimens were also collected for histological analysis. Glucose, cholesterol, triglycerides, low density lipoprotein (LDL) and C-reactive protein (CRP) were significantly increased, while insulin was decreased in diabetic rats as compared to the normal control group ($p<0.05$). Low dose pumpkin significantly decreased glucose, triglycerides, LDL and CRP as compared to diabetic group and high dose pumpkin decreased cholesterol ($p<0.05$).

Wang *et al*. (2017) studied the extraction and purification of pumpkin Pumpkins (*C. moschata*) polysaccharides (PPs) and their hypoglycemic effect. The PPs were administered by intraperitoneal injection to the alloxan-induced diabetic male ICR mice. The mice used for the experiment were randomly divided into 10 groups of 5 each as follows: Group I: normal mice as control group and injected with saline (0.86% NaCl), Group II: alloxan-induced diabetic mice, the model group and injected with 0.86% NaCl, Group III: alloxan-induced diabetic mice were administered Xiaoke pill (the Chinese medicine pill is widely usedin

the clinical treatment of diabetes in China) at 750 mg/kg of BW in 0.86% NaCl, as positive control group. Group IV: alloxan-induced diabetic mice administered 150 mg/kg of BW PPs by intraperitoneal injection, and Group V ~ X: alloxan-induced diabetic mice administered 150 mg/kg were administered for 7 h. The result showed the PPs significantly hypoglycemic effect ($p < 0.01$) and significantly increase insulin level ($p < 0.05$). Analysis of sugar composition showed the PPs composed of rhamnose, ara-binose, glucose, galactose, and little amount of inositol could maintain the blood glucose at a low level in diabetic mice and could even last for more than 24 h.

Makni et al. (2011) studied the effects of flax and pumpkin powder seed mixture on alloxan induced diabetes in Wistar rats. Animals were into 3 groups of 6 rats each: a control group (CD), diabetic group (DD) and diabetic rats fed with flax and pumpkin seed mixture (DMS) group. The diabetic rats (DD) presented a significant increase in glycemia, plasma and liver lipid parameters such as total lipid, total cholesterol and triglycerides compared to the control group (CD). The present study revealed a significant increase in the activities of aspartate aminotransferase and alanine aminotransferase on diabetic rat. The administration of flax and pumpkin seed mixture attenuated the increased levels of the plasma enzymes produced by the induction of diabetes and caused a subsequent recovery towards normalization comparable to the control group animals.

Sharmin *et al*. (2013) studied on the effects hypoglycemic and hypolipidemic of Cucumber, white pumpkin and ridge gourd in alloxan induced diabetic rats. A total of 18 rats were randomly into 6 groups of 3 each as follows: Group I and II served as non-diabetic and diabetic control group, respectively. Group III stands for metformin control group in which metformin was administered as a single intraperitoneal dose of 150 mg/ kg body weight. Group IV, V and VI received cucumber, white pumpkin and ridge gourd extracts, respectively as a single intraperitoneal dose of 200 mg/kg body weight. The blood samples were analyzed for blood glucose content at 0, 4, 8, and 12 hours, respectively. Screening results suggested that among the tested fruits the hypoglycemic potency follows: cucumber > white pumpkin > ridge gourd. These three fruit-extracts were further investigated for their hypoglycemic, hypolipidemic and

glycogenesis effects. Cucumber, white pumpkin and ridge gourd extracts reduced blood glucose level by 67, 65 and 51%, respectively at 12 hours after single intraperitoneal injection; while reduced the low density lipoprotein (LDL) level to 13, 28 and 86%, respectively in AIDRs. The maximum reduction 87% was observed by cucumber extract. Cucumber, white pumpkin and ridge gourd extracts reduced total cholesterol level to 29, 15 and 38%, respectively comparing with the diabetic control group. Here the maximum reduction of 85% was observed by white pumpkin extract. Cucumber, white pumpkin and ridge gourd also reduced triglyceride levels to 72, 68 and 80%, respectively.

Prasad *et al*. (2014) studied the phytochemicals in the seeds extracts of four plants belonging to the family Cucurbitaceae such as *Citrullus lanatus* (Watermelon), *Cucumis melo* (muskmelon), *Lagenaria siceraria* (bottle gourd), *Cucurbita pepo* (pumpkin) by preliminary phytochemical analysis and DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity assay. Extracts were prepared using powder of shade dried seeds with methanol, ethanol and hexane as solvents. The results revealed that all studied species showed high presence of Carbohydrates, proteins alkaloids and terpenoids. All three solvents showed different degree of phytochemical extraction as presence of terpenoids, cardiac glycosides and alkaloids was seen only in hexane. The extracts were found to have different levels of antioxidant activity in the systems tested. Highest DPPH radical scavenging activities were found in methanol extracts of *Cucumis melo* (muskmelon) with IC_{50} value 0.44mg/ml followed by *Citrullus lanatus* (Watermelon) with IC_{50} value of 0.47 mg/ml. According to results, methanol was considered to be the suitable solvent for extracting antioxidants whereas hexane extracted more types of phytochemicals than other solvents.

Zang *et al*. (2013) studied the effects of polysaccharide from pumpkin on biochemical indicator and pancreatic tissue of the diabetic rabbits. A water-soluble polysaccharide (PCE-CC) was obtained from pumpkin which belongs to the family Cucubitaceae by the water and ethanol extract, organic solvent fractional extraction and deproteinization. Alloxan-induced diabetic rabbits were injected with PCE-CC for 21 days to assess effects on islet tissue morphology. After 21 days, the weights of the alloxan-induced diabetic and non-diabetic rabbits fed with diet

contained PCE-CC were significantly increased as compared to the negative group. The data of blood glucose (BG), total cholesterol (TC), total triglyceride (TG) and glycosylated hemoglobin (HbA1c) indicated that PCE-CC had beneficial effects on the improvement in the control of blood glucose, serum lipid and glycosylated hemoglobin levels. Observing the pancreatic tissue of the diabetic rabbits revealed that PCE-CC could promote the regeneration of damaged pancreatic islets by stimulating cell proliferation, which was accompanied by a decrease in plasma glucose levels. PCE-CC was further separated and purified to obtain PCE-CCH by ion exchange and gel chromatography. PCE-CCH was a heteropolysaccharide and consisted of glucose, galactose, arabinose, rhamnose and little amount of hexuronic acid, with a molecular weight of 1.15×10^5 Da.

Latha and Kolavali (2016) studied the effect of the methanolic extract of Cucurbita maxima on blood pressure of both normotensive and hypertensive (egg-feed and glucose- induced) rats. The effect of the extract on systolic, diastolic, mean blood pressures and heart rate were evaluated by using non-invasive blood pressure measurement apparatus (NIBP). The extract at doses of 200 and 400 mg/kg (p.o) exhibited a significant decrease in blood pressure and heart rate of normotensive rats. While at the dose of 200mg/kg (p.o) it produced less significant effect than 400 mg/kg (p.o). The 400mg/kg of the extract produced a highly significant effect was selected for antihypertensive effect in egg feed and glucose treated hypertensive rats. A significant antihypertensive was observed at 400 mg/kg (p.o) in both hypertensive models. In addition, a non -significant decrease in ALT, AST, and ALP but significant decrease in total cholesterol, triglycerides, LDL and increase in HDL levels were observed in the serum of the extract treated animals.

Song et al. (2013) studied the effect of acetylation on antioxidant and cytoprotective activity of polysaccharides isolated from pumpkin (Cucurbita pepo, lady godiva). Acetylation of pumpkin (Cucurbita pepo, lady godiva variety) polysaccharide using acetic anhydride with pyridines as catalyst under different conditions was conducted to obtain different degrees of acetylationon a laboratory scale. Furthermore, antioxidant activities and cytoprotective effects of pumpkin polysaccharide and its acetylated derivatives were investigated employing various

established in vitro systems. Results showed that addition of pyridine as catalyst could increase the degree of substitution, whereas volume of acetic anhydride had little effect. The acetylated polysaccharides in DPPH scavenging radical activity assay, superoxide anion radical activity assay and reducing power assay exhibited higher antioxidant activity than that of unmodified polysaccharide. H_2O_2-induced oxidative damages on rat thymic lymphocyte were also prevented by pumpkin polysaccharide and its acetylated derivatives and the derivatives presented higher protective effects. On the whole, acetylated polysaccharide showed relevant antioxidant activity both in vitro and in a cell system.

Zhang *et al*. (2016) studied antioxidant acitivity in vitro and in vivo of polysaccharide isolated from pumpkin (PP-e). Determination of antioxidant activity of PP-e in vitro, the antioxidant activity study employing DPPH· free radical-scavenging activity test, superoxide radical-scavenging activity test and hydroxyl radical scavenging activity test: Determination of antioxidant activity of PP-e in vivo: Animal selection and experimental design: A total of 18 mice were equally divided into 3 groups randomly including normal control group (NCG) were given 15 mL/kg (body weight) physiological saline solution (0.9% w/v), alloxan model control group (ACG) and PP-e group were given physiological saline solution and PP-e by a rapid intravenous injection, respectively. In vitro antioxidant assay, PP-e showed strong inhibition of superoxideradical, hydroxyl radical and DPPH radical. For antioxidant testing in vivo, PP-e was administrated by intraperitoneal injection with the dosage of 150 mg/kg in alloxan-induced mice model. PP-e could significantly inhibit the formation of malondialdehyde and nitric oxide in mice livers and raised the activities of antioxidant enzymes in mice livers and serums.

2.3. Diabetic mellitus

2.3.1 Definition of diabetic mellitus

Diabetes mellitus (DM) describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.

Several pathogenetic processes are involved in the development of diabetes. These include processes which destroy the beta cells of the pancreas with consequent insulin deficiency, and others that result in resistance to insulin action. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin (America Diabetes Association, 2010).

The effects of diabetes mellitus include long– term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma, in absence of effective treatment, and death. Often symptoms are not severe, or may be absent, and consequently hyperglycemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease.

2.3.2 Etiologic types

The type of diabetes is based on the presumed etiology. The two most common types of diabetes: are type 1 and type 2 diabetes. (Grant *et al*., 2009)

Type 1 diabetes, is caused by a lack of insulin due to the destruction of insulin-producing beta cells in the pancreas. In type 1 diabetes an autoimmune disease, the body's immune system attacks and destroys the beta cells. Normally, the immune system protects the body from infection by identifying and destroying bacteria, viruses, and other potentially harmful foreign substances. But in autoimmune diseases, the immune system attacks the body's own cells. Beta cell destruction may take place over several years, but symptoms of the disease usually develop over a short period of time.

Type 1 diabetes typically occurs in children and young adults, though it can appear at any age. In the past, type 1diabetes was called juvenile diabetes or insulin-dependent diabetes mellitus. Latent autoimmune diabetes in adults (LADA) may be a

slowly developing kind of type 1 diabetes. Diagnosis usually occurs after age 30. In LADA, as in type 1 diabetes, the body's immune system destroys the beta cells. At the time of diagnosis, people with LADA may still produce their own insulin, but eventually most will need insulin shots or an insulin pump to control blood glucose levels.

Type 2 diabetes, the most common form of diabetes, is caused by a combination of factors, including insulin resistance, a condition in which the body's muscle, fat, and liver cells do not use insulin effectively. Type 2 diabetes develops when the body can no longer produce enough insulin to compensate for the impaired ability to use insulin. Symptoms of type 2 diabetes may develop gradually and can be subtle. Some people with type 2 diabetes remain undiagnosed for years.

Type 2 diabetes develops most often in middle-aged and older people who are also overweight or obese. The disease, once rare in youth, is becoming more common in overweight and obese children and adolescents. Scientists think genetic susceptibility and environmental factors are the most likely triggers of type 2 diabetes.

2.3.3 Diagnostic criteria

2.3.3.1 Change in diagnostic value for fasting plasma/blood glucose concentrations

The major change recommended in the diagnostic criteria for diabetes mellitus is the lowering of the diagnostic value of the fasting plasma glucose concentration to 7.0 mmol l^{-1} (126 mg dl^{-1}) and above (WHO, 1985), from the former level of 7.8 mmol l^{-1} (140 mg dl^{-1}) and above. For whole blood the proposed new level is 6.1 mmol l^{-1} (110 mg dl^{-1}) and above, from the former 6.7 mmol l^{-1} (120 mg dl^{-1}).

The new fasting criterion is chosen to represent a value which is at the upper end of the range that corresponds in diagnostic significance in many persons to that of the 2–h post–load concentration, which is not changed.

2.3.3.2 Epidemiological studies

For population studies of glucose intolerance and diabetes, individuals have been classified by their blood glucose concentration measured after an overnight fast and/or 2 h after a 75 g oral glucose load. Since it may be difficult to be sure of the

fasting state, and because of the strong correlation between fasting and 2–h values, epidemiological studies or diagnostic screening have in the past been restricted to the 2–h values only. Whilst this remains the single best choice, if it is not possible to perform the OGTT (e.g. for logistical or economic reasons), the fasting plasma glucose alone may be used for epidemiological purposes. It has now been clearly shown, however, that some of the individuals identified by the new fasting values differ from those identified by 2–h post glucose challenge values (Franklin *et al*., 2006). The latter include the elderly and those with less obesity, such as many Asian populations. On the other hand, middle-aged, more obese patients are more likely to have diagnostic fasting values. Overall population prevalence may or may not be found to differ when estimates using fasting and 2–h values are compared (Franklin *et al*., 2006; Amiel *et al*., 2002).

2.3.4 Insulin

The function of insulin (Magkos *et al*., 2010)

Insulin is the major anabolic hormone whose action is essential for appropriate tissue development, growth, and maintenance of glucose homeostasis. Insulin is secreted by the pancreatic βcells in response to increased circulating levels of glucose and amino acids after a meal. Insulin suppresses hepatic glucose production (via decreased gluconeogenesis and glycogenolysis), stimulates glucose uptake in muscle, suppresses adipose tissue lipolysis and fatty acid release from adipose tissue into the blood stream, suppresses hepatic apolipoprotein B-100 and triglyceride secretion, reduces lipoprotein lipase activity in adipose tissue, and inhibits protein breakdown. Adequate insulin action on adipose tissue lipolysis prevents fatty acid-induced insulin resistance in β-cells, muscle, and the liver.

a) Increased glycogen synthesis, insulin forces storage of glucose in liver (and muscle) cells in the form of glycogen; lowered levels of insulin cause liver cells to convert glycogen to glucose and excrete it into the blood. This is the clinical action of insulin which is directly useful in reducing high blood glucose levels as in diabetes.

b) Increased fatty acid synthesis, insulin forces fat cells to take in blood lipids which are converted to triglycerides; lack of insulin causes the reverse.

c) Increased esterification of fatty acids that forces adipose tissue to make fats (i.e. triglycerides) from fatty acid esters; lack of insulin causes the reverse.

d) Decreased proteolysis resulting in decreasing the breakdown of protein.

e) Decreased lipolysis which forces reduction in conversion of fat cell lipid stores into blood fatty acids; lack of insulin causes the reverse.

f) Decreased gluconeogenesis which decreases production of glucose from non- sugar substrates, primarily in the liver (The vast majority of endogenous insulin arriving at the liver never leaves the liver); lack of insulin causes glucose production from assorted substrates in the liver and elsewhere.

g) Increased amino acid uptake which forces cells to absorb circulating amino acids; lack of insulin inhibits absorption.

2.3.5 Causes of Diabetes (Scottish Intercollegiate Guidelines Network (SIGN), 2010)

2.3.5.1 Type 1 diabetes

1. Genetic Susceptibility

Heredity plays an important part in determining who is likely to develop type 1 diabetes. Genes are passed down from biological parent to child. Genes carry instructions for making proteins that are needed for the body's cells to function. Many genes, as well as interactions among genes, are thought to influence susceptibility to and protection from type 1 diabetes. The key genes may vary in different population groups. Variations in genes that affect more than 1 percent of a population group are called gene variants. Certain gene variants that carry instructions for making proteins called human leukocyte antigens (HLAs) on white blood cells are linked to the risk of developing type 1 diabetes. The proteins produced by HLA genes help determine whether the immune system recognizes a cell as part of the body or as foreign material. Some combinations of HLA gene variants predict that a person will be at higher risk for type 1 diabetes, while other combinations are protective or have no effect on risk. While HLA genes are the major risk genes for type 1 diabetes, many additional risk genes or gene regions have been found. Not only can these genes help identify people at risk for

type 1 diabetes, but they also provide important clues to help scientists better understand how the disease develops and identify potential targets for therapy and prevention. Genetic testing can show what types of HLA genes a person carries and can reveal other genes linked to diabetes. However, most genetic testing is done in a research setting and is not yet available to individuals. Scientists are studying how the results of genetic testing can be used to improve type 1 diabetes prevention or treatment.

2. Autoimmune Destruction of Beta Cells

In type 1 diabetes, white blood cells called T cells attack and destroy beta cells. The process begins well before diabetes symptoms appear and continues after diagnosis. Often, type 1 diabetes is not diagnosed until most beta cells have already been destroyed. At this point, a person needs daily insulin treatment to survive. Finding ways to modify or stop this autoimmune process and preserve beta cell function is a major focus of current scientific research. Recent research suggests insulin itself may be a key trigger of the immune attack on beta cells. The immune systems of people who are susceptible to developing type 1 diabetes respond to insulin as if it were a foreign substance, or antigen. To combat antigens, the body makes proteins called antibodies. Antibodies to insulin and other proteins produced by beta cells are found in people with type 1 diabetes. Researchers test for these antibodies to help identify people at increased risk of developing the disease. Testing the types and levels of antibodies in the blood can help determine whether a person has type 1 diabetes, LADA, or another type of diabetes.

3. Environmental Factors

Environmental factors, such as foods, viruses, and toxins, may play a role in the development of type 1 diabetes, but the exact nature of their role has not been determined. Some theories suggest that environmental factors trigger the autoimmune destruction of beta cells in people with a genetic susceptibility to diabetes. Other theories suggest that environmental factors play an ongoing role in diabetes, even after diagnosis.

4. Viruses and infections

A virus cannot cause diabetes on its own, but people are sometimes diagnosed with type 1 diabetes during or after a viral infection, suggesting a link

between the two. Also, the onset of type 1 diabetes occurs more frequently during the winter when viral infections are more common. Viruses possibly associated with type 1 diabetes include coxsackievirus B, cytomegalovirus, adenovirus, rubella, and mumps. Scientists have described several ways these viruses may damage or destroy beta cells or possibly trigger an autoimmune response in susceptible people. For example, anti-islet antibodies have been found in patients with congenital rubella syndrome, and cytomegalovirus has been associated with significant beta cell damage and acute pancreatitis-inflammation of the pancreas. Scientists are trying to identify a virus that can cause type 1 diabetes so that a vaccine might be developed to prevent the disease.

2.3.5.2 Type 2 diabetes

1. Genetic Susceptibility

Genes play a significant part in susceptibility to type 2 diabetes. Having certain genes or combinations of genes may increase or decrease a person's risk for developing the disease. The role of genes is suggested by the high rate of type 2 diabetes in families and identical twins and wide variations in diabetes prevalence by ethnicity. Type 2 diabetes occurs more frequently in African Americans, Alaska Natives, American Indians, Hispanics/Latinos, and some Asian Americans, Native Hawaiians, and Pacific Islander Americans than it does in non-Hispanic whites. Recent studies have combined genetic data from large numbers of people, accelerating the pace of gene discovery. Though scientists have now identified many gene variants that increase susceptibility to type 2 diabetes, the majority have yet to be discovered. The known genes appear to affect insulin production rather than insulin resistance. However, even in those with the variant, diet and physical activity leading to weight loss help delay diabetes, according to the Diabetes Prevention Program (DPP), a major clinical trial involving people at high risk. Genes can also increase the risk of diabetes by increasing a person's tendency to become overweight or obese. One theory, known as the "thrifty gene" hypothesis, suggests certain genes increase the efficiency of metabolism to extract energy from food and store the energy for later use. This survival trait was advantageous for populations whose food supplies were scarce or unpredictable and could help keep people alive during famine. In modern times,

however, when high-calorie foods are plentiful, such a trait can promote obesity and type 2 diabetes.

2. Obesity and Physical Inactivity

Physical inactivity and obesity are strongly associated with the development of type 2 diabetes. People who are genetically susceptible to type 2 diabetes are more vulnerable when these risk factors are present. An imbalance between caloric intake and physical activity can lead to obesity, which causes insulin resistance and is common in people with type 2 diabetes. Central obesity, in which a person has excess abdominal fat, is a major risk factor not only for insulin resistance and type 2 diabetes but also for heart and blood vessel disease, also called cardiovascular disease (CVD). This excess “belly fat” produces hormones and other substances that can cause harmful, chronic effects in the body such as damage to blood vessels.

3. Insulin Resistance

Insulin resistance is a common condition in people who are overweight or obese, have excess abdominal fat, and are not physically active. Muscle, fat, and liver cells stop responding properly to insulin, forcing the pancreas to compensate by producing extra insulin. As long as beta cells are able to produce enough insulin, blood glucose levels stay in the normal range. But when insulin production falters because of beta cell dysfunction, glucose levels rise, leading to prediabetes or diabetes.

4. Abnormal Glucose Production by the Liver

In some people with diabetes, an abnormal increase in glucose production by the liver also contributes to high blood glucose levels. Normally, the pancreas releases the hormone glucagon when blood glucose and insulin levels are low. Glucagon stimulates the liver to produce glucose and release it into the bloodstream. But when blood glucose and insulin levels are high after a meal, glucagon levels drop, and the liver stores excess glucose for later, when it is needed. For reasons not completely understood, in many people with diabetes, glucagon levels stay higher than needed. High glucagon levels cause the liver to produce unneeded glucose, which

contributes to high blood glucose levels. Metformin, the most commonly used drug to treat type 2 diabetes, reduces glucose production by the liver.

5. Metabolic Syndrome

Metabolic syndrome, also called insulin resistance syndrome, refers to a group of conditions common in people with insulin resistance, including

a) blood glucose levels higher than normal

b) increased waist size due to excess abdominal fat

c) high blood pressure

d) abnormal levels of cholesterol and triglycerides in the blood

People with metabolic syndrome have an increased risk of developing type 2 diabetes and cardiovascular disease (CVD). Many studies have found that lifestyle changes, such as being physically active and losing excess weight, are the best ways to reverse metabolic syndrome, improve the body's response to insulin, and reduce risk for type 2 diabetes and CVD.

6. Beta Cell Dysfunction

Scientists think beta cell dysfunction is a key contributor to type 2 diabetes. Beta cell impairment can cause inadequate or abnormal patterns of insulin release. Also, beta cells may be damaged by high blood glucose itself, a condition called glucose toxicity. Scientists have not determined the causes of beta cell dysfunction in most cases. Single gene defects lead to specific forms of diabetes called maturity-onset diabetes of the young (MODY). The genes involved regulate insulin production in the beta cells. Although these forms of diabetes are rare, they provide clues as to how beta cell function may be affected by key regulatory factors. Other gene variants are involved in determining the number and function of beta cells. But these variants account for only a small percentage of type 2 diabetes cases. Malnutrition early in life is also being investigated as a cause of beta cell dysfunction. The metabolic environment of the developing fetus may also create a predisposition for diabetes later in life.

2.3.6 Signs and symptoms (Grant *et al*., 2009)

Early warning signs for type 1 and type 2 Diabetes a blood glucose level should be checked if one or more of these symptoms is present:

Increased urination

Increased thirst

Increased appetite

Unexplained weight loss

2.3.7 Managements and control of diabetic mellitus

Medical nutrition treatment guidelines for medical nutrition therapy (American Diabetes Association, 2012) have been established by the American Dental Association (ADA). The primary focus of these guidelines is targeted to improve outcomes including glycemic control, weight reduction (as appropriate), blood pressure control, and a favorable lipid profile. There is clear evidence that excess saturated fat in the diet has a detrimental effect on lipid profiles, and therefore restriction of saturated fat is recommended. The data supporting absolute restriction of carbohydrates are not robust, so the ADA guidelines allow flexibility in intake of carbohydrates and no saturated fat. Separate guidelines have been published about the carbohydrate content and composition of the diet.

The most important variable in prandial glycemic excursion is total carbohydrate intake. Low glycemic index foods consumed alone result in lower prandial glucose excursion than do high glycemic index foods. However, in the context of a mixed meal, differences between low and high glycemic index foods are attenuated. The amount and source of carbohydrates are important determinants of postprandial glucose levels (Gannon *et al*., 1989; Sheard *et al*., 2004).

Restriction of alcohol and sodium is generally advised. Nutritional supplements are not necessary in patients who are otherwise consuming a well-balanced diet. Many recommendations for weight management propose restriction of calories based on the degree of obesity and propose 30 to 45 minutes of exercise 3 to 5 days a week. Exercise is an important component of any regimen for weight reduction and glycemic control. Other nutritional guidelines for patients with diabetes are generally consistent with the ADA guidelines (Clark *et al*., 2000; Grundy *et al*., 2002).

2.3.7.1 Pharmacologic Treatment

1. Metformin

Available since the late 1950s, metformin can trace its roots back to medieval Europe, where biguanides in the form of French lilac were used in diabetes

treatment. Its primary mechanism of action is suppression of hepatic glucose output, but it also enhances insulin sensitivity of muscle and fat. It affects primarily fasting glycaemia; however, some decreases in postprandial glucose concentrations, especially after the midday meal, can also be seen. Metformin is well tolerated, with the most common side effect being gastrointestinal (GI) complaints, such as diarrhea, nausea, abdominal discomfort, and a metallic taste. All of these improve with time and dose reduction. Metformin causes a small increase in basal and postprandial lactate concentrations in the blood, leading to potential to produce very rare but life-threatening lactic acidosis (<1 in 100,000). It is best to avoid use in patients with hepatic impairment. The use of metformin is contraindicated in patients with a serum creatinine 1.5 mg/dL or higher in male patients or 1.4 mg/dL or higher in female patients.

The major benefits of metformin are that it usually does not lead to hypoglycemia when used as monotherapy. It can lead to weight loss, and it has been shown to decrease plasma triglycerides concentration (10%-20%). Dosing is typically twice daily; however, it can be dosed three times daily or once daily (extended release). The typical starting dose is 500 mg daily. The maximum dose is 2,550 mg/day. Gradual titration of metformin, starting at 500 mg with breakfast and increasing by 500 mg in weekly intervals until a dose of 1,000 mg with breakfast and dinner is reached help to prevent GI side effects. (American Diabetes Association, 2009; Nathan *et al*., 2006)

2. Thiazolidinediones

Thiazolidinediones (TZDs) are agonists of peroxisome proliferator-activated receptor gamma (PPARγ) and primarily enhance sensitivity of muscle and fat, and mildly of the liver, to exogenous and endogenous insulin. TZDs lower fasting and postprandial blood glucose levels. Major side effects include weight gain, with an increase in subcutaneous adiposity, and fluid retention which typically manifests as peripheral edema, but heart failure has been shown to occur on occasion. These agents should be avoided in patients with functional class III or IV heart failure. These effects are mostly seen at higher doses. The PROactive trial (PROspective pioglitAzone Clinical Trial In macroVascular Events) showed that compared with placebo, pioglitazone does not increase cardiovascular risks. TZDs have been shown to have an association with an increased risk of fractures, particularly in women. The TZDs do not

cause hypoglycemia when used as monotherapy. Pioglitazone use leads to lowering triglycerides, increasing high-density lipoprotein cholesterol (HDL), and increasing the low-density lipoprotein cholesterol (LDL) particle size. Dosing is once a day. It takes 2 to 12 weeks for TZDs to become fully effective. For rosiglitazone, starting dose is 4 mg/day and maximum dose is 8 mg/day. For pioglitazone, the starting dose is 7.5 mg/day and the maximum dose is 45 mg/day (American Diabetes Association, 2009).

3. Glibenclamide

Glibenclamide, also known as glyburide (USAN), is an antidiabetic drug in a class of medications known as sulfonylureas, closely related to sulfonamide antibiotics. People with diabetes need treatment to control the amount of sugar in their blood. This is because good control of blood sugar levels reduces the risk of complications later on. Some people can control the sugar in their blood by making changes to the food they eat but, for other people, medicines like glibenclamide are given alongside the changes in diet. Glibenclamide works by increasing the amount of insulin that your pancreas produces. This helps to reduce the amount of sugar in your blood. The drug works by binding to and inhibiting the ATP-sensitive potassium channels (KATP) inhibitory regulatory subunit sulfonylurea receptor 1 (SUR1) (Serrano *et al*., 2006) in pancreatic beta cells. This inhibition causes cell membrane depolarization, opening voltage-dependent calcium channels. This results in an increase in intracellular calcium in the beta cell and subsequent stimulation of insulin release.

After a cerebral ischemic insult, the blood–brain barrier is broken and glibenclamide can reach the central nervous system. Glibenclamide has been shown to bind more efficiently to the ischemic hemisphere. Moreover, under ischemic conditions SUR1, the regulatory subunit of the KATP- and the NCCa-ATP-channels, is expressed in neurons, astrocytes, oligodendrocytes, endothelial cells and by reactive microglia (Ortega *et al*., 2012).

4. Sulfonylureas

Sulfonylureas lower fasting and postprandial glucose levels. Main adverse effects include weight gain (about 2 kg upon initiation) and hypoglycemia. The hypoglycemia episodes can be significant (leading to need for assistance, coma, or seizure) and are seen more often in the elderly. The benefits include a 25% reduction in

microvascular complications with or without insulin found by a UKPDS trial. Dosing is typically once or twice daily. Caution should be used in patients with liver or kidney dysfunction or patients who often skip meals. (American Diabetes Association, 2009; Nathan *et al*., 2006; Nathan, 2002)

5. Glinides

Glinides work in a manner similar to sulfonylureas; however, they have a more-rapid onset of action and a short duration of action, so they are a good option for patients with erratic timing of meals. They have a lower risk of hypoglycemia than sulfonylureas; they have a similar to lower risk of weight gain with initiation of therapy. Caution must be used in patients with liver dysfunction. Dosing is before meals. (American Diabetes Association, 2009; Fonseca *et al*., 2008)

6. Alpha Glucosidase Inhibitors

Alpha glucosidase inhibitors competitively block the enzyme alpha glucosidase in the brush borders of the small intestine, which delays absorption of carbohydrates (absorbed in the mid and distal portions of the small intestine instead). They primarily target postprandial hyperglycemia without causing hypoglycemia. GI complaints, such as bloating, abdominal cramps, flatulence, and diarrhea are the main side effects. Use should be avoided in patients with severe hepatic or renal impairment. Dosing must be prior to carbohydrate-containing meals. (American Diabetes Association, 2009; Fonseca *et al*., 2008; Nathan, 2002)

7. Incretins

Incretin-based therapies can be used as injections (GLP-1 analogs) or as pills (DPP-4 inhibitors). All incretin-based medications carry increased risk of acute pancreatitis. Patients must be warned about this risk and be advised to stop taking these medications and seek medical evaluation if acute abdominal pain develops. These medications should not be given to the individuals who have a history of medullary thyroid carcinomas or have multiple endocrine neoplasia syndrome type 2. This is because increased incidence of the thyroid C-cell tumors have been observed with these medications in the mice and rats. So far, there is no increased risk in humans but the above groups of individuals should not use these medication

2.3.8 Mechanisms of Glucose Lowering

2.3.8.1 The Roles of Insulin and Glucagon in Normal Blood Glucose Regulation (Scottish Intercollegiate Guidelines Network (SIGN), 2009)

A healthy person's body keeps blood glucose levels in a normal range through several complex mechanisms. Insulin and glucagon, two hormones made in the pancreas, help regulate blood glucose levels:

a) Insulin, made by beta cells, lowers elevated blood glucose levels.

b) Glucagon, made by alpha cells, raises low blood glucose levels. When blood glucose levels rise after a meal, the pancreas releases insulin into the blood.

c) Insulin helps muscle, fat, and liver cells absorb glucose from the bloodstream, lowering blood glucose levels.

d) Insulin stimulates the liver and muscle tissue to store excess glucose. The stored form of glucose is called glycogen.

e) Insulin also lowers blood glucose levels by reducing glucose production in the liver. When blood glucose levels drop overnight or due to a skipped meal or heavy exercise, the pancreas releases glucagon into the blood.

f) Glucagon signals the liver and muscle tissue to break down glycogen into glucose, which enters the bloodstream and raises blood glucose levels.

g) If the body needs more glucose, glucagon stimulates the liver to make glucose from amino acids.

h) Insulin and glucagon help regulate blood glucose levels.

i) Alpha-glucosidase inhibitors

Alpha–glucosidase inhibitors are oral glucose-lowering agents that specifically inhibit alpha-glucosidases in the brush border of the small intestine. These enzymes are essential for the release of glucose from more complex carbohydrates. The evidence for alpha-glucosidase inhibitors was obtained from three high quality systematic reviews and one further RCT (Bolen *et al*., 2007). The majority of data reviewed examined alpha-glucosidase inhibitors as monotherapy in the management of patients with type 2 diabetes. Few studies were long term in determining the impact of a therapy for a chronic condition. The largest evidence base for the use of alpha-glucosidase inhibitors is with acarbose. There are no peer-reviewed data available on

the long term effects of alpha-glucosidase inhibitors in terms of mortality, morbidity and quality of life (Pan *et al*., 2008).

2.4 Free radical

2.4.1 Introduction to free radical

Radicals (often referred to as free radicals) are atoms (e.g. oxygen, nitrogen), molecules or ions with at least one unpaired electron in the outermost shell, and is capable of independent existence. A free radical is easily formed when a covalent bond between entities is broken and one electron remains with newly formed atom. Free radicals are highly reactive due to the presence of unpaired electron (Halliwell and Gutteridge, (2005). Any free radical involving oxygen can be referred to as reactive oxygen species (ROS). A major consequence of oxidative stress is damage to nucleic acid bases, lipids, and proteins, which can severely compromise cell functioning and viability or induce a variety of cellular responses through generation of secondary reactive species, ultimately leading to cell death by necrosis or apoptosis (Halliwell, 2001; Klaunig and Ka-mendulis, 2004).

2.4.2 Sources of free radicals

Free radicals come from two major sources: endogenous and exogenous. Endogenous free radicals are produced in the body by the following different mechanisms: Exogenous sources of free radicals include air pollution, of which industrial waste and cigarette smoke (both active and passive), radiation (from industry, sun exposure, cosmic rays, and medical X-rays). The trace metals, notably lead, mercury, iron and copper are also major sources of free radical generation. Alcohol, unsaturated fat and normal diets containing plant foods with large quantities of certain compounds such as phenols and even caffeine may contribute to the exogenous supply of oxidants to the body. The body produces several antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase, that neutralize many types of free radicals. In addition to enzymes, many vitamins and minerals act as antioxidants in their own right, such as vitamin C, vitamin E, beta-carotene, lutein,

vitamin B2, coenzyme Q10, and cysteine (an amino acid). Herbs, such as bilberry, turmeric (curcumin), grape seed or pine bark extracts, and ginkgo can also provide powerful antioxidant protection for the body.

2.4.3 Types of free radicals

Most free radicals are coming from oxygen atoms and are called reactive oxygen species (ROS), such as superoxide ion, hydroxyl radical, hydrogen peroxide and singlet oxygen (Yang *et al*., 2010). Superoxide ion (or reactive oxygen species) is an oxygen molecule with an extra electron. This free radical can cause damage to mitochondria DNA and other molecules. Human body can neutralize superoxide ions by using superoxide dismutase (Kim *et al*., 2010). Hydroxyl radical is formed by the reduction of an oxygen molecule in the electron transport chain. It is a neutral (not charged) form of the hydroxide ion. Hydroxyl radicals are highly reactive and form an important part of radical biochemistry. Unlike superoxide, the hydroxyl radical cannot be eliminated by an enzymatic reaction. It has a very short half-life and will only react with molecules its vicinity. Because of its high reactivity it will damage most organic molecules such as carbohydrates, DNA, lipids and proteins (Luo *et al*., 2009). Singlet oxygen is formed by immune system. Singlet oxygen causes oxidation of LDL cholesterol. Hydrogen peroxide is not a free radical but it is involved in the production of many reactive oxygen species. It is a by product of oxygen metabolism and is neutralized by peroxidases. Sometimes reactive nitrogen atoms are involved and these free radicals grouped under reactive nitrogen species (RNS). Nitric acid is the most important RNS. Some transitional metals, such as iron and copper, have many numbers of unpaired electrons and can also act as free radicals. These metals do not have strong electron affinity but can easily accept and donate electrons (Yabuta *et al*., 2010). The example of the free radicals are listed in Table 2.1

Table 2.1 Radicals and related substances (Chattopadhyay *et al*., 2008).

Radicals	Related substance
Reactive oxygen species (ROS)	
Alkoxyl ($RO^{\bullet}$)	Organic peroxides (ROOH)
Carbon dioxide ($CO_2^{-\bullet}$)	Peroynitrous acid (ONOOH)
Carbonate ($CO_3^{-\bullet}$)	Peroxynitrite ($ONOO^-$)
Hydroperoxyl ($HO_2^{\bullet}$)	Hypochlorous acid (HOCl)
Hydroxyl ($HO^{\bullet}$)	Hypobromous acid (HOBr)
Peroxyl ($RO_2^{\bullet}$)	Singlet oxygen ($^1O_2$)
Superoxide, Superoxide anion ($O_2^{-\bullet}$)	H_2O_2, Ozone (O_3)
Reactive nitrogen species (RNS)	
Nitric oxide ($NO^{\bullet}$)	Nitrous acid (HNO_2)
Nitrogen dioxide ($NO_2^{\bullet}$), ($NO_2^{-\bullet}$)	Nitrosyl cation (NO^+), Nitroxyl anion (NO^-)
Dinitrogen tetroxide (N_2O_4)	Dinitrogen trioxide (N_2O_3)
	Peroxynitrite ($ONOO^-$)
	Peroynitrous acid (ONOOH)

2.5 Antioxidant

2.5.1 Introduction to antioxidant

A substance that reduces damage due to oxygen, such as that caused by free radicals. Well-known antioxidants include enzymes and other substances, such as vitamin C, vitamin E, and beta carotene, which are capable of counteracting the damaging effects of oxidation**.** The main function of antioxidant is radical scavengers, and helps in converting the radicals to less reactive species. A variety of antioxidant is found in dietary sources like fruits, vegetables and tea, etc. (Mandal *et al*, 2009).

2.5.2 Sources of antioxidant

In general, antioxidant divided into 2 main groups: natural and synthetic antioxidants.

2.5.2.1 Natural antioxidants

Sources of natural antioxidant are mainly found in plant (fruits, vegetables), animal or microorganism (Van Acker *et al*., 2000). Various types of the antioxidant have been reported. Among them, naturally occurring antioxidant can be grouped to high and low molecular weight. Plants have been proposed as main natural antioxidant sources (Hwang *et al*., 2006). The natural antioxidants occurred in all parts of plant (Rastogi *et al*., 2010). Previous works showed that many kinds of plant derived antioxidants were found in both non-enzymatic system such as vitamin C, α-tocopherol, tannin, gallic acid, catechin etc. and enzymatic system including superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) , ascorbate peroxidase (APX), glutathione reductase (GA) and polyphenol oxidase ect. (Nagayama *et al*., 2002). In current, the study on medicinal plant was considerly focused. This was due to the medicinal plants having been suggested about their phytochemical components. Until now, some plant derived antioxidants have been used for treatment of viral, bacterial, amoeboid (Luo *et al*., 2009) and diabetic diseases (Luka and Mohammed, 2012). The structures of some natural antioxidants are shown in Figure 2.1.

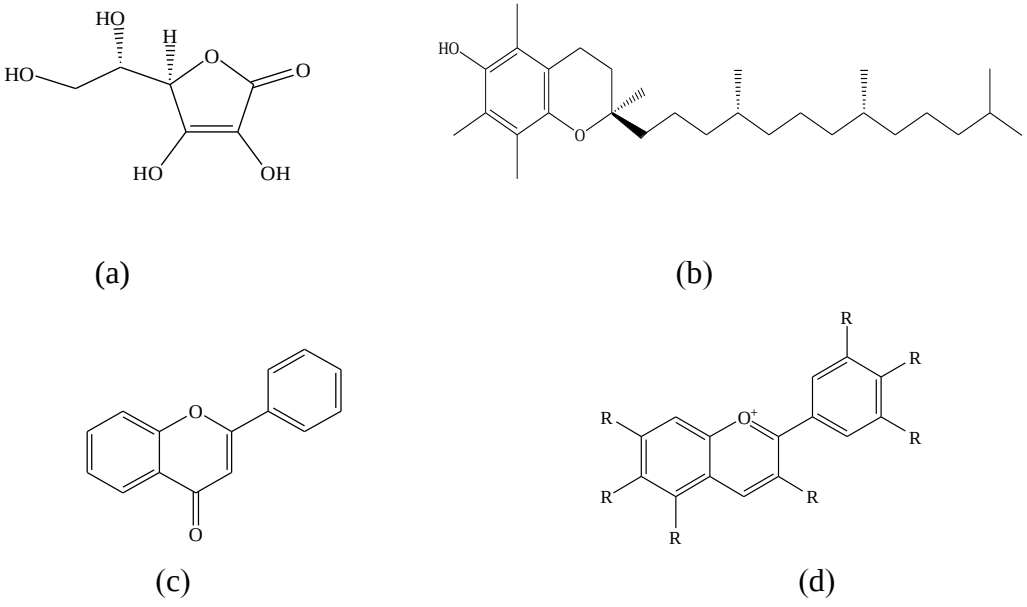

Figure 2.1: Chemical structures of natural antioxidants: (a) ascorbic acid, (b) α-tocopherol, (c) beta-carotene and (d) flavonoid

2.5.2.2 Synthetic antioxidants

Most synthetic antioxidants are chemically synthesized since they do not found in nature. Those of radical terminators are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), and gallates such as propyl gallate (PG), dodecyl gallate (DG) and octyl gallate (OG). The examples of oxygen scavengers are glucose oxidase and ascorbyl palmitate. Polyphosphatases and ethylene diamine tetra acetic acid (EDTA) are chelating agent.

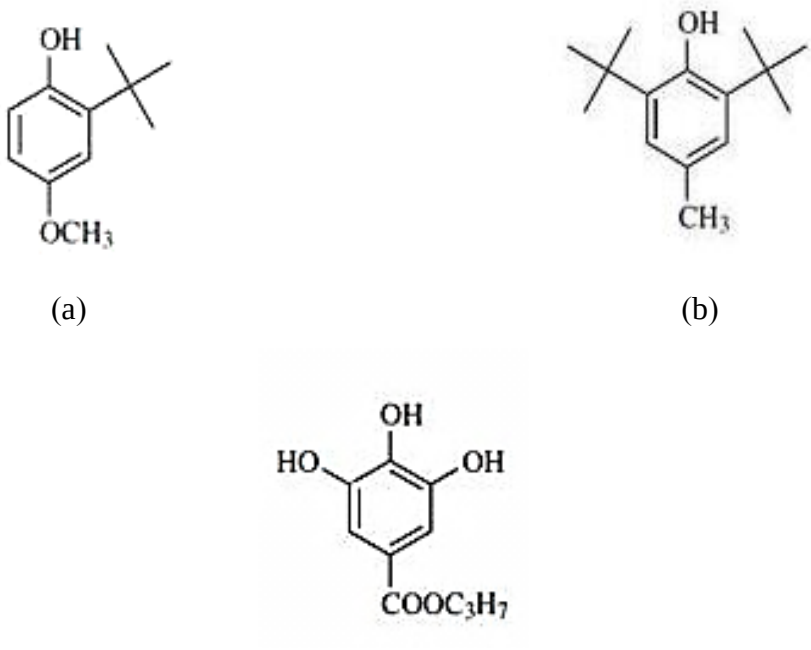

Figure 2.2 : Chemical structures of Synthetic antioxidants: (a) butylated hydroxyanisole (BHA), (b) butylated hydroxytoluene (BHT) and (c) propyl gallate (PG)

2.5.3 Assay for antioxidant activity

2.5.3.1. Diphenylpicrylhydrazyl (DPPH) radical.

The DPPH radical absorbs at 517 nm and in a second substrate-free system, antioxidant activity can be determined by monitoring the decrease in this absorbance. Results were reported as the EC_{50} that is the amount of antioxidant to decrease by 50% the initial DPPH concentration. The time taken to reach the steady

state to EC50 concentration (TEC50) was also calculated. In recognition of the effect of both parameters on antiradical capacity, a new parameter, namely antiradical efficiency, which combined both factors, was defined (Antolovich et al., 2002).

It is worth reiterating that the ABTS and DPPH methods are substrate-free. Their popularity can be attributed to simplicity and speed of analysis, but this is achieved at a potential price and the relevance of data generated with these procedures must be considered carefully.

2.5.3.2 ABTS assay

The assay measures ABTS.+ radical cation formation induced by metmyoglobin and hydrogen peroxide. Trolox [6-Hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid], a water soluble vitamin E analog, serves as a positive control inhibiting the formation of the radical cation in a dose dependent manner. The antioxidant activity in biological fluids, cells, tissues, and natural extracts can be normalized to equivalent Trolox units to quantify the composite antioxidant activity present.

A ferryl myoglobin radical is formed from metmyoglobin and hydrogen peroxide. The ferryl myoglobin radical can oxidize ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) to generate a radical cation, $ABTS^{\cdot+}$, that is green in color and can be measured by absorbance at 734 nm. Antioxidants suppress this reaction by electron donation radical scavenging and inhibit the formation of the colored ABTS radical. The concentration of antioxidant in the test sample is inversely proportional to the ABTS radical formation and 734 nm absorbance. (Antolovich *et al*., 2002).

2.5.3.3 FRAP assay

The ferric reducing antioxidant power (FRAP) method (Benzie *et al*., 1996) is based on the reduction of a ferroin analog, the Fe^{3+} complex of tripyridyltriazine Fe(TPTZ) $^{3+}$, to the intensely blue coloured Fe^{2+} complex $Fe(TPTZ)^{2+}$ by antioxidants in acidic medium. Results are obtained as absorbance increases at 593 nm and can be expressed as micromolar Fe^{2+} equivalents or relative to an antioxidant standard. The authors claim the method to be simple and rapid and both manual and automated procedures have been described. We are in agreement with Frankel and

Meyer, however, that the measured reducing capacity does not necessarily reflect antioxidant activity. It provides instead a very useful ‘total’antioxidant concentration, without measurement and summation of the concentration of all antioxidants involved. The method was originally applied to plasma but has been extended to other biological fluids, foods, plant extracts, juices, etc.

CHAPTER III

RESEARCH METHODS

3.1 Plant Materials

Fresh fruits of *C. moschata* Duch. were collected from natural resource in Kho Wang District, Yasothon Province and *C. maxima* Duch from local market in Nam Pong district, Khon Kaen Province, Northeast of Thailand. The specimen was identified and authenticated in the Department of Biology, Faculty of Science, Mahasarakham Univesity, Thailand. Voucher specimen (Code: MSU-SAMOS, MSU-SAMAX) of the plants has been deposited at the Department of Biology, Faculty of Science, Mahasarakham Univesity, Thailand.

3.2 Preparation of fruit pulp extracts

Fresh fruits of *C. moschata* and *C. maxima* were washed, peeled, cut into small pieces and dried in a hot air oven at 50 ^{o}C. The specimens were dried and powdered fruit (4,000 g) was separately suspended in distilled water at ratio of 1 g powder per 20 ml of water. The suspension of each sample was stirred on the water bath at 45 ^{o}C for 16 h before cooling, followed by centrifugation at 4,800 rpm for 25 min. The supernatant was concentrated to a quarter of the original volume by evaporation under reduced pressure at 45 ^{o}C filtered to remove any residues. The crude extract from *C. moschata* (PCMOS) and *C. maxima* (PCMAX) were precipitated from the filtrates by the addition of three volumes of ethanol (95%). The precipitated materials from pumpkin were collected by centrifugation at 4,800 rpm for 25 min and pellet was washed with ethanol and freeze-dried. Each preparation was then dissolved in distilled water (1 g/50 ml), dialysed against distilled water for 72 h at 4 ^{o}C, and freeze-dried. The precipitate was collected, freeze-dried and PCMOS, PCMAX of a brownish substance was obtained. The obtained fruit pulp extract as stored at 4 ^{o}C until be used. This method was modified procedure from Li *et al*. (2005).

The yield of the extract from *C. moschata* (PCMOS) and *C. maxima* (PCMAX) were 1.15 and 1.10% DW, respectively.

3.3 Measurement of total polysaccharide content

The total polysaccharide content was estimated by using the phenol-sulfuric analysis method (Dubois *et al*., 1956). A 2 mL aliquot of the extract solution is mixed with 1 mL of 5% aqueous solution of phenol in test tubes. Subsequently, 5 mL of concentrated sulfuric acid is added rapidly to the mixture. After allowing the test tube to stand for 10 min, they are vortexed for 30 s and placed for 20 min in a water bath at room temperature (27 ^{o}C) for color development. Then, light absorption at 490 nm is recorded on a spectrophotometer. Reference solutions are prepared in identical manner as above, except that the 2 mL aliquot of carbohydrate is replaced by DDI water. The phenol used in this procedure was redistilled and 5% phenol in water (w/w) was prepared immediately before the measurements.

3.4 Experimental animals

The animals used in this study were male albino Wistar rats weighing 200-250 g purchasing from the Laboratory Animal Centre Suranaree University of Technology, Thailand. The rats were acclimatized in an air conditioned room at 23±2 ^{o}C, 12-h light/12-h dark cycle and 50-55% relative humidity. They were given a standard chow and water ad *libitum* for 7 days prior to the commencing experiment. The rats were maintained in accordance with the guidelines of the Committee Care and Use of Laboratory Animal Resource, National Research Council Thailand, and performed in accordance with the advice of the Institutional Animal Care and Use Committee, Mahasarakham University, Thailand (Appoval number: 0020/2017).

3.5 Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of 65 mg/kg b.w. streptozotocin (Sigma Chemicals, St. Louis, MO) freshly prepared in cold 20 mM citrate buffer pH 4.5. After STZ injection, the rats were provided with 2% sucrose solution for 48 h to alleviate the discomfort after initiating the hypoglycemic phase. Three days after STZ injection, fasting blood glucose (FBG) of the rats were examined to confirm the diabetic stage. The rats with FBG at or higher than 126 mg/dL were used as diabetic rats in experimentation (Talubmook, 2008).

3.6 Antidiabetic activity study

3.6.1 Experimental designs

The rats were randomly divided into 5 groups with 6 rats in each

Group 1 (normal control rats): treated with 0.5% Tween 80

Group 2 (diabetic control rats): treated with 0.5% Tween 80

Group 3 (diabetic rat): treated with PCMOS (500, mg/kg b.w.)

Group 4 (diabetic rats): treated with PCMAX (500, mg/kg b.w.)

Group 5: (standard): treated with glibencamide (0.5, mg/kg b.w.)

PCMOS and PCMAX were suspended in 0.5% Tween 80 and treated orally once daily to the rats for 6 weeks using an orogastric tube. The volume of administration was 10 ml/kg. b.w.

3.6.2 Determination of blood glucose level and body weight

Fasting blood glucose level of each animal was monitored weekly for 6 weekly. A drop of fasting blood glucose collecting from the tail vein of each rat was measured using Accu-chek Adventage II (Roche, Germany). Inital and final body weight was recorded.

3.6.3 Determination of biochemical values, hematological values, serum insulin, lipid profiles and histological study of pancreatic tissues

After 6 weeks of treatment, the rats were fasted overnight and sacrificed by an over dose of chloroform. The blood samples were then drawn from the rat hearts for the determination of biochemical values for the renal function t and hapatic function, hematological values, serum insulin, and lipid profiles using an automatic blood chemical analyzer (BT 2000 plus, Germany). Pancreas was dissected from each rat for histological study.

3.6.3.1 Biochemical values

3.6.3.1.1 Biochemical values for renal function

Biochemical values for renal function including total protein (TP), blood urea nitrogen (BUN), creatinine (Crea) were determined.

3.6.3.1.2 Biochemical values for hepatic function

Biochemical values for hepatic function including aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined.

3.6.3.2 Hematological values

Hematological values including White blood cells (WBC), Red blood cell (RBC), Hemoglobin (Hb), Hematocrit (Hct), Neutrophil (Neu), Lymphocyte (Lym), Monocyte (Mono), and Platelet (Plt) were determined.

3.6.3.3 Lipid profiles

Lipid profile including Triglyceride (TG), Cholesterol (CHO), High-density lipoprotein (HDL) and Low- density lipoprotein (LDL) were determined.

3.6.3.4 Serum insulin

The serum insulin levels were measured after the centrifugation of the blood at 3000 rpm for 15 min and detected using an automatic gamma counter (Wallac 1470 Wizard; Perkin Elmer Instrument; Über-lingen, Germany).

3.6.3.5 Histological feature of pancreatic tissues

The pancreas was fixed in 10% formaldehyde and embedded in paraffin. The specimens in paraffin blocks were cut using a microtome. Sections of approximately 4 μm thickness were stained with hematoxylin and eosin (H&E). The

histopathological changes in the stained sections were observed under a light microscope Leica DM 750 (Leica Microsystems (SEA) Pte Ltd, Singapore). The histological feature of the pancreatic tissues such as the shape and size of Islet of Langerhans among the treatment groups was observed under the light microscope and compared.

3.7 Antioxidant activity study

3.7.1 DPPH free radical scavenging assay

Free radical scavenging activity of the fruit pulps extract from PCMOS and PCMAX were determined by using a stable 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay following a modified method by Krasaetep (2012). The method consisted of spectrophotometric measurement of the intensity of the color change in solution depending on the amount of DPPH. The 0.1, 0.2, 0.4, 0.6, 0.8, and 1 mg/mL of the fruit pulps extracts (PCMOS and PCMAX) were mixed with 3.0 mL of 0.1 mM DPPH solution. The mixture was shaken well and incubated at room temperature for 30 min at dark place. An absorbance was measured at 517 nm using UV-Visible spectrophotometer. The antioxidant activity of PCMOS and PCMAX was calculated as follows:

$$\% \text{ inhibition} = \left(\frac{\text{A control - A sample}}{\text{A control}}\right) \text{ x } 100$$

$A_{control}$ = absorbance of control

A_{sample} = absorbance of sample

The ascorbic acid which was used a reference standard of DPPH scavenging activity assay. The antioxidant activity, IC_{50}, defined as the concentration of sample showing 50% DPPH radical scavenging activity was determined. IC_{50} of Ascorbic acid and these extracts were estimated from a graph of concentration vs. % inhibition.

3.7.2 Ferric reducing antioxidant power (FRAP) assay

FRAP assay was conducted with a modified method described by Benzie and Strain (1996). The 100 µL of 10 mg/mL of the fruit pulps extract from PCMOS and PCMAX solution were mixed with 3 mL of FRAP solution (300 mM acetate buffer (pH 3.6): 10 mM tripyridyltriazine solution: 20 mM ferric chloride solution (in 40 mM HCl) of 10: 1: 1 by volume) and 300 µL of deionized water, then incubated at 37 ˚C for 4 min. The absorbance was then measured at 593 nm using an UV-Visible spectrophotometer. The results were expressed as millimolar ferrous sulphate per grams of dry weight (mM Fe (II) g^{-1} of DW).

3.8 Determination of phytochemical components

3.8.1 Total phenolic content

The total phenolic contents were spectrophotometric method using the Folin-Ciocalteu reagent according to the modification method of Bonli *et al*. (2004). Fifty microliters of of the fruit pulps extract each solution was (10 mg/mL) was mixed with 1.5 mL of 10% Folin-Ciocalteu reagent (diluted 10 folds with distilled water). The mixture solution incubated at room temperature for 15 min, and then 1.5 mL of 10% (w/v) sodium carbonate solution was added. The mixture was shaken and incubated at room temperature for 15 min. The absorbance of all samples was measured at 750 nm using an UV-Visible spectrophotometer. The total phenolic content was analyzed against gallic acid standard calibration curve with triplicate and averages of values content. The results were expressed as milligrams of gallic acid equivalents (mg GAE) per gram of dry weight (g DW).

3.8.2 Total flavonoid content

The total flavonoid content was determined according to the modified method of Yang *et al*. (2009). The 250 µL of 10 mg/mL of the fruit pulps extract olutions were mixed with 1.25 mL of deionized water, 75 µL of 5% sodium nitrite ($NaNO_2$) solution and allowed to stand for 5 min at room temperature. One hundred and fifty microliters of 10% aluminium chloride ($AlCl_3$) was added to the mixture solution and allowed to react for 6 min at room temperature. The 500 µL of 1 M sodium

hydroxide (NaOH) and 775 µL of distilled water was added to the mixture. The absorbance of all samples was immediately measured at 510 nm using an UV-Visible spectrophotometer. Total flavonoid content was calculated using the standard curve of (±)-catechin, and expressed as milligrams of catechin equivalents (mgCE) per gram of fresh weight (g FW).

3.9 Statistical analyses

Data were subjected to analysis using the Statistical Package for Social Sciences (SPSS), version 17.0. The results were expressed as the mean±SEM. Variable between groups was analyzed using One-way analysis of variance (One-way ANOVA) test. Subsequent multiple comparisons between the different groups were analyzed by using Duncan's Multiple comparison test. P-value of less than was 0.05 were considered to indicate a significant difference between treatments.

CHAPTER IV

RESULTS AND DISCUSSIONS

The present study was aimed to investigated the antidiabetic and antioxidant activities of the fruit pulps extract from *C. moschata* (PCMOS) and *C. maxima* (PCMAX). The results found in the study were shown as follow

4.1 Total polysaccharides content

Determination of total polysaccharides content using phenol-sulfuric analysis method (Dubois *et a*l., 1956) revealed that the total polysaccharides content from PCMOS and PCMAX were 33.41 and 36.99 %, respectively.

4.2 Antidiabetic activity

4.2.1 Body weight

At initial stage, the body weight of all rats groups were not different (Table 4.1).

The final body weight of normal controls was 301.50±21.28g, but it was significantly ($p<0.05$) decreased in the diabetic controls (361.33±10.95 g) compared to normal controls. The body weight of diabetic rats treated with PCMOS (354.16±4.74 g) and PCMAX (359.66±7.74 g) significantly ($p<0.05$) increased compared to diabetic controls. The body weight of diabetic rats treated with glibenclamide (354.16±18.96 g) also significantly ($p<0.05$) increased compared to diabetic controls. However, the body weight of diabetic rats treated with PCMOS and PCMAX and glibenclamide was not different (Table 4.1).

Table 4.1 Body weight of normal controls, diabetic controls, diabetic rats treated with PCMOS, diabetic rats treated with PCMAX and diabetic rats treated with glibencamide

Groups	Body weight (g)	
	initial	final
Normal controls	244.66±7.48[a]	361.33±10.95[b]
Diabetic controls	236.00±4.73[a]	301.50±21.28[a]
Diabetic + PCMOS 500 mg/kg	238.33±7.85[a]	354.16±4.74[b]
Diabetic + PCMAX 500 mg/kg	240.00±8.90[a]	359.66±7.74[b]
Diabetic + Glibencamide 0.5 mg/kg	238.66±9.56[a]	354.16±18.96[b]

The values represent the mean±SEM within the same column followed by the different superscript letters (a,b) are significantly different at $p<0.05$.

4.2.2 Fasting blood glucose levels

At initial stage, the fasting blood glucose level of normal control was 89.16±2.25 mg/dl, but it was significantly ($p<0.05$) higher in the diabetic controls (141.66±13.73 mg/dl), diabetic rats treated with PCMOS (138.83±10.40 mg/dl), PCMAX (139.66±15.89 mg/dl) and glibenclamide (140.66±18.27 mg/dl) compared to normal controls. However, the initial fasting blood glucose levels of diabetic rats treated with PCMOS, PCMAX and glibenclamide were not different (Table 4.2).

The final fasting blood glucose level of normal control was 83.00±1.70 mg/dl, but it was significantly ($p<0.05$) higher in the diabetic controls (186.66±25.70 mg/dl) compared to normal controls. The fasting blood glucose level of the diabetic rats treated with PCMOS (100.16±3.20 mg/dl), PCMAX (96.00±3.05 mg/dl) and glibenclamide (96.66±3.32) significantly ($p<0.05$) decreased compared to diabetic controls. However, the fasting blood glucose levels of diabetic rats treated with PCMOS, and PCMAX were not different and not different from that in the diabetic rats treated with glibenclamide (Table 4.2).

Table 4.2 Fasting blood glucose levels of normal controls, diabetic controls, diabetic rats treated with PCMOS, diabetic rats treated with PCMAX and diabetic rats treated with glibencamide

Groups	Fasting blood glucose levels (mg/dl)	
	initial	final
Normal controls	89.16 ± 2.25^{aA}	83.00 ± 1.70^{aA}
Diabetic controls	141.66 ± 13.73^{bB}	186.66 ± 25.70^{bA}
Diabetic + PCMOS 500 mg/kg	138.83 ± 10.40^{bB}	100.16 ± 3.20^{aA}
Diabetic + PCMAX 500 mg/kg	139.66 ± 15.89^{bA}	96.00 ± 3.05^{aB}
Diabetic + Glibencamide 0.5 mg/kg	140.66 ± 18.27^{bA}	96.66 ± 3.32^{aB}

The values represent the mean±SEM within the same column followed by the different superscript letters (a,b) are significantly different at $p<0.05$.

4.2.3 Serum insulin levels

The serum insulin level of normal control was 23.88±1.38 uIU/ml, but it was significantly ($p<0.05$) decreased in the diabetic controls (12.14±0.34 uIU/ml) compared to normal controls. The serum insulin level of diabetic rats treated with PCMAX was 16±3.20 uIU/ml significantly ($p<0.05$) increased compared to diabetic controls. The serum insulin level of the diabetic rats treated with PCMOS (13.00±0.20 uIU/ml) was also increased but not reach a significant difference compared to the diabetic controls. In addition, the serum insulin level of diabetic rats treated with glibenclamide (24.80±0.50 uIU/ml) significantly ($p<0.05$) increased compared to that in the diabetic controls, and closed to that in the normal controls (Table 4.3).

Table 4.3 Serum insulin levels of normal controls, diabetic controls, diabetic rats treated with PCMOS, diabetic rats treated with PCMAX and diabetic rats treated with glibencamide

Groups	Serum insulin (uIU/ml)
Normal controls	23.88±1.38[c]
Diabetic controls	12.14±0.34[a]
Diabetic + PCMOS 500 mg/kg	13.00±0.20[a]
Diabetic + PCMAX 500 mg/kg	16.60±0.06[b]
Diabetic + Glibencamide 0.5 mg/kg	24.80±0.50[c]

The values represent the mean±SEM within the same column followed by the different superscript letters (a,b,c) are significantly different at $p<0.05$.

4.2.4 Biochemical values for a determination of renal function

TP level of normal controls was 6.65.±0.81 g/dl, but it was significantly ($p<0.05$) decreased in the diabetic controls (5.65±0.32 g/dl) compared to that in the normal controls. TP levels of diabetic rats treated with PCMAX (6.73±0.43 g/dl) and PCMOS (6.40±0.60 g/dl) significantly ($p<0.05$) increased compared to diabetic controls. TP level of diabetic rats treated with glibenclamide (6.00±0.56 g/dl) was not different compared to diabetic controls. However, the TP levels of diabetic rats treated with PCMAX, PCMOS, and glibenclamide were not different (Table 4.4).

BUN and Crea levels of all rats were not different (Table 4.4).

Table 4.4 Biochemical values for renal function of normal controls, diabetic controls, diabetic rats treated with PCMOS, diabetic rats treated with PCMAX and diabetic rats treated with glibencamide

Groups	Biochemical values		
	TP (g/dl)	**BUN (mg/dl)**	**Crea (mg/dl)**
Normal controls	6.65.±0.81[b]	16.00±1.39[a]	0.58±0.13[a]
Diabetic controls	5.65±0.32[a]	15.00±1.47[a]	0.55±0.16[a]
Diabetic + PCMOS 500 mg/kg	6.73±0.43[b]	18.00±2.33[a]	0.53±0.12[a]
Diabetic + PCMAX 500 mg/kg	6.40±0.60[b]	20.66±2.91[a]	0.53±0.0[a]
Diabetic + Glibencamide 0.5 mg/kg	6.00±0.56[ab]	18.33±2.91[a]	0.53±0.12[a]

The values represent the mean±SEM within the same column followed by the different superscript letters (a,b) are significantly different at $p<0.05$.

TP = Total protein; BUN = Blood urea nitrogen; Crea = Creatinine

4.2.5 Biochemical values for a determination of hepatic function

AST and ALT levels of all rats were not different (Table 4.5).

ALP level of normal controls was 100.16±3.42 U/L, but it was significantly ($p<0.05$) increased in the diabetic controls (149.16.±25.29 U/L g/dl) compared to that in the normal controls. ALP levels of the diabetic rats treated with PCMAX (104.83±2.34 U/L) and PCMOS (95.83±2.73 U/L) significantly ($p<0.05$) decreased compared to that in the diabetic controls. ALP level of the diabetic rats treated with glibenclamide (93.00±3.99 U/L) significantly ($p<0.05$) decreased compared to that in the diabetic controls. However, the ALP levels of diabetic rats treated with PCMAX, PCMOS and glibenclamide were not different (Table 4.5).

Table 4.5 Biochemical values for a determination of hepatic function of normal controls, diabetic controls, diabetic rats treated with PCMOS, diabetic rats treated with PCMAX and diabetic rats treated with glibencamide

Groups	Biochemical values		
	AST (U/L)	**ALT (U/L)**	**ALP (U/L)**
Normal controls	126.16±3.36[a]	43.00±2.42[a]	100.16±3.42[a]
Diabetic controls	148.50±6.81[a]	50.83±3.32[a]	149.16.±25.29[b]
Diabetic + PCMOS 500 mg/kg	122.83±8.55[a]	46.00±6.53[a]	104.83±2.34[a]
Diabetic + PCMAX 500 mg/kg	130.66±11.20[a]	43.33±6.81[a]	95.83±2.73[a]
Diabetic + Glibencamide 0.5 mg/kg	139.33±10.42[a]	48.00±3.13[a]	93.00±3.99[a]

The values represent the mean±SEM within the same column followed by the different superscript letters (a,b) are significantly different at $p<0.05$.

AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; and ALP = Alkaline phosphatase

4.2.6 Hematological values

WBC, RBC, Hb, Hct, Neu, Lym and Mono of all rats groups were not different (Table 4.6).

Plt of normal controls was 76.45±22.61 $\times 10^6$cell/mm^3, but it was significantly ($p<0.05$) increased in the diabetic controls (90.28±11.21 $\times 10^6$cell/mm^3), diabetic rats treated with PCMAX (96.98±13.43 $\times 10^6$cell/mm^3), PCMOS (92.65±12.31 $\times 10^6$cell/mm^3) and glibenclamide (88.50±15.1$\times 10^6$cell/mm^3). Plt level of diabetic rats treated with PCMAX significantly ($p<0.05$) increased compared to that in the normal controls (Table 4.6)

Table 4.6 Hematological values of normal controls, diabetic controls, diabetic rats treated with PCMOS, diabetic rats treated with PCMAX and diabetic rats treated with glibencamide

Groups	Hematological values							
	RBC ($\times 10^6$ µL)	WBC ($\times 10^3$ cell/mm^3)	Hb (g/dl)	Hct (%)	Neu (%)	Lym (%)	Mono (%)	Plt ($\times 10^6$ cell/mm^3)
Normal controls	9.51±0.12^a	7.65±3.45^a	16.83±0.31^a	50.28±0.59^a	11.33±0.49^a	82.33±0.49^a	4.83±0.70^a	76.45±22.61^a
Diabetic controls	9.32±0.19^a	5.83±1.38^a	16.45±0.91^a	48.78±1.03^a	13.33±1.22^a	80.83±1.24^a	4.83±0.60^a	90.28±11.21^{ab}
Diabetic + PCMOS 500 mg/kg	9.71±0.15^a	8.90±2.22^a	16.86±0.67^a	49.48±1.17^a	15.00±1.93^a	80.00±2.69^a	3.66±0.84^a	92.65±12.31^{ab}
Diabetic + PCMAX 500 mg/kg	9.80±0.17^a	8.10±2.31^a	16.80±0.51^a	48.71±0.83^a	13.16±1.79^a	82.00±1.59^a	3.33±0.80^a	96.98±13.43^b
Diabetic + Glibencamide 0.5 mg/kg	9.80±0.08^a	6.90.±2.12^a	16.80±0.72^a	48.58±1.21^a	10.50±1.74^a	85.16±1.51^a	3.33±0.33^a	88.50±15.12^{ab}

The values represent the mean±SEM within the same column followed by the different superscript letters (a,b) are significantly different at $p<0.05$.WBC = White blood cells; RBC = Red blood cell; Hb = Hemoglobin; Hct = Hematocrit; Neu = Neutrophil; Lym = Lymphocyte; Mono = Monocyte; Plt = Platel

4.2.7 Lipid profiles

Table 4.7 showed hematological values of normal controls, diabetic controls, diabetic rats treated with PCMOS, diabetic rats treated with PCMAX and diabetic rats treated with glibencamide.

TG level of all rats groups were not different**.**

CHOL level normal controls was 62.00±8.69 mg/dl, but it was significantly ($p<0.05$) increased in the diabetic controls (77.66±12.32 mg/dl) compared to that in the normal controls. CHOL levels in the diabetic rats treated with PCMOS (75.83±12.43), PCMAX (74.83±5.67 mg/dl) and glibenclamide (69.66±12.37 mg/dl) were not different and were not different from that in the diabetic controls. However, CHOL levels of diabetic rats treated with PCMAX and glibencamide were closely to that in the normal controls.

LDL levels of normal controls was 15.16±5.23 mg/dl, but it was significantly ($p<0.05$) increased in the diabetic controls (21.5±3.98 mg/dl) and the diabetic rats treated with glibenclamide ((21.83±3.06 mg/dl) compared to that in the normal controls. LDL levels of diabetic rats treated with PCMOS (18.33±1.63 mg/dl) and PCMAX (17.83±2.22 mg/dl) slightly decreased compared to that in the diabetic controls. LDL level of diabetic rats treated with glibencamide slightly decreased compared to that in the diabetic controls. However, LDL levels of diabetic rats treated with PCMOS, PCMAX and glibencamide was not different.

HDL level of normal controls was 60.83±7.16 mg/dl, but it was significantly ($p<0.05$) decreased in the diabetic controls (49.50±10.65 mg/dl) compared to that in the normal controls. HDL levels of diabetic rats treated with PCMOS (56.50±5.78), PCMAX (57.00±7.87 mg/dl) and glibencamide (53.66±8.57 mg/dl) slightly increased compared to that in the diabetic controls. However, HDL levels of diabetic rats treated with PCMOS, PCMAX and glibencamide were not different.

Table 4.7 Lipid profiles of normal controls, diabetic controls, diabetic rats treated with PCMOS, diabetic rats treated with PCMAX and diabetic rats treated with glibencamide

Group	Lipid profiles			
	CHO (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Normal controls	62.00±8.69^{a}	128.33±32.30^{a}	15.16±5.23^{a}	60.83±7.16^{b}
Diabetic controls	77.66±12.32^{b}	121.66±21.36^{a}	21.5±3.98^{b}	49.50±10.65^{a}
Diabetic + PCMOS 500 mg/kg	75.83±12.43^{b}	111.16±15.91^{a}	18.33±1.63^{ab}	56.50±5.78^{ab}
Diabetic + PCMAX 500 mg/kg	74.83±5.67^{ab}	101.83±13.87^{a}	17.83±2.22^{ab}	57.00±7.87^{ab}
Diabetic + Glibencamide	69.66±12.37^{ab}	117.00±11.24^{a}	21.83±3.06^{b}	53.66±8.57^{ab}

The values represent the mean±SEM within the same column followed by the different superscript letters (a,b) are significantly different at $p<0.05$.

TG = Triglyceride; CHO = Cholesterol; LDL = Low- density lipoprotein; HDL = High-density lipoprotein

4.2.8 Histological feature in the pancreatic tissues

Histological observation in the pancreatic tissues of normal controls, diabetic controls, diabetic rats treated with PCMOS, diabetic rats treated with PCMAX and diabetic rats treated with glibencamide was shown in Figure 4.1. After the treatment of the extracts to the rats for 6 weeks, the normal controls showed normal β-cell architecture (Fig.1A) whilst Streptozotocin administration elicited significant morphological changes in the diabetic controls with severe injury of pancreatic islets by decreasing the islets β-cell numbers, cell damage, and cell death (Fig. 1 B).

The diabetic rats treated with PCMOS, PCMAX and glibenclamide showed β-cell architecture similar to that in the normal controls (Fig.C-E)

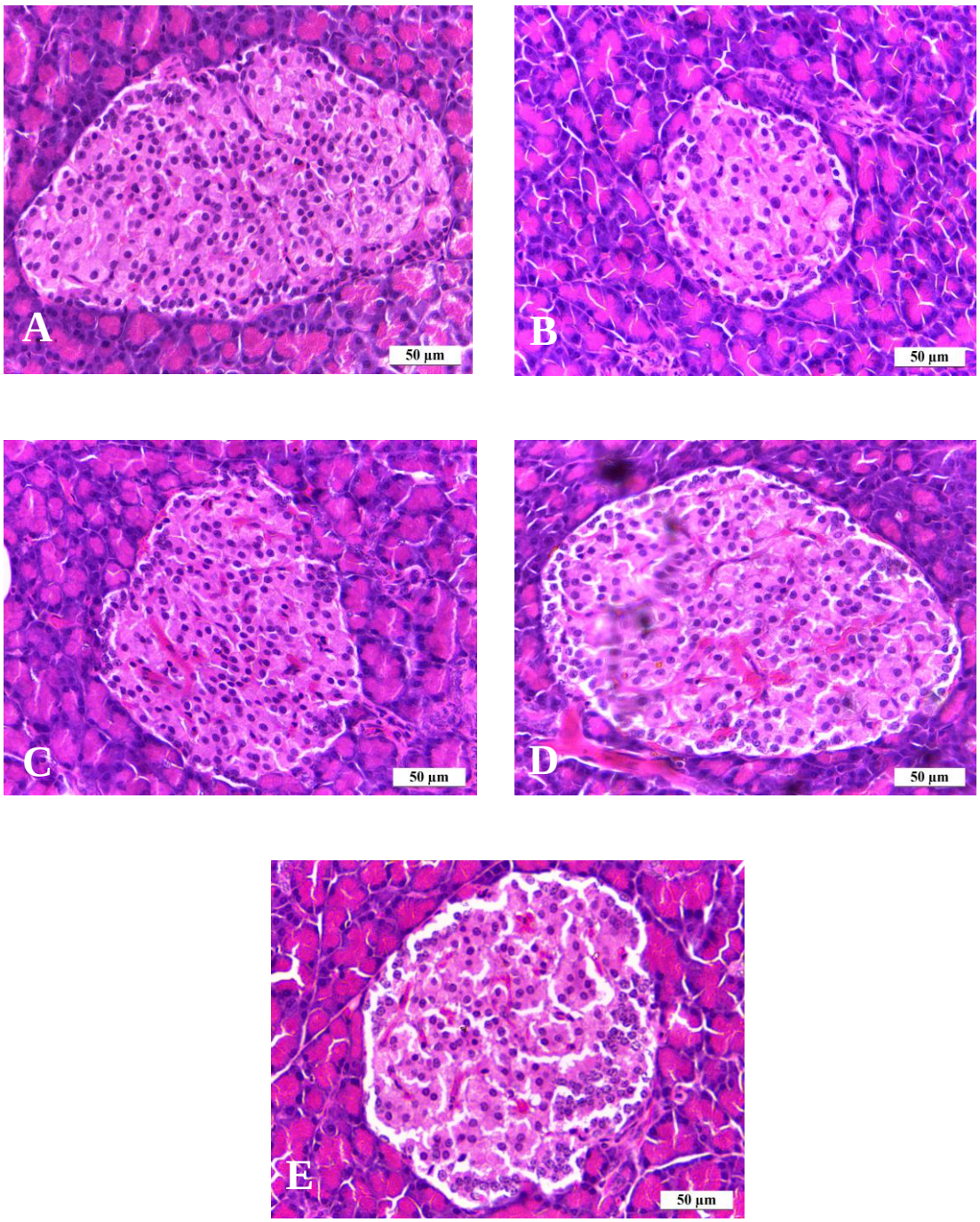

Figure 4.1 Pancreatic islets in the normal controls (A), diabetic controls (B), diabetic rats treated with PCMOS (C), diabetic rats treated with PCMAX (D) and diabetic rats treated with glibenclamide (E)

4.3 Antioxidant activity

4.3.1 DPPH free radical scavenging assay

Table 4.8 showed the antioxidant activity using DPPH assay measured at 517 nm using spectrophotometer revealed that PCMAX exhibited potent antioxidant activity higher than PCMOS with IC_{50} of 8.0396 vs. 13.6973 µg/ml, but was less potent than Ascorbic acid (0.0010 µg/ml).

4.3.2 Ferric reducing antioxidant power (FRAP) assay

Antioxidant activity using FRAP assay measured at 593 nm using spectrophotometer revealed that PCMAX also exhibited potent antioxidant activity higher than PCMOS with the value of 42.66 vs. 31.28 mM Fe(II)/g DW (Table 4.8).

Table 4.8 Antioxidant activity of PCMOS and PCMAX using DPPH assay, comparing to Ascorbic acid, and FRAP assay

Sample	DPPH assay (IC_{50} µg/ml)	FRAP assay (mM Fe(II)/g DW)
PCMOS	13.69 ± 0.4534^{c}	31.28 ± 3.19^{a}
PCMAX	8.03 ± 0.9544^{b}	42.66 ± 0.78^{b}
Ascorbic acid	0.0010 ± 0.0002^{a}	

*The values represent the mean±SEM within the same column followed by the different superscript letters (a,b) are significantly different at $p<0.05$.

4.4 Phytochemical components

Phytochemical components including total phenolic content and total flavonoid content of PCMOS and PCMAX were shown in Table 4.9.

4.4.1 Total phenolic content

The amount of total phenolic content was measured at 750 nm using an UV-Visible spectrophotometer. The total phenolic content of PCMOS and PCMAX were shown in Table 4.7. The total phenolic content obtaining from PCMAX was higher than that from PCMOS with the values of 11.2365 mg GAE/g DW vs. 5.2851 mg GAE/g DW.

4.4.2 Total flavonoid content

The amount of total flavonoid content of PCMOS and PCMAX were measured at 510 nm using an UV-Visible spectrophotometer. The results showed that the total flavonoid content obtaining from in PCMAX was also higher than that from PCMOS with the values of 55.04 mg CE/g DW vs.20.85 mg CE/g DW.

Table 4.9 Total phenolic content and total flavonoid content of PCMOS and PCMAX

Sample	Total phenolic content (mg GAE/g DW)	Total flavonoid content (mg CE/g DW)
PCMOS	5.2851 ± 0.36^{a}	20.85 ± 0.08^{a}
PCMAX	11.2365 ± 0.17^{b}	55.04 ± 2.25^{b}

*The values represent the mean±SEM within the same column followed by the different superscript letters (a,b) are significantly different at $P<0.05$

Discussion

Streptozotocin (STZ) induced hyperglycemia is a useful experimental model to study the antidiabetic activity, because of its structural features. STZ gets selective entry into the beta-cells of the islets of Langerhans via the low affinity glucose transporter GLUT2 in its plasma membrane and causes the destruction of beta-cells, leading to a reduction insulin, which inturn results in a rise in blood glucose concentration, i.e. hyperglycemia (Elsner *et al.*, 2000). In the present study, STZ at a dose of 65 mg/kg

was carried out to evaluate the antidiabetic activity of the fruit pulp extracts from *C. moschata* (PCMOS) and *C. maxima* (PCMAX) on STZ-induced diabetic rats. Results obtaining in this study indicated that the administration of PCMOS and PCMAX at an oral dose of 500 mg/kg to rats once daily for 6 weeks exhibited the antidiabetic activity by significantly decreasing the blood glucose in the diabetic treated rats with a similar potent to glibenclamide. Increase serum insulin was found in the diabetic rats treated with PCMAX whilst slightly increasing serum insulin was found in the diabetic rats treated with PCMOS. These findings indicate insulin stimulating activity of the extracts from pumpkins, *C. moschata* and *C. maxima.* These results were in line with the study by Li *et al*. (2005) who found that protein-bound polysaccharides from the fruit of pumpkin *(C. moschata)* could obviously increase the levels of serum insulin leading to decrease the blood glucose levels. Polysaccharide and terpenoids from *Phellinus linteus* lowered the blood glucose levels in streptozotocin-induced diabetic rats (Ketwong and Talubmook, 2010). It has also been shown that powderd pumpkin from *C. pepo* has hypoglycemic activity properties in type 1 diabetes sufferes (Sedigheh *et al*., 2011) which was shown to be due to polysaccharide components (Caili *et al*., 2006; Li *et al*., 2010; Sedigheh *et al*., 2011; Wang *et al*., 2017). Polysaccharide content was found in PCMOS and PCMAX. Therefore, polysaccharide content presenting in PCMOS and PCMAX are responsible for lowering the blood glucose levels. Moreover, previous studies found that flavonoid extracted from Cucurbitaceae, with antioxidant activity also possess hypoglycemic effect in diabetic rats (Rauter *et al*., 2010). The fruit pulp extracts from pumpkin, PCMOS and PCMAX, in the present study possess hypoglycemic activity partly by increasing the levels of serum insulin. PCMOS and PCMAX possessed total flavonoid content, the hypoglycemic activity of PCMOS and PCMAX found in this study is therefore partialy due to the the presence of flavonoid content.

Induction of diabeteswith STZ is associated with the characteristic loss of body weight, which is due to the increased muscle wasting and loss of tissue proteins (chatterjea and Shinde, 2002). The body weight in the diabetic rats is lower than that in the normal controls as the diabetic rats break down protein and fat to produce energy instead of blood glucose which leading to loss the body weight. PCMOS and PCMAX increased the body weight in the diabetic treated rats. In the present study is consistent with the report by Baldi *et al*., (2010) who found that the pumpkin concentrate prepared

caused significantly increased the body weight of the diabetic rats. And it has also been shown that a water-soluble polysaccharide (PCE-CC) intake resulted in a significant increase the body weight of the diabetic treated rabbits as compared to the diabetic control rabbits (Zhang *et al*., 2013). This phenomenon found in the present study was similar to that of other plant extracts such as *Antidesma bunius* (L.) Spreng (Chowtivannakul *et al*., 2016). These findings indicate that PCMOS and PCMAX increasing the body weight in the diabetic rats may be due to PCMOS and PCMAX possess hypoglycemic activity by using blood glucose leading to a prevention of the break down of fat and protein to produce energy.

Streptozotocin (STZ) effectively destroys pancreatic islet of β-cells which produces insulin and causes persistent hyperglycaemia. In the present study, the islet β -cells optical microscope picture of experimental animals suggest that the oral dose of PCMOS and PCMAX using in the present study was potentially effective after STZ damage, as evidenced from the histological observations where the damage of the pancreas tissues was recovered in PCMOS and PCMAX treated rats. This helped to prevent disorders involving excessive islet cell damage, for preventing STZ-induced toxicity in pancreatic islet cells of the rats, which was consistent with other studies (Zhang *et al*., 2013; Sedigheh *et al*., 2011; Makni *et al*., 2011). The mechanism of antidiabetic action of PCMOS and PCMAX may act by stimulating insulin release from the pancreatic β-cells (Mahomed and Ojewole, 2003) or/and reduction of the absorption of intestinal glucose, the conversion to glucose is glycogen storage on the liver or muscle, and to inhibit glycogen breakdown into sugar. Based on this research, it is possible that PCMOS and PCMAX prevented the destruction of β-cells of islets in the pancreas as shown in the histological examination. The mechanism by which these extracts, PCMOS and PCMAX bring about their antidiabetic action may be by potentiating the insulin effect of plasma by stimulating insulin release from the pancreatic β-cells. Histological study indicating PCMOS and PCMAX stimulate pancreatic β-cells proliferation. Therefore, PCMOS and PCMAX possess antidiabetic activity by lowering the blood glucose levels via a stimulation of pancreatic β-cells proliferation leading to increase serum insulin release.

Diabetes mellitus (DM) is one of the most common human metabolic diseases, and derangements in lipid metabolism in diabetic subjects are often important

determinants of the course and status of the disease. DM mainly increased the levels of cholesterol or LDL-C is important risk factor in the initiation and progression of atherosclerotic lesions (Harrison *et al*., 2003). The administration of PCMOS and PCMAX at an oral dose of 500 mg/kg b.w. to the rats once daily for 6 weeks showing a significant decrease cholesterol, LDL and increase HDL levels in the diabetic treated rats. In continence with the present data, other works reported that the rats in the treated pumpkin (*C. maxima*) at a dose of 400 mg/kg (p.o) shows significantly decrease in total cholesterol, triglycerides, LDL and increase in HDL levels in the diabetic rats (Latha and kolavali, 2016), Administration of the pumpkin polysaccharide significantly lowered the total cholesterol, triglycerides and HbA1c level in the diabetic rabbits (Zhang *et al*., 2013), and also it has been shown that the flax and pumpkin seed mixture extract has strong hypotriglyceridemic and hypocholesterolemic effects on rats with a decrease in plasma LDL-C and increase in HDL-C levels (Makni *et al*., 2011). Furthermore, a fiber-rich diet reduces triglyceride levels by suppressing lipogenesis in the liver (Lecumberri *et al*., 2007). The presence of unsaturated fatty acids, such as oleic acid and linoleic acid in pumpkin seed reduce cholesterol levels in rats (Takada *et al*., 1994). These data indicated that the lipid reducing effects of PCMOS and PCMAX is probably due to its fibers. These substances reduce plasma LDL levels by inhibiting the absorption of bile acids and cholesterol and enhancing the activity of LDL receptors.

Serum enzymes including ALT, AST and ALP are used in the evaluation of hepatic disorders. An increase in these enzyme activities effects active liver damage (Achliya *et al*., 2004; Saha *et al*., 2017). The present study revealed a significant decrease in the activities of ALP, but not AST and ALT on diabetic rats, indicating considerable hepatocellular injury comparable to the normal control animals. The alteration of these enzymes observed in the present study using 500 mg/kg of PCMOS and PCMAX, indicated that the extract can affect hepatic function. The decreased in ALP in the diabetic rats treated with the PCMOS and PCMAX agree with Latha and Kolavali. (2016) who reported that the methanolic extract of *C. maxima* reduced serum enzymes ALP. In this study, a reduction of ALP probably results from the prevention of PCMOS and PCMAX from cellular and tissue damage in a diabetic stage (Ragava and Krishnakumari, 2006).

Platelets are fragment of cells that participates in blood clotting, they initiate repair of blood vessels walls and are also considered as an acute phase reactant to infection or inflammation; plateletcrits show cases the precise method of determining the degree of acute blood loss while mean platelet volume (MPV) is used when investigating the ability of a drug to enhance blood clotting (Ganong et al., 1999). The implication of the slightly increases in Plt levels in the diabetic treated with PCMOS at an oral dose of 500 mg/kg b.w. when compared to that in the diabetic controls. but significant increases ($p< 0.05$) in Plt level in the diabetic rats treated with PCMAX at an oral dose of 500 mg/kg b.w. when compared to that in the normal controls. The results in the present study is consistent with the report by Edet *et al*., (2013) on the ability of extracts and fractions of *Nauclea lafiloia* in increasing Plt closed to that in normal controls, suggesting that PCMAX may not cause thrombosis.

The antioxidant activity study revealed that PCMOS and PCMAX have antioxidant activity. DPPH assay revealed that PCMAX exhibited potent antioxidant activity higher than PCMOS with IC_{50} of 8.0396 and 13.6973 µg/ml, respectively, but less potent than ascorbic acid (0.0010 µg/ml). FRAP assay revealed that PCMAX also exhibited potent antioxidant activity higher than PCMOS with values of 42.66 vs. 31.28 mM Fe(II)/g DW). The antioxidant activity in the plants is mainly attributed to their phenolic (Puravankara *et al*., 2000) and falvonoids compounds (Rauter *et al*., 2010). In the present study, PCMOS and PCMAX possess antioxidant activity is likely due to the presence of total phenolic and total flavonoid contents.

CHAPTER V

CONCLUSIONS

The fruit pulp extracts from *C. moschata* (PCMOS) and *C. maxima* (PCMAX)

5.1 possess polysaccharide content of 33.41 and 36.99 %, respectively

5.2 possess total phenolic content of 11.2365 mg GAE/g DW and 5.2851 mg GAE/g DW, respectively and flavonoid content of 55.04 mg CE/g DW and 20.85 mg CE/g DW, respectively.

5.3 possess antidiabetic and antioxidant activities

5.4 at an oral dose of 500 mg/kg giving daily for 6 weeks increased the body weight, serum insulin and HDL cholesterol, but decreased the blood glucose level, total cholesterol and LDL cholesterol, but reduced hepatic function by decreasing ALP enzyme in the diabetic treated rats.

5.5 at an oral dose of 500 mg/kg giving daily for 6 weeks recovered the histopathological feature of pancreatic islet induced by decreasing the size of islet of Langerhans and the number of pancreatic islet β-cells.

5.6 lower the blood glucose level of the diabetic treated rats

5.6.1 via a stimulation of pancreatic islet β-cell proliferation leading to increase serum insulin release.

5.6.2 by the presence of flavonoid content and polysaccharide

5.7 possess antioxidant activity

5.7.1 by free radical scavenging with the IC_{50} of 8.0396 and 13.6973 μg/ml, respectively.

5.7.2 by reducing antioxidant power with the values of 42.66 and 31.28 mM Fe(II)/g DW, respectively.

5.7.3 via the presence of flavonoid content of 55.04 mg CE/g DW and 20.85 mg CE/g DW, respectively.

5.8 at an oral dose of 500 mg/kg giving daily for 6 weeks did not affect hematological values including WBC, RBC, Hb, Hct, Neu, Lym and Mono but increased Plt in the diabetic treated rats.

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APPENDICES

APPENDIX A

Certificate

คณะกรรมการกำกับและส่งเสริมการดำเนินการต่อสัตว์เพื่องานทางวิทยาศาสตร์ มหาวิทยาลัยมหาสารคาม (คกส. มมส)

Institutional Animal Care and Use Committee, Mahasarakham University (IACUC-MSU), Thailand

ใบรับรองการอนุมัติ

(Certificate of Approval)

เลขที่การรับรอง : 0020 / 2560

Approval number : 0020/ 2017

ชื่อโครงการวิจัย : ฤทธิ์ลดระดับน้ำตาล, กลไก และฤทธิ์ต้านอนุมูลอิสระของพอลิแซ็กคาไรด์จากฟักทอง Cucorbita moschata Duch. และ Cucorbita maxima Duch.

Research Title : Antidiabetic, mechanism action and antioxidant activities of polysaccharides from Cucorbita moschata Duch. and Cucorbita maxima Duch.

ผู้วิจัยหลัก : นางสาวอภิญญา สุวรรณพงษ์

Principal Investigator : Ms. Apinya Suwannapong

คณะ/หน่วยงาน : คณะวิทยาศาสตร์ มหาวิทยาลัยมหาสารคาม

Affiliation : Faculty of Science, Mahasarakham University

สถานที่ดำเนินการวิจัย : จังหวัดมหาสารคาม

Research Site : Mahasarakham Province

วันที่รับรอง : 25 กรกฎาคม 2560 **วันหมดอายุ :** 25 กรกฎาคม 2561

Date of Approval : 25 July 2017 **Date of Expiration :** 25 July 2018

โครงการวิจัยนี้ได้รับการพิจารณาและอนุมัติจากคณะกรรมการกำกับและส่งเสริมการดำเนินการต่อสัตว์เพื่องานทางวิทยาศาสตร์ มหาวิทยาลัยมหาสารคาม (คกส. มมส.) ให้ดำเนินการศึกษาวิจัยเรื่องข้างต้นได้บนพื้นฐานของโครงการวิจัยที่คณะกรรมการฯ ได้รับการพิจารณา คณะผู้วิจัยต้องปฏิบัติตามจรรยาบรรณการใช้สัตว์และพระราชบัญญัติสัตว์เพื่องานทางวิทยาศาสตร์ รวมทั้งข้อกฎหมายต่างๆ ที่เกี่ยวข้องอย่างเคร่งครัด หากมีการเปลี่ยนแปลงใดๆ ในโครงการวิจัย ผู้วิจัยหลักจักต้องแจ้งต่อคณะกรรมการฯ ทันที และต้องยื่นขอรับการพิจารณาโครงการวิจัยใหม่

This research project has been reviewed and approved by the Institutional Animal Care and Use Committee, Mahasarakham University (IACUC-MSU). The IACUC-MSU approval is limited to the project as approved. All researchers who associated with this research project must act strictly in accordance with the ethical principles, guidelines and act of parliament for the use of animals in scientific works, including all relevant policies and laws. Any subsequent changes to the consent form, the principal investigator must notify the IAUAC-MSU immediately and re-submit the research application to the IACUC-MSU.

.....

(ศาสตราจารย์สัมพันธ์ ฤทธิเดช)

(Prof. Dr. Sampan Rittidech)

ประธานคณะกรรมการกำกับและส่งเสริมการดำเนินการต่อสัตว์เพื่องานทางวิทยาศาสตร์

(The Institutional Animal Care and Use Committee's Chairman)

APPENDIX B

Graph of concentration vs.% inhibition

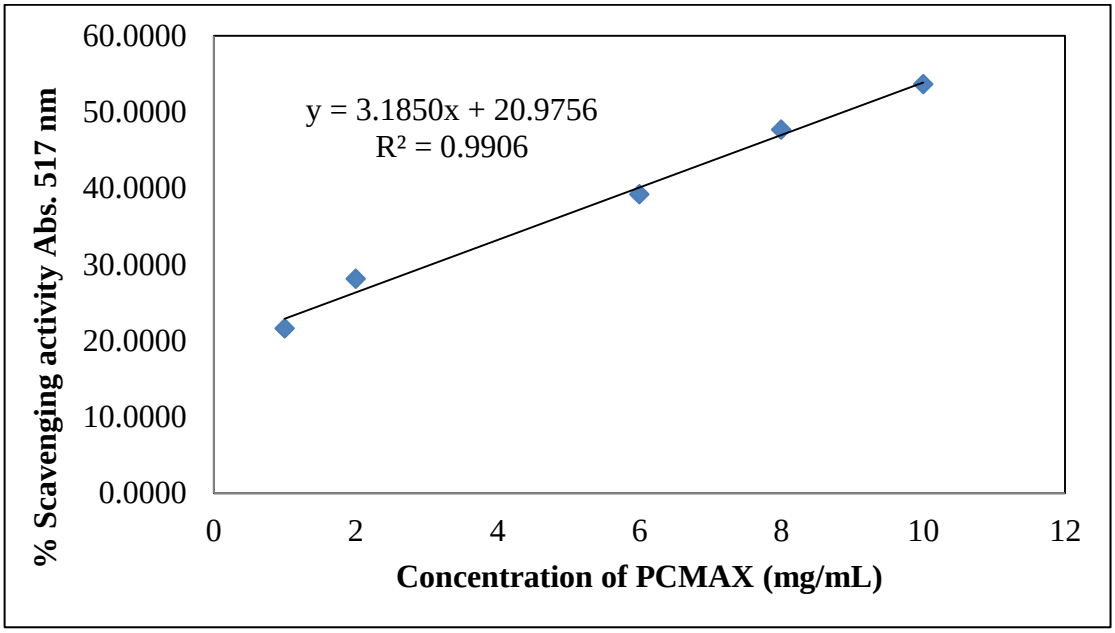

Figure B-1 Graph of concentration vs.% inhibition of PCMAX, repetitive 1.

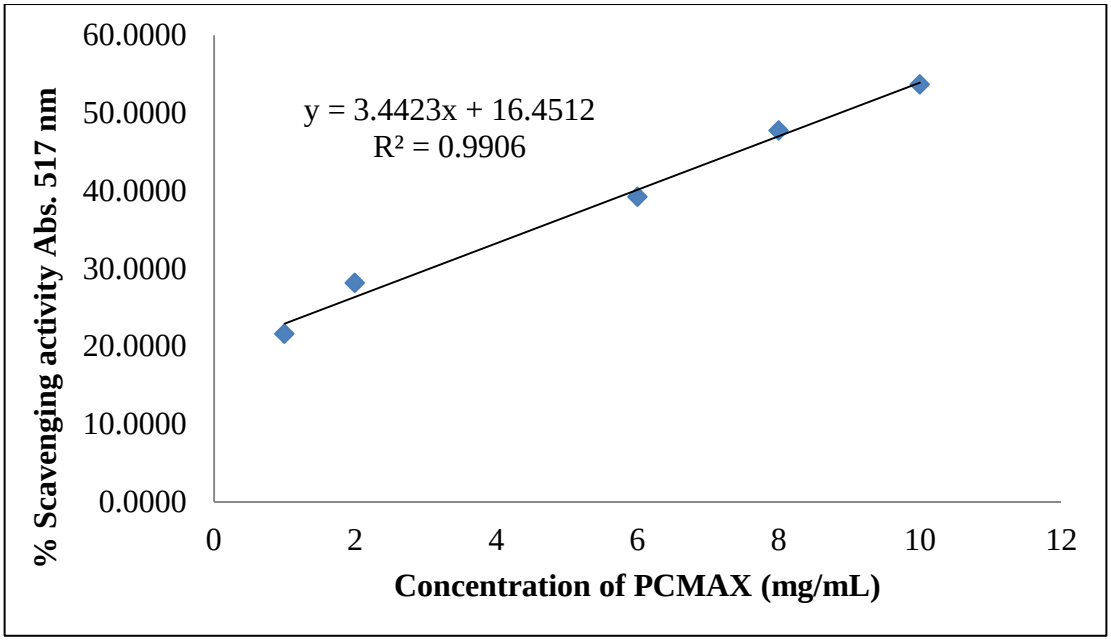

Figure B-2 Graph of concentration vs.% inhibition of PCMAX, repetitive 2.

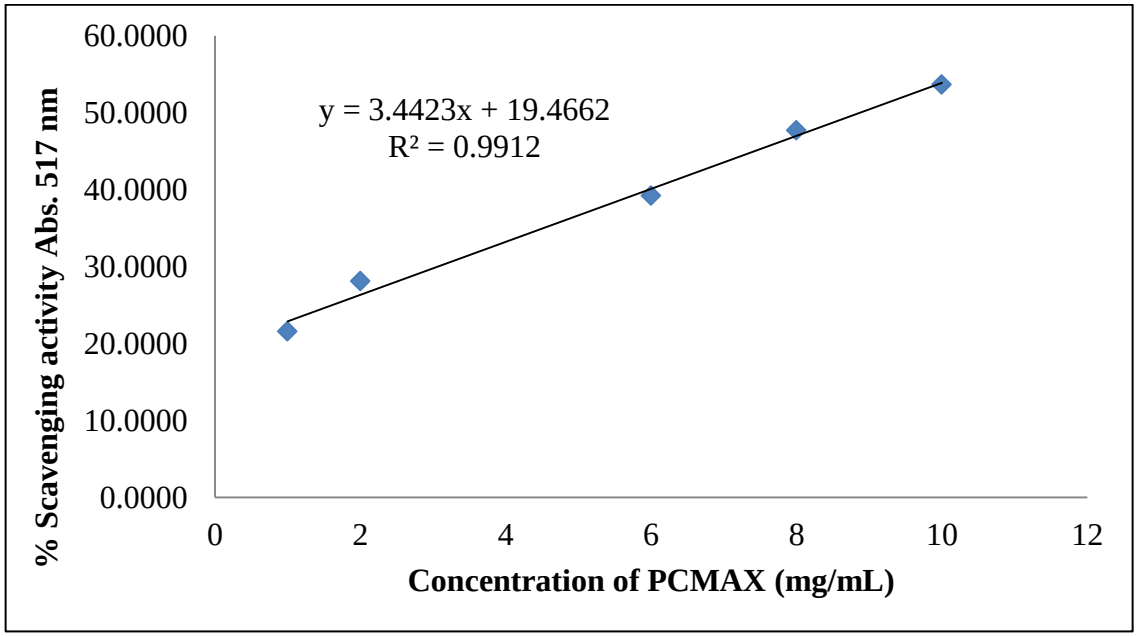

Figure B-3 Graph of concentration vs.% inhibition of PCMAX, repetitive 3.

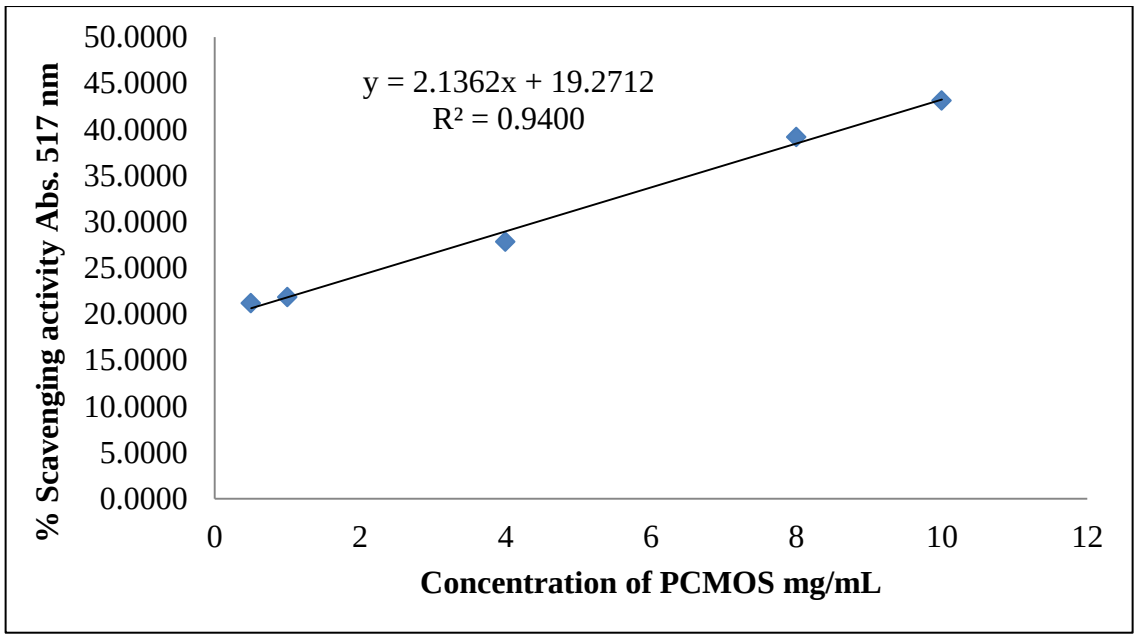

Figure B-4 Graph of concentration vs.% inhibition of PCMAX, repetitive 1.

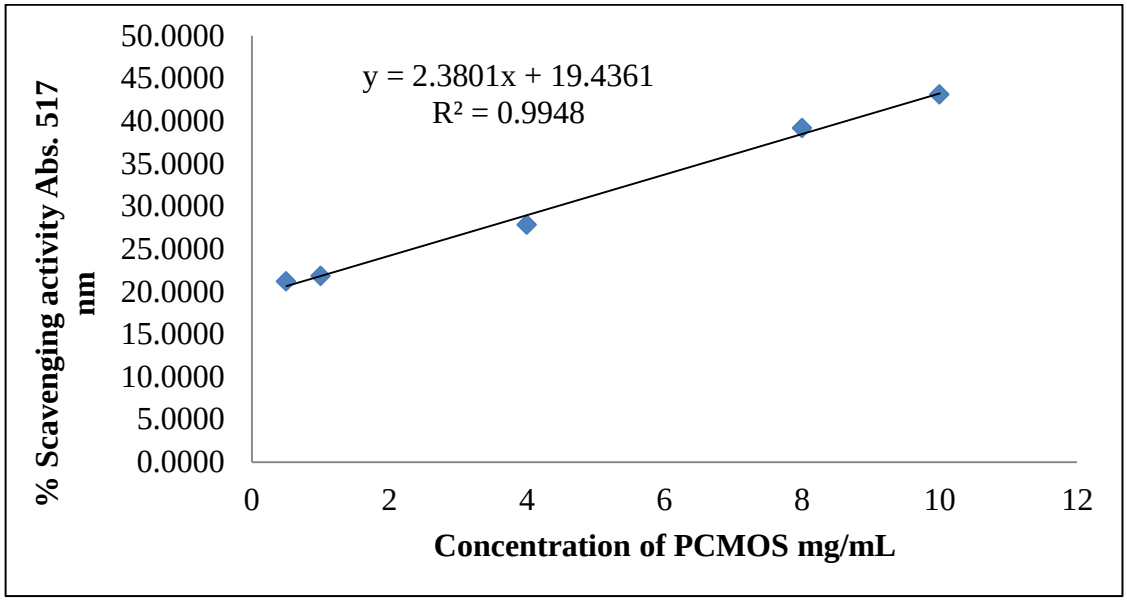

Figure B-5 Graph of concentration vs.% inhibition of PCMOS, repetitive 2.

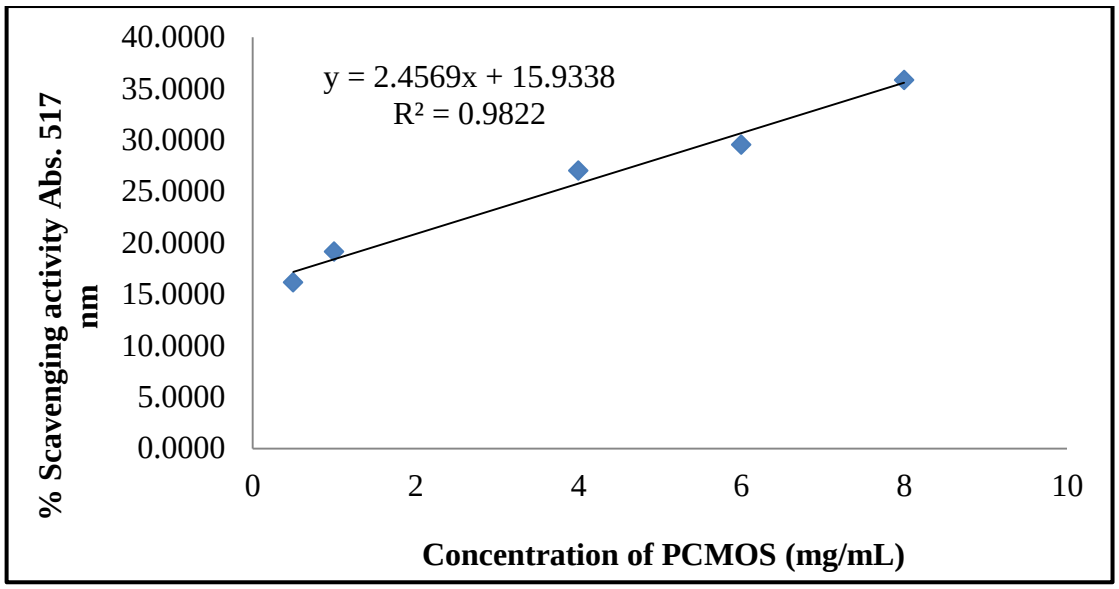

Figure B-6 Graph of concentration vs.% inhibition of PCMOS, repetitive 3.

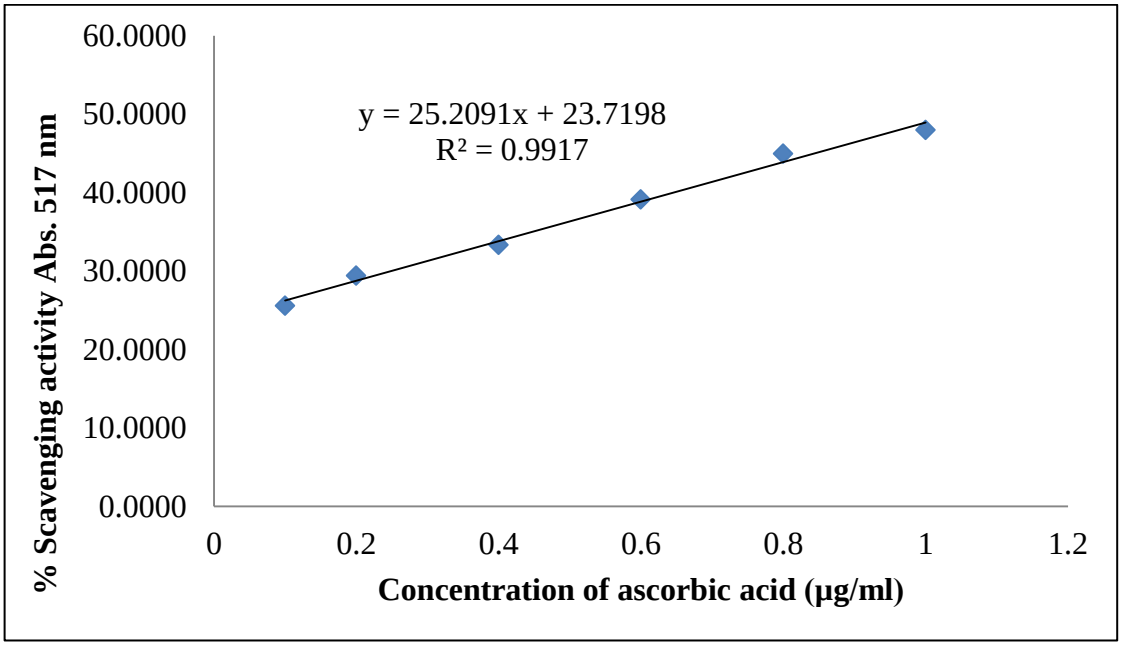

Figure B-7 Graph of concentration vs.% inhibition of ascorbic acid, repetitive 1.

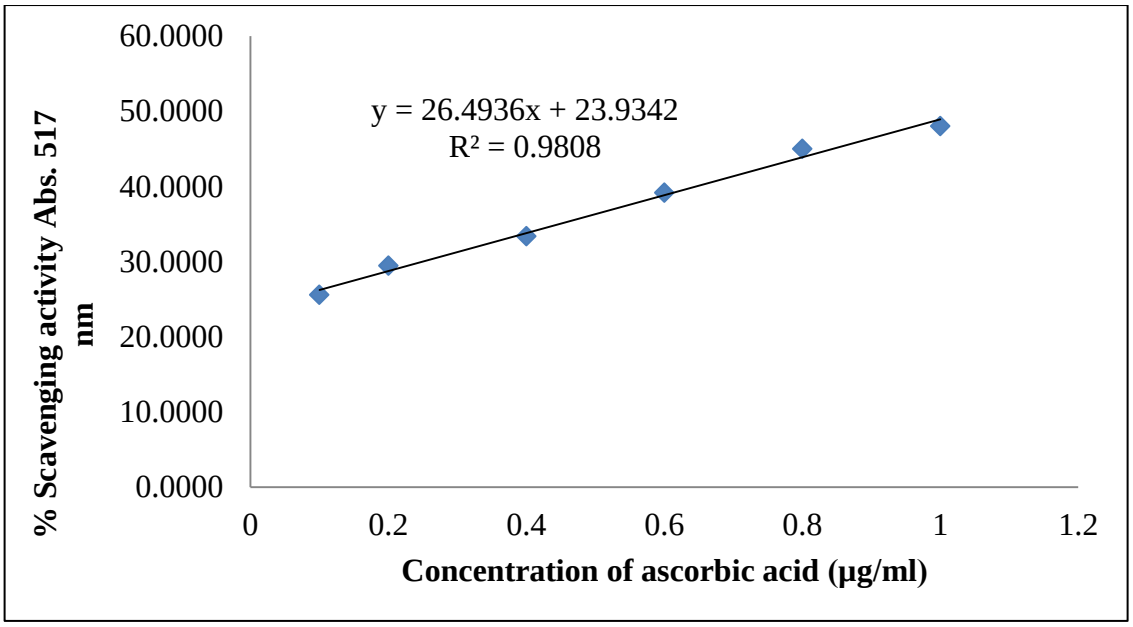

Figure B-8 Graph of concentration vs.% inhibition of ascorbic acid, repetitive 2.

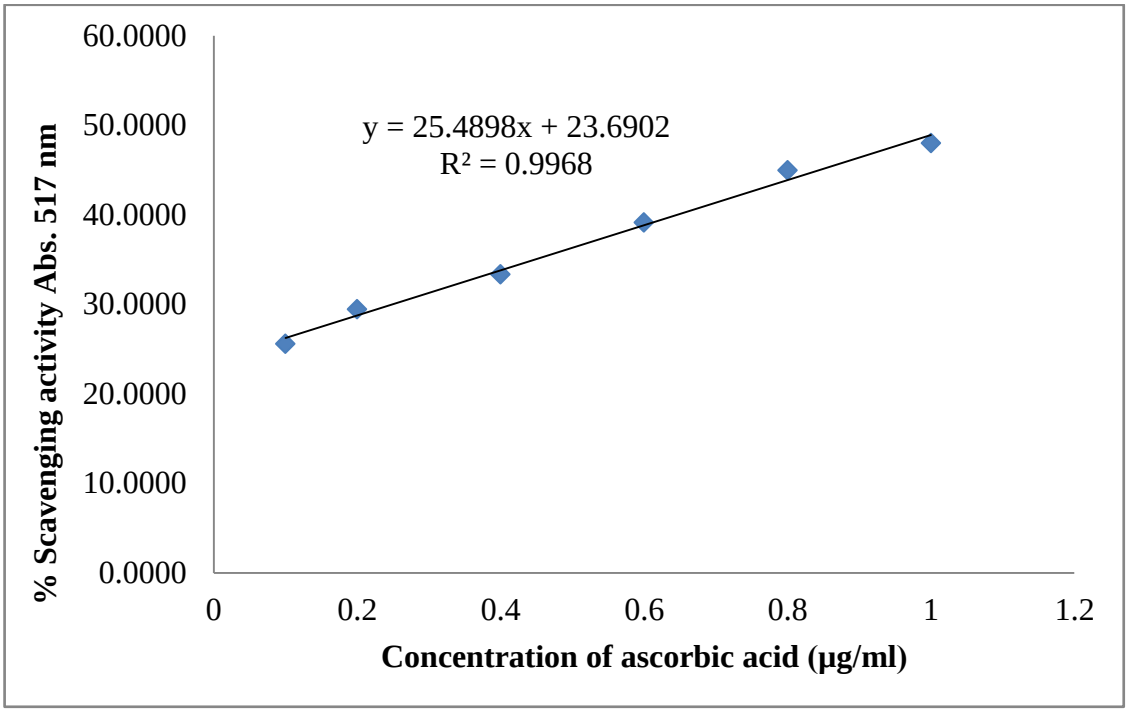

Figure B-9 Graph of concentration vs.% inhibition of ascorbic acid, repetitive 3.

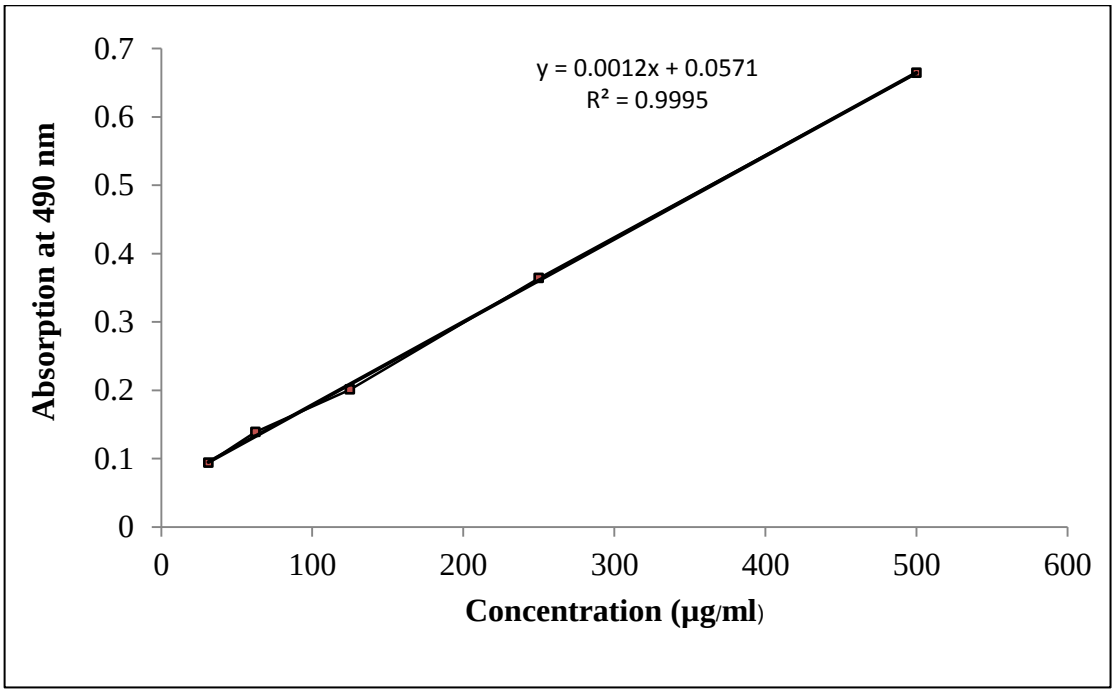

Figure B-10 Glucose standard curve and straight line equation

VITA

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Research grant & awards

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- National Research Council of Thailand (NRCT)" Academic Year 2017.

Research output

Proceedings

- Apinya Suwannapong , Chusri Talubmook and Wilawan Promprom. Antioxidant activity of polysaccharides from *Cucurbita moschata* Duch. and *Cucurbita maxima* Duch. Proceedings of the International Conference on Science and Technology 2017, Faculty of Science and Technology, RMUTT, 7-8 December, 2017. (Oral presentation)