PROFILING OF ANTI-DIABETIC AGENT FROM *PERESKIA BLEO* (KUNTH)

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FACULTY OF SCIENCE
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ABSTRACT

Pereskia bleo (Kunth) belongs to the family of Cactaceae and locally known as jarum tujuh bilah. It has been used traditionally by native Malaysian for the treatment of diabetes. However, the lack of scientific data to validate such claim have motivates researcher to study for its anti-diabetic activities. In this study, the leaves, stems and roots of *Pereskia bleo* crude extracts were extracted with hexane, petroleum ether, chloroform, methanol and aqueous. The separation and detections of bioactive compounds were done using Thin Layer Chromatography (TLC) and Liquid Chromatography Mass spectrometry combined with Mass Spectrometry (LCMS/MS) methods. The TLC results showed the presence of terpenoid, alkaloid, phenolics acid and flavonoids in *Pereskia bleo* crude extracts. While in LCMS/MS analysis has revealed the presence of phenolics acid, amino acid, jasmonic acid or dihydroxymellein, Chrysin, Apigenin based compounds, Apigenin 6 C Glucoside, and Apigenin 7 Rutinoside in *Pereskia bleo* crude extracts. The total phenol content were highest in stem aqueous extract, root chloroform extract and leaves methanol extract at 6.06$\pm$0.1 mg GAE/g, 3.64$\pm$0.1 mg GAE/g and 3.00$\pm$0.1 mg GAE/g respectively. Whereas, the total flavonoids content were highest in stems , leaves and root chloroform extract at 11.07$\pm$0.7 mg QE/g, 5.2$\pm$0.7 mg QE/g and 3.40$\pm$0.7 mg QE/g respectively. The Glycogen Phosphorylase Inhibition bioassay (GPi) showed the highest percentage of GPi activity from roots, leaves and stems methanol extract of *Pereskia bleo* at 89.9%, 82.3% and 78.3% respectively. The acute toxicity test was performed using non-diabetic Sprague Dawley (SD) rats have showed none of the toxic signs occur such as anxiety, urination or defecation, pain response and death when administrated with 250, 500 and 1000mg/kg of *Pereskia bleo* aqueous extract. In Oral Glucose Tolerance Test (OGTT), the administration

of *Pereskia bleo* aqueous extracts showed the improved of glucose tolerance in non-diabetic rats. The roots aqueous extracts of *Pereskia bleo* also showed a maximum reduction of blood glucose level when administered with 250mg/kg and 500mg/kg at 120 minutes with 20% and 21%. Followed by leaves and stem aqueous extracts of *Pereskia bleo* with 11% and 14% of reduction, respectively. In treatments of diabetic rats, the alloxan-induced diabetic rats showed that 500mg/kg of the leaves, stems and roots aqueous extracts of *Pereskia bleo* showed the significantly reduction in fasting plasma glucose levels by 66%, 65 and 58% ($p<0.05$) respectively on 25^{th} day compared to diabetic control group. The treatments of *Pereskia bleo* aqueous extracts to diabetic rats showed the significantly ($p<0.05$) improved in body weight and decreased in liver enzymes levels (ALT, AST and ALP), renal function test (creatinine and urea) and serum lipid profile (Total cholesterol, triglyceride, LDL) compared to diabetic group. It is also showed a significantly ($p<0.05$) increased in HDL levels compared to diabetic group. Thus, this study discovered that bioactive compounds found in crude extract of *Pereskia bleo* might be useful as anti-hyperglycaemic agents and these finding might be a fundamental to the further research to prove the claimed made by traditional practitioner that has consumed *Pereskia bleo* as diabetes treatment.

ABSTRAK

Pereskia bleo (Kunth) terdiri daripada famili Cactaceae dan dikenali oleh masyarakat tempatan sebagai pokok ' Jarum tujuh bilah ' . Ia telah digunakan secara tradisional oleh orang melayu dalam rawatan penyakit diabetis. Walau bagaimanapun, kekurangan data saintifik untuk mengesahkan dakwaan ini telah menggalakkan penyelidik untuk mengkaji aktiviti anti-diabetis pokok ini. Dalam kajian ini ekstrak mentah daun, batang dan akar *Pereskia bleo* ini diekstrak dengan heksana, petroleum eter, kloroform, metanol dan akues. Pengasingan dan pengesanan sebatian bioaktif telah dilakukan dengan menggunakan kaedah Thin Layer Chromatography (TLC) dan Liquid Chromatography Mass Spectrometry bergabung dengan Massa spektrometri (LCMS/MS). Keputusan TLC menunjukkan kehadiran terpenoid, alkaloid , asid phenolic dan flavonoid di dalam *Pereskia bleo* ekstrak mentah. Manakala di dalam analisis LCMS/MS telah mengesan kehadiran asid fenolik, asid amino, asid jasmonic atau dihydroxymellein, Chrysin, sebatian berasaskan apigenin, apigenin 6 C Glucoside, dan apigenin 7 Rutinoside di dalam ekstrak mentah *Pereskia bleo*. Jumlah kandungan fenol paling banyak di batang ekstrak akueus, ekstrak kloroform akar dan ekstrak methanol daun pada 6.06$\pm$0.1 mg GAE/g, 3.64$\pm$0.1 mg GAE/g dan 3.00$\pm$0.1 mg GAE/g, masing-masing. Manakala jumlah kandungan flavonoid yang paling tinggi di dalam ekstrak kloroform batang, daun dan akar pada 11.07$\pm$0.7 mg QE/g , 5.2$\pm$0.7 mg QE/g dan 3.40$\pm$0.7 mg QE/g masing-masing. Ujian bioesei Glikogen Phosphorylase Perencatan (GPI) menunjukkan peratusan yang tertinggi aktiviti GPI adalah dari ekstrak metanol akar, daun dan batang *Pereskia bleo*, dengan masing-masing 89.9%, 82.3% dan 78.3%. Ujian ketoksikan akut telah dilakukan dengan menggunakan tikus Sprague Dawley (SD) bukan diabetis telah menunjukkan tiada tanda-tanda toksik yang

berlaku seperti keresahan, kencing atau membuang air besar, tindak balas kesakitan dan kematian apabila diberi dengan 250, 500 dan 1000mg/kg ekstrak akueus *Pereskia bleo*. Di dalam Toleransi Glukosa Oral Test (OGTT), ekstrak akueus *Pereskia bleo* menunjukkan toleransi glukosa yang baik pada tikus bukan diabetis. Akar ekstrak akueus *Pereskia bleo* juga menunjukkan pengurangan maksimum tahap glukosa darah apabila diuji dengan dos 250mg/kg dan 500mg/kg pada 120 minit dengan 20% dan 21%. Diikuti dengan daun dan batang ekstrak akueus *Pereskia bleo* dengan 11% dan 14%, masing-masing. Manakala di dalam rawatan ke atas tikus diabetis, pada dos 500mg/kg daun, batang dan akar ekstrak akueus *Pereskia bleo* menunjukkan pengurangan paras glukosa plasma puasa signifikan ($p < 0.05$) sebanyak 66% , 65% dan 58%, masing-masing pada hari-hari ke-25 berbanding dengan kumpulan kawalan diabetis. Rawatan dengan ekstrak akueus *Pereskia bleo* kepada tikus diabetis menunjukkan peningkatan yang signifikan ($p < 0.05$) pada berat badan dan pengurangan di dalam paras enzim hati (ALT, AST dan ALP), ujian fungsi buah pinggang (creatinine dan urea) dan profil lipid serum (Jumlah kolesterol, trigliserida, LDL) berbanding dengan kumpulan diabetis. Ia juga menunjukkan peningkatan yang signifikan ($p < 0.05$) di dalam paras HDL berbanding dengan kumpulan diabetis. Oleh itu, kajian ini mendapati bahawa sebatian bioaktif yang terdapat dalam ekstrak mentah daripada *Pereskia bleo* mungkin berguna sebagai agen anti- hyperglyceamic dan penemuan ini mungkin menjadi asas kepada penyelidikan lebih lanjut untuk membuktikan tuntutan yang telah dibuat oleh pengamal tradisional yang menggunakan *Pereskia bleo* di dalam rawatan diabetes .

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LIST OF ABBREVIATIONS

%	Percentage
:	Ratio
±	Plus minus
<	Less then
>	More then
µg/ml	microgram per millilitre
µM	Micro molar
µmol/L	Micromoles per litre
A1C	Glycated haemoglobin
$AlCl_3$	Aluminium Chloride
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransaminase
ANOVA	Analysis Of Variance
AST	Aspartate Aminotransaminase
ATP	Adenosine Triphosphate
BSLA	Brine Shrimp Lethality Assay
CC	Column Chromatography
cm	Centimetre
cps	Counts per second
CrCl	Creatinine clearance rate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid

EDTA	Ethylenediaminetetraacetic Acid
ESI	Electrospray ionization
et al.	Et alia (and other)
g	Gram
g/kg	Gram per kilogram
g/ml	Gram per millilitre
G6P	Glucose-6-phosphate
GAE	Gallic acid equivalents
GDM	Gestational Diabetes Mellitus
GFR	Glomerular filtration rate
GLP1	Glucagon-like-peptide-1
GP	Glycogen phosphorylase enzyme
GPa	Glycogen phosphorylase a
h	Hour
HCl	Hydrochloric acid
HDL	High Density Cholesterol
IC_{50}	Half maximal inhibitory concentration
ICR mice	Imprinting Control Region mice
IU	International Units
IU/L	International Units Per Litre
KCl	Potassium Chloride
LC	Leaves chloroform extracts
LC_{50}	Lethality concentrations
LCMS/MS	Liquid Chromatography Mass Spectrometry with Mass Spectrometry

LDL	Low Density Cholesterol
LH	Leaves hexane extracts
LM	Leaves methanol extracts
LP	Leaves petroleum ether extracts
LW	Leaves aqueous extracts
m/z	Mass to Charge Ratio
mg/dL	Milligram per decilitre
mg/kg	Milligram per kilogram
mg/ml	Milligram per millilitre
$MgCl_2$	Magnesium chloride
mM	Millimolar
mm	Millimetre
mmol/L	Millimoles per litre
n	total number
N_2	Nitrogen
$NaNO_2$	Sodium nitrate
NaOH	Sodium hydroxide
NIDDM	Non-insulin-dependent diabetes mellitus
Nm	Nanometres
^oC	Degree of Celsius
OGTT	Oral glucose tolerance test
PCOS	Polycystic Ovarian Syndrome
Psi	Pounds Per Square Inch
QE	Quarcetin equivalents

r^2	Correlation of Determination
RC	Roots chloroform extracts
R_f	Retardation factor
RH	Roots hexane extracts
RM	Roots methanol extracts
ROS	Reactive oxygen species
RP	Roots petroleum ether extracts
RW	Roots aqueous extracts
SC	Stems chloroform extracts
SD	Standard deviation
SH	Stems hexane extracts
SM	Stems methanol extracts
SP	Stems petroleum ether extracts
SPSS	Statistical Package for the Social Sciences
SW	Stems aqueous extracts
T.CHOL	Total cholesterol
TLC	Thin Layer Chromatography
TRIG	Triglyceride
U/L	Units Per Litre
UDP	Uridine diphosphate
US$	Dollar America
USA	United States America
UV	Ultra violet
v/v	volume per volume

WHO	World Health Organization
α	Alpha
β	Beta

Diabetes mellitus or generally known as diabetes is one of the chronic disorder. It is occurred either because of lack of insulin production by the pancreas or the insulin cannot be used by our body effectively. Various complications developed due to the effect of metabolism dysfunction in diabetes, (Auslander *et al.*, 2002) including the damage to the heart, kidney, blood vessels, eyes and nerves. Therefore, diabetes is categorized as a third killing disease after cancer and cardiovascular disease (Li *et al.*, 2004). Diabetes and its complications have a significant economic impact on health system as well as to families and countries. There are no medications that can cure this disorder. However with a proper diet, exercise, and use of appropriate medications such as oral medications and insulin, blood glucose can be manage close to normal level without causing hypoglycaemia. The oral medications varies with their classes, depends on the type of diabetes, ages, the individual itself and other factors. Many research has been done on finding the treatment of diabetes thus lead to the synthesis of oral medications, still, the medications were found to cause side effects (Fowler, 2007). As a result, these serious side effects have been reported worldwide (WHO, 2002). Therefore, studies of medicinal plant has been a great interest among researcher as these herbs were claimed to be safe, low cost and effective, hence suitable to be used as one of the alternative treatment (Pari *et al*., 1999).

The *Pereskia bleo* (Kunth) DC tree is a species belongs to the botanical family of Cactaceae (Tan *et al.*, 2004). It is commonly known as “jarum tujuh bilah” or “pohon jejarum” by Malaysian. This species was believed as the origin of other cacti. They are the only cactus genus that has a persistent non-succulent leaves. This species may grow to 7

meter high. *Pereskia bleo* has thinner, corrugated leaves, and oranges-red flower with shorter spines.

A preliminary survey were done to study the effectiveness of *Pereskia bleo* consumed by villagers in Kelantan, Malaysia that was reported that this plant was used as a health supplement, cancer treatment, muscular ache, hypertension, diabetes mellitus, internal problem, cyst and treating boils (Khor *et al.*, 2013). It was verified by various studies reported that this plant has anti-tumours, anti-rheumatic, anti-ulcer, anti-inflammatory activities (Sim *et al.*, 2010; Tan *et al.*, 2004). Furthermore, *Pereskia bleo* can be eaten raw as it was found to be non-toxic (Sim *et al.*, 2010). However, there are no scientific data reported regarding the anti-diabetic agents from this plant.

2.1 Diabetes Mellitus

Diabetes mellitus is a common chronic disorder. It is characterized by hyperglycaemia (high blood glucose level)c due to carbohydrate metabolism disorder with an insulin deficiency or insulin resistance or both (Anonymous, 2009). This disease is a third killer disease, after cancer and cardiovascular disease (Li *et al.*, 2004). It is the most prevalent metabolic disorder for all age-group in the world (Saladin, 2010). The World Health Organizations (WHO) has characterized this disorder as pandemic due to the spread of diabetes globally. It is about more than 347 million people of worldwide were estimated to have diabetes (Danaei *et al.*, 2011). In 2005, 1.1 million people were estimated to die from diabetes and almost 80% of them are from low and middle-income countries (Mathers *et al.*, 2006). Furthermore, 50% of them are people at age 70 years, while 55% of deaths case was women. Total of death in the next 10 years are projected to rise more than 50%. By the year 2030, WHO has predicted that this disorder will become the seventh leading cause of death.

The untreated diabetes mellitus can cause many complications. Acute complications are characterized by elevated blood glucose level (hyperglycaemia) and abnormally low blood glucose level due to medications. While chronic complications are characterized by diabetes with kidney, eyes, heart, nerves and feet damage. Diabetes is associated with cardiovascular disease. Patients with diabetes have a risk to suffer from heart disease and stroke. About 50% of diabetes patients has died due to cardiovascular disease (Candido *et al.*, 2003; Morrish *et al.*, 2001). Patients with

diabetes mainly type 2 diabetes are often have unhealthy cholesterol level including high LDL-cholesterol (Grundy *et al.*, 1990), high triglyceride and low HDL-cholesterol. In addition, the decreased in body weight and insulin concentrations due to diabetes may lead to increasing of cardiovascular risk. Lipid profiles have been used as an indicator of cardiovascular disease (Huang *et al.*, 1988). It has showed a correlation between increasing in plasma LDL-cholesterol, total cholesterol and the risk to have heart disease (Kamisah *et al.*, 2005). Type 2 diabetes associated with alteration in plasma lipid and lipoproteins are recognized as predictor for coronary heart disease (Craig *et al.*, 1998; Otamere *et al.*, 2011). Previous study reported that the inhibition of GP cause the reduction of lipid parameter such as total cholesterol and triglyceride level (Jasem *et al.*, 2012; Parthiban *et al*., 2013). Diabetes mellitus associated with lipid changed, has attributed to an increasing of fatty acid flux into liver. The accumulation of fatty acid are converted to triglycerides, thus reduce the level of HDL-cholesterol and increase in small dense of LDL-cholesterol (Mbaka *et al.*, 2012; Mooradian, 2009; Shih *et al.*, 1997).

Liver is a big organ that has a main function in managing carbohydrate, lipids, and protein metabolism. Liver disease occurred as consequences of diabetes mellitus. It is one of the main causes of death in type 2 diabetes. The death risk cause by liver disease is higher compared to the risk cause by cardiovascular disease (Ragavan *et al.*, 2006). Previous study reported that diabetes caused by to alloxan administration has deleterious effects on liver and kidneys (Giannini *et al.*, 2005). The increased in hepatic glucose due to glycogenolysis or glyconeogenesis was detected by hepatic enzyme; alanine aminotransaminase (ALT), serum aspartate aminotransaminase (AST) and alkaline

phosphatase (ALP) (Nahla *et al.*, 2012; Rawi *et al.*, 1998; Shibib *et al.*, 1993; WHO, 2002). The activities of these hepatic enzymes were used as marker to observe any damage in liver tissue (Eze *et al.*, 2012; Ravikumar *et al.*, 2010; Uboh *et al.*, 2010), organ and body. The damage in liver function is demonstrated with the increasing of ALT and AST (Zafar *et al.*, 2009). In alloxan-induced diabetes, the damage of β-cells cause the reduction in insulin produced, leads to organelles injury, thus released ALT and AST (Muhammad *et al.* , 2008; Sallie *et al.*, 1991; Senthil *et al.*, 2003).

Diabetes also associated with diabetic nephropathy. It caused damage to the kidney which can lead to chronic renal failure (Nelson *et al.*, 1995; Olimpia *et al.*, 2005). It is most likely to develop in diabetes type 1 compared to diabetes type 2. The kidney acts as a filter of the blood and removes wastes from the blood. The damage occurred in blood vessel walls of kidney filter due to prolong diabetes, initially will become leaking to larger protein such as albumin. Previous study shows that the treatment of diabetes by controlling their blood glucose level and blood pressure may prevent onset of chronic renal failure (Micheal *et al.*, 2005). Urea and creatinine are the parameters in diagnosis of kidney functions. The increase in creatinine and urea level indicates the dysfunction of kidney (Alagammal *et al.*, 2012; Almdal *et al.*, 1988; Kakadiya *et al.*, 2010).

Other complications that occur due to diabetes are diabetic retinopathy and diabetic neuropathy. Diabetic retinopathy is a damage of blood vessel in retina of the eye. This damage can lead to visual symptoms, reduce in vision, and potentially

become blindness. About 80% of patients who had diabetes more than 10 years will be affected by this disease (Eliott, 2007). While diabetic neuropathy is damage that occurs in nervous system, mostly cause numbness, pain in feet and skin damage. This disease has affected about 131 million people in 2010 which is 1.9% of the world population (Vos, 2012).

2.1.1 Type 1 Diabetes

Type I diabetes was also known as childhood-onset diabetes, insulin-dependent, or juvenile. It is characterized by lacking of insulin production cause by the failure of the pancreatic beta cells. It can also occur due to damage of pancreas by disease, alcohol or surgery. Most patient developed this type of diabetes are healthy, and it was termed as juvenile as the majority of the patients are children. Patients with type I diabetes have symptoms that includes weight loss, blurred vision, fatigue, frequent hunger and thirsty (polydipsia) and extremely urine discharge (polyuria). Diabetes type I occur because of some factors such as genetics, environmental, virus, diet and drugs (Knip *et al.*, 2005). This type of diabetes is a polygenic disease, which is the disease that occurred because of contributions of multiple genes. It is depends on locus or combination of loci regardless it is dominant, recessive or in between. A newborn child with father or sibling having diabetes type 1 has a 10% risk to develop type 1 diabetes, 4% if the mother aged below 25 years old and 1% if the mother aged above 25 years old. Environmental factors including identical twin, where one of the twin had diabetes type 1, the other one had about 30-50% risk to develop diabetes type 1. While in diabetes caused by virus as a factor, diabetes type 1 also

had proposed as a virus-triggered autoimmune response that happened when the immune system attacks virus-effected cells along with beta cells in pancreas (Delisa *et al.*, 2002). However, not everyone infected by this virus has developed type 1 diabetes. The other factor caused diabetes is diet where the antibodies against cow milk protein are found to influence the autoimmune response. Vitamin D at dose of 2000IU given during the first year of a child was found to cause a reduction about 80% in the risk of getting diabetes type 1 later in life. Furthermore, a period of breastfeeding also known to be associated with this factor among Czech children (Hana *et al.*, 2005). Other than that, the usage of drugs sometimes caused the destruction of the pancreatic cells. For examples, streptozotocin used as an antibiotic and antineoplastic agent in chemotherapy of pancreatic cancer, can kills beta cells. Trauma, pancreatitis or tumour also can lead to the fall in insulin productions. Type 1 diabetes can be treated with insulin, either via injection, pumps or inhaled of insulin. Other alternatives treatments are pancreatic transplants and pancreatic islet cell transplant

2.1.2 Type 2 Diabetes

Type II diabetes also known as non-insulin-dependent or adult-onset. Usually patients above 45 years old were suffered from this type of diabetes. It is a metabolic disorder and characterized by insulin resistance and insulin deficiency (Kumar *et al.*, 2005). Insulin produces by beta cells of pancreas are important in regulating carbohydrate and fat metabolism in body. Insulin resistance is a condition which cells fails to response normally to insulin. Insulin worked as a glucose transporter, deliver glucose to cells in livers, skeletal muscles, and fat tissue to absorb glucose from blood in order to provide energy. Due to

insulin resistance, cells are unable to receive glucose, amino acid, and fatty acid. Thus, glucose levels in blood will rise. At least 90% of patients with diabetes suffered from diabetes type 2. It is increased over 50 years in parallel of obesity. In 2010, approximately 285 million people suffered with these disease, compared to 30 million in 1985 (Smyth *et al.*, 2006). Diabetes type 2 always associated with cardiovascular, blindness and kidney failure (Ripsin *et al.*, 2009). Diabetes type 2 occurs because of some factors such as lifestyle, genetic factors (Ripsin *et al.*, 2009; Riserus *et al.*, 2009), diet, lack of sleep and development of foetal during pregnancy that altered DNA methylation (Christian *et al.*, 2010). It is usually controlled by proper diet, regular exercise (Raina *et al.*, 2008), maintaining ideal body weight and oral medication.

2.1.3 Gestational Diabetes

Gestational diabetes (GDM) was recognized by occurrence of hyperglycaemia during pregnancy especially in third semester. It is due to changes in growth hormone and cortisol secretion, secretion of human placenta lactogen and insulinase. Human placenta lactogen is produced by placenta and affects fatty acids and glucose metabolism, thus promotes lipoysis and decreases glucose uptake. The insulinase is produced by placenta and facilitates the insulin metabolism. About 2-5% of pregnancy women may have this disease and will have normal blood glucose reading after baby delivery of baby (Gilmartin *et al.*, 2008). However, 20-50% of affected women mostly will develop to type 2 diabetes in the future. Pregnancy women with previous history of macrosomia (birth weight more than 4000g), strong family history of diabetes, and

obesity have higher percentage to get GDM (Gilmartin *et al.*, 2008). Other risks factors for GDM includes ethnic group with higher rate of type 2 diabetes, polycystic ovarian syndrome (PCOS), hypertension, history of spontaneous abortions and unexplained stillbirths, and age older than 25 years (Marion *et al.*, 2008).

Gestational diabetes can be diagnose during prenatal screening as glucose tolerance was not present or recognized (Beckmann *et al.*, 2005). Untreated gestational diabetes can cause risks of some disorder including macrosomia (high birth weight), congenital cardiac, abnormality in central nervous system and skeletal muscle malformations to foetus or the mother.

If untreated, it can cause seizures or still birth. The early treatment of GDM shows reducing in foetal complication such as macrosomia (‘fat’ baby). Macrosomia develops due to extra glucose that goes inside the placenta, thus cause baby’s pancreas to works harder in making extra insulin that will reduce the high blood glucose levels. Therefore, newborn baby tents to have very low blood glucose level at birth and breathing problems (Diabetes Malaysia, 2007).

The treatment of GDM includes the management of glycaemia control (Marion *et al.*, 2008) such as the quality nutritional intake daily (Jovanovic-Peterson *et al.*, 1992), scheduled physical activity, daily blood glucose testing and insulin injections (Crowther *et al.*, 2005). The uses of oral hypoglycaemic agent such as glyburide and metformin are recommended in management of GDM when diet alone was insufficient. Previous study of

comparison between metformin and insulin in GDM patient has showed that it did not vary in neonatal complication (Rowan *et al.*, 2008). However, the less severe hypoglycaemia in infants and preterm birth were common in GDM women treated with metformin (Gilmartin *et al.*, 2008).

2.2 Carbohydrate Metabolism

Carbohydrate metabolism indicates in many biochemical processes such as the formation, breakdown and interconversion of carbohydrate in living organism. Glucose is the most important carbohydrate. Glucose is a simple monosaccharide that is important in cell body as a source of energy and metabolic intermediate. It is selected due to its lower tendency that can react with the amino groups of protein that can cause a glycation. Glycation of protein or lipids is a result from covalent bonding of this substrate with sugar molecule such as fructose. It can destroy the function of many enzymes and leads to varies disease such as renal failure, blindness and peripheral neuropathy in diabetes mellitus (American Diabetes Association, 2010).

Cells body need glucose for proper functions. Glucose metabolism is a process which converts glucose into energy cells needed which is glycogen. There are three hormones from pancreas that is important in blood glucose regulations which are insulin, glucagon and epinephrine. Insulin is released from pancreatic beta cells when blood glucose concentration in body is too high. These rises may be due to a few factors such as the speed up of breakdown of glucose by glycolisis, the increase in glycogen synthesis in

liver and skeletal muscle or the increase of synthesis of lipids and protein. Insulin is important in stimulating the conversion of glucose into glycogen in liver or muscles via glycogenesis. Insulin will keep the glycogen synthase which an active enzyme for glycogenesis, in active state (Alvin, 1970; Vickram *et al.*, 2011). Insulin also important in inhibits the glycogen phosphorylase (Vickram *et al.,* 2011). When glucose enters a cell from bloodstream, it will convert into glucose-6-phosphate by glucokinase or hexokinase. This both enzymes plays important roles in facilitates phosphorylation of glucose. Glucose-6-phosphate then isomerized to glucose-1-phosphate and then attached to uridine diphosphate (UDP).In muscle cells, the degradation of glycogen provides an immediate source of G6P for glycolysis which is important to provide energy for muscle contraction. In liver cells, this process important for release glucose into bloodstream for other cells uses.

In contrast, the glucagon and epinephrine are released from pancreatic alpha cells when blood glucose in body falls. It can be due to the slower glucose enter the cell, the faster in liver glycogen breakdown, the increase of gluconeogenesis in glucose synthesis to breakdown the lipids and protein, or accelerates of glucogenesis. These hormones stimulate the conversion of glycogen to glucose via glycogenolysis (Sowka *et al.*, 2001)

Glycolysis occurs when body needs glucose immediately to supply energy. The end products of this process are pyruvic acid and ATP. Pyruvic acid later converted to acetyl CoA then citric acid in citric acid cycle. During contraction of muscle, pryruvic acid is converted to lactic acid and converted back to pyruvic acid during rest time. The pyruvic

acid was then converted back to glucose via gluconeogenesis. However, if the body does not require glucose, it will be converted into glycogen via glycogenesis.

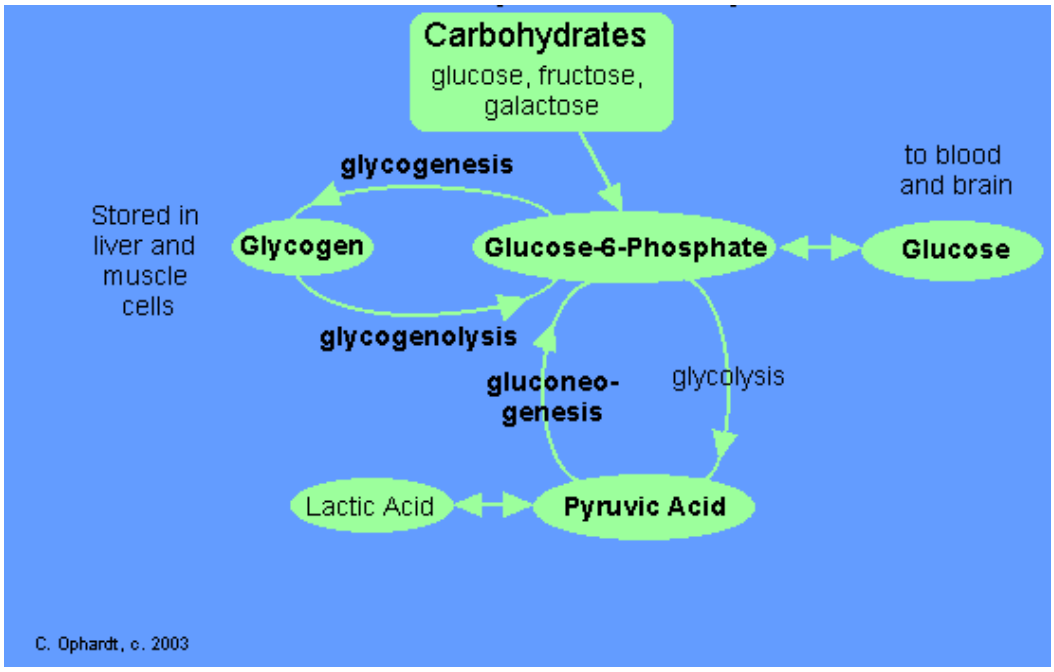


Figure 2. 1 Glucose metabolisms mechanism (Ophardt, 2003)**.**

Glycogen contains many types of glucoses, providing a quick source of glucose when body needed. Glycogen stored in liver is a main source of energy supply to body tissue. It is considered as a main buffer of blood glucose levels. A second source is stored in skeletal muscle. However, muscle lack of glucose-6-phophatase enzyme, thus not available to other tissues even though the total of glycogen stored in muscle in about twice from liver. Other minor storage of glycogen is in kidney and intestines. Brain used 75% of glucose consumption daily via aerobic pathway. Body obtains glucose directly either from amino acids and lactate or diet via gluconeogenesis. These sources either remain soluble in body fluids or stored as glycogen.

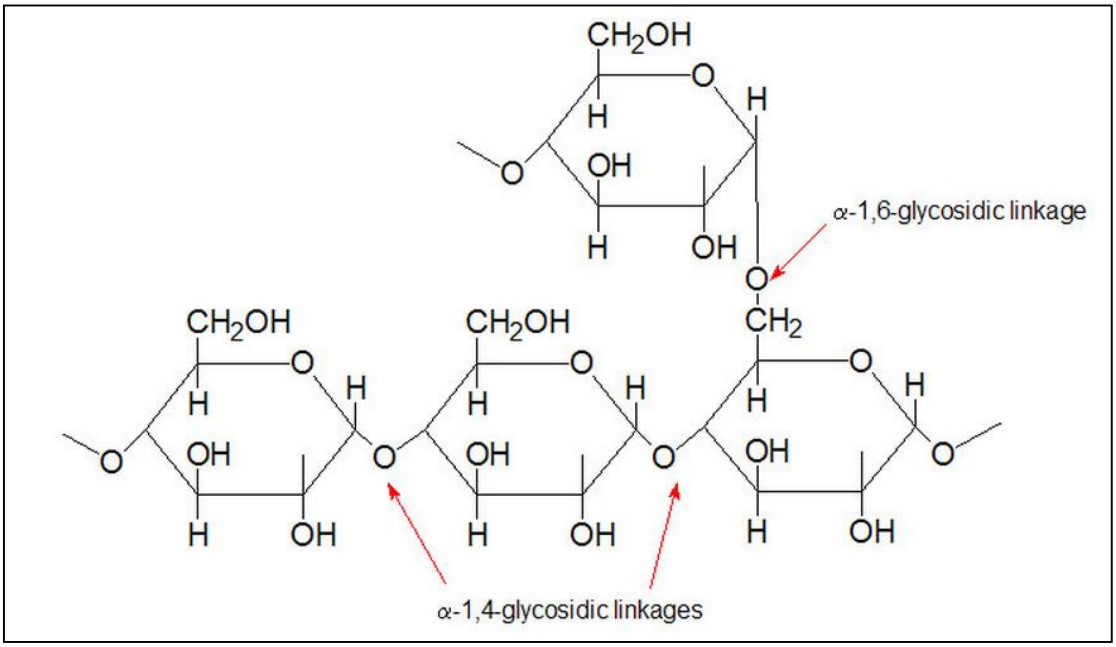


Figure 2. 2 Glycogen structure showing α-1,4- and β-1,6-glycosidic linkages.

Approximately up to 120,000 glucose residue linked by α-(1,4)- and α-1,6)-glycosidic bonds in glycogen. These links are occurred in every 8-12 of glucose residues. The branches occurred between α-(1,4)- and α-1,6)- are important in degradation process of glycogen to glucose. More branches causing more glucose released at once. The degradation process also known as a glycogenolysis process. This process occurs through the action of glycogen Phosphorylase.

2.2.1 Glycogen Phosphorylase Enzymes

Glycogen phosphorylase is a phosphorylase enzyme that is important in the catalyzes or breakdown of glycogen in liver and tissue with high energy demands (David *et al.*, 2005). This enzyme has been proposed in the treatment of diabetes type 2 via the inhibition of released glucose in the liver (Martin *et al.*, 1998; Ogawa *et al.*, 2003; Somsak *et al.*, 2003; Treadway *et al.*, 2001). The term phosphorylase is not derived from phosphorylation process, but from breaking a bond in glycogen. Glycogen phosphorylase form in 2 types which are phosphorylated phosphorylase b and phosphorylase a. The differences between these 2 enzymes are phosphorylase a form (Ser-PO42-) whereas b forms (Ser-OH). The b form is less active and transformed to a form which is more active by phosphorylase kinase. The glycogen phosphorylase a form is subsequently dephosphorylated by the action of protein phosphatases, it returned to less active b form (David *et al.*, 2005).

The breakdown or degradation of glycogen, known as glycogenolysis, remove single glucose residues from α-(1,4)-linkages and release glucose-1-phosphate (G1P) as a product reaction which then converted to glucose-6-phosphate (G6P) by phosphoglucomutase. G6P derived from glycogen is important in (1) continuing on the glycolysis pathway and use as energy for anaerobic or aerobic metabolism such as in muscle. (2) dephosphorylated back to glucose by glucose-6-phosphotase enzyme in gluconeogenesis pathway of liver and kidney and released into blood. (3) Produce NADPH and 5-carbon sugars in pentose phosphate pathway via glucose-6-phosphate

dehydrogenase enzyme (Berg *et al.*, 2002). In muscle cells (myocytes), degradation of glycogen is important to provide source of glucose-6-phosphate for glycolysis which is important in supplying energy for muscle contraction. While in liver cells (hepatocytes), the breakdown of glycogen is important so that glucose can uptake by other cells.

2.2.2 Glycogen Phosphorylase Inhibitor

Glycogen Phosphorylase (GP) inhibitor have been developed and studied as potential therapy of hyperglycaemia (Moller, 2001; Somsak *et al.*, 2003). Administration of GP inhibitor showed to reduce liver glycogen phosphorylase activity and attenuate hyperglycaemia without producing hypoglycaemia, a potential side effect by many antidiabetic agents (Martin *et al.*, 1998; Ogawa *et al.*, 2003). Previous research also showed that GP inhibitor are effective in reducing hepatic glucose output in presence of higher glucose contents (Kasvinsky *et al.*, 1978) and also cardio protective (Huang *et al.*, 1988). This situation important in protecting against rebound hypoglycaemia and reduce fatality cause by cardiovascular disease especially in type 2 diabetes patients (Oikonomakos *et al.*, 2000). The administration of glibenclamide in alloxan-induced diabetic rats was reported to have hypoglycaemic effects by increasing the insulin secretion, therefore stimulates the glycogen synthase and inhibit the glycogen phosphorylase activity (Gerich, 1989; Harrison, 2001; Nidhi *et al.*, 2010).

2.3 Anti-hyperglyceamic Agents

Management of diabetes mellitus consists of exercise, diet, and administration of common drug such as biguanides and sulfonylureas. These anti-hyperglyceamic drugs were categorized in different classes and were use depends on the type of diabetes, age, and condition of patients. Patients with diabetes type I required injections of insulin due to lack of insulin. It is because type 1 diabetes results from absolute deficiency of pancreatic β cells, thus most oral agents are not required. While for patients with diabetes type 2 typically required oral agents due to insulin resistance (Fowler, 2007). There oral agents either works (1) increased the amount of insulin secretion by pancreas (2) increase the sensitivity of target organ to insulin, or (3) decrease rate of glucose absorbance from gastrointestinal tract. Now a day, a lot of drugs are available. Metformin is the first drug prescribed in diabetes therapy. When metformin combined with diet and lifestyle intervention is not enough to reach the glycaemia target, other options are available such as sulfonylureas, pioglitazone, α glucosidase inhibitors and others (Derosa *et al.* 2012)

2.3.1 Insulin

Insulin is commonly used in the treatment of diabetes Type 1 as the lack of insulin produced is the cause of the disease. Insulin usually will be injected subcutaneously using syringe needles with insulin pump and also using repeated-use insulin pump with needles. In acute-care disease, insulin is given intravenously. It cannot be consume orally as it will be reduce to fragment in gastrointestinal tract and

lots their activity. Insulin is manufactured using DNA recombinant technology. Research by SembioSys Genetics company in 2007 has reported the successfully production of human insulin derived from seed of safflower as the global demand for the hormone grows (Levinson, 2007). This technique is believed to reduce the cost of insulin productions. The insulin is characterizes based on their metabolism rate; rapid acting insulin, intermediate acting insulin and long acting insulin. The rapid acting insulin have an effect as fast as 15 minutes due to mutation in sequence that prevent this type of insulin forming dimers and hexamer which can rapidly absorb. It is including Humulin R and Novolin R (Regular insulin), Humalog (lispro), Novolog (aspart), Apidra (glusine) and Semilente (insulin zinc). The intermediate acting insulin includes Humulin N and Novolin N, and Lente. While a long acting insulin includes Ultralente (extended insulin zinc), Lantus (glargine) and Levemir (detemir). These types of insulin have an effect that extended from 18 to 24 hours.

2.3.2 Sensitizer

Insulin sensitizers consist of two classes of drugs which are biguanide and thiazolidinedione. These types of drug are used in the treatment of diabetes type 2. Biguanides such as metformin (Glucophage) is commonly used for type 2 diabetes. Research reported that this drug is the best to consumed for patients who have heart failure (Eurich *et al.,* 2007) with certain procedures. Metformin also showed to be safe and effective to gestational diabetes. Previous research reported that newborn babies with mother given metformin showed a healthier in neonatal period (Balani *et al.*,

2009; Palshetkar *et al.*, 2011; Tertti *et al.*, 2008). Moreover, this drug also reported can prevents cardiovascular complications of diabetes via reducing LCL cholesterol and triglyceride level and at the same time is not causing weight gain (Bolen *et al.*, 2007; Maharani, 2010). While phenformin (DBI) and buformin, was withdrawn due to lactic acidosis risk (Fimognari *et al.*, 2006; Verdonck *et al.*, 1981).

Thiazolidinediones also known as glizones, is an anti-hyperglyceamic drug in the treatment of diabetes type 2. It acts in reducing insulin resistance by activating PPAR-y in fats and muscle. This type of drug consists of rosiglitazone (avandia), pioglitazone (actos) and Troglitazone (rezulin). Recent studies reported that rosiglitazone increase the risk of coronary heart disease and heart attacks (Home *et al.*, 2009). In contrast, research on pioglitazone shows that this drug decrease the incidence of cardiac event in patient with diabetes type 2 with history of heart attack (Erdman *et al.*, 2007).

2.3.3 Secretagogues

Insulin secretagogues are a substance that triggered the secretion of insulin. It is consists of sulfonylureas and meglitinides (non-sulfonylurea). Both drugs used in the treatment of diabetes type 2 which they bind to the ATP-dependent K^+ (K_{ATP}) channel on cell membrane of pancreatic beta cells. Thus, insulin is secreted. Sulfonylureas are safe to use as treatment of neonatal diabetes (Greeley *et al.*, 2010; Oztekin *et al.*, 2012). Study had shows that this drug has no effect on blood pressure (Rodger, 1999). In high dose and patient is fasting, this drug can cause hypoglycaemia due to excesses in insulin release. Thus, it can lead to weight gain due to high glucose uptake. Glycated

haemoglobin (A1C) is use in second-generation of sulfonylureas. The high amount of glycated haemoglobin indicates poorer control in blood glucose level and associated with cardiovascular disease, nephropathy, and retinopathy. The reductions in haemoglobin by using second-generation of sulfonylureas are 1-2%. The second-generation agent of sulfonylureas consists of glipizide, glibenclamide, gliclazide, glycopyramide and gliquidone. Meglitinide also known as Glinides and it has similar mechanism as sulfonylureas but have separate binding site. Contrast to sulfonylureas, this drug also can lead to weight gain and hypoglycaemia but the chance are lesser. Glycated haemoglobin (A1C) shows about 0.5-1% reduction during treatment with this drug. Meglitinides consists of repaglinide and nateglinide.

2.3.4 Alpha-glucosidase Inhibitor

The alpha-glucosidase inhibitor includes Arcabose, Miglitol and Voglibose. This drug used in the treatment of diabetes type 2. It is also is the best in treating impaired glucose tolerance at earliest stages. It is not a technically hypoglycaemic agent because this drug did not have direct effect on insulin secretion. This drug acts as preventing the digestion of carbohydrate such as starch and sugar (Bischoff, 1994). However, since the carbohydrate remain longer in the intestine, bacteria will digest this carbohydrate and causing gastrointestinal side effect such as flatulence (78% of patients) and diarrhoea (14% of patients) (Derosa *et al.*, 2012). The alpha-glucosidase inhibitors also can found in natural products (Benalla *et al.*, 2010; Ji *et al.*, 2010). Research showed that this inhibitor also can

be found in plant such as mushroom maitake *Grifola frondosa* (Lo *et al.*, 2008) and soybean (Lee *et al.*, 2001).

2.4 Medicinal Plants

WHO currently encourage, facilitate, promotes and provide international standards guidelines in traditional medicine (Bodeker *et al.*, 2000; World Health Organization, 2002). The WHO traditional medicine strategy program was introduced in early 2002 and the main objectives of this program are to discuss the role of this traditional medicine in healthcare system, challenges and their opportunities. Traditional medicine is widely used in Asia and Latin America due to their historical circumstances and cultural beliefs. For example in China, around 40% of health care used traditional medicine. In the developing country where the drugs are expensive and unaffordable by majority of diabetic patients, a wide range of medicinal plants have been used as an alternative therapy (Adesokan *et al.*, 2009). At least 48% in Australia, 70% in Canada, 42% in USA, 38% in Belgium and 75% in France used this alternative treatment. In Malaysia, WHO (2005) estimated US$ 500 million had spent on this treatment, and this showed that this treatment growing rapidly and become more popular.

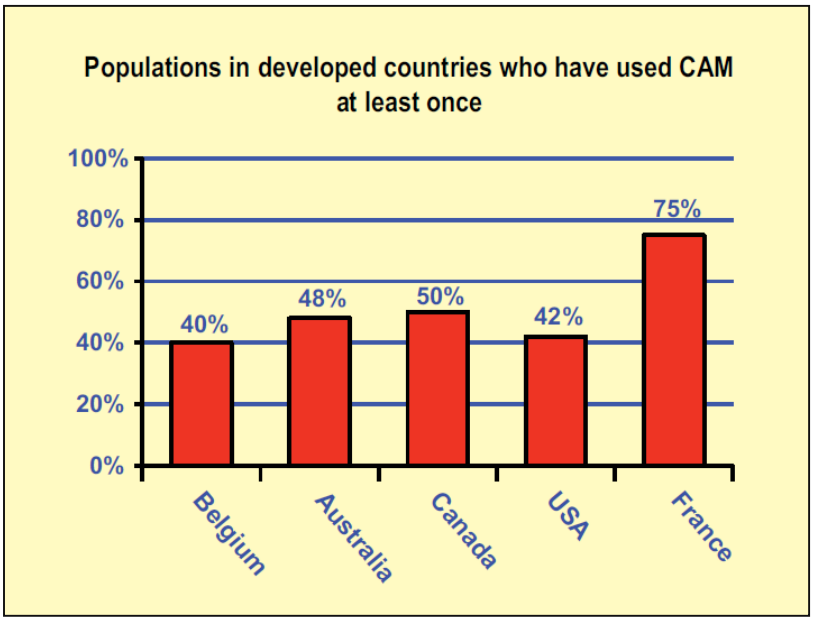


Figure 2. 3 Percentages of populations in developed country

(Source: WHO Traditional Medicine Strategy: 2002-2005)

Medicinal plants have been known since ancient times in the treatment of the disease. Today, this treatment has been accepted as an important component of healthcare system. The worldwide has reported that the drug currently used in clinical practice have serious side effects. This situation makes people turn to medical plants as it is free from side effect, low cost and has significant effects (Pari *et al.*, 1999). Other than the population rise, insufficient drugs supplies and development of resistance of infectious diseases to currently modern medicine, have led to the increasing of the usage of plant materials as an alternative treatments.

There are 350,000 species of higher plants in the world and about 35,000 to 70,000 of this species are used worldwide for traditional medicinal (Haidar *et al.*, 2009; Lewington, 1990). This treatment is a traditional medicinal that are based on the use of

plants crude extract. There are a lot of scopes for this medicine, include bee product, minerals, fungal and certain animal parts (Acharya *et al.*, 2008). Asia countries likes Malaysia, China and India already consistently used this medicinal treatment especially among ethnic people. Meanwhile countries in Europe are beginning to explore these types of treatment. In Peninsular Malaysia, the unique values showed by the presence of three main races that are already having medicinal plant knowledge: the indigenous Malay medicinal knowledge, the Ayurvedic (Indian) school of medicine and the Oriental (Chinese) medicinal knowledge. This makes the field of medicinal plant are highly recommended and interesting. Estimated about 12,500 species existed in the forest and about 2000 species have been used in medicinal treatment in Malaysia (Bidin, 1995).Previous study reported that approximately 2,000 species from 14,500 flowering plants have been reported to contains medicinal properties and many have been scientifically proven in Malaysia (Nardiah *et al.*, 2010). The large scale of processing some of the important medicinal product has been started here. In 1999, about 1,546 of traditional medicine companied have been registered (Anonymous., 2009). In 1996, Malaysia Traditional & Complementary Medicine Unit was formed and registered.

2.5 Hyperglyceamic Plants Compounds.

According to WHO, approximately 80% of populations from the third countries are depends on medicinal plants as traditional therapies. Over 400 plants used in the treatment of diabetes mellitus in almost two thirds of the world population were observed (Musbah, 1994; Singh *et al.*, 2011). The earliest study of hyperglyceamic plants was reported that

about 343 plants showed a potential in reducing blood glucose levels and has been classified (Atta-Ur-Rahman, 1989). Furthermore, Bailey and Day (1989) reported that 29 isolated compounds were obtained and classified to 14 polysaccharides, 5 alkaloids, 4 glycosides and 6 other compounds. Similarly, the earliest previous study also reported that there are four chemical groups of 78 different isolated compounds founds in hypoglycaemic plants (Ivorra *et al.*, 1989), which are;

i. Alkaloid (7 compounds)
ii. Flavonoids and related compounds (5 compounds)
iii. Terpenoid and steroids (7 compounds)
iv. Polysaccharides and proteins (59 compounds)

A single medicinal plant contain thousands of bioactive compounds, thus may have multiple effects in the treatment of diseases. The uses of medicinal plant, alone or in combination with other medicinal plants are believed can be a good combinations of therapy (Kaur *et al.*, 2013). In hypoglycaemic plants, this combination may have multiple actions either in insulin action, insulin production or both (Chang *et al.*, 2013).

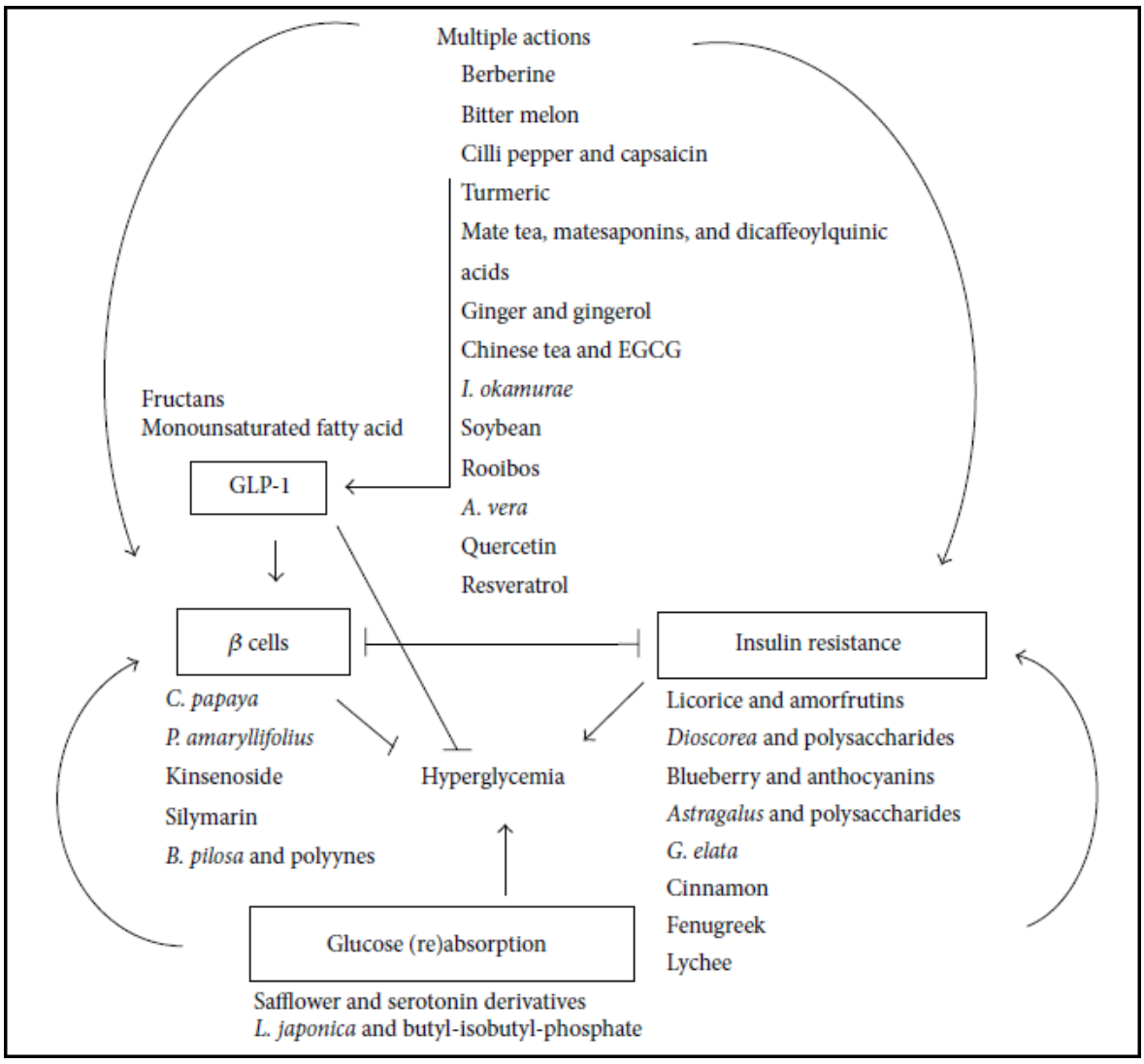


Figure 2. 4 Mechanism actions of hypoglycaemic plants. The selected plants and compounds with their multiple anti-hyperglycaemic reactions; insulin resistance, β cell function, GLP1 or glucose reabsorption (Chang *et al.*, 2013)

A review study reported that the antidiabetic activities of bioactive compounds found in medicinal plants involved either in regulate insulin resistance, β cell function, GLP-1 homeostasis, glucose absorption in the gut, or with multiple antidiabetic actions (Chang *et al.*, 2013).

Table 2. 1 Active compounds and biological actions of antidiabetic plants

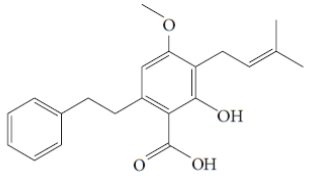
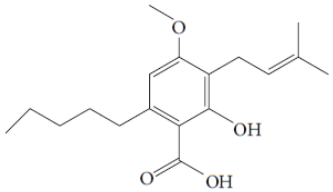
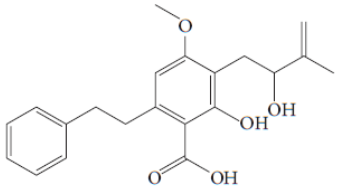
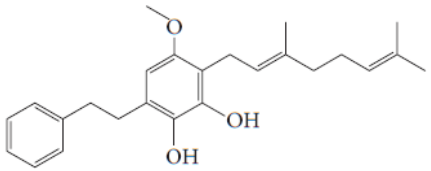
Antidiabetic Mechanism	Plants	Compounds	Chemical Structure	Reference
Regulate insulin resistance	*G. uralensis*	Amorfrutin 1		(Weidner *et al.*, 2012)
		Amorfrutin 2		
		Amorfrutin 3		
		Amorfrutin 4		

Table 2.1 'Continued'

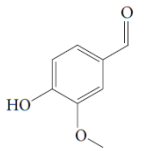
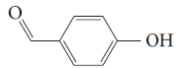
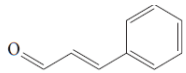
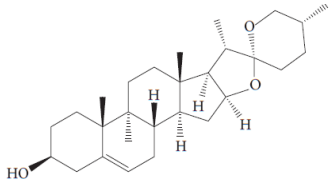
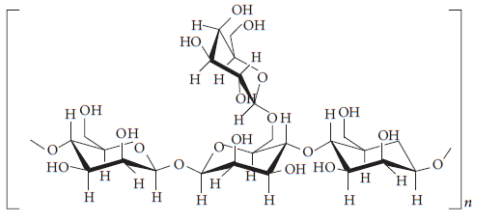
Antidiabetic Mechanism	Plants	Compounds	Chemical Structure	Reference
Reduce insulin resistance	*G.elata*	Vanillin		(Park *et al.*, 2011)
		4-hydroxybenzaldehyde		
Regulate insulin resistance	*C.verum* *C.zeylanicum* *C.aromaticum*	Cinnamaldehyde		(Liu *et al.*, , 2012)
Reduce insulin resistance	*T. foenum-graecum*	Diosgenin		(Puri *et al.*, 2002; Uemura *et al.*, 2010)
		Galactomannan		(Puri *et al.*, 2002)

Table 2.1 'Continued'

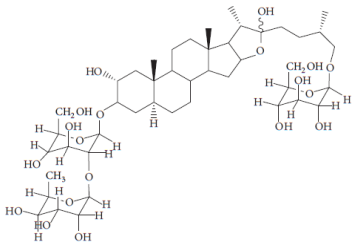
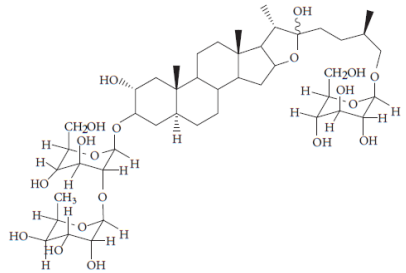
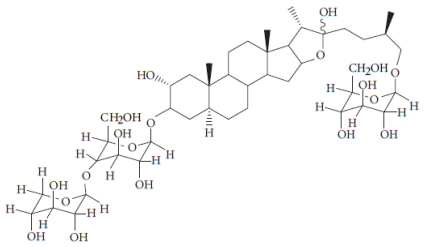
Antidiabetic Mechanism	Plants	Compounds	Chemical Structure	Reference
Reduce insulin resistance	*T. foenum-graecum*	Trigoneoside Xa		(Puri *et al.*, 2002)
		Trigoneside Xb		
		Trigoneoside Xlb		

Table 2.1 'Continued'

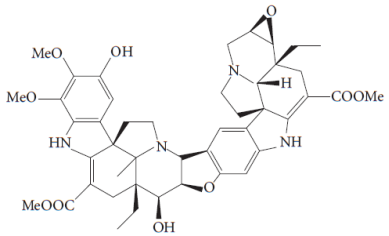
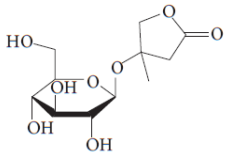
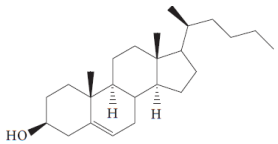
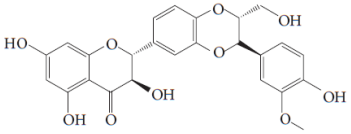
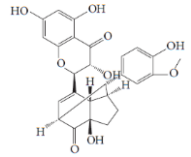
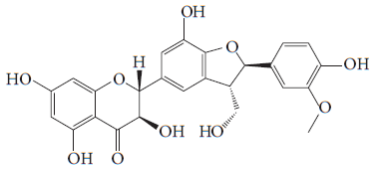
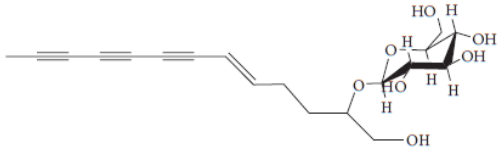
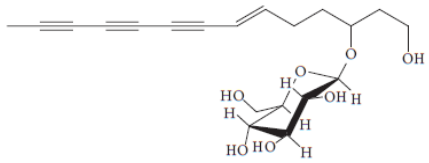
Antidiabetic Mechanism	Plants	Compounds	Chemical Structure	Reference
Regulate β cell function	*C. papaya* *P. amaryllifolius*	Flavonoid, alkaloids, saponin, and tannins	N.A	(Sasidharan *et al.*, 2011)
	T. divaricate *E. nicrophylla*	Conophyline		(Kawakami *et al.*, 2010; Kodera *et al.*, 2009; Ogata *et al.*, 2004; Saito *et al.*, 2012)
	A.roxyburghii	Kinsenoside		(Zhang *et al.*, 2007)
	N. stellata	Nymphayol		(Subash-Babu *et al.*, 2009)
	S. marianum	Silybin		(Jose *et al.*, 2011; Soto *et al.*, 2010; Vengerovskii *et al.*, 2007)

Table 2.1 'Continued'

Antidiabetic Mechanism	Plants	Compounds	Chemical Structure	Reference
Regulate β cell function	*S. marianum*	Silydianin		(Jose *et al.*, 2011; Soto *et al.*, 2010; Vengerovskii *et al.*, 2007)
		Silychristin		
	B.pilosa	3-*β*-D-glucopyranosyl-1-hydroxy-6(*E*)-tetradecene-8,10,12-triyne		(Chien *et al.*, 2009; Ubillas *et al.*, 2000)
		2-*β*-D-glucopyranosyloxy-1-hydroxy-5(*E*)-tridecene-7,9,11-triyne		
		2-*β*-D-glucopyranosyloxy-1-hydroxytrideca-5,7,9,11-tetrayne (cytopiloyne)		(Liu *et al.*, 2012)

'Table 2.1 'Continued'

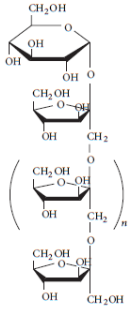
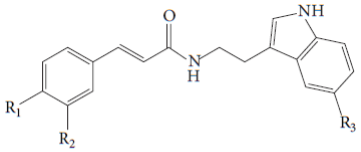
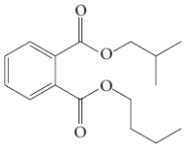
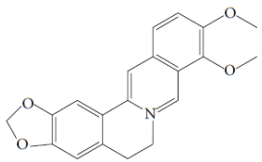
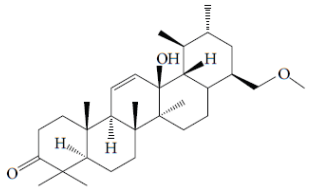
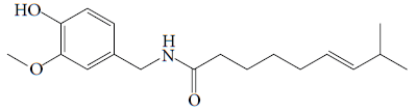
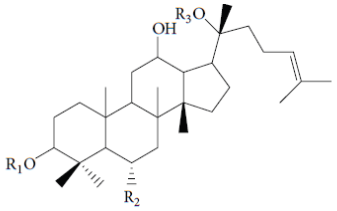
Antidiabetic Mechanism	Plants	Compounds	Chemical Structure	Reference
Regulate GIP-1 homeostasis	*A.tequilana*	Inulin/Raftilose		(Urlas-Silvas *et al.*, 2008)
	Olive oil	Monounsaturated Fatty acids	N.A	(Gentilcore *et al.*, 2006)
Regulate glucose absorption in guts	*C. tinctorius*	Serotonin derivatives	 1 R_1 = OH; R_2 = H; R_3 = OH 2 R_1 = OH; R_2 = OMe; R_3 = OH	(Takahashi *et al.*, 2012)
	L.japonica	Butyl-isobutyl-phthalate		(Akar *et al.*, 2011)

Table 2.1 Continued

Antidiabetic Mechanism	Plants	Compounds	Chemical Structure	Reference
Regulate two or more pathways	*B. vulgaris*	Berberine		(Dong *et al.*, 2012; Jeong *et al.*, 2009)
	M. charantia	Momordicin		(Singh *et al.*, 2011)
	Capsicum plants	Capsaidin		(Gram *et al.*, 2007; Hwang *et a*l., 2005)
	P. ginseng	Ginsenoside Rb1 Ginsenoside Rb2 Ginsenoside Rc Ginsenoside Rd Ginsenoside Re Ginsenoside Rf Ginsenoside Rgf		(Cho *et al.*, 2006; Kao *et al.*, 2007)

2.6 Studied Plant – *Pereskia bleo*

Pereskia bleo (Kunth) DC tree is a species belongs to the botanical family Cactaceae (Tan *et al*., 2004). It is commonly known as "jarum tujuh bilah" or "pohon jejarum" by Malaysian. It is also commonly known as leaf cactus, rose cactus and wax rose in English. While in Chinese, this plant known as cak sing cam.

Kingdom:	Plantae (Plants)
Subkingdom:	Viridaeplantae (Green plants)
Division:	Tracheophyta (Vascular plants)
Subdivision:	Spermatophytina (Seed plants)
Class:	Magnoliopsida
Order :	Caryophyllales
Family:	CactaceaeJuss (Cactus, cacti)
Genus:	*Pereskia* Mill
Species:	*P.bleo*

Pereskia bleo is originates from Paraguay, Argentina and Korea (Khor *et al.*, 2013; Yusof, 2010). It has been cultivated in Malaysia for traditional medicine and also as decorative plant. This plant does not look much like other types of cacti, having substantial leaves and thin stems. They are the only cactus genus that has persistent non-succulent leaves. It is believed that this is the origin of other cacti. Most of the *Pereskia* species

correspond in dry forests or thorny scrub, in tropical climates with a dry season and very dry forest life except for *Pereskia bleo* which is lives in higher annual rainfall (Erika *et al.*, 2006).

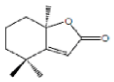
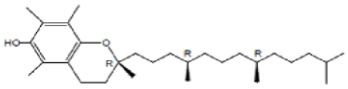
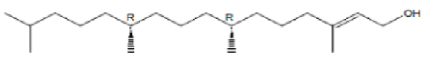
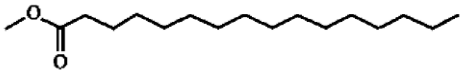
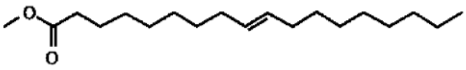
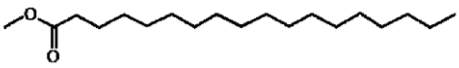
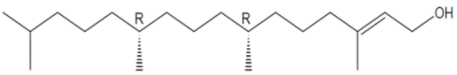
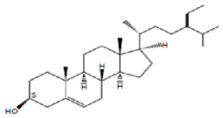
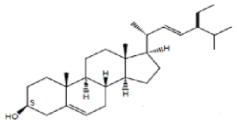
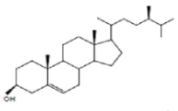
Figure 2. 5 *Pereskia bleo* (Kunth) DC; leaves, flower, fruit and stem**.**

This species may grow 2-8 meter high. Their leaves are large, bright green and privet-like leaves. They have long spiny stems and the young branches in red. It spines in fascicles of 5 or 6, but young shoots often bear but 1 to 4. These spines show development from areoles. The orange-red Flowers may appear alone or in clusters. They usually resemble roses and reach a diameter of 1 to 5 cm. Colours of the flower depends on the species and vary from white, yellow to magenta or red (Yusof, 2010). Fruits are ordinarily waxy spherical green with big and black colour. It will turn to yellow when ripe (Khor *et al*., 2013).

2.6.1 Chemical Constituents

Doetsch, (1998) has reported that the alkaloids isolation from *Pereskia bleo* were 3,4-dimethroxy-β-phenethylamine, mescaline, 3-methoxytyramine and tyramine. Whereas, Goh (2000) reported that the isolated compounds presence in *Pereskia bleo* were stigma sterol, β-sitosterol, dihydroactinidiolide and campesterol. Ethyl acetate extract of *Pereskia bleo* was reported to contains β-sitosterol, 2,4-ditert-butylphenol, α-tocopherol and phytol (Sri Nurestri *et al.,* 2008).

Table 2. 2 Chemical compounds reported found in *Pereskia bleo.*

Chemical compounds	Chemical Structure	Reference
Dihydroactinidiolide		Appalaraju *et al.*, 2013; Goh, 2000
A-tocopherol		Appalaraju *et al.*, 2013; Sri Nurestri *et al.*, 2008
Phytol		Sri Nurestri *et al.*, 2004
Methyl palmitate		Sri Nurestri *et al.*, 2009
Methyl oleate		Sri Nurestri *et al.*, 2009
Methyl stearate		Sri Nurestri *et al.*, 2009
Phytol		Sri Nurestri *et al.*, 2009; Sri Nurestri *et al.*, 2008
B-sitosterol		Appalaraju *et al.*, 2013; Goh, 2000; Sri Nurestri *et al.*, 2008
2,4-ditert-butylphenol		Appalaraju *et al.*, 2013, Sri Nurestri *et al.*, 2008
Stigma sterol		Appalaraju *et al.*, 2013; Goh, 2000
Campesterol		Appalaraju *et al.*, 2013; Goh, 2000

2.6.2 Medicinal Uses

In Malaysia, the leaves of *Pereskia bleo* were used as a natural medicine and can be eaten as salad (Tan *et al.*, 2004). Similarly, *Pereskia grandifolia*, one of medicinal plant under the same family with *Pereskia bleo* also has been traditionally used by local Malaysia as natural medicine in cancer-related disease, high blood pressure, diabetes, rheumatism, inflammation and ulcer (Goh, 2000; Sri-Nurestri *et al.*, 2009). The leaves of this plant also used as traditional treatment for headache, gastric pain, haemorrhoids, atopic dermatitis, ulcers and also used to refresh the body (Goh, 2000; Rahmat, 2004; Tan *et al.*, 2004). In previous cytotoxic study, this plant has been reported to have cytotoxic effects on various cancer cell lines but not in normal cell lines. (Sim *et al.*, 2010). Furthermore, previous study of acute oral toxicity on experimental ICR mice also showed that there are no mortality occurred when ICR mice were induced with both *Pereskia bleo* and *Pereskia grandifolia* (Sim *et al.*, 2010). Current drugs used in the treatment of diabetes are always associated with several side effects. Therefore, oral hyperglyceamic agents that are safe, and effective are needed (Murthy, 1995; Nidhi *et al*., 2010). *Pereskia bleo* also reported commonly used as antidiabetic and hypertension treatment for certain ethnic in Malaysia (Tan *et al*., 2004). However, there are no scientific reports indicates that this plant contained anti-diabetic effects (Khor *et a l.*, 2013).

2.7 Research Objectives

The main objectives of the study are:

1. To investigate the profile of organic compounds from *Pereskia*
2. To assay the glycogen phosphorylase inhibitory activity of the plant crude extracts and isolated compounds
3. To evaluate the effect of *Pereskia bleo* aqueous crude extracts as antidiabetic agents in Sprague Dawley (SD) rats
4. To evaluate the toxicity of *Pereskia bleo* using Brine Shrimp Lethality Assay (BSLA)

3.1 Experimental Materials

Figure 3. 1 Samples of *Pereskia bleo* (Jarum Tujuh Bilah)

Samples of leaves, stems and roots of *Pereskia bleo* were collected at Rimba Ilmu, University Malaya. It taxonomic identification was verified by Prof Ong Hean Chooi from Institute Science Biology, Faculty of Science, University Malaya. Mice were purchased from Animal House, University Malaya. Sprague Dawley (SD) rats were purchased from Bumi Kenyalang Sdn. Bhd. Glipizide and glycogen was purchased from Fisher Scientific (M) Sdn. Bhd. All chemical and reagents were analytical grade and purchased from Fisher Scientific (M) Sdn. Bhd. Silica gel 60 F254 plates (20x20 cm, 0.5mm thickness) were purchased from Merck Sdn. Bhd. Silica gel 60 (0.063-0.200mm) for column Chromatography were purchased from Megalab Supplies.

3.2 Extraction of Plant Chemical Compounds

Samples of leaves, stem and roots were clean and dried into constant weight in oven at 40^{o}C and ground to become powder form. These dried powder of each samples were exhaustively extracted in ratio 1:10 with four different solvents which are hexane, petroleum ether, chloroform, and methanol. Extractions were done separately using soxhlet apparatus started with hexane and followed by more polar solvents. Extraction for each samples were done for 72 hours at 30^{o}C to ensure that all active compounds were completely extracted out. After 72 hours, the crude extracts were filtered with Whatman Filter Paper Grade 1. Aqueous extraction was done by using conventional method. Samples were soaked with distilled water and were place in aqueous bath at room temperature for 3 hours. All these samples were evaporated into concentrated volume using a rotary evaporator at 40^{o}C. These concentrated crude extract were then placed in a freeze drier and were left to run under pressure. The dried extracts were collected and stored at -20^{o}C for further test.

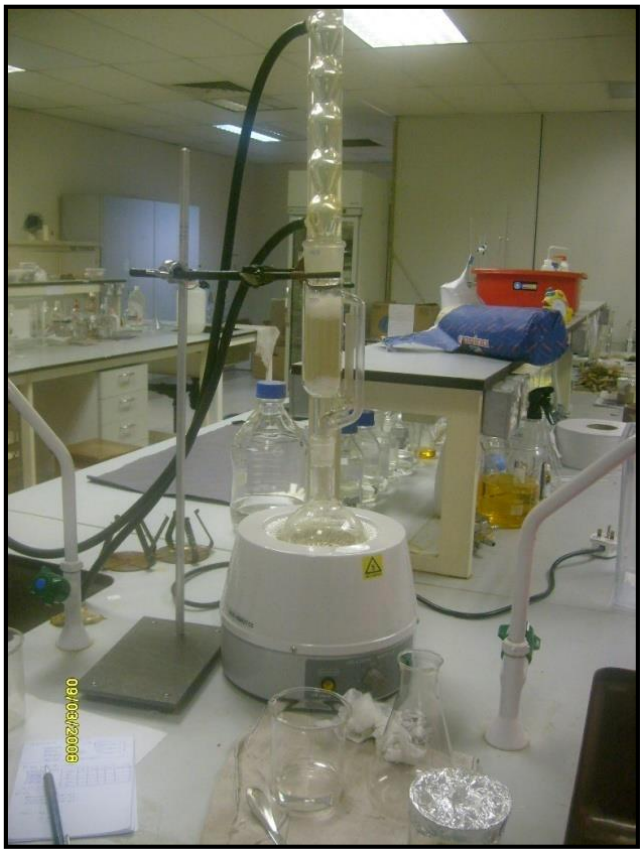


Figure 3. 2 Soxhlet Apparatus

3.3 Separation And Detection Of Chemical Compounds

3.3.1 Thin Layer Chromatography (TLC)

Samples are prepared in $0.01gml^{-1}$. Heparins capillaries were used to spot sample on TLC plate. The samples were spotted in one line about 1cm for couple of times to ensure it is visible.

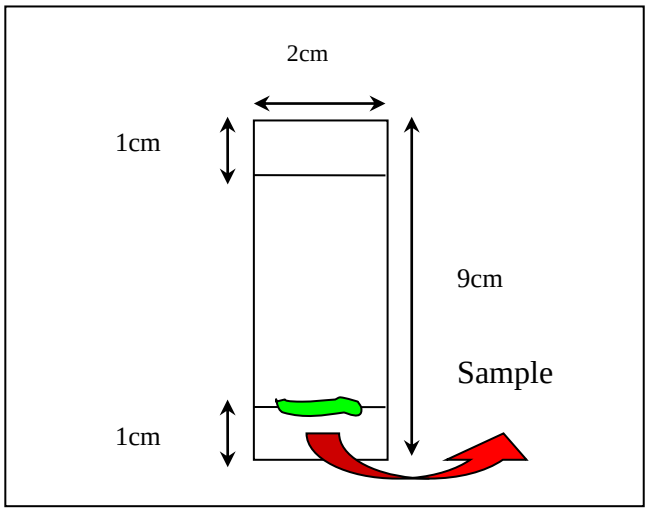


Figure 3. 3 Illustration of sample's draw on TLC plates.

TLC separation was carried out on silica gel 60 F254 plates (20x20 cm, 0.5mm thickness) with a combination of Toluene:ethyl acetate (30:70, v/v), Toluene:ethyl acetate (7:93, v/v) and Methanol:chloroform (1:9, v/v) as a mobile phase. But only Toluene:ethyl acetate (30:70, v/v) was selected as a mobile phase for further test. The reason was explained in chapter 5. Development glass jar was filled with small amount of mobile phase approximately less than 1 cm in depth. Development glass jar was then covered with watch glass to create an atmosphere saturated with mobile phase. TLC plates that were drawn with a line of sample were placed in the development glass jar and were left for a few minutes until the solvents risen up on top of the TLC plate (Figure 3.4). After the solvent risen up on top of the TLC plate, TLC plate was removed and a front line was drawn across the plate. These front lines are important for R_f calculations. TLC plates were observed for any coloured band presence with visible light and under UV light. Detection of compounds presence was further tested

using chemical reagents. Difference chemical reagents were used to detect difference chemical compounds.

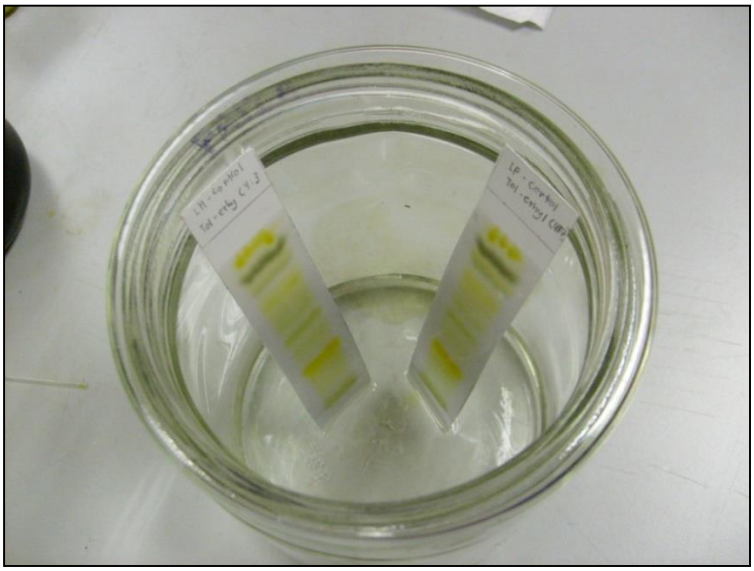


Figure 3. 4 TLC plates were placed in the development glass jar.

Two spray reagents were used were Dragendorff reagent and Vanillin-sulphuric acid reagent. Dragendorff reagent was used in the detection of alkaloid compound. This reagent was prepared by adding 1.7 g basic bismuth nitrate with 20 g tartaric acid and 80ml distilled water for solution A. While in solution B, 16g potassium iodide was added with 40ml distilled water. Both solutions were then mixed together in ratio 1:1 as a stock solution.

The vanillin-sulphuric acid reagent was used in the detection of terpenoid compound. This reagent was prepared by mixing 0.5g vanillin powder with 100ml sulphuric acid. After sprayed with vanillin reagent, the TLC plates were then heated at

120^{o}C until maximum colour appeared. After sprayed with both reagents, TLC plates were observed under visible and UV light for any coloured band arises.

3.3.2 Column Chromatography (CC)

Hexane, petroleum ether, chloroforms and methanol crude extracts of stem, root and leaves of *Pereskia bleo* were prepared in 0.01g/ml. This concentration was used as a standard concentration of samples in further test. Silica gel was used as a stationary phase and combinations of Toluene-ethyl acetate (30:70, v/v) as a mobile phase. While for aqueous crude extract of leaves, stems and roots of *Pereskia bleo*, column chromatography was developed using combination of butane, acetic acid and distilled water at ratio 60:15:25 as mobile phases. The mixed of silica gel and solvents yield a thick white slurry. A plug of glass wool was placed at the end of burette as prevention from any contamination of fractions collected from silica gel. Then 18 ml mixture of silica gel and solvents was loaded into burette slowly to avoid any air bubble. 0.5 ml of prepared samples was loaded at top of packed column. Samples were eluted in column at flow rate of 1ml per minutes. Ten fractions were collected with 5 ml each. These fractions were left dried in fume hood before stored at -20^{o}C for further test according to Harbone (1973) with slightly modifications.

3.3.3 Liquid Chromatography Mass Spectrometry (LCMS/MS)

An AB Sciex 3200QTrap mass spectrometer (AB Sciex California, USA) was used to determine the bioactive compound of *Pereskia bleo* with column Phenomenex aqua C18-50mm x 2.0mm x 5µM. All data were analyzed using electrospray ionization (ESI) source in negative ion mode with settings; capillary voltage of ESI-MS -4500kV and temperature 500^{o}C, collision energy spread at 35eV +/- 15eV, purified Nitrogen gas (N_2) 99.9995% as nebulisation and desolvation gas (Gas 1 40 psi, Gas 2 40 psi), declustering potential -40V. Full scan data acquisitions were performed for identification of unknown compounds at m/z range 100-1200 in profile mode. MS/MS experiments (product ion scan) were performed to confirm the identity of bioactive compounds presence in plant crude extracts. The buffer A contained distilled water with 0.1% formic acid and 5mM ammonium formate, while buffer B contained Acetonitrile with 0.1% formic acid and 5mM ammonium formate. The gradient was run in 10% A and increased to 90% B from duration 0.01 minutes to 8.0 minutes. After 3 minutes, gradient was switched back to 10% A in 0.1 minutes. Column was equilibrated for 5 minutes before next injection. All data were obtained and processed using AB Sciex Analyst 1.5 software.

3.4 Determinations Of Total Phenolics Contents

The total phenolics contents were determined by Follin-Ciocalteau method with slightly modification (Amin *et al.*, 2006). 10mg/ml of standard concentration samples in

methanol (1:1) were mixed with 5.0 ml Follin-Ciocalteau reagent (1:10 diluted with distilled water) and 4.0ml 1M Na_2CO_3. The mixtures were then incubated at 450^oC for 15 minutes. Absorbance was measured at 765nm using spectrophotometer. Total phenolics was determined using a standard curve with gallic acid (0-125mg/ml) and was expressed in mg of gallic acid equivalents (GAE) per gram of dry sample.

3.5 Determinations Of Total Flavonoids Contents

Flavonoids contents were determined using aluminium chloride colorimetric method with slightly modification (Liu *et al*., 2007). 10mg/ml of standard concentration samples in methanol (1:1) were mixed with 0.3ml 5% $NaNO_2$ and incubated for 5 minutes at 370^oC. 0.3ml 10% $AlCl_3$ was added and incubated 6 minutes at 370^oC. 2.0ml NaOH and 10ml distilled water were added and absorbance was measured at 510nm using spectrophotometer using methanol as a blank. Total flavonoids were determined using a standard curve of quarcetin (0-2.5mg/ml). Total flavonoids content was expressed as mg of quarcetin equivalent (QUE) per gram of dry sample.

3.6 Brine Shrimp Lethality Bioassay (BSLA)

The cytotoxic activity of *Pereskia bleo* extracts were measured using *Brine shrimp* lethality bioassay according to Meyer (1982) with slightly modifications. The artificial sea water was prepared by dissolving 38g sea salt into 1 litre of distilled water. This sea water was loaded in small tank and divided into 2 sides. One of these sides was loaded with half

of spoon shrimp eggs and covered with aluminium foil. After 2 days, the shrimps were hatched and matured as *Brine shrimp* nauplii. The lamp was placed to another side, to attract *Brine shrimp* nauplii to go through the aluminium foil hole. The 100 µg/ml concentration of samples stocks were prepared by adding *Pereskia bleo* samples with dimethyl sulfoxide (DMSO) solutions and diluted with artificial sea water. Serial dilutions of samples at concentration 10, 100 and 1000 µg/ml were prepared from these stocks. Samples and ten live nauplii were placed in each of vials. After 24 hours, nauplii in each vial were observed using magnifying glass and the number of survived nauplii were counted and recorded. The lethal concentrations (LC50) were determined at 95% confidence intervals by analyzing the data on Finney Programmer (Olaleye, 2007). The bioassay was done in triplicate.

3.7 Antidiabetic

3.7.1 Glycogen Phosphorylase Inhibitor Bioassay (GP)

The enzymatic inhibition of phosphorylase activity was monitored using microplate reader (Bio-Rad) following the methods as described by Martin, (1998) with slight modification. GPa activity was measured in the direction of glycogen synthesis by the release of phosphate from glucose-1-phosphate. Each compound was dissolved in DMSO and diluted at five different concentrations for IC50 determination. The enzymes were added into the 1L buffer with compounds dissolved in a mixture of 50 mM Hepes (pH 7.2), 100 mM KCl, 2.5 mM MgCl2, 0.5 mM glucose-1-phosphate, and 1 mg/mL glycogen in 96-well microplates (Costar). Then, lL of 1 M HCl

containing 10 mg/mL ammonium molybdate and 0.38 mg/mL malachite green were added to the mixture. Reactions were run at 22°C for 25s min. The phosphate absorbance was measured at 655 nm. The IC_{50} values were estimated by fitting the inhibition data to a dose-dependent curve using a logistic derivative equation.

3.7.2 Treatments on Sprague Dawley (SD) Rats

3.7.2.1 Experimental Animals

Both healthy male and female Sprague-Dawley (SD) rats ages between 12 to 15 weeks and weight between 100 to 150g was supplied from Animal House University Malaya, Kuala Lumpur. SD rats were used after acclimatized for 7 days and were housed individually in separate cages under standard conditions which were 12 hours light and 12 hours dark, under room temperature. SD rats were provided with standard pellets diet that were also supplied by Animal house, University Malaya and given water *ad libitium.* Animal study was approved by Animal House, University Malaya with Animal Care and Use Committee (ACUC) with ethic number ISB/05/05/2010/NAMD (R).

3.7.2.2 Acute Toxicity Study

The acute toxicity test was carried out according to Barik, (2008) with slightly modifications. Both healthy male and female Sprague Dawley rats were fasted overnight and divided into 7 groups, with 6 rats in each group. These rats were administrated with *Pereskia bleo* aqueous extract in increased dose levels from 250,

500 and 1000 mg/kg body weight by oral gavages. SD rats were observed continuously for 2 hours, 24 hours, 72 hours and 7 days under the following profiles:

i. Behavioral; fearfulness, irritability
ii. Autonomic profile; Urination, defecation.
iii. Neurological profile; Pain response, spontaneous activities.
iv. Mortality

3.7.2.3 Oral Glucose Tolerance Test (OGTT)

OGTT was performed based on Shiwaikar, (2006) with slightly modifications. The healthy rats' averages weights between 150 to 200g were fasted overnight for 8 to 12 hours before the test started. Eight groups contained of six SD rats were divided according to the samples and doses below (Table 3.1). A dose of *Pereskia bleo* extracts was prepared at 250mg/kg and 500mg/kg body weight. Each group was administrated with saline 0.9% and *Pereskia bleo* extracts. A standard dose of glucose was prepared at 3g/kg body weight and administrated by oral gavages to all SD rats after 30 minutes. Small amount of blood samples taken from the tail at 0, 30, 60, 90 and 120 minutes were used for blood glucose level (mg/dL) measurement using glucometer (Accu-Check PERFOMA). The results were recorded and the curves obtained from graph were observed to determine the responds of rats to glucose assumptions.

Table 3. 1 Groups were divided into each samples and dosage.

Groups (n=6)	**Samples**	**Dose**
I	Control (saline 0.9%)	-
II	Standard reference (Glipizide)	3mg/kg
III	Leaf aqueous extract	250mg/kg
IV	Leaf aqueous extract	500mg/kg
V	Stem aqueous extract	250mg/kg
VI	Stem aqueous extract	500mg/kg
VII	Roots aqueous extract	250mg/kg
VIII	Roots aqueous extract	500mg/kg

3.7.2.4 Induction of non-insulin dependent diabetes mellitus (NIDDM)

Both male and female SD rats were induced to become diabetic using previous methods with slightly modifications (Mukhtar *et al.*, 2004). Both healthy male and female SD rats weight between 100 to 150g were fasted overnight and induced by a single intraperitoneal injection of 120mgkg^{-1} body weight of alloxan monohydrate. After 72 hours, the rise of blood glucose level which was more than 150mg/dl were considered as diabetic. SD rats with permanent NIDDM for 7 days were used for the anti-diabetic study.

3.7.2.5 Anti-diabetic effect of *Pereskia bleo* aqueous extracts.

SD rats were divided into nine groups, consisting of six rats per group. Glipizide was used as standard drug. The extracts and standard drug were administrated for 25 days to each group as below;

Group 1: Non diabetic rats administrated with saline.

Group 2: Diabetic rats administrated with saline.

Group 3: Diabetic rats administrated with Glipizide 3 mg/kg.

Group 4: Diabetic rats administrated with leaves aqueous extract 250mg/kg.

Group 5: Diabetic rats administrated with leaves aqueous extract 500mg/kg.

Group 6: Diabetic rats administrated with stem aqueous extract 250mg/kg

Group 7: Diabetic rats administrated with stem aqueous extract 500mg/kg.

Group 8: Diabetic rats administrated with root aqueous extract 250mg/kg.

Group 9: Diabetic rats administrated with root aqueous extract 500mg/kg.

The effects of *Pereskia bleo* extracts in diabetic rats were observed and compared with a group given with standard drug (Glipizide). Fasting blood glucose was estimated on days 0, 5, 10, 15, 20 and day 25. Their blood glucose levels were measured using Glucometer (Accu-Check PEFORMA).

3.7.2.6 Biochemical Parameters

On day 25, SD rats were sacrificed by cervical dislocation after overnight fasting. Bloods were collected and stored in EDTA blood tubes to further test for their lipid profiles, liver function tests, and renal function tests that were measured in a Biohealth Science laboratory, Institute of Biological Sciences, Faculty of Science, University of Malaya.

3.7.2.7 Statistical Analysis

All data were collected and statistically evaluated using one-way ANOVA, followed by Dunnett test using the SPSS software, version 2.0. The values $p<0.05$ were considered significant.

4.1 Extractions of Plant Chemical Compounds.

Hexane, petroleum ether, chloroform and methanol extract were obtained using a Soxhlet apparatus as described in the methodology. While aqueous extract was obtained by soaking with distilled water and incubated in a water bath at 40-50^{o}C for 3 hours. These extracts were then evaporated under pressure and stored in -20^{o}C for further tests. The extracts from different parts of *Pereskia bleo* gave different colours and percentages of yield were summarized in Table 4.1.

Table 4.1 Samples of *Pereskia bleo* crude extracts at five different solvent systems.

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Samples	**Colours / Yield (%)**				
	Hexane	**Petroleum Ether**	**Chloroform**	**Methanol**	**Aqueous**
Leaves	Dark green^{++} 3.32g	Light green^{+++} 2.08g	Dark green^{+++} 2.11g	Light green^{+++} 2.49g	Dark green^{+++} 8.4g
Stem	Light green^{++} 2.1g	Light green^{+} 1.7g	Light green^{++} 1.83g	Light green^{+} 2.51g	Brown^{++} 7,15g
Root	Colourless 1.32g	Colourless 1.08g	Colourless 1.21g	Light green^{+} 2.39g	Brown^{+++} 3.63g

Indications of intensity of colour: +++ Strong ++ Medium + Weak

4.2 Separation and Detections of Chemical Compounds

4.2.1 Thin Layer Chromatography (TLC) of Leaves *Pereskia bleo*

Based on Table 4.2, 6 compounds were detected in leaves *Pereskia bleo* hexane crude extract. These compounds were identified as alkaloid, phenol and terpenoid according to the colour appeared after sprayed with vanillin-sulphuric acid and dragendorff reagents. However, 7 compounds could not be detected by chemical reagents and were classified as unknown.

19 compounds were detected in leaves *Pereskia bleo* petroleum ether crude extract. These compounds were identified as alkaloid, flavonoids and terpenoid according to the colour appeared after TLC plates were sprayed with vanillin-sulphuric acid and dragendorff reagents. Five unknown compounds were detected (Table 4.3).

In Thin Layer Chromatography (TLC) of leaves *Pereskia bleo* chloroform crude extract, 12 compounds were detected as alkaloid, phenol, flavonoids and terpenoid based on the colour appeared after sprayed with vanillin-sulphuric acid and dragendorff reagents. While five unknown compounds were detected (Table 4.4).

Table 4.5 showed the Thin Layer Chromatography (TLC) analysis of leaves *Pereskia bleo* methanol crude extract. 14 compounds were detected and revealed as phenol, flavonoids and terpenoid according to the colour appeared after sprayed with vanillin-sulphuric acid and dragendorff reagents. While one compound was detected as unknown compound due to could not be detected by reagents.

Table 4.2 TLC analysis of leaves *Pereskia bleo* hexane crude extract.

Labels	R_f	Colour		Reagents		Comment
Compounds	Value	Visible light	UV light	Dragendorff	Vanillin-H_2SO_4	
LH_1	0.986	O +++	-	O+++	-	Alkaloid
LH_2	0.950	D.G+++	-	D.G+++	D.G++	Unknown
LH_3	0.907	L.G++	-	L.G+	Pr++	Terpenoid
LH_4	0.879	D.G+	-	L.G+	Pr ++	Terpenoid
LH_5	0.836	Y+	-	++	Pr ++	Terpenoid
LH_6	0.800	Y++	-	Y+	-	Alkaloid
LH_7	0.750	O ++	-	O+	-	Alkaloid
LH_8	0.743	O ++	-	-	Pr+++	Terpenoid
LH_9	0.707	L.G++	-	L.G+	Pk+	Phenol
LH_{10}	0.693	L.G++	-	L.G+	-	Unknown
LH_{11}	0.629	-	-	-	Pr +++	Terpenoid
LH_{12}	0.586	L.G++	-	L.G+	Pk+	Phenol
LH_{13}	0.543	L.G++	-	L.G+	-	Unknown
LH_{14}	0.514	O +	-	L.G+	-	Unknown
LH_{15}	0.493	L.G+	-	L.G+	-	Unknown
LH_{16}	0.464	O +	-	-	-	Unknown
LH_{17}	0.386	O +++	-	O+++	-	Alkaloid
LH_{18}	0.371	O +++	-	-	Pr ++	Terpenoid
LH_{19}	0.329	O ++	-	O++	-	Alkaloid
LH_{20}	0.271	O++	-	O++	-	Alkaloid
LH_{21}	0.207	L.G+	-	L.G+	-	Unknown

Indications: +++ Strong ++ Medium + Weak

Colour:

-	No colour	Pr	Purple
O	Orange	Pk	Pink
L.G	Light green	L.B	Light blue
D.G	Dark green	D.B	D.Blue
Y	Yellow	D.BF	Dark Blue Fluorescent
OF	Orange Fluorescent	PrF	Purple Fluorescent
RF	Red Fluorescent		

Table 4. 3 TLC analysis of leaves *Pereskia bleo* petroleum ether crude extract.

Label	R_f	Colour		Reagents		Comment
Compound	Value	Visible light	UV light	Dragendorff	Vanillin-H_2SO_4	
LP $_1$	1.000	O+++	-	-	Pr +++	Terpenoid
LP $_2$	0.986	O+++	-	O+++	-	Alkaloid
LP $_3$	0.900	D.G+++	-	D.G+++	D.G+++	Unknown
LP $_4$	0.836	Y+	-	Y++	L.G++	Alkaloid
LP $_5$	0.871	-	-	-	Pr++	Terpenoid
LP $_6$	0.814	D.G+	-	L.G+	L.G++	Unknown
LP $_7$	0.714	Y+	-	-	Pr+	Terpenoid
LP $_8$	0.657	O+	-	O+	Pr++	Terpenoid/ Alkaloid
LP $_9$	0.600	O+	OF++	O+	-	Alkaloid/ Flavonoids
LP $_{10}$	0.571	L.G+	-	-	Pr+++	Terpenoid
LP $_{11}$	0.500	L.G++	-	Y+	-	Unknown
LP $_{12}$	0.471	-	-	-	Pr++	Terpenoid
LP $_{13}$	0.414	O+	-	-	Pr+++	Terpenoid
LP $_{14}$	0.329	L.G+	-	-	-	Unknown
LP $_{15}$	0.257	O+++	-	O+++	-	Alkaloid
LP $_{16}$	0.229	Y+	-	O+	-	Alkaloid
LP $_{17}$	0.200	Y+	-	-	Pr+	Terpenoid
LP $_{18}$	0.143	-	-	L.G++	-	Unknown
LP $_{19}$	0.129	-	-	-	Pr++	Terpenoid

Indications: +++ Strong ++ Medium + Weak

Colour:

-	No colour	Pr	Purple
O	Orange	Pk	Pink
L.G	Light green	L.B	Light blue
D.G	Dark green	D.B	D.Blue
Y	Yellow	D.BF	Dark Blue Fluorescent
OF	Orange Fluorescent	PrF	Purple Fluorescent
RF	Red Fluorescent		

Table 4. 4 TLC analysis of leaves *Pereskia bleo* chloroform crude extract.

Label	R_f	Colour		Reagents		Comment
Compound	Value	Visible light	UV light	Dragendorff	Vanillin-H_2SO_4	
LC_1	0.920	D.G+++	-	D.G+++	Pk++	Phenol
LC_2	0.840	L.G+++	OF++	O+	-	Alkaloid/ Flavonoids
LC_3	0.800	D.G++	-	D.G+	-	Unknown
LC_4	0.740	L.G++	OF++	L.G+	Pk+	Phenol/ Flavonoids
LC_5	0.670	D.G+++	-	D.G+++	-	Unknown
LC_6	0.640	D.G+++	-	O++	-	Alkaloid
LC_7	0.570	L.G+++	OF++	D.G+	Pk+	Phenol/ Flavonoids
LC_8	0.510	D.G++	-	-	Pk+	Phenol
LC_9	0.450	L.G++	-	-	-	Unknown
LC_{10}	0.340	L.G++	-	-	-	Unknown
LC_{11}	0.260	L.G++	-	-	-	Unknown
LC_{12}	0.190	L.G++	-	-	Pr++	Terpenoid

Indications: +++ Strong ++ Medium + Weak

Colour:

-	No colour	Pr	Purple
O	Orange	Pk	Pink
L.G	Light green	L.B	Light blue
D.G	Dark green	D.B	D.Blue
Y	Yellow	D.BF	Dark Blue Fluorescent
OF	Orange Fluorescent	PrF	Purple Fluorescent
RF	Red Fluorescent		

Table 4. 5 TLC analysis of leaves of *Pereskia bleo* methanol crude extract.

Labelled Compound	R_f Value	Colour		Reagents		Comment
		Visible light	UV light	Dragendorff	Vanillin-H_2SO_4	
LM 1	1.000	-	-	-	Pr+	Terpenoid
LM 2	0.886	D.G++	-	D.G++	Pk++	Phenol
LM 3	0.800	L.G++	-	L.G+	-	Unknown
LM 4	0.707	D.G+	-	L.G+	Pk++	Phenol
LM 5	0.643	L.G+	OF++	L.G+	-	Flavonoids
LM 6	0.607	L.G+	-	L.G+	Pr ++	Terpenoid
LM 7	0.586	L.G+	-	L.G+	Pk +	Phenol
LM 8	0.500	-	-	-	Pr +	Terpenoid
LM 9	0.470	L.G+	-	L.G+	Pk +	Phenol
LM 10	0.386	L.G+	-	L.G+	Pr +	Terpenoid
LM 11	0.371	L.G+	-	L.G+	Pk +	Phenol
LM 12	0.314	-	-	-	P +++	Terpenoid
LM 13	0.271	L.G+	-	L.G+	Pk +	Phenol
LM 14	0.243	L.G+	-	L.G+	Pk +	Phenol

Indications: +++ Strong ++ Medium + Weak

Colour:

-	No colour	Pr	Purple
O	Orange	Pk	Pink
L.G	Light green	L.B	Light blue
D.G	Dark green	D.B	D.Blue
Y	Yellow	D.BF	Dark Blue Fluorescent
OF	Orange Fluorescent	PrF	Purple Fluorescent
RF	Red Fluorescent		

4.2.2 Thin Layer Chromatography (TLC) of Stems *Pereskia bleo*

Table 4.6 showed the Thin Layer Chromatography (TLC) analysis has revealed the presence of 13 compounds identified as alkaloid, phenol, flavonoids and terpenoid while 1 unknown compound detected in stem *Pereskia bleo* hexane crude extract.

In stem *Pereskia bleo* petroleum ether crude extract, 9 compounds were revealed in Thin Layer Chromatography (TLC). These compounds were identified as terpenoid, flavonoids and alkaloid according to colour appeared after sprayed with vanillin-sulphuric acid and dragendorff reagents. While three compounds were identified as unknown compound (Table 4.7).

In stem *Pereskia bleo* chloroform crude extract, 14 compounds were revealed in Thin Layer Chromatography (TLC). These compounds were identified as alkaloid, phenol and terpenoid according to colour appeared after sprayed with vanillin-sulphuric acid and dragendorff reagents. No unknown compound was detected (Table 4.8).

While in stem *Pereskia bleo* methanol crude extract, 6 compounds were revealed in Thin Layer Chromatography (TLC). These compounds were identified as terpenoid and phenol according to colour appeared after sprayed with vanillin-sulphuric acid and dragendorff reagents. No unknown compound was detected (Table 4.9).

Table 4. 6 TLC analysis of stem *Pereskia bleo* hexane crude extract.

Labelled Compound	R_f Value	Colour		Reagents		Comment
		Visible light	UV light	Dragendorff	Vanillin-H_2SO_4	
SH 1	0.97	Y+	OF+++	-	Pr+++	Terpenoid Flavonoids
SH 2	0.964	Y+	OF+++	O++	-	Alkaloid Flavonoids
SH 3	0.905	D.G+++	-	-	Pk +++	Phenol
SH 4	0.846	L.G++	OF+++	-	Pk ++	Phenol Flavonoids
SH 5	0.78	Y++	-	-	Pr++	Terpenoid
SH 6	0.677	-	Y+	-	Pr +++	Terpenoid
SH 7	0.596	-	Pk+	-	R++	Phenol
SH 8	0.537	-	Y+	L.G+	Pr ++	Terpenoid
SH 9	0.368	Y+++	-	-	Pr +++	Terpenoid
SH 10	0.304	-	-	-	-	Unknown
SH 11	0.177	L.G++	Y+++	-	Pr ++	Terpenoid
SH 12	0.118	O+++	OF++	-	Pk+	Phenol Flavonoids
SH 13	0.063	D.G++	Pk++	-	Pr+	Terpenoid

Indications: +++ Strong ++ Medium + Weak

Colour:

-	No colour	Pr	Purple
O	Orange	Pk	Pink
L.G	Light green	L.B	Light blue
D.G	Dark green	D.B	D.Blue
Y	Yellow	D.BF	Dark Blue Fluorescent
OF	Orange Fluorescent	PrF	Purple Fluorescent
RF	Red Fluorescent		

Table 4. 7 TLC analysis of stem *Pereskia bleo* petroleum ether crude extract.

Labelled Compound	R_f Value	Colour		Reagents		Comment
		Visible light	UV light	Dragendorff	Vanillin-H_2SO_4	
SP $_1$	1.000	-	-	-	Pr +++	Terpenoid
SP $_2$	0.985	Y++	-	-	-	Unknown
SP $_3$	0.933	D.G++	-	-	-	Unknown
SP $_4$	0.88	D.G ++	OF++	-	-	Flavonoids
SP $_5$	0.603	Y++	OF+	-	-	Flavonoids
SP $_6$	0.682	-	-	-	Pr+++	Terpenoid
SP $_7$	0.545	-	-	-	Pr ++	Terpenoid
SP $_8$	0.230	O+++	-	O+++	-	Alkaloid Flavonoids
SP $_9$	0.097	L.G++	Y+	-	-	Unknown

Indications: +++ Strong ++ Medium + Weak

Colour:

-	No colour	Pr	Purple
O	Orange	Pk	Pink
L.G	Light green	L.B	Light blue
D.G	Dark green	D.B	D.Blue
Y	Yellow	D.BF	Dark Blue Fluorescent
OF	Orange Fluorescent	PrF	Purple Fluorescent
RF	Red Fluorescent		

Table 4. 8 TLC analysis of stem *Pereskia bleo* chloroform crude extract.

Labelled Compound	R_f Value	Colour		Reagents		Comment
		Visible light	UV light	Dragendorff	Vanillin-H_2SO_4	
SC 1	0.986	Y+	-	Y+	Pr+++	Terpenoid
SC 2	0.879	D.G+++	D.BF+++	D.G++	Pk+++	Phenol
SC 3	0.815	L.G+++	D.BF++	L.G+++	Pk+++	Phenol
SC 4	0.729	L.G+	-	O+	-	Alkaloid
SC 5	0.272	Y+++	-	L.G+	Pr +	Terpenoid
SC 6	0.086	O+++	-	O+++	Y+++	Alkaloid
SC 7	0.043	D.G+++	PrF+++	D.G+++	Pk+++	Phenol
SC 8	0.029	L.G+++	-	D.G++	Pk+++	Phenol
SC 9	0.586	-	D.BF++	-	O++	Phenol
SC 10	0.643	-	D.BF++	Y+	Pr +++	Terpenoid Phenol
SC 11	0.513	-	-	-	Pr +++	Terpenoid
SC 12	0.443	-	-	-	Pr+++	Terpenoid
SC 13	0.229	-	-	-	Pr+++	Terpenoid
SC 14	0.186	-	-	-	Pr++	Terpenoid

Indications: +++ Strong ++ Medium + Weak

Colour:

-	No colour	Pr	Purple
O	Orange	Pk	Pink
L.G	Light green	L.B	Light blue
D.G	Dark green	D.B	D.Blue
Y	Yellow	D.BF	Dark Blue Fluorescent
OF	Orange Fluorescent	PrF	Purple Fluorescent
RF	Red Fluorescent		

Table 4. 9 TLC analysis of stem *Pereskia bleo* methanol crude extract.

Labelled Compound	R_f Value	Colour		Reagents		Comment
		Visible light	UV light	Dragendorff	Vanillin-H_2SO_4	
SM 1	0.956	-	-	-	Pr +++	Terpenoid
SM 2	0.926	-	D.BF+	-	-	Phenol
SM 3	0.853	-	-	-	Pr++	Terpenoid
SM 4	0.676	-	-	-	Pr+	Terpenoid
SM 5	0.485	-	-	-	Pr +++	Terpenoid
SM 6	0.441	-	D.BF+	-	-	Phenol

Indications: +++ Strong ++ Medium + Weak

Colour:

-	No colour	Pr	Purple
O	Orange	Pk	Pink
L.G	Light green	L.B	Light blue
D.G	Dark green	D.B	D.Blue
Y	Yellow	D.BF	Dark Blue Fluorescent
OF	Orange Fluorescent	PrF	Purple Fluorescent
RF	Red Fluorescent		

4.2.3 Thin Layer Chromatography (TLC) of Roots *Pereskia bleo*

Table 4.10 showed 11 compounds were detected in root *Pereskia bleo* hexane crude extract. Thin Layer Chromatography (TLC) analysis has revealed that these compounds were phenol, flavonoids and terpenoid due to colour appeared after TLC plates were sprayed with vanillin-sulphuric acid and dragendorff reagents.

In root *Pereskia bleo* petroleum ether crude extract, 12 compounds were revealed in Thin Layer Chromatography (TLC) analysis. The coloured band appeared once the TLC plates were sprayed with vanillin-sulphuric acid and dragendorff reagent showed the presence of phenol, flavonoids and terpenoid (Table 4.11).

While the Thin Layer Chromatography (TLC) analyses of root *Pereskia bleo* chloroform crude extract has detected the presence of 10 compounds. These compounds were identified as alkaloid and terpenoid according to colour appeared after sprayed with vanillin-sulphuric acid and dragendorff reagents. Two unknown compounds were identified due to could not be detected by reagents (Table 4.12).

Furthermore, 12 compounds were detected in Thin Layer Chromatography (TLC) analysis of roots *Pereskia bleo* methanol crude extract. These compounds were identified as alkaloid, phenol and terpenoid according to colour appeared after TLC plates were sprayed with vanillin-sulphuric acid and dragendorff reagents. While 2 unknown compounds were identified due to could not be detected by reagents (Table 4.13).

Table 4. 10 TLC analysis of root *Pereskia bleo* hexane crude extract.

Labelled Compound	R_f Value	Colour		Reagents		Comment
		Visible light	UV light	Dragendorff	Vanillin-H_2SO_4	
RH_1	0.971	Y+	Y++	D.G+	Pr+++	Terpenoid
RH_2	0.929	-	-	-	Pr+++	Terpenoid
RH_3	0.913	L.G+	O++	D.G++	Pk++	Phenol/ Flavonoids
RH_4	0.797	L.G++	RF++	-	Pk+	Phenol
RH_5	0.736	L.G+	L.BF+++	-	Pk++	Phenol
RH_6	0.670	Y+	L.BF++	-	Pr+++	Terpenoid/ Phenol
RH_7	0.614	-	-	-	Pk+++	Phenol
RH_8	0.550	-	-	-	Pr+	Terpenoid
RH_9	0.500	-	-	-	P+	Terpenoid
RH_{10}	0.304	Y++	-	-	Pr+	Terpenoid
RH_{11}	0.057	-	-	-	Pk+++	Phenol

Indications: +++ Strong ++ Medium + Weak

Colour:

-	No colour	Pr	Purple
O	Orange	Pk	Pink
L.G	Light green	L.B	Light blue
D.G	Dark green	D.B	D.Blue
Y	Yellow	D.BF	Dark Blue Fluorescent
OF	Orange Fluorescent	PrF	Purple Fluorescent
RF	Red Fluorescent		

Table 4. 11 TLC analysis of root *Pereskia bleo* petroleum ether crude extract.

Labelled Compound	R_f Value	Colour		Reagents		Comment
		Visible light	UV light	Dragendorff	Vanillin-H_2SO_4	
RP 1	0.993	Y++	-	-	Pr+++	Terpenoid
RP 2	0.884	L.G++	OF+	L.G++	Pr++	Terpenoid/ Flavonoids
RP 3	0.819	Y+++	OF+++	-	Pr+	Terpenoid/ Flavonoids
RP 4	0.732	G+	L.BF+	-	-	Phenol
RP 5	0.679	-	-	-	Pr+	Terpenoid
RP 6	0.594	L.G++	-	-	Pk+	Phenol
RP 7	0.571	-	-	-	Pr+++	Terpenoid
RP 8	0.536	-	-	-	Pr++	Terpenoid
RP 9	0.493	L.G++	-	-	Pk+	Phenol
RP 10	0.308	Y+	-	-	Pr++	Terpenoid
RP 11	0.091	-	-	-	Pr++	Terpenoid
RP 12	0.057	-	-	-	Pk++	Phenol

Indications: +++ Strong ++ Medium + Weak

Colour:

-	No colour	Pr	Purple
O	Orange	Pk	Pink
L.G	Light green	L.B	Light blue
D.G	Dark green	D.B	D.Blue
Y	Yellow	D.BF	Dark Blue Fluorescent
OF	Orange Fluorescent	PrF	Purple Fluorescent
RF	Red Fluorescent		

Table 4. 12 TLC analysis of root *Pereskia bleo* chloroform crude extract.

Labelled Compound	R_f Value	Colour		Reagents		Comment
		Visible light	UV light	Dragendorff	Vanillin-H_2SO_4	
RC 1	0.884	D.G+	OF++	L.G++	-v	Alkaloid/ Flavonoids
RC 2	0.824	Y+	OF++	O++	Y+	Alkaloid/ Flavonoids
RC 3	0.78	Y+	Y++	-	Pr++	Terpenoid
RC 4	0.614	-	PR+	-	-	unknown
RC 5	0.518	Y+	Y+	-	Pr+++	Terpenoid
RC 6	0.543	-	-	-	Pr+	Terpenoid
RC 7	0.314	Y+	-	-	-	Unknown
RC 8	0.139	Y+	L.BF+	-	-	Phenol
RC 9	0.088	O++	OF+	O+	Y+	Alkaloid/ Flavonoids
RC 10	0.037	D.G+++	OF+	D.G++	Pr++	Alkaloid/ Flavonoids/ Terpenoid

Indications: +++ Strong ++ Medium + Weak

Colour:

-	No colour	Pr	Purple
O	Orange	Pk	Pink
L.G	Light green	L.B	Light blue
D.G	Dark green	D.B	D.Blue
Y	Yellow	D.BF	Dark Blue Fluorescent
OF	Orange Fluorescent	PrF	Purple Fluorescent
RF	Red Fluorescent		

Table 4. 13 TLC analysis of roots *Pereskia bleo* methanol crude extract.

Labelled Compound	R_f Value	Colour		Reagents		Notes
		Visible light	UV light	Dragendorff	Vanillin-H_2SO_4	
RM $_1$	0.906	D.G+++	OF++	-	-	Flavonoids
RM $_2$	0.856	Y++	OF++	-	-	Flavonoids
RM $_3$	0.758	-	Y+	-	-	Unknown
RM $_4$	0.66	-	Pr+	-	-	Unknown
RM 5	0.628	D.G++	OF+	-	Pk+	Phenol/ Flavonoids
RM $_6$	0.562	L.G+	OF++	-	Pr+++	Terpenoid/ Flavonoids
RM $_7$	0.497	D.G+	Y+	-	Pr++	Terpenoid
RM $_8$	0.258	O++	OF+	Pr+	Pk+	Phenol/ Flavonoids
RM $_9$	0.196	Y+	Y+	-	-	Unknown
RM $_{10}$	0.145	Pr+++	OF+++	O+	Pk++	Alkaloid /Flavonoids/ Phenol
RM $_{11}$	0.098	D.G++	OF++	L.G+	Pk+	Phenol/ Flavonoids
RM $_{12}$	0.058	L.G+++	OF+	L.G+	Pr++	Terpenoid Flavonoids

Indications: +++ Strong ++ Medium + Weak

Colour:

-	No colour	Pr	Purple
O	Orange	Pk	Pink
L.G	Light green	L.B	Light blue
D.G	Dark green	D.B	D.Blue
Y	Yellow	D.BF	Dark Blue Fluorescent
OF	Orange Fluorescent	PrF	Purple Fluorescent
RF	Red Fluorescent		

4.2.4 Liquid Chromatography Mass Spectrometry combined with Mass Spectrometry (LCMS/MS) of *Pereskia bleo* Leaves.

Initial investigation showed the presence of phenolics acid and flavonoids in aqueous crude extract of leaves *Pereskia bleo*. Further test was done using Liquid Chromatography Mass Spectrometry combined with Mass Spectrometry (LCMS/MS) has showed that *Pereskia bleo* leaves aqueous extract contained five expected chemical compounds which were one fatty acid (Jasmonic acid/dihydrocymellein) and four flavonoids (Chrysin, Apigenin based compounds, Apigenin 6 C Glucoside and Apigenin 7 Rutinoside).

In figure 4.2 showed the chromatogram of MS/MS fragmentation of Jasmonic acid or dihydrocymellein compound at mass spectrum 209.16 m/z. Other major peaks of m/z were 165.0, 149.0 and 191.0. The retention time of this compound was 2.091 minutes and the count per second of tallest peak was 7.3e6 cps.

In figure 4.3 showed the chromatogram of MS/MS fragmentation of Chrysin compound at mass spectrum 253.09 m/z. Other major peaks of m/z were 165.0, 147.0, 191.0, 209.0, 149.0, 173.0 and 161.0. The retention time and count per second of the tallest peak were 2.202 minutes and 6.9e6 cps.

While in figure 4.4, showed the chromatogram of MS/MS fragmentation of Apigenin based compounds at mass spectrum 739.17 m/z. Other major peaks of m/z were

310.9, 576.9 and 283.0. The retention time and count per second of the tallest peak were 2.648 minutes and 3.5e6 cps.

The chromatogram of MS/MS fragmentation of Apigenin 6 C Glucoside at mass spectrum 431.28 m/z was showed in figure 4.5. Other major peaks of m/z were showed at 310.9 and 283.0. While the retention time and count per second of the tallest peak was 3.095 minutes and 2.1e7 cps.

In addition, in figure 4.6 showed the chromatogram of MS/MS fragmentation of Apigenin 7 rutinoside at mass spectrum 577.22 m/z. Other major peaks of m/z were showed at 282.9 and 310.0. While the retention time was 2.759 minutes and the count per second of the tallest peak was 3.1e6 cps.

The detection of compounds contained in leaves *Pereskia bleo* aqueous crude extract using LCMS/MS method also showed the presence of 12 unknown compounds. These compounds were identified as unknown due to the standard compounds were not available in database system. Details MS/MS fragmentation of unknown compounds were showed in Appendix

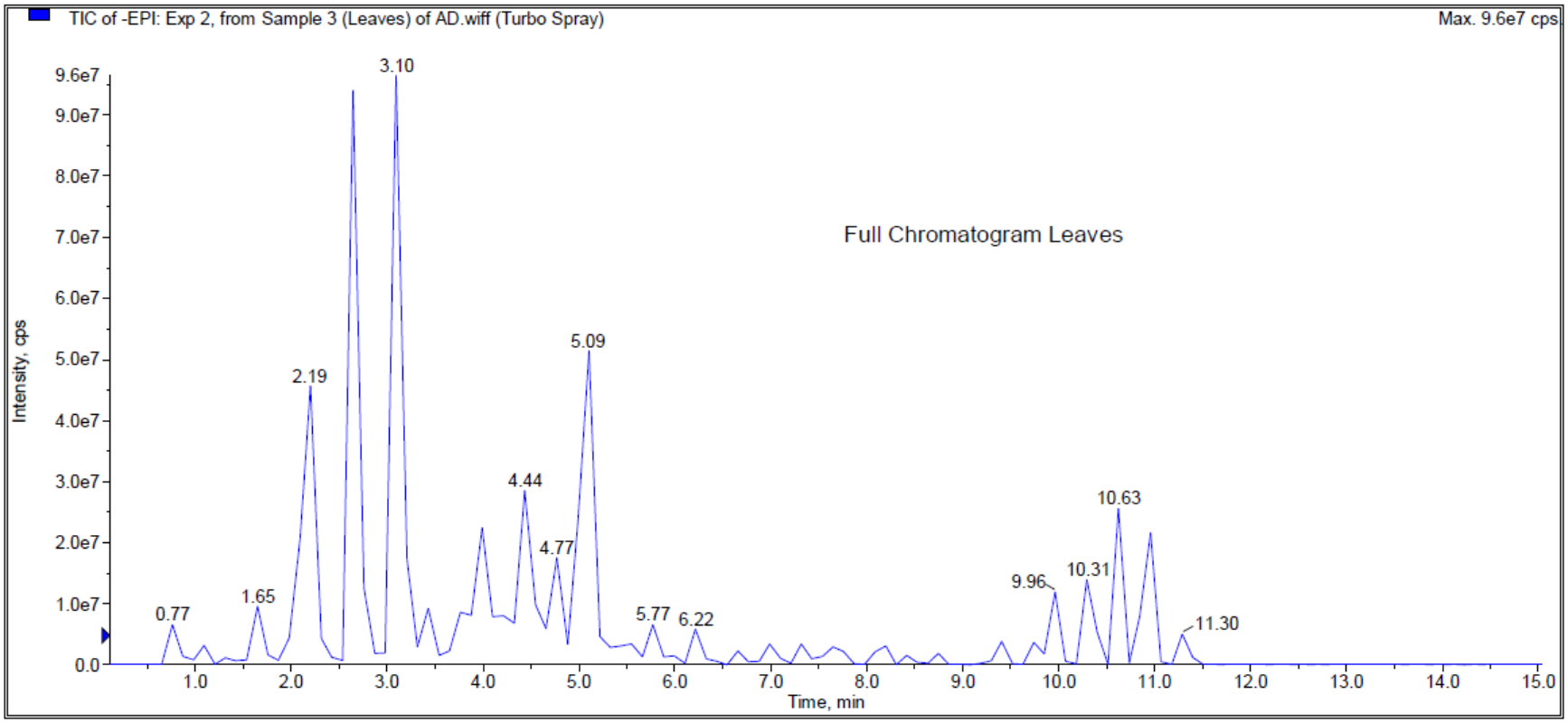

Figure 4. 1 Full Chromatogram of LCMS/MS of *Pereskia bleo* leaves aqueous extract.

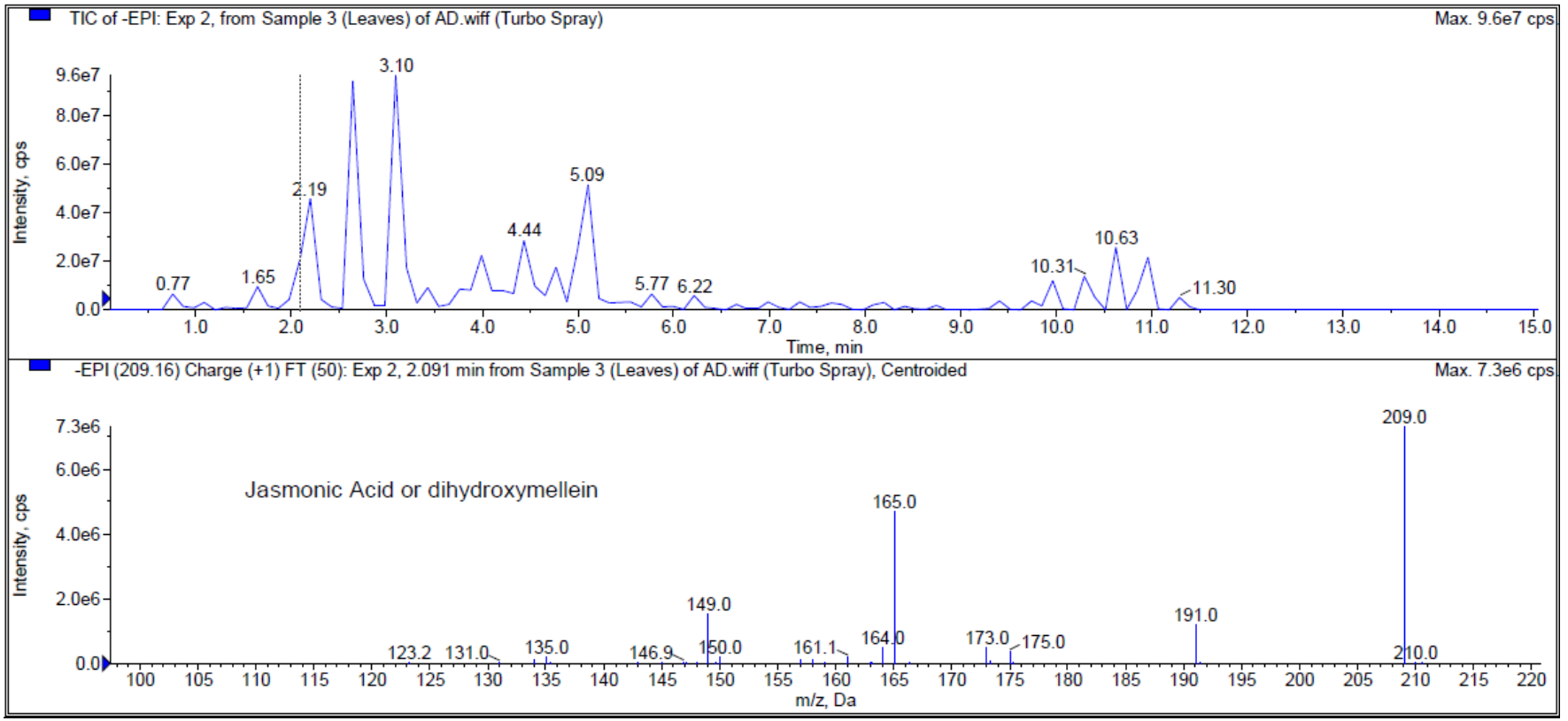

Figure 4.2 Full chromatogram of LCMS/MS Jasmonic acid or dihydroxymellein from *Pereskia bleo* leaves aqueous extract.

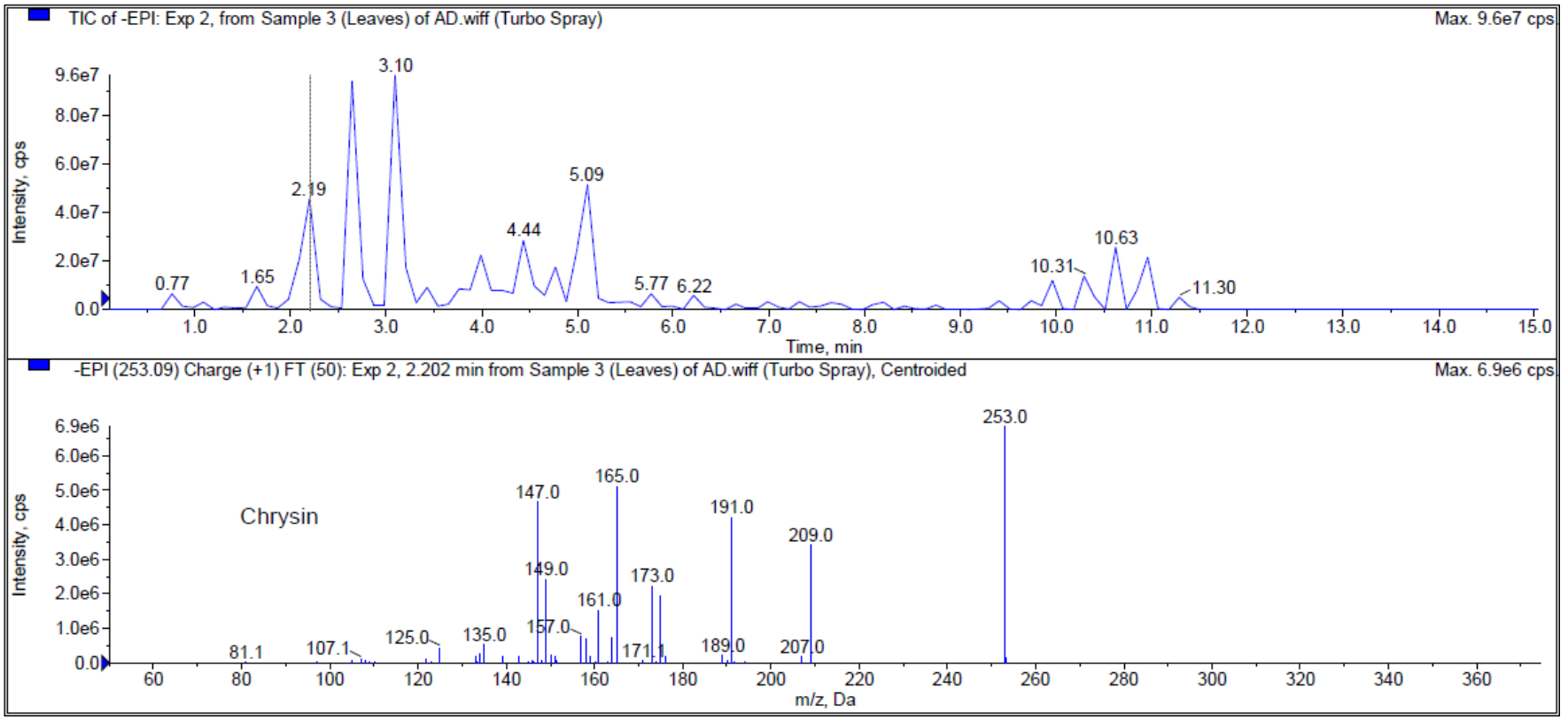

Figure 4. 3 Full chromatogram of LCMS/MS chrysin from *Pereskia bleo* leaves aqueous extract.

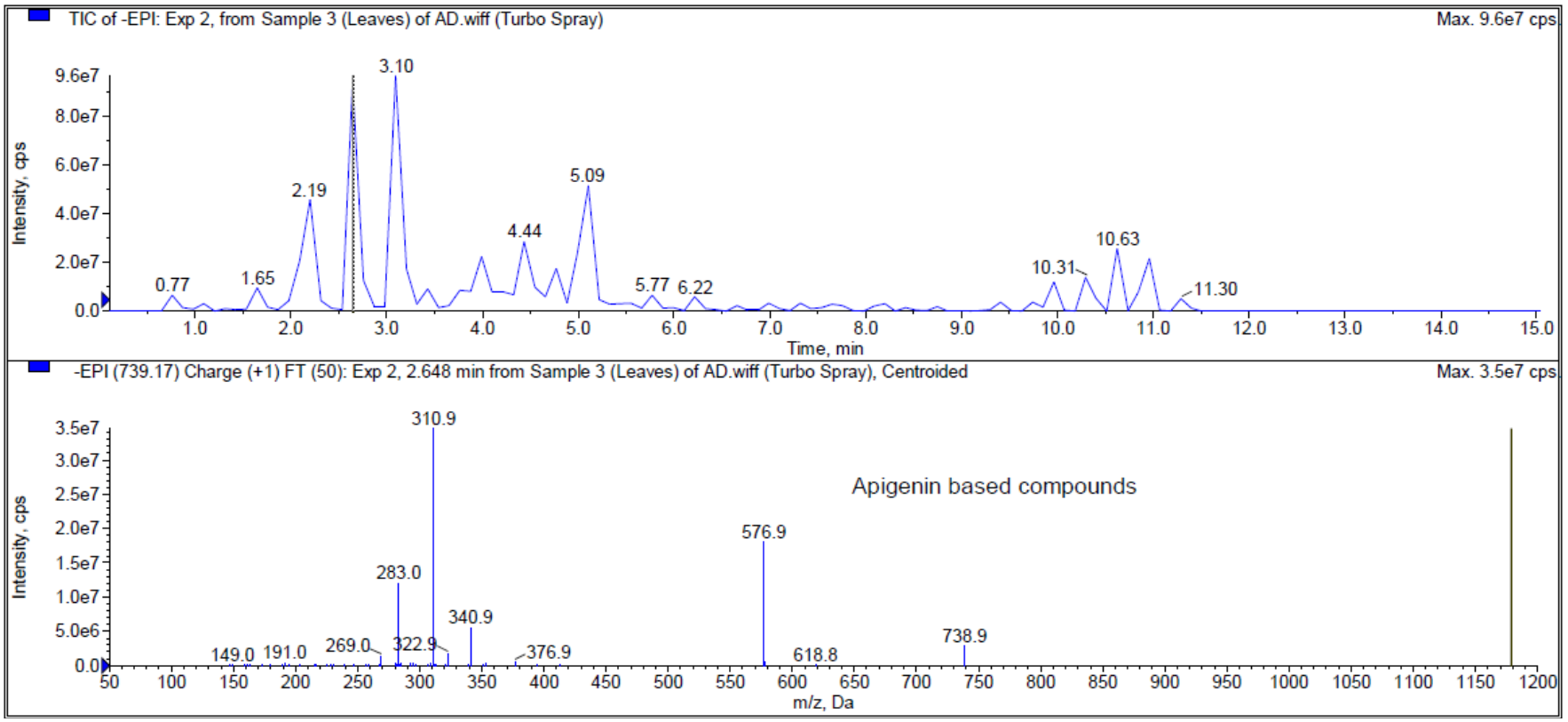

Figure 4. 4 Full chromatogram of LCMS/MS apigenin based compounds from *Pereskia bleo* leaves aqueous extract.

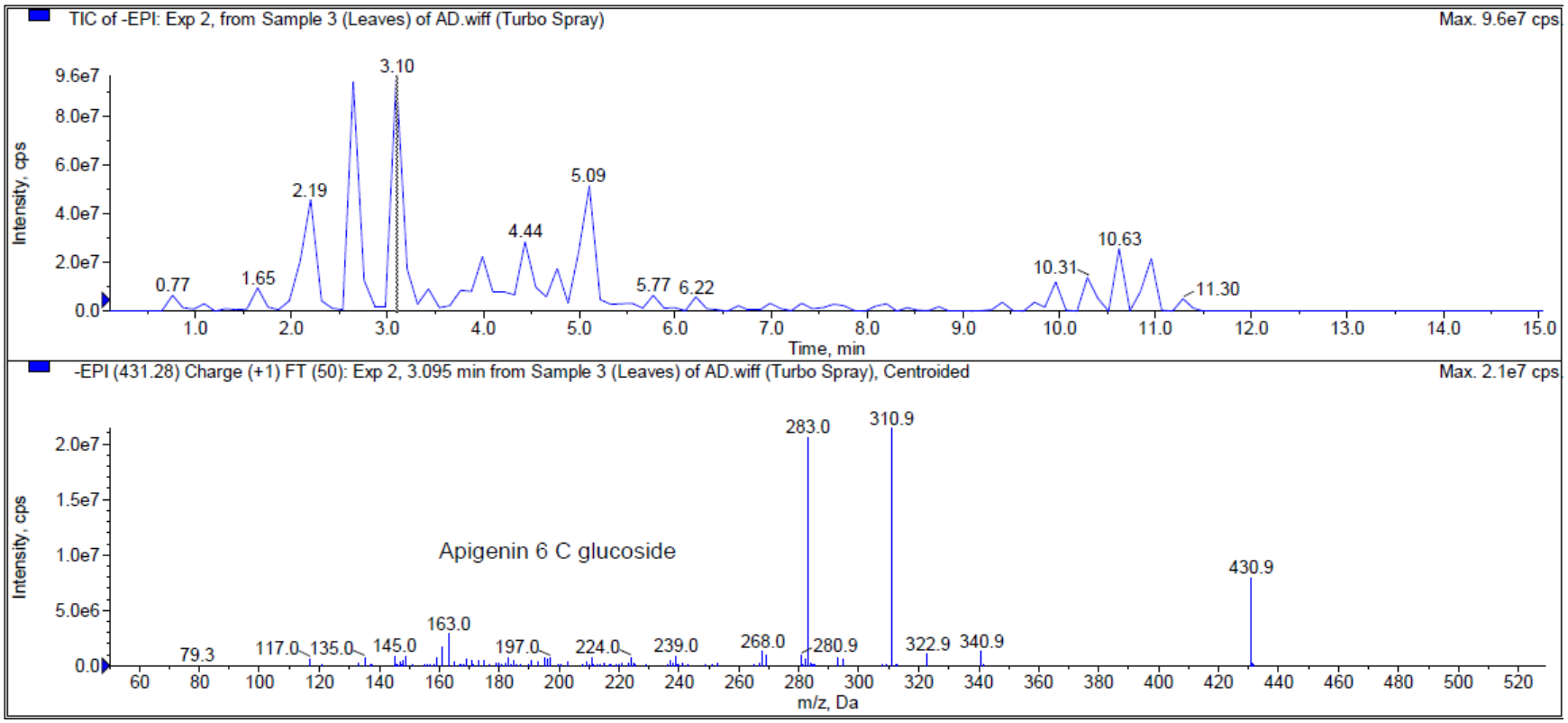

Figure 4. 5 Full chromatogram of LCMS/MS Apigenin 6 C glucoside from *Pereskia bleo* leaves aqueous extract.

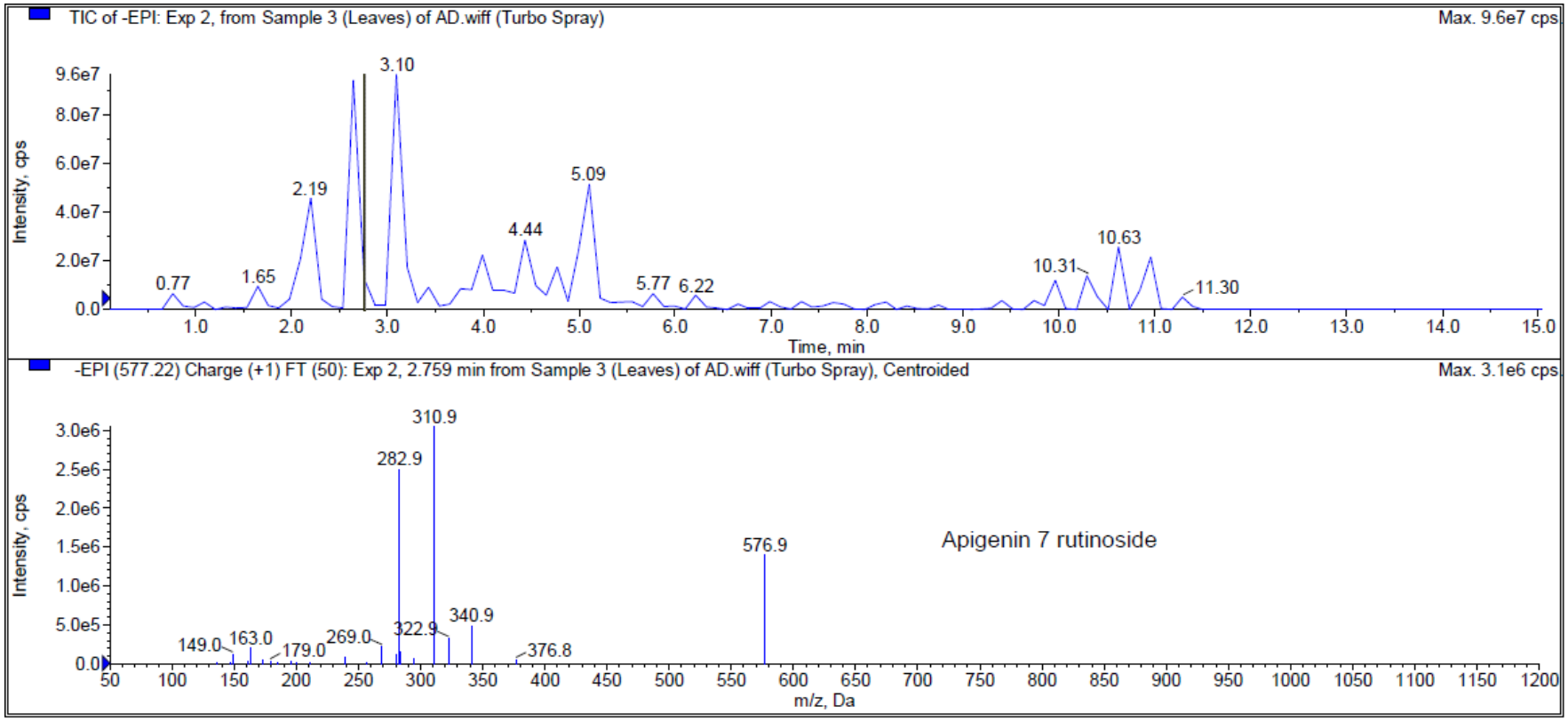

Figure 4. 6 Full chromatogram of LCMS/MS apigenin 7 rutinoside from *Pereskia bleo* leaves aqueous extract.

4.2.5 Liquid Chromatography Mass Spectrometry combined with Mass Spectrometry (LCMS/MS) of *Pereskia bleo* Stems.

Initial investigation of aqueous crude extracts from stems *Pereskia bleo* has detected the presence of phenolics and flavonoids compounds. Liquid Chromatography Mass Spectrometry combined with Mass Spectrometry (LCMS/MS) analysis showed the MS spectrum of stems *Pereskia bleo* aqueous crude extract contained three expected compounds which were 2 phenolics compound (phenolics acid and 3,30-di-O-methyl ellagic acid) and amino acid (Tryptophan).

Figure 4.8 showed chromatogram of MS/MS fragmentation of Phenolics Acid compound at mass spectrum 243.13 m/z. Other major peaks of m/z were 136.0 and 199.1 m/z. The retention time of this compound was 3.189 minutes and the count per second of tallest peak was 1.9e6 cps.

While in figure 4.9 showed the chromatogram of MS/MS fragmentation of Tryptophan at mass spectrum 203.03 m/z. Other major peaks of m/z were 116.0, 142.0, 159.1, and 186.0 m/z. The retention time and the count per second of tallest peak were 1.422 minutes and 2.0e6 cps.

In addition, in figure 4.10 showed the chromatogram of MSMS fragmentation of 3,30-di-O-methyl ellagic acid at mass spectrum 329.31 m/z. Other major peaks of m/z

were 171.0 and 211.1 m/z. The retention time and the count per second of tallest peak were 4.739 minutes and 1.4e7 cps.

LCMS/MS of stems *Pereskia bleo* aqueous crude extract also found 14 unknown compounds. These compounds were identified as unknown compounds due to unavailable data of the standard compounds in database system. Details MS/MS fragmentation of unknown compounds were showed in Appendix.

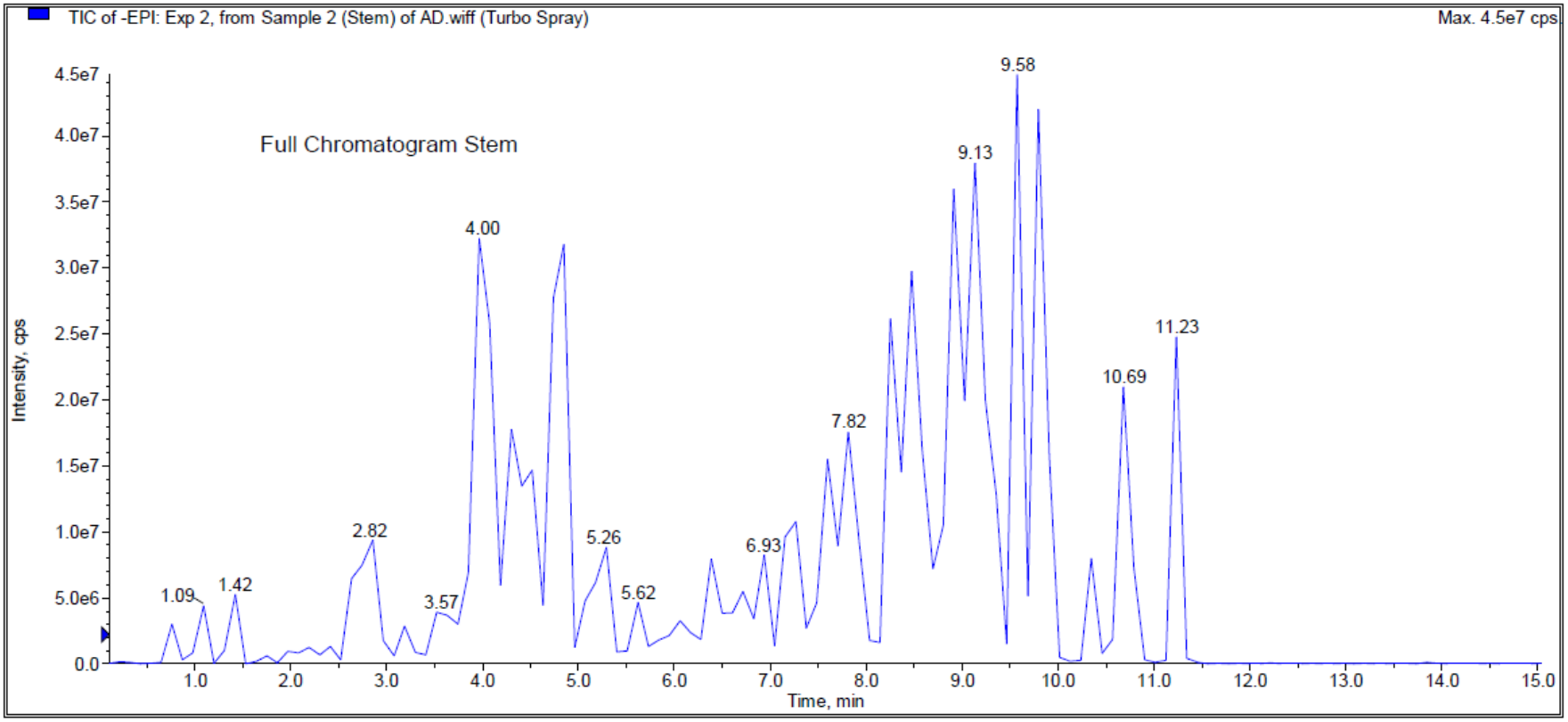

Figure 4. 7 Full Chromatogram LCMS/MS of *Pereskia bleo* stems aqueous extract.

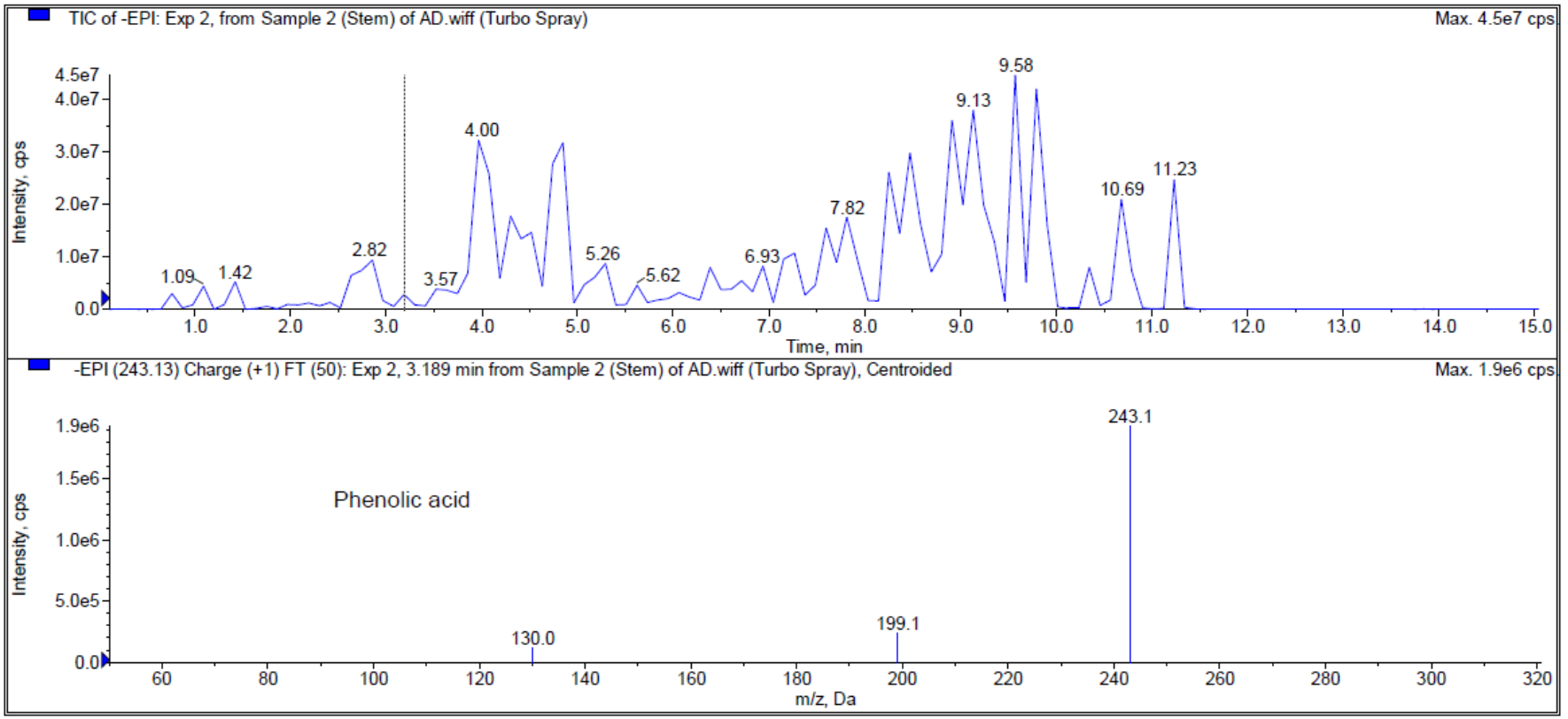

Figure 4. 8 Full chromatogram of LCMS/MS phenolics acid from *Pereskia bleo* stems aqueous extract.

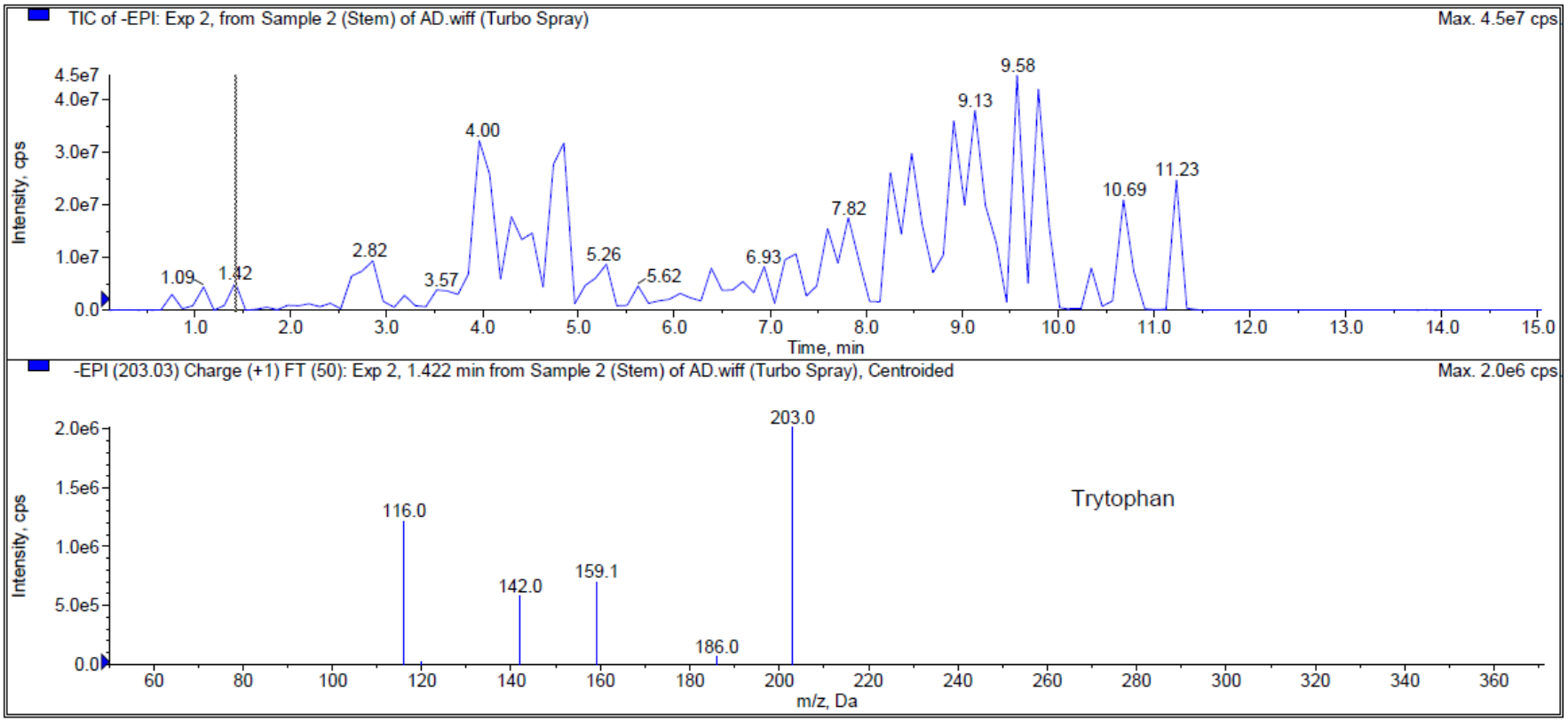

Figure 4. 9 Full chromatogram of LCMS/MS trytophan from *Pereskia bleo* stems aqueous extract.

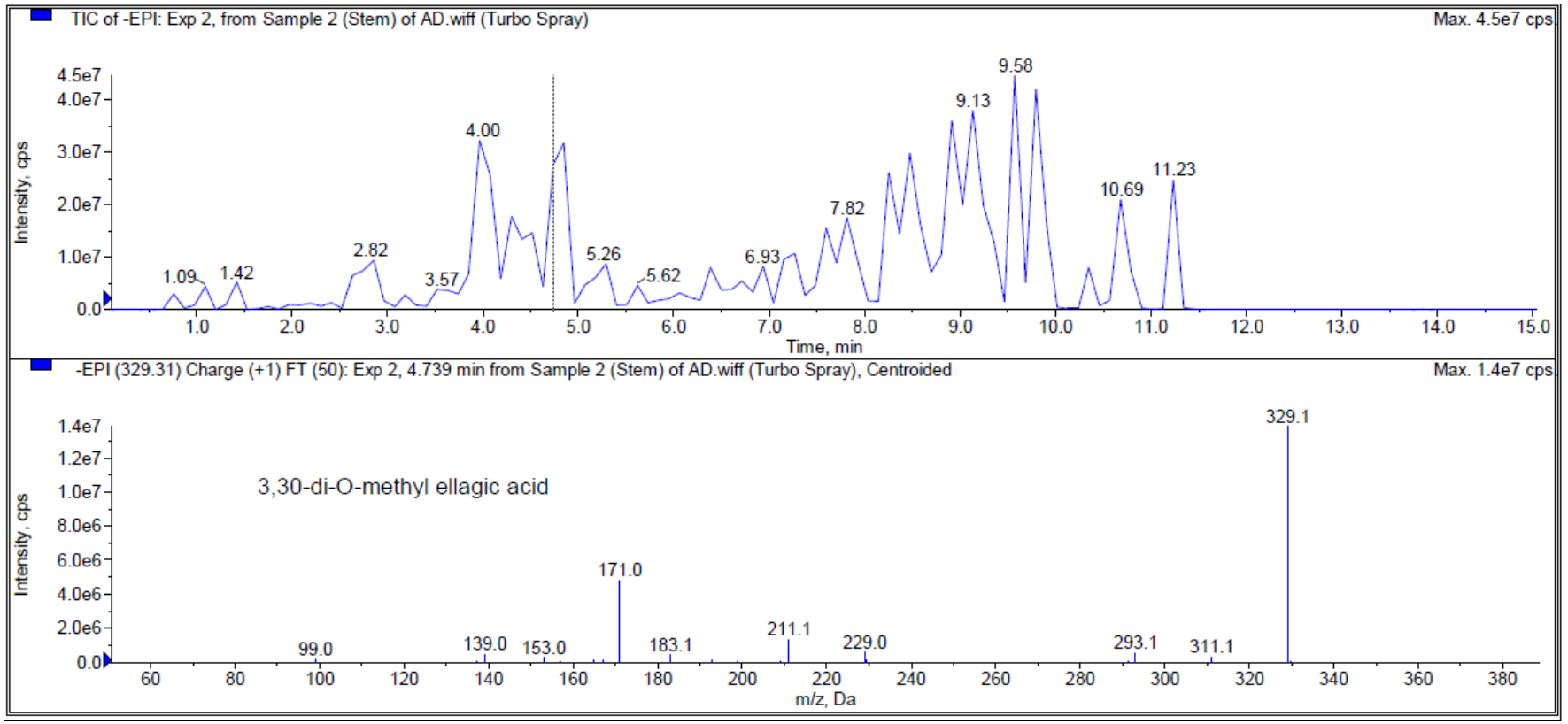

Figure 4. 10 Full chromatogram of LCMS/MS 3,30-di-O-methyl ellagic acid from *Pereskia bleo* stems aqueous extract.

4.2.6 Liquid Chromatography Mass Spectrometry combined with Mass Spectrometry (LCMS/MS) of *Pereskia bleo* Roots.

Initial investigation revealed that aqueous crude extract of roots *Pereskia bleo* contained phenolics and flavonoids compounds. The further test using Liquid Chromatography Mass Spectrometry combined with Mass Spectrometry (LCMS/MS) showed the MS spectrum of *Pereskia bleo* roots aqueous crude extract contained 9 expected compounds. However, these compounds were identified as unknown compounds due to unavailable data of the standard compounds in database system. Details MS/MS fragmentation of unknown compounds were showed in Appendix.

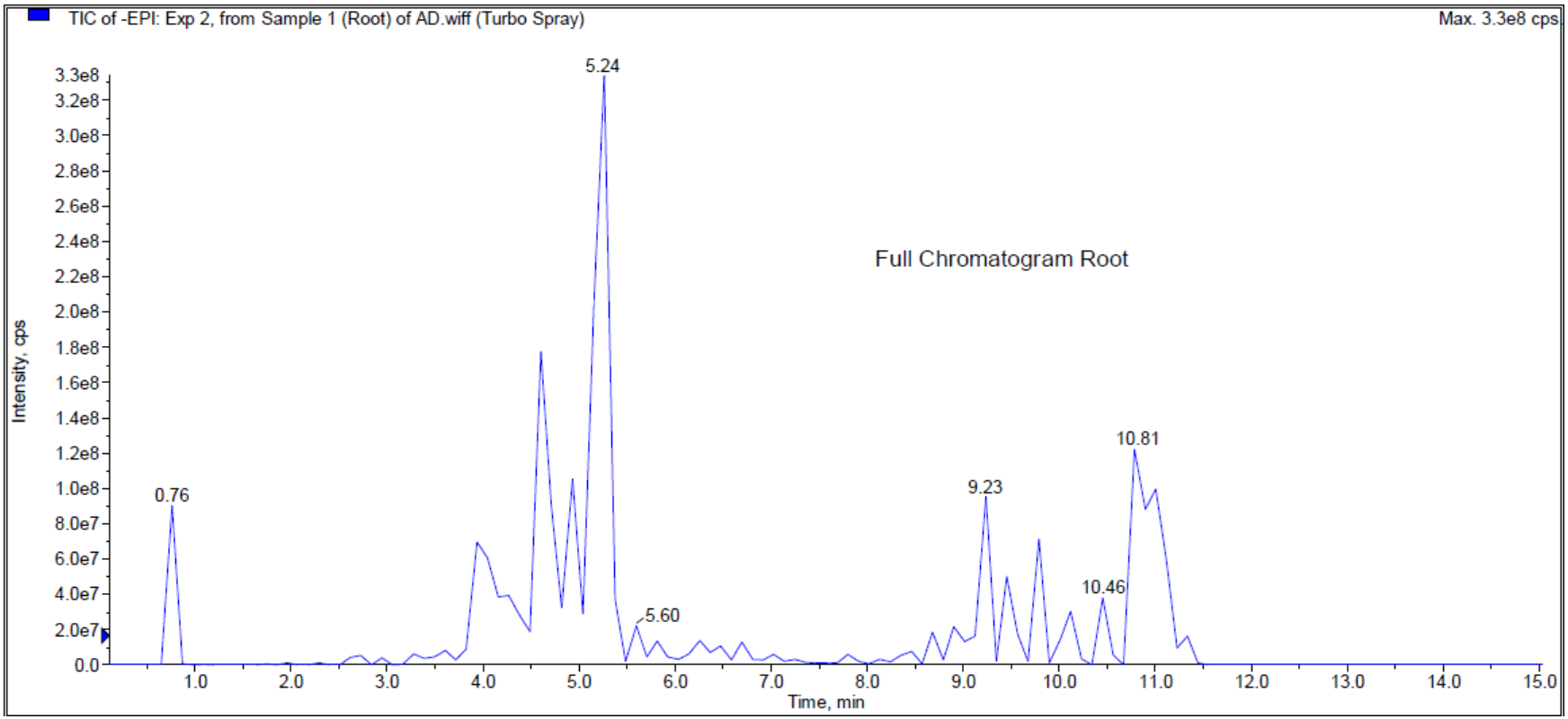

Figure 4. 11 Full Chromatogram LCMS/MS of *Pereskia bleo* roots aqueous crude extract.

4.3 Determination of Total Phenolics Contents.

Gallic acid was used as a standard reference in order to determine the total phenolics contents in leaves, stems and roots *Pereskia bleo* crude extracts. The standard curve of gallic acid was prepared at five different concentrations which were 0 µg/ml, 25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml and 125µg/ml. The total phenolics contents in *Pereskia bleo* crude extracts were expressed as milligram of gallic acid equivalent (GAE) per gram of dry sample.

Table 4. 14 Absorbance of gallic acid in total phenolics contents.

Gallic acid	**Absorbance at 765nm**		**Mean ± SD**
(µg/ml)	**1**	**2**	
0	0.052	0.048	0.050± 0.003
25	0.440	0.452	0.446 ± 0.008
50	0.818	0.811	0.815 ± 0.005
75	1.157	1.168	1.163 ± 0.008
100	1.370	1.420	1.39 ± 0.04
125	1.727	1.721	1.720± 0.001

Absorbance was expressed as means ±standard deviation (SD), n=2

Figure 4.12 above showed a standard curve of gallic acid with linear trend line at zero intercept. The equation of linear standard curve was y=0.014x, which y was a absorbance reading at 725nm and x was a gallic acid concentration in µg/ml. The R-squared (r^2) value was 0.982.

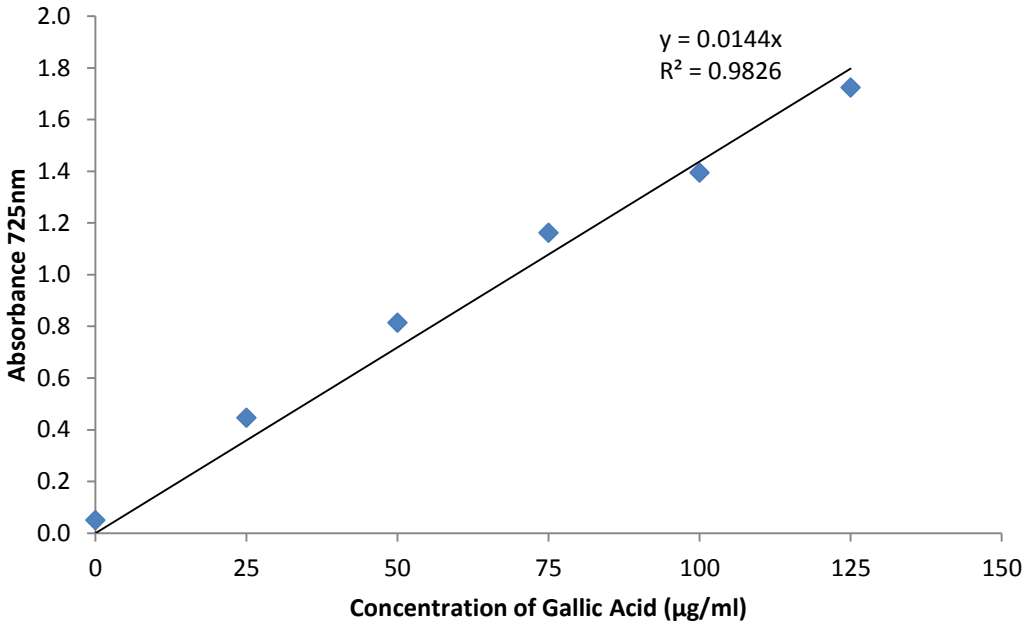

Figure 4.12 Standard curve of Gallic acid (standard) in total phenolics contents.

Samples of leaves, stems and roots of *Pereskia bleo* crude extracts were prepared at 10mg/ml concentration. Figure 4.13 showed both stems and roots of *Pereskia bleo* aqueous and chloroform crude extracts revealed a higher amount of total phenolics content. The stems of *Pereskia bleo* aqueous and chloroform crude extracts revealed the highest of total phenolics content with 6.06 mg GAE/g and 5.69 mg GAE/g followed by roots *Pereskia bleo* chloroform and aqueous crude extracts with 3.64 mg GAE/g and 3.51 mg GAE/g. While leaves of *Pereskia bleo* methanol showed the highest in total phenolics content with 3.00 mg GAE/g.

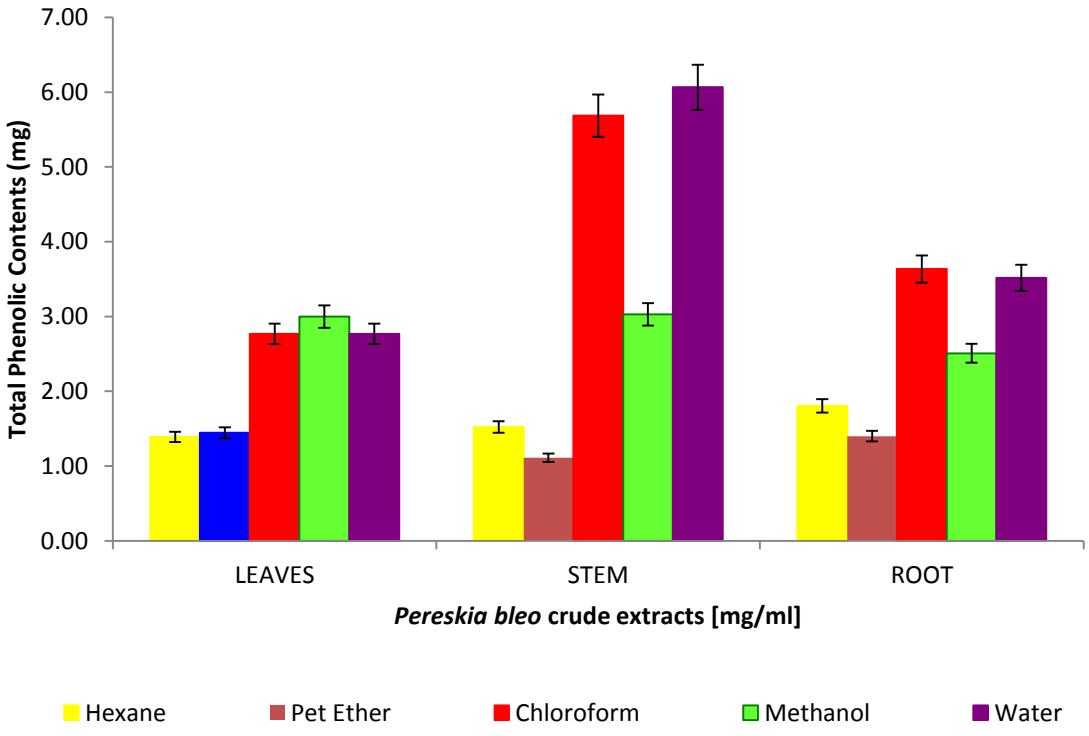

Figure 4.13 Total Phenol Content of *Pereskia bleo* crude extracts.

4.4 Determination Of Total Flavonoids Contents

Quarcetin was used as a standard reference in order to determine the total flavonoids contents in leaves, stems and roots of *Pereskia bleo* crude extract. This standard curve of quarcetin was prepared at five different concentrations which were 0 µg/ml, 0.5 µg/ml, 1.0µg/ml, 1.5 µg/ml, 2.0 µg/ml and 2.5µg/ml. The total of flavonoids contents in *Pereskia bleo* crude extracts were expressed as milligram of quarcetin equivalent (QUE) per gram of dry sample.

Table 4.15 Absorbance of Quarcetinin total flavonoids content.

Quarcetin (mg/ml)	Absorbance at 765nm		Mean±SD
	1	2	
0.0	0.008	0.012	0.01±0.003
0.5	0.064	0.059	0.062±0.004
1.0	0.110	0.101	0.106±0.006
1.5	0.168	0.159	0.164±0.006
2.0	0.202	0.215	0.209±0.009
2.5	0.245	0.259	0.252±0.010

Absorbance was expressed as means ±standard deviation (SD), n=2

Figure 4.14 showed a standard curve of quercetin with linear trend line at zero intercept. The equation of linear standard curve was y=0.014x, which y was an absorbance reading at 725nm and x was a quercetin concentration in µg/ml. The R-squared (r^2) value was 0.982.

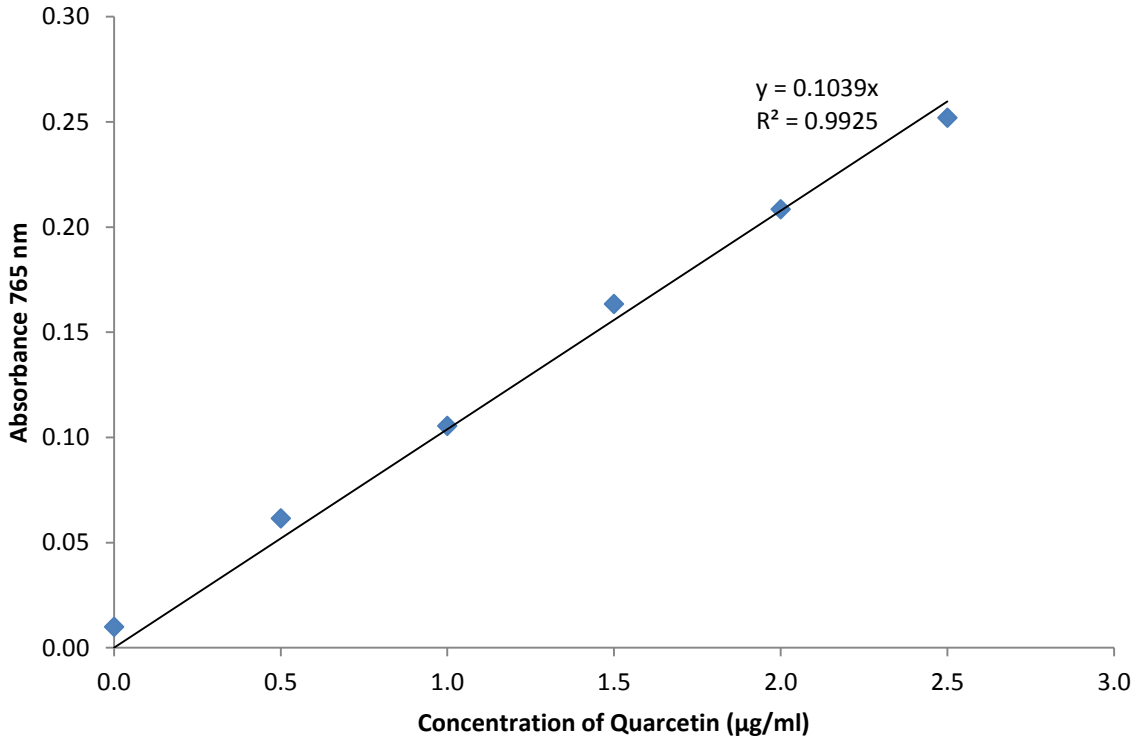

Figure 4.14 Standard curve of quarcetin in total flavonoids content.

Samples of leaves, stems and roots of *Pereskia bleo* crude extracts were prepared at 10mg/ml concentration. Figure 4.15 showed chloroform, methanol and aqueous crude extracts of stem *Pereskia bleo* revealed the highest total flavonoids content with 11.07 mg QE/g, 8.51 mg QE/g and 7.86 mg QE/g followed by both leaves and roots of *Pereskia bleo* chloroform crude extracts with 5.2 mg QE/g and 3.40 mg QE/g.

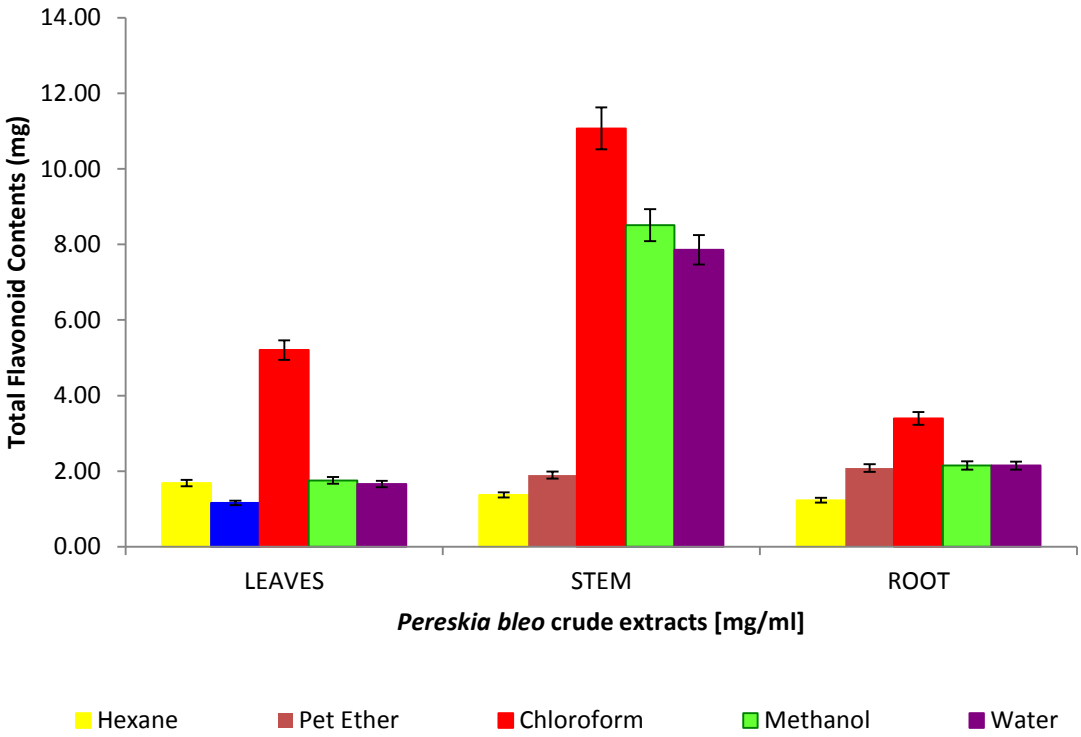

Figure 4.15 Total Flavonoids content of *Pereskia bleo* crude extract.

4.5 Brine Shrimp Lethality Assay (BSLA)

The toxicity of *Pereskia bleo* aqueous crude extracts were investigated using Brine Shrimp Lethality Assay (BSLA) technique. Table 4.16 demonstrated the number of shrimps exposed to the *Pereskia bleo* aqueous crude extracts and the number of shrimp dead.

Table 4.16 Total of shrimps dead in *Pereskia bleo* aqueous crude extracts.

Concentration samples (ug/ml)	Total number of shrimp			Total number of dead		
	Leaves	Stem	Root	Leaves	Stem	Root
10	10	10	10	2	2	2
100	10	10	10	3	4	3
1000	10	10	10	4	5	3

The lethal dose concentration LC_{50} was determined using Finney program. The higher value of LC_{50} means the lower toxicity of plant crude extracts. Tables below showed the LC_{50} of leaves, stems and roots *Pereskia bleo* aqueous crude extracts.

Table 4.17 Probit analysis of *Pereskia bleo* aqueous crude extract in BSLA.

Sample [ug/ml]	Number of Exposed	Number of dead	Percentage mortality	LC50 [ug/ml]	95 percents Confidence
Leaves					
1000	10	4	40		3.83×10^{-9} –
100	10	3	30	2.51	1.65×10^{9}.
10	10	2	20		
Stem					
1000	10	5	50		7.98×10^{-7} –
100	10	4	40	3.55	1.58×10^{7}.
10	10	2	20		
Root					
1000	10	4	30		3.83×10^{-9} –
100	10	3	30	2.51	1.65×10^{9}
10	10	2	20		

4.6 Antidiabetic Studies

4.6.1 Glycogen Phosphorylase Inhibitor Bioassay (GP) Of *Pereskia bleo* Crude Extracts.

Glipizide was used as a standard reference in glycogen phosphorylase (GP) inhibitory activity. This drug was used to compare the antidiabetic activity of *Pereskia bleo* with common diabetic drug used nowadays. Five different concentrations of glipizide were prepared at 2, 4, 6, 8, and 10µgml^{-1}.

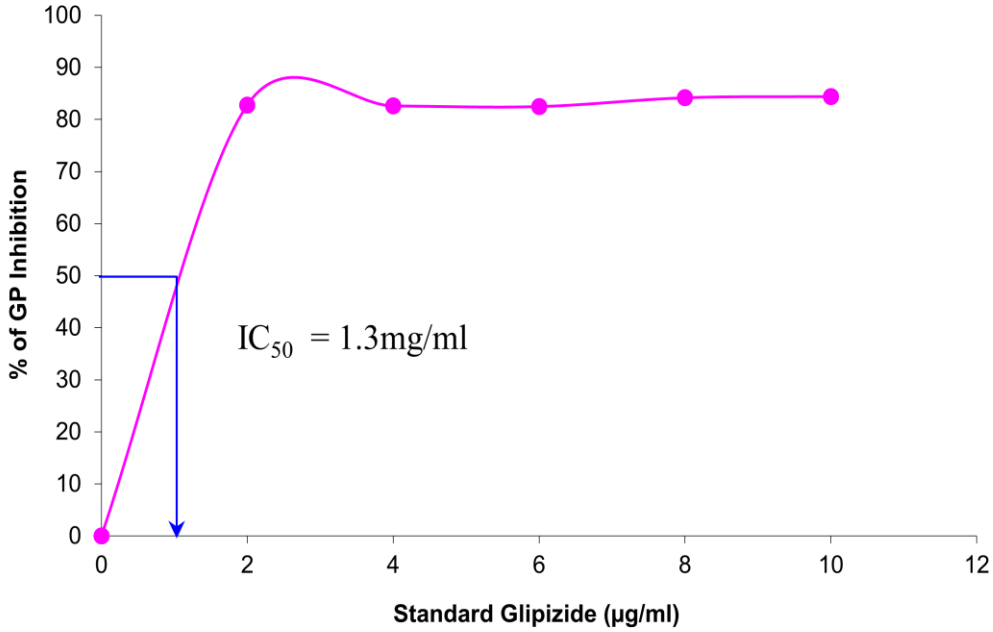

Figure 4.16 Percentage of GP Inhibition and IC_{50} value of Glipizide.

Figure 4.16 showed the glycogen phosphorylase activity of glipizide increased while the concentrations of glipizide increase. The percentage of GP inhibition at all glipizide concentrations showed more than 80% inhibitions. At concentration of 2 µg/ml, 4

µg/ml, 6 µg/ml, 8 µg/ml and 10 µg/ml, the percentage of GP inhibition were 82.8%, 82.6%, 82.5%, 84.2% and 84.4%, respectively.

Leaves, stems and roots of *Pereskia bleo* crude extracts were prepared and tested to determine the percentage inhibition of glycogen phosphorylase activity. The highest percentage of GP inhibition indicated that more of GP activity was inhibited by crude extract. Figure 4.17 showed the percentage of GP inhibitions in *Pereskia bleo* crude extracts increased while the concentrations of crude extracts increased. At concentration of 10mg/ml *Pereskia bleo* crude extracts, the highest GP inhibitions in three samples of leaves, stems and roots crude extracts came from the methanol extracts. The highest percentage of GP inhibition was from roots methanol crude extract, with the percentage of GP inhibition was 89.9% followed by leaves methanol crude extract with percentage of GP inhibition was 82.3%. While stems methanol crude extracts showed percentage of GP inhibition was 78.3%. These percentages of GP inhibitions were compared to glipizide as a standard reference.

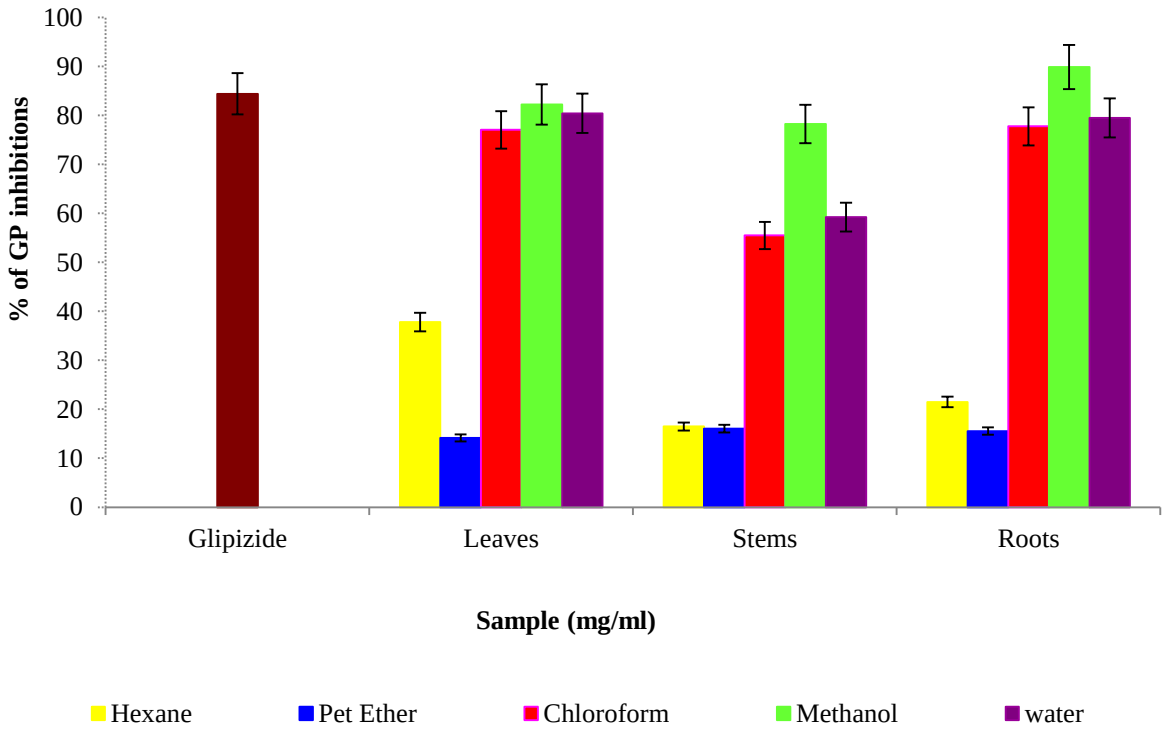

Figure 4.17 Percentage of GP Inhibition of *Pereskia bleo* crude extracts compared to Glipizide.

Leaves, stems and roots of *Pereskia bleo* crude extracts were prepared at five different concentrations at 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml and 10mg/ml. The half maximal inhibitory activity (IC_{50}) for each crude extracts were calculated and showed in Table 4.18. IC_{50} is important to calculate the concentration of inhibitor at half of inhibitor activity. This value was obtained from graph extrapolation. The IC_{50} of glipizide was 1.1 µg/ml. However, the $IC_{50\ of}$ hexane and petroleum ether for leaves, stems and roots of *Pereskia bleo* crude extracts could not be determined due to the percentage of inhibition of these samples did not reach 50% even at the highest concentration. Details of absorbance recorded for all samples and standard drug were attached in appendix.

Table 4.18 The IC_{50} for all *Pereskia bleo* crude extracts.

Samples	IC_{50} Values (µg/ml)
Glipizide	1.1
Leaves	
Hexane	-
Petroleum ether	-
Chloroform	1.4
Methanol	1.6
Aqueous	2.0
Stems	
Hexane	-
Petroleum ether	-
Chloroform	3.2
Methanol	1.6
Aqueous	2.3
Roots	
Hexane	-
Petroleum ether	-
Chloroform	2.9
Methanol	1.2
Aqueous	1.9

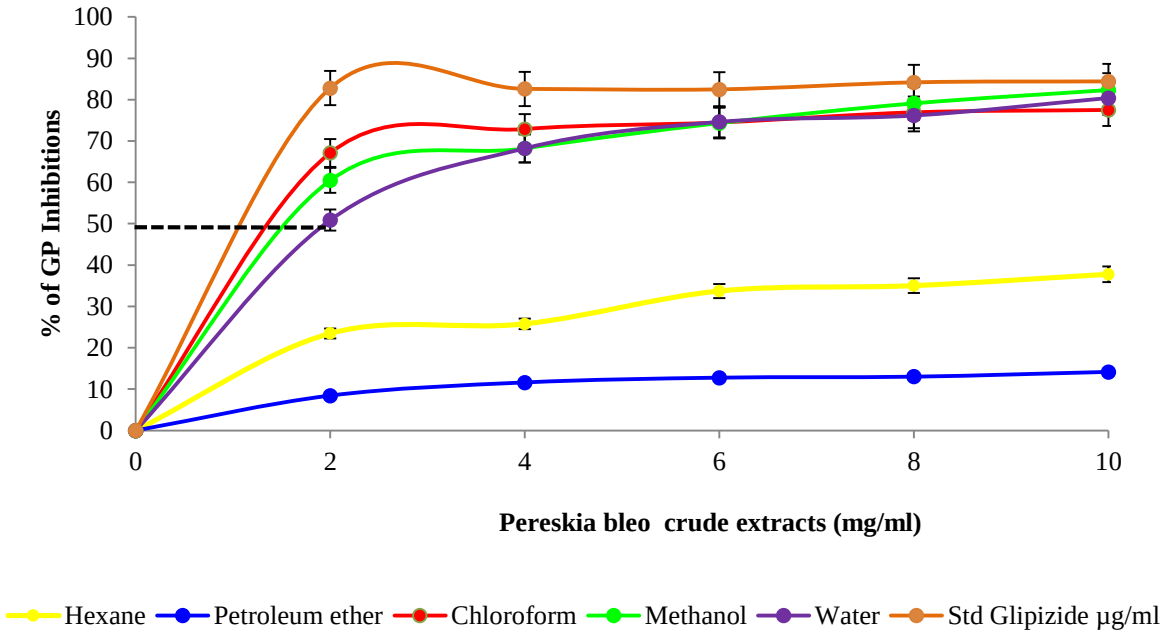

Figure 4.18 The percentage of GP inhibitions and$IC_{50}$of *Pereskia bleo* leaves crude extracts

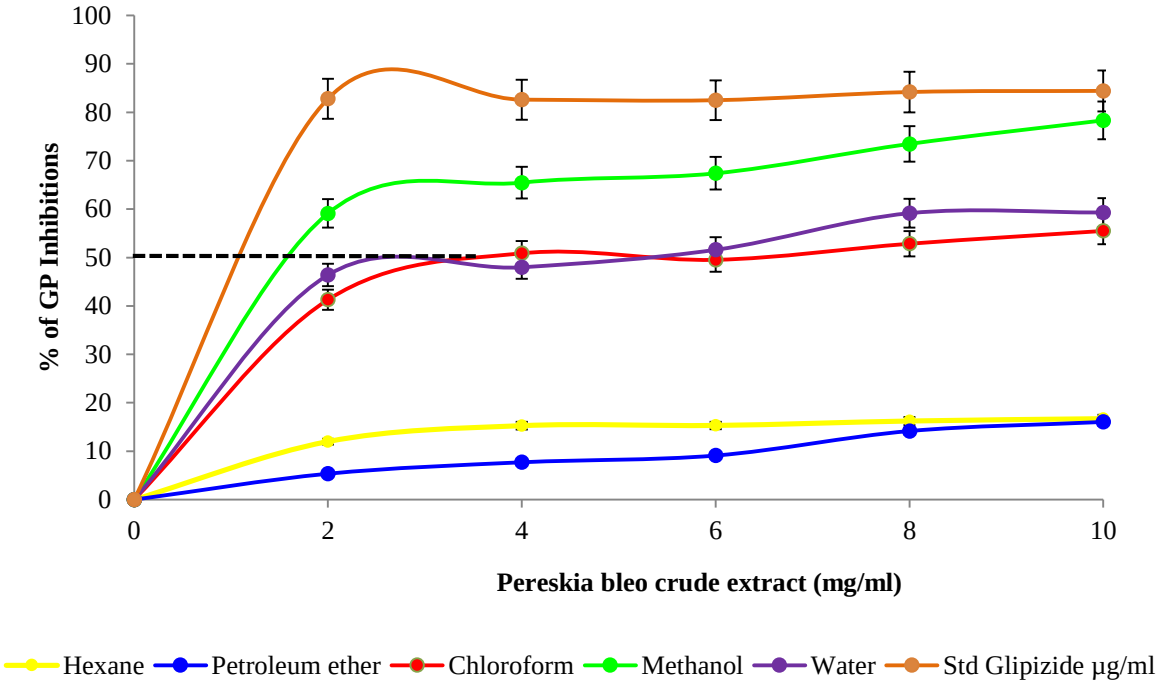

Figure 4.19 The percentage of GP inhibitions and IC_{50} of *Pereskia bleo* stems crude extracts .

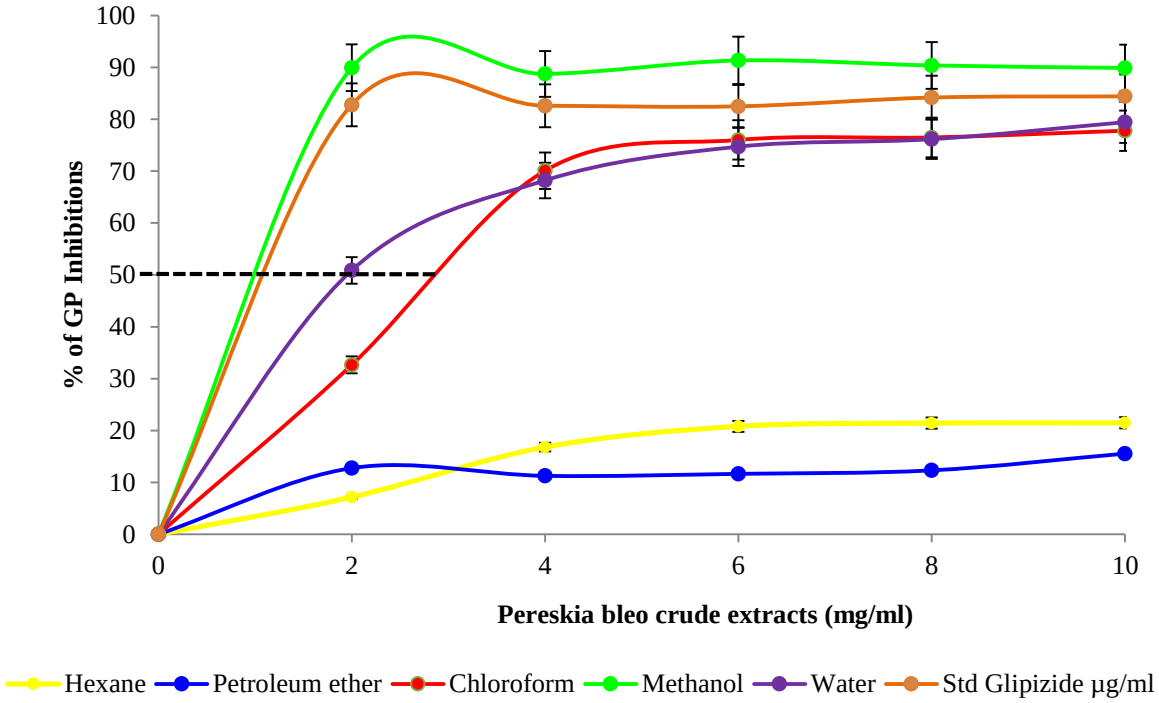

Figure 4.20 The percentage of GP inhibitions and IC_{50} of *Pereskia bleo* roots crude extracts

4.6.2 Glycogen Phosphorylase Inhibition Bioassay (GP) Of *Pereskia bleo* Fractions.

The leaves, stems and roots of *Pereskia bleo* crude extracts were fractioned with column chromatography using Toluene-ethyl acetate as mobile phase in a gradient way, range 30:70 (v/v) in order to categorize the compounds in plant extracts based on their polarities. Each of fractions was prepared at 1mg/ml concentration before further tested with glycogen phosphorylase inhibition (GP) bioassay. Figure 4.21 to 4.35 showed percentage of GP inhibitions of *Pereskia bleo* fractions. The absorbance recorded for all samples and standard drug (Glipizide) was attached in appendix section.

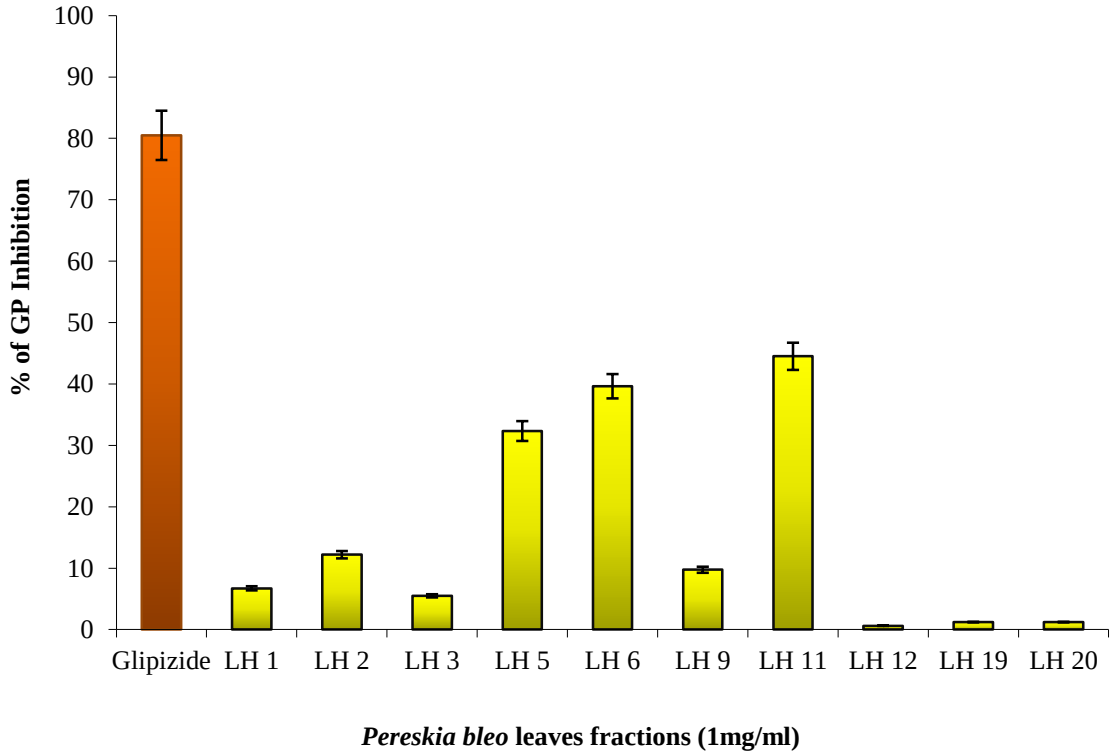

Figure 4.21 Percentage of GP Inhibitions of *Pereskia bleo* leaves hexane fractions

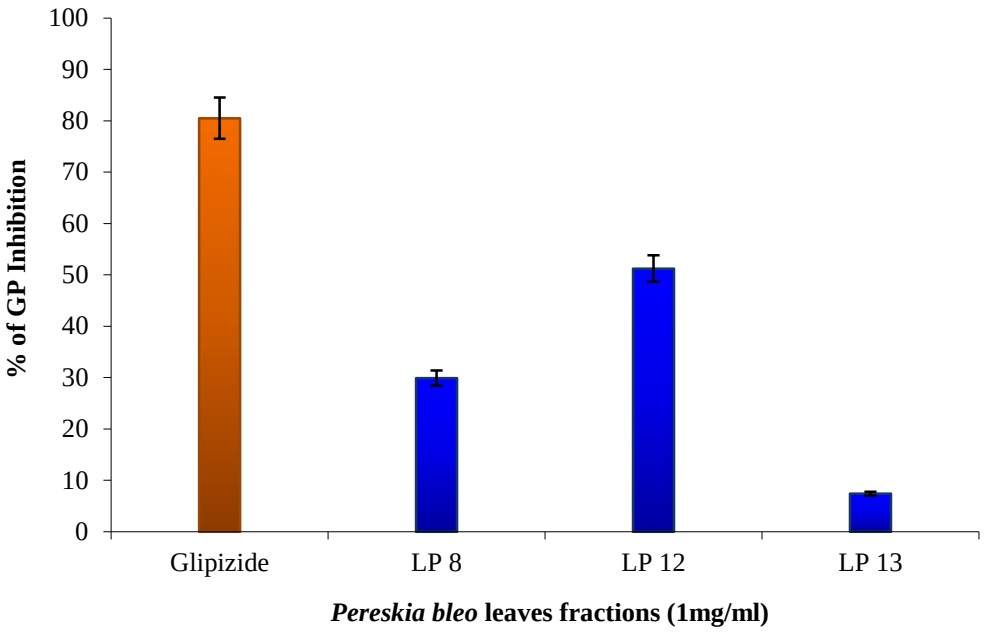

Figure 4.22 Percentage of GP Inhibitions of *Pereskia bleo* leaves petroleum ether fractions

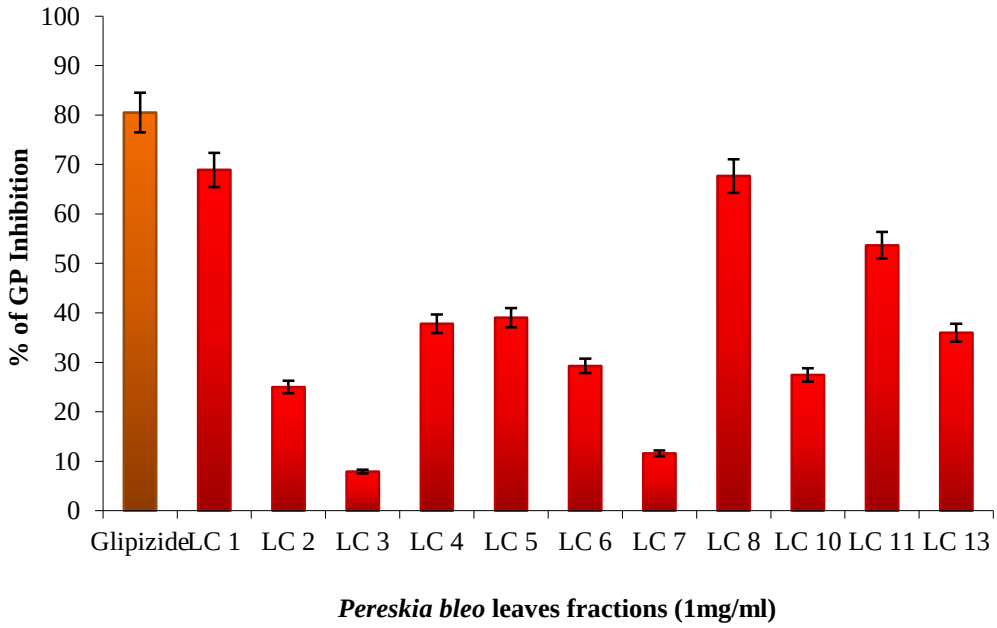

Figure 4.23 Percentage of GP Inhibitions of *Pereskia bleo* leaves chloroform fractions.

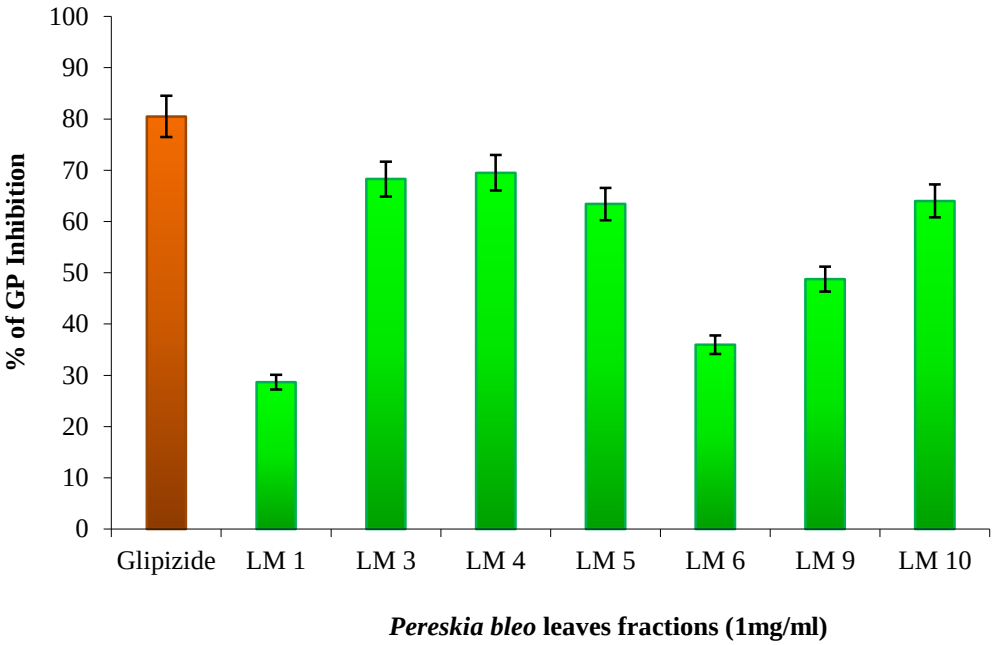

Figure 4.24 Percentage of GP Inhibitions of *Pereskia bleo* leaves methanol fractions.

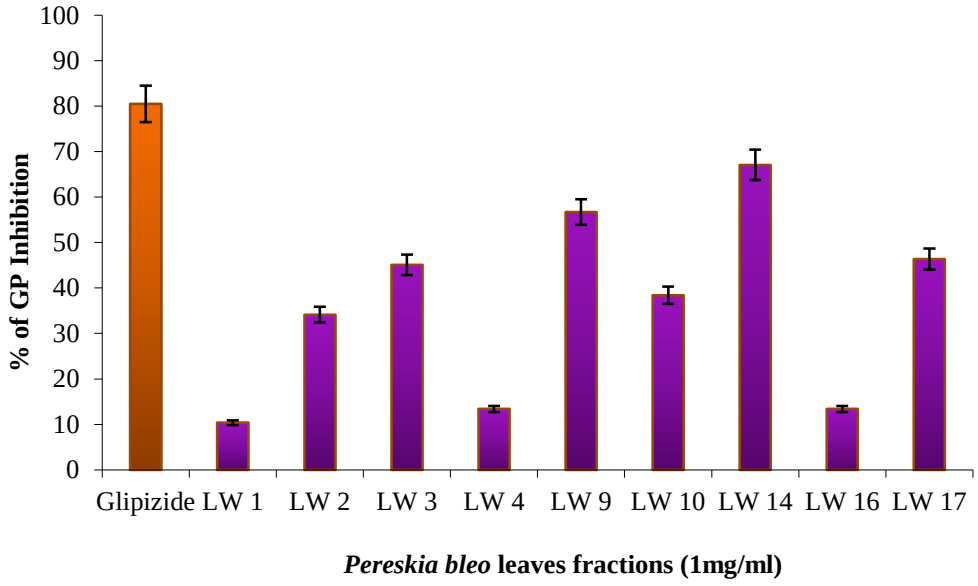

Figure 4.25 Percentage of GP Inhibitions of *Pereskia bleo* leaves aqueous fractions

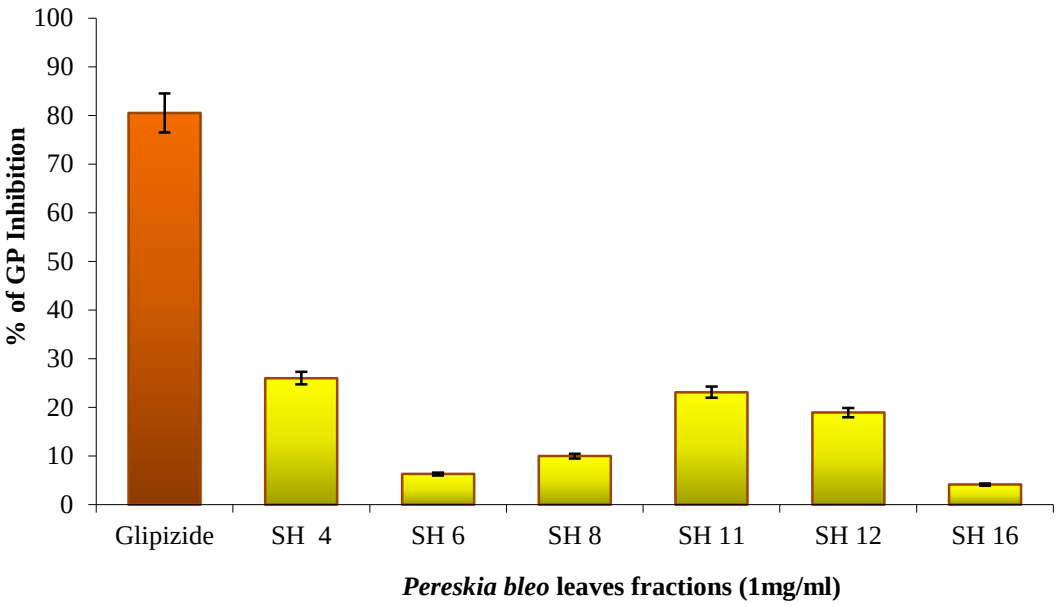

Figure 4.26 Percentage of GP Inhibitions of *Pereskia bleo* stems hexane fractions.

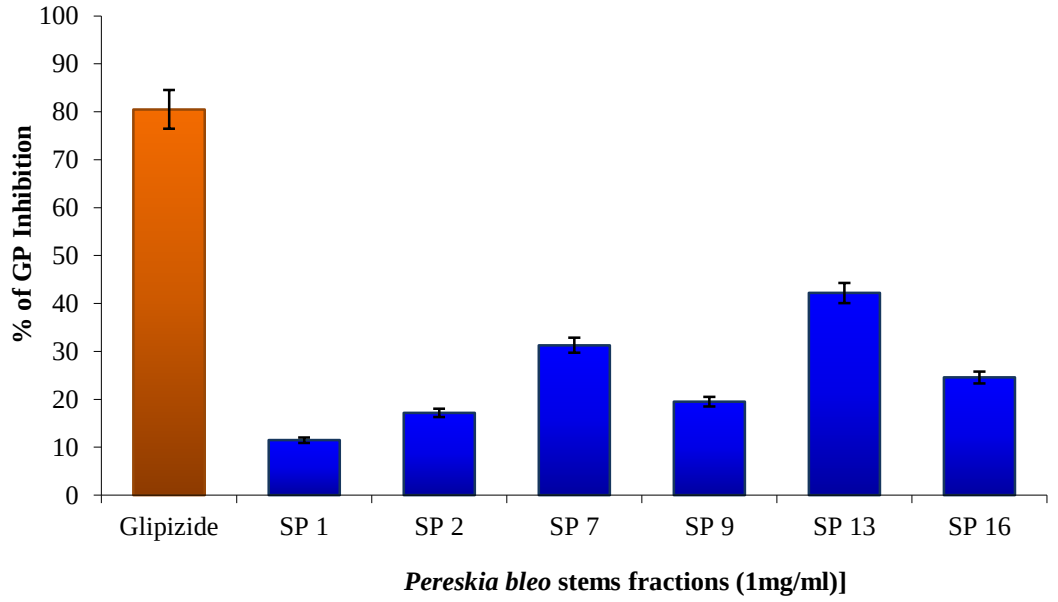

Figure 4.27 Percentage of GP Inhibitions of *Pereskia bleo* stems petroleum ether fractions

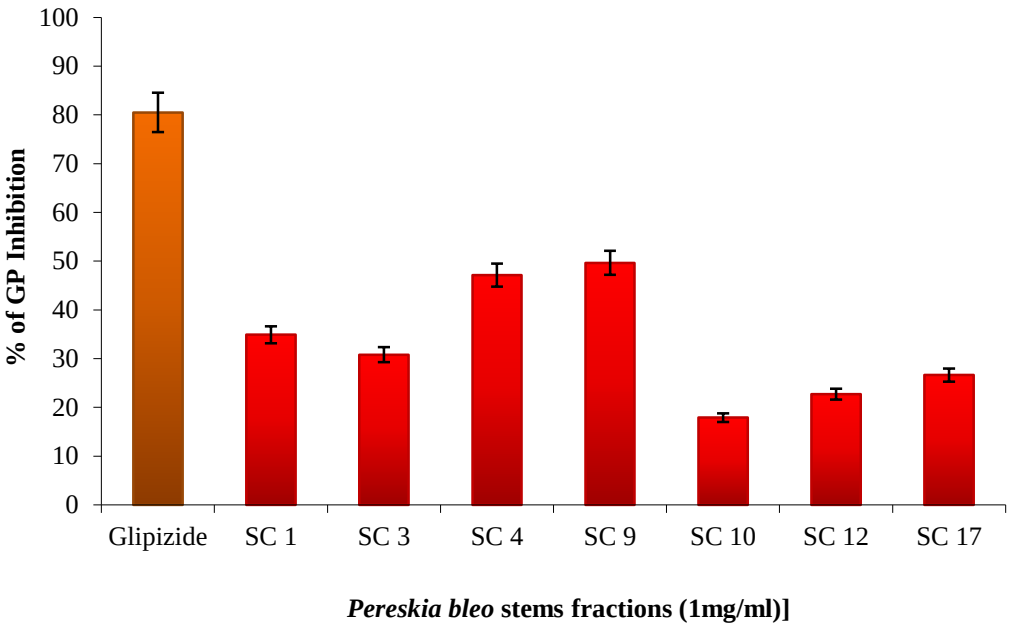

Figure 4.28 Percentage of GP Inhibitions of *Pereskia bleo* stems chloroform fractions .

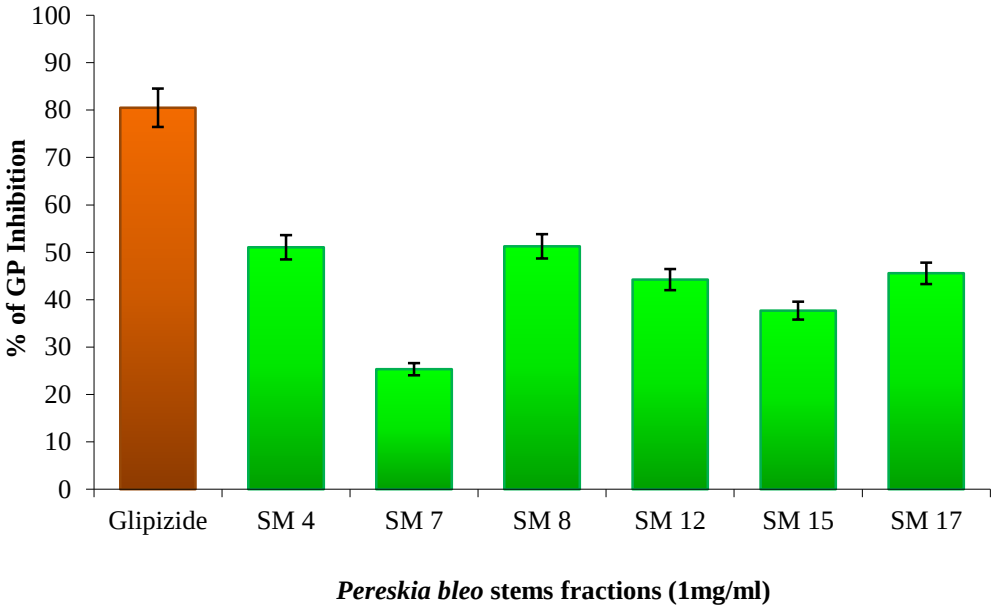

Figure 4.29 Percentage of GP Inhibitions of *Pereskia bleo* stems methanol fractions.

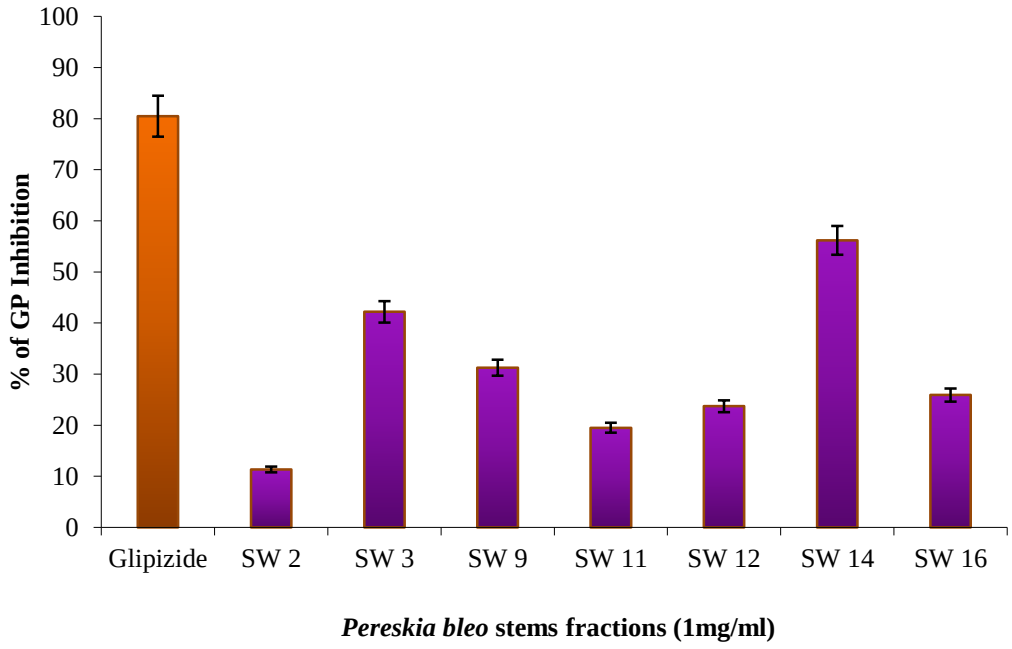

Figure 4.30 Percentage of GP Inhibitions of *Pereskia bleo* stems aqueous fractions.

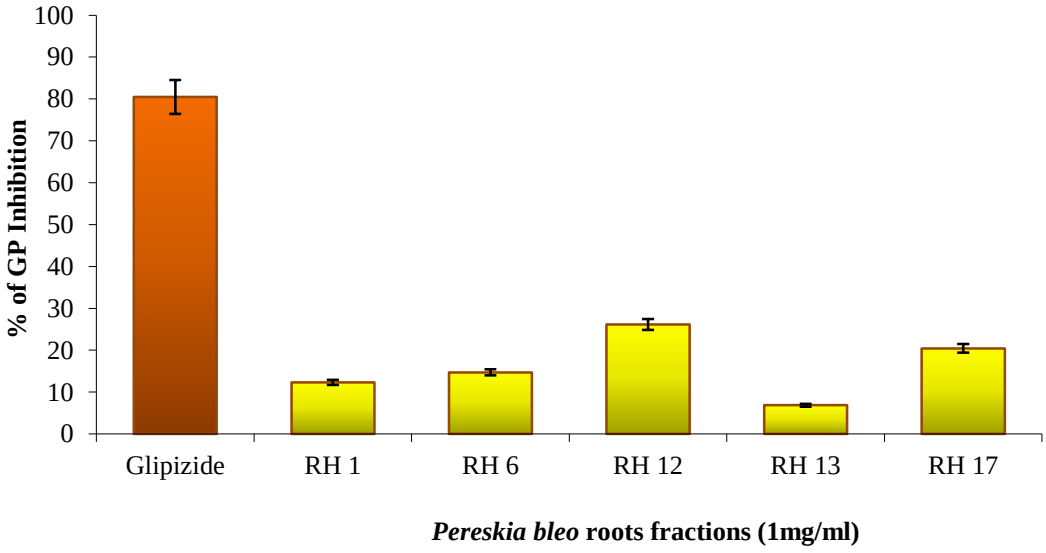

Figure 4.31 Percentage of GP Inhibitions of *Pereskia bleo* roots hexane fractions.

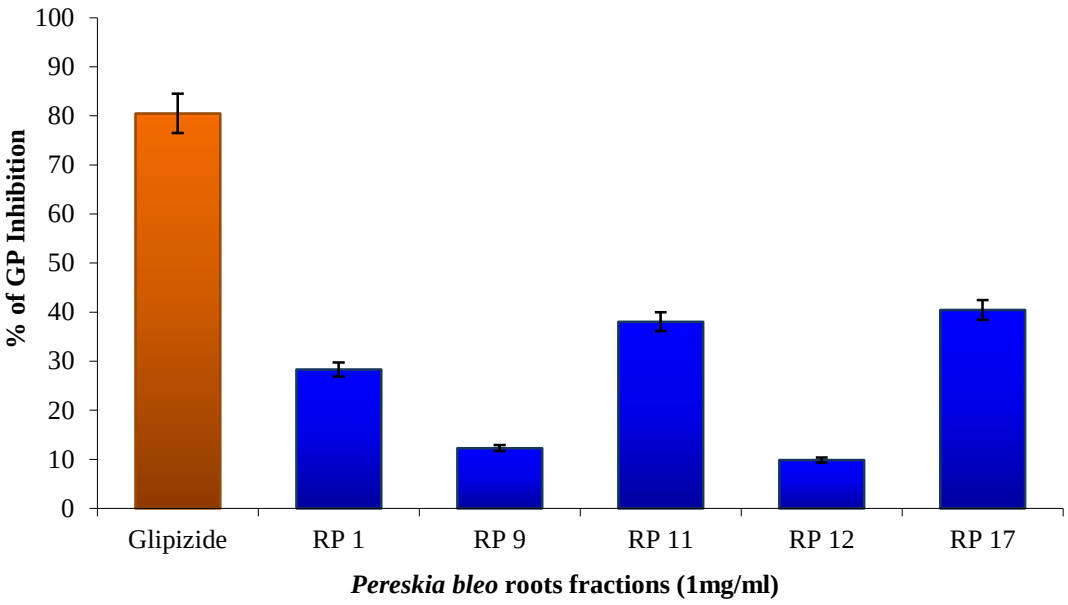

Figure 4.32 Percentage of GP Inhibitions of *Pereskia bleo* roots petroleum ether fractions.

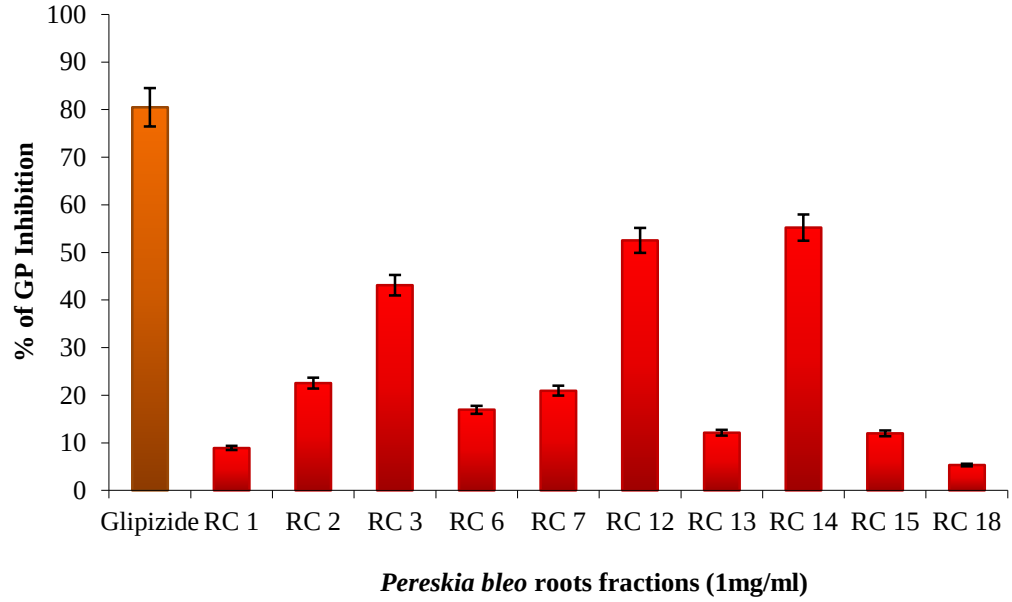

Figure 4.33 Percentage of GP Inhibitions of *Pereskia bleo* roots chloroform fractions .

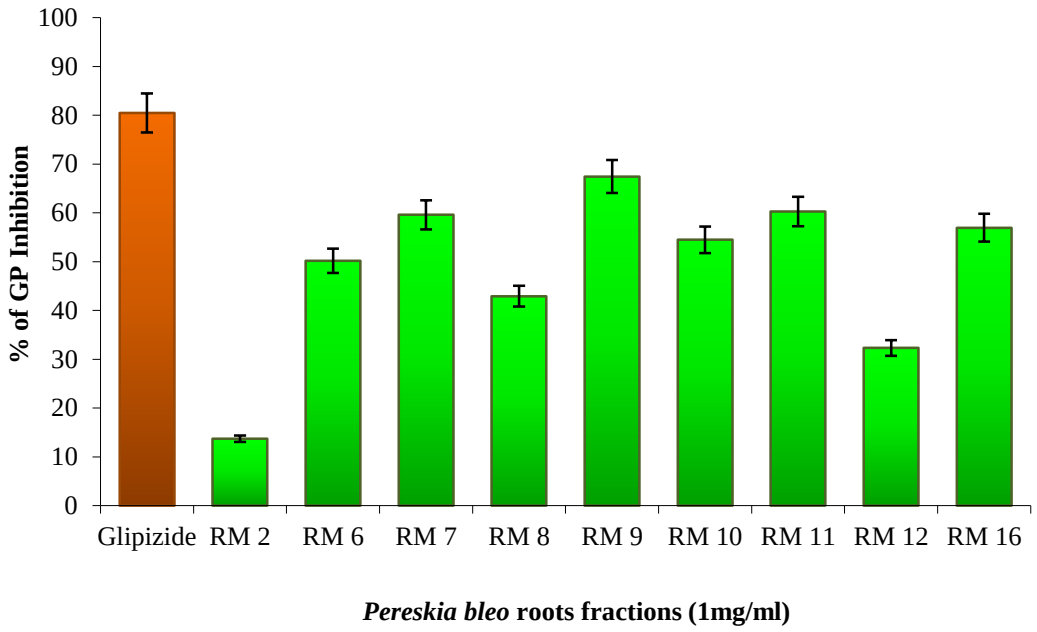

Figure 4.34 Percentage of GP Inhibitions of *Pereskia bleo* roots methanol fractions.

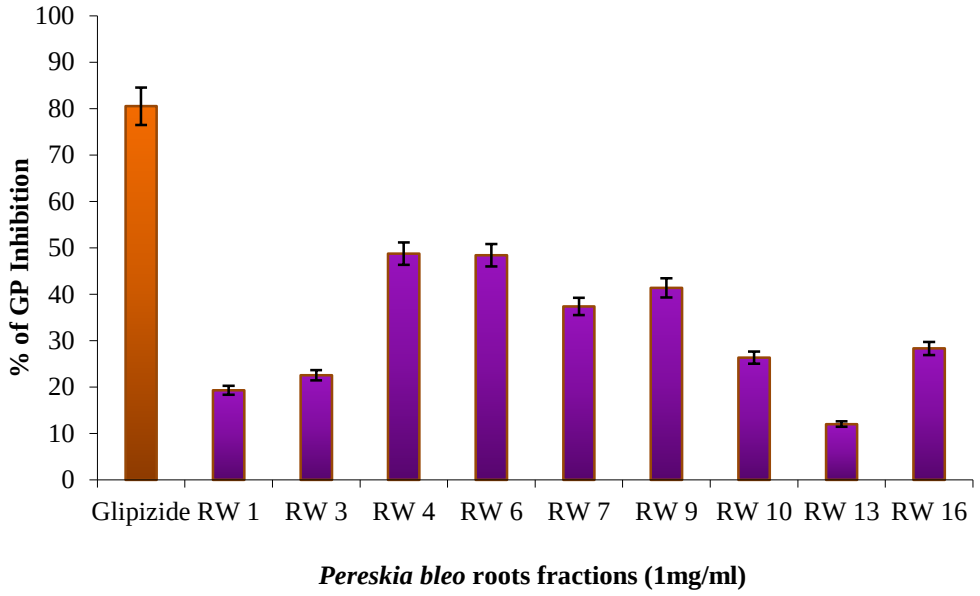

Figure 4.35 Percentage of GP Inhibitions of *Pereskia bleo* roots aqueous fractions.

4.6.3 Experimental Animals

4.6.3.1 Acute Toxicity

Both male and female of Sprague dawley (SD) rats was administrated with leaves, stems and roots of *Pereskia bleo* aqueous crude extract via intraperitoneal at doses of 250mg/kg, 500mg/kg and 1000mg/kg. Table 4.19 showed that all samples of *Pereskia bleo* aqueous crude extracts did not show any lethality or any toxic reaction at all doses in14 days.

Table 4.19 Number of exposed and survived after administrated with *Pereskia bleo* aqueous crude extracts.

Sample	Dose (mg/kg)	Number of exposed	Number of survived			Toxic Reactions
			24h	72h	14days	
Leaves	250	6	6	6	6	-
	500	6	6	6	6	-
	1000	6	6	6	6	-
Stem	250	6	6	6	6	-
	500	6	6	6	6	-
	1000	6	6	6	6	-
Root	250	6	6	6	6	-
	500	6	6	6	6	-
	1000	6	6	6	6	-

4.6.3.2 Oral Glucose Tolerance Test (OGTT)

OGTT was performed to measure the body's ability of SD rats to consumed glucose as a body's main source of energy. SD rats were fasted overnight before administrated with glucose at dose 3g/kg body weight by oral gavages in oral glucose tolerance test (OGTT). The reductions of blood glucose levels were compared with control group.

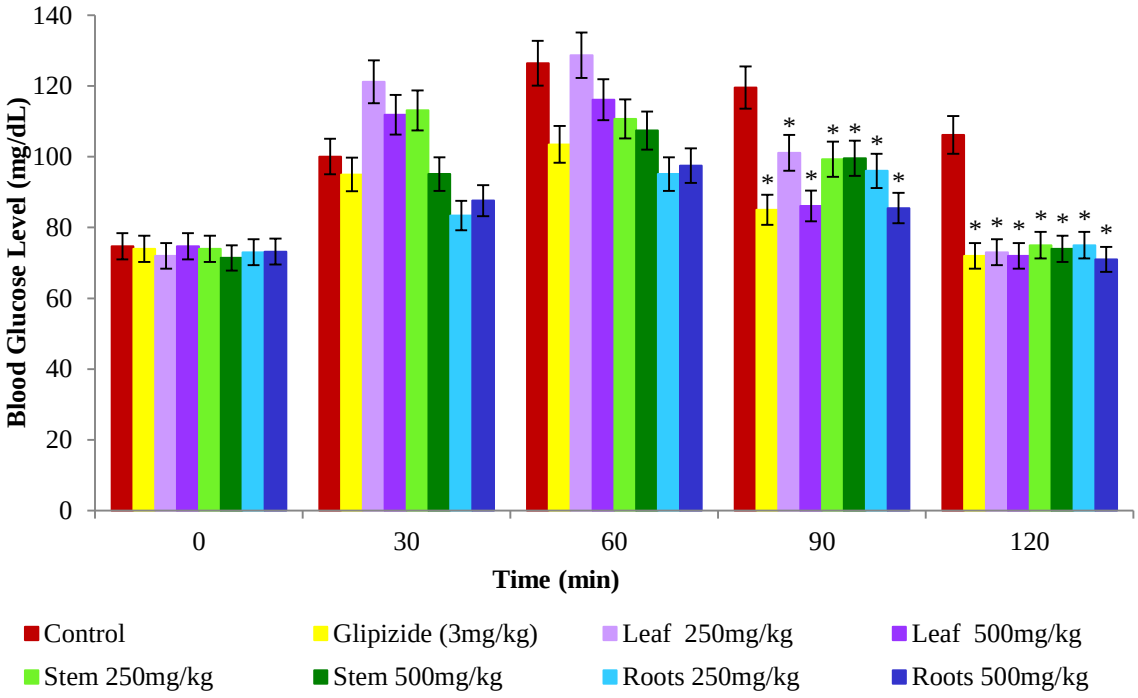

Figure 4.36 Blood glucose level of SD rats after administrated with *Pereskia bleo* aqueous crude extracts and glipizide. The sign (*) means significantly different from the control value of diabetic group at $p < 0.05$.

Figure 4.36 showed an increased in blood glucose level for all groups from 0 minutes until 60 minutes. The high dose of 500mg/kg body weight for leaves, stems and roots of *Pereskia bleo* aqueous crude extracts showed a significant maximum falls of blood glucose levels at 120 minutes as compared to control group. The roots of *Pereskia bleo* aqueous crude extract at dose of 500mg/kg showed a significantly reduction of blood glucose levels at 120 minutes with 21.5% of reduction. In addition, a standard drug glipizide showed a maximum fall of blood glucose level with 18.3%.

4.6.3.3 Effect of *Pereskia bleo* aqueous crude extracts on blood glucose level.

An alloxan-induced Sprague dawley (SD) rats with blood glucose levels more than 200 mg/dL after 7 days were considered as diabetic and were selected for antidiabetic study. The hyperglyceamic effects of *Pereskia bleo* extracts on fasting blood glucose levels of diabetic induced rats were showed in Table 4.20. There was a significantly decreased in blood glucose level in diabetic group as compared to normal group. While diabetic groups treated with leaves, stems and roots of *Pereskia bleo* aqueous crude extracts showed a significantly decreased in blood glucose level compared to diabetic control group.

Table 4. 20 Effect of *Pereskia bleo* aqueous extracts in fasting blood glucose level of diabetic rats

Groups	Blood Glucose Level (mg/dL)					
	Day 0	**Day 5**	**Day 10**	**Day 15**	**Day 20**	**Day 25**
Normal Control	75±11	78.5±13	74.5±10	76.8±14	79.2±11	78±10
Diabetic Control	284±17	312±15	359±11	375±10	385±16	380±15*
Glipizide 3mg/kg	285±11	280±19	260 ±19	270±19	265±12	260±14*
Leaf 250mg/kg	284±16	231±17	185+13	128±16	119±13	115±10*
Leaf 500mg/kg	286±13	263±17	205±18	160±14	99±10	97±10*
Stem 250mg/kg	281±11	178±13	193±11	187±14	160±12	129±11*
Stem 500mg/kg	284±13	182±19	173±12	149±10	119±19	99±12*
Root 250mg/kg	283±12	207±19	190±12	181±18	146±10	131±16*
Root 500mg/kg	285±15	195±18	126±16	113±16	120±10	120±19*

The sign (*) indicated values significantly different from diabetic control group at $p < 0.05$.

4.6.3.4 Effect of *Pereskia bleo* aqueous crude extracts on body weights.

The hyperglyceamic effects of *Pereskia bleo* aqueous crude extracts on body weight of diabetic rats were shown in figure 4.37. There was a significantly decreased in body weight for diabetic rats groups treated with *Pereskia bleo* and glipizide (standard drug) as compared to diabetic control group. The group of diabetic rats treated with glipizide showed 51% significantly increase in body weight as compared to diabetic control group. The highest significant increase in body weight for diabetic rats treated with *Pereskia bleo* were from leaves at dose of 500mg/kg by 35%.

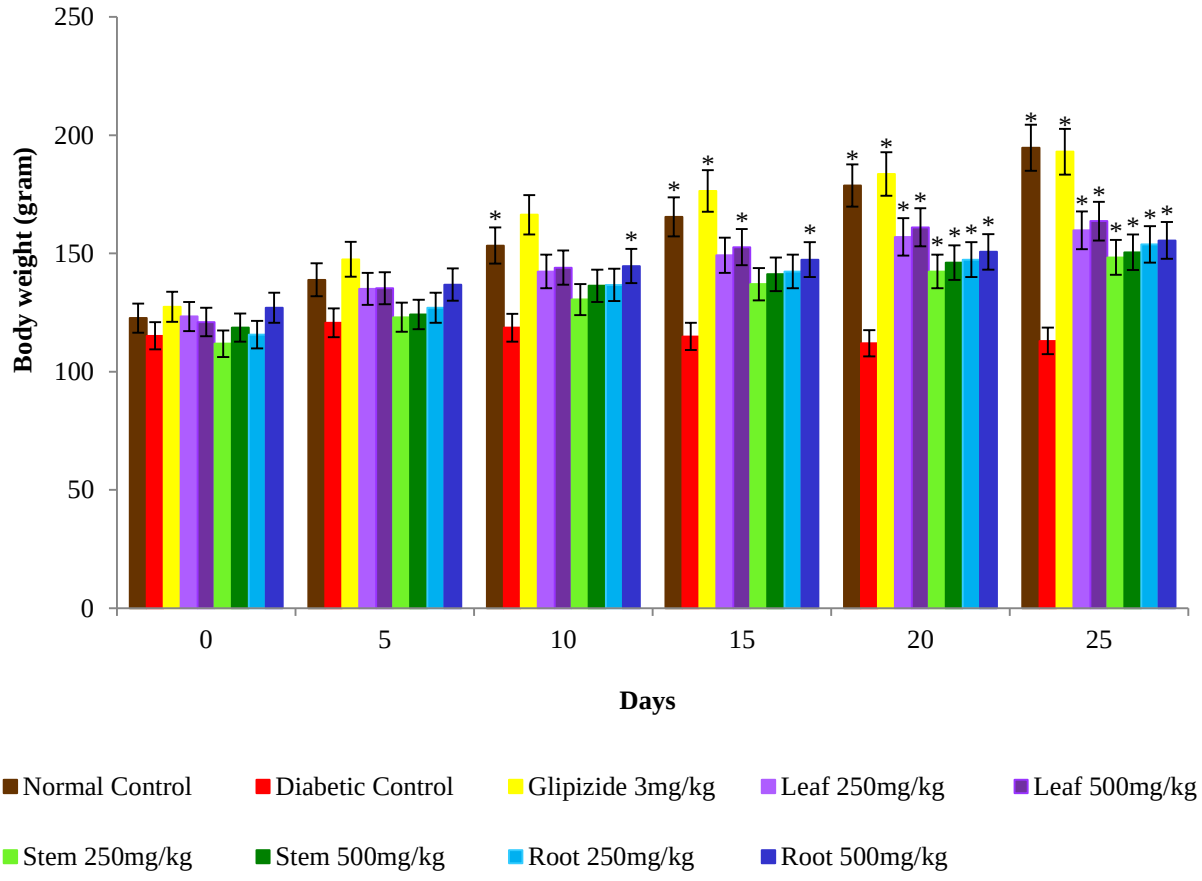

Figure 4.37 Effect in body weight after administrated with of *Pereskia bleo* aqueous extract and Glipizide as standard drug. The sign (*) indicated values significantly different from diabetic control group at $p < 0.05$.

4.6.3.5 Effects of *Pereskia bleo aqueous* crude extracts on Liver Function Test

Liver functions test were used to determine the normal level of ALP, ALT and AST contains in liver. An abnormal result of liver function test showed problems existed in the liver. Table 4.21 showed that the oral administrations of leaves, stems and roots of *Pereskia bleo* aqueous extracts had a significant reduction ($p<0.05$) on serum level of Alanine Transaminase (AST), Aspartate Aminotransferase (AST*) and Alkaline phosphatase (ALP) as compared to diabetic control group.

Table 4.21 Liver functions test on diabetic rats treated with *Pereskia bleo* aqueous extracts.

Sample	Liver Func. Test (U/L)		
	ALT	AST	ALP
Normal Control	54±8*	40±2*	39±5*
Diabetic Control	97±8	94±4	89±3
Glipizide 3mg/kg	59±3*	44±5*	37±2*
Leaf 250mg/kg	52±3*	47±3*	39±4*
Leaf 500mg/kg	59±6*	41±6*	34±6*
Stem 250mg/kg	62±5*	44±5*	36±5*
Stem 500mg/kg	54±9*	49±3*	36±3*
Root 250mg/kg	58±4*	46±6*	33±6*
Root 500mg/kg	60±6*	42±3*	32±3*

The sign (*) indicated values significantly different from diabetic control group at $p < 0.05$.

4.6.3.6 Effect of *Pereskia bleo* aqueous crude extracts on Lipid Profile Test.

Lipid profile test were used to measure any abnormalities in lipid such as in cholesterol and triglyceride. These abnormalities identify any risk of cardiovascular disease, inflammation of pancreas (pancreatitis) or other diseases. Table 4.27 showed the triglycerides (TRIG), total cholesterol (T.CHOL) and low-density lipoprotein (LDL) level in alloxan-induced diabetic SD rats treated with *Pereskia bleo* aqueous crude extracts significantly ($p<0.05$) decreased as compared to diabetic control group. While high-density lipoprotein (HDL) level showed significantly ($p<0.05$) increased as compared to diabetic control group.

Table 4.22 Lipid Profile Test on diabetic rats treated with *Pereskia bleo* aqueous crude extracts.

Sample	Lipid Profile Test (mmol/L)			
	TRIG	T.CHOL	HDL	LDL
Normal Control	0.7±0.05*	1.5±0.2*	1.3±0.1*	0.5±0.1*
Diabetic Control	1.9±1	2.3±0.1	0.5±0.1	1.1±0.1
Glipizide 3mg//kg	0.5±1*	1.5±0.2*	1.5±0.1*	0.3±0.1*
Leaf 250mg/kg	0.6±0.1*	1.7±0.3*	1.9±0.1*	0.6±0.1*
Leaf 500mg/kg	0.4±0.1*	1.6±0.1*	1.2±0.2*	0.5±0.2*
Stem 250mg/kg	0.6±0.1*	1.7±0.3*	1.9±0.1*	0.6±0.1*
Stem 500mg/kg	0.6±0.1*	1.6±0.1*	1.1±0.2*	0.5±0.1*
Root 250mg/kg	0.7±0.1*	1.8±0.1*	1.8±0.1*	0.7±0.1*
Root 500mg/kg	0.6±0.2*	1.5±0.1*	1.8±0.1*	0.5±0.1*

The sign (*) indicated values significantly different from diabetic control group at $p < 0.05$.

4.6.3.7 Effect of *Pereskia bleo* aqueous crude extracts on Renal Function Test

Renal functions test were used to detect any renal disease and define the clinical state of renal dysfunction. Table 4.23 showed the creatinine and urea levels in alloxan-induced diabetic rats increased significantly ($p<0.05$) as compared with normal control group. Whereas, the alloxan-induced diabetic groups treated with leaves, stems and roots of *Pereskia bleo* aqueous crude extracts and glipizide as a standard drug showed a significant ($p<0.05$) decreased in creatinine and urea levels as compared with the diabetic group.

Table 4.23 Creatinine and urea levels on diabetic rats treated with *Pereskia bleo* extracts and Glipizide as a standard drug**.**

Sample	Renal Functions Test	
	Urea (mmol/L)	Creatinine (umol/L)
Normal Control	3.7±0.8	37±2
Diabetic Control	9.5±3	99±7
Glipizide 3mg/kg	3.2±0.7	29±5.8
Leaf 250mg/kg	3.9±0.4	41±3
Leaf 500mg/kg	3.2±0.6	34±2
Stem 250mg/kg	4.1±1	42±3
Stem 500mg/kg	3.6±0.6	37±6
Root 250mg/kg	4.8±0.7	33±5
Root 500mg/kg	4.5±0.5	32±6

Samples of *Pereskia bleo* were prepared by dried it under room temperature. The dried samples were then grinded to become powder. An extraction is a processes that are widely used to obtain crude extract contain bioactive compounds of plants samples (Chirinos *et al.*, 2007). In this study, a serial of extensive extraction method was used involving the extractions with non-polar solvent (hexane) to more polar solvent (methanol) to ensure a wide polarity range of bioactive compounds could be extracted. For aqueous extraction, it was done by soaking in aqueous bath at room temperature for 3 hours. The extracts obtained recorded as in Table 4.1. During the extraction, temperature is one of the most important factors to control as different bioactive compound has different stability that easily affected by temperature. To avoid from loss of these active compounds due to thermal decomposition, the temperature was carefully controlled and maintain the optimum temperature which is 40^{o}C. Other factors that are important in order to get a quality extracted bioactive compounds were the type and concentration of solvent, size of plant samples, pH and extraction time (Liyana-Pathirana, 2005). The leaves, stems and roots of *Pereskia bleo* crude extracts were then evaporated to dryness under reduced pressure to give solid residues and further stored at -20^{o}C for subsequent experiment. These extracts were standardized prepared at 10mg/g to ensure the same amount of bioactive compounds presence during every test.

Separation and detection of chemical compounds were carried out using thin layer chromatography (TLC). TLC is one of the chromatography separation methods that are useful to separate various bioactive compound based on polarity or size. It is a common natural product analysis, stability test of samples and sample quality control (Cimpoiu, 2006). This method also was the simplest and inexpensive compared to other

chromatography method (Nyiredy, 2001).The different ratio of mobile phase are important to produced solvents at varied polarities, thus allowed the different polarities of compounds to flows up on the TLC plate surface by capillary action at different rate. The compounds that are more soluble to solvent will travel faster as compared to less soluble compounds. In this study, the separations of bioactive compound were initially done by using combinations of toluene and ethyl acetate (30:70), Toluene:ethyl acetate (7:93) and Methanol:Chloroform (1:9) as a mobile phase. All three mobile phases showed the same presence of chemical compounds. But only Toluene:ethyl acetate (30:70,) was showed the highest total of separated bands presences in each samples after tested. Thus this combination was selected as a mobile phase for further test. The separated bands of compounds were observed under visible light. The specific classes of each bands were determined by sprayed the TLC plates with two chromogenic spray reagents; vanillin-sulphuric acid and dragendorff reagents. Sometimes, bioactive compounds have a same colour and R_f values. Therefore, these reagents are very useful as alternative bioactive compound indicators. R_f (retardation factor) values are a measurement of flow rate of compounds. It is the relative distance of compound had travel along TLC plate surface relative to distance of mobile phase had travel. Visualization of TLC plate under UV light showed flavonoids compounds present in orange-yellow colour and phenolics compounds present in blue fluorescent colour (Males *et al.*, 2001). In dragendorff reagent, the presence of alkaloid compound was identified by the development of orange colour. While in vanillin-sulphuric acid reagent, the presence of terpenoid compound was identified by the development of purple colour and phenols compound by red or other colours (Mehri *et al.*, 2011). The TLC analysis has revealed that terpenoid was detected in all crude extracts of *Pereskia bleo* leaves, stems and roots. Leaves of *Pereskia bleo* extracts showed the

presence of alkaloid compounds in hexane, petroleum ether, chloroform and methanol extract. Phenolics compound was detected in hexane, chloroform and methanol extract. In addition, flavonoids was detected in petroleum ether, chloroform and methanol extract (Table 4.2, Table 4.3, Table 4.4 and Table 4.5). Stems of *Pereskia bleo* extracts showed the presence of alkaloid in hexane, petroleum ether and chloroform extract. Phenol was detected in hexane and chloroform extracts, while flavonoids were detected in hexane and petroleum ether extract (Table 4.6, Table 4.7, Table 4.8, and Table 4.9). Roots of *Pereskia bleo* extracts showed the presence of alkaloid in chloroform and methanol extracts. Phenol was detected in hexane, petroleum ether and methanol extracts, while flavonoids was detected in all crude extracts (Table 4.10, Table 4.11, Table 4.12 and Table 4.13).

Liquid Chromatography Mass Spectrometry combined with Mass Spectrometry (LCMS/MS) is frequently used in many drug developments such as in natural product, impurity identifications, *in vivo* drug screening and many more (Mike *et al.*, 1999). It is a useful technique that has highly sensitivity and selectivity in detection and identification of chemical compounds. Moreover, this technique helps in the determination of chemical compound structure based on its fragmentation. In this study, LCMS/MS analysis was conducted on an AB Sciex 3200QTrap mass spectrometer. The full scan was done to evaluate the unknown compounds targets. Thus, the collision energy used was in range of 35eV +/- 15eV. Initial investigation using LCMS/MS showed all leaves, stems and roots of *Pereskia bleo* aqueous extracts contained high amount of phenolics acid and flavonoids compounds. These results were in agreement with the results obtained from total phenolics and total flavonoids contents test. The LCMS/MS results showed that eight compounds were detected in *Pereskia bleo* aqueous extracts which were apigenin based compounds,

apigenin 7 rutinoside, Apigenin 6 C glucoside, chrysin, jasmonic acid or dehydrocymellein, dimenthyl ellagic acid, tryptophan and phenolics acid. Whereas, other 33 compounds were detected but classified as unknown compounds due to the unavailable reference data in databases. All the observed known and unknown compounds were determined based on journal reference and ACD/Labs advanced chenometrics mass fragmentations predictive software. The unknown compounds were classified as unknown due to the data were not available in database. Further confirmation can be done for compounds presence usig reference standard or complementary data from various analytical instumentations in future. Chromatogram and spectrum mass of *Pereskia bleo* aqueous extracts were showed in Figure 4.1 to Figure 4.11.

In previous research of phytochemical study reported by Doetsch (1980) using GCMS analysis, *Pereskia bleo* was claimed to contained alkaloids (3,4-dimenthoxy-β-phenethylamine, mescaline,3-methoxytyramine and tyramine). Sri Nurestri (2008 & 2009) reported the presence of phytol, phenolics (α-tocophrol and 2,4-di-tert-butylphenol), dihydroactinidiolide and sterols (campesterol, stigmasterol, and β-sitosterol) in *Pereskia bleo*. The previous study reported that β-sitosterol, vitamin E (Sri-Nurestri *et al.*, 2008), phytone (Uechi *et al.*, 2007), 2,4-ditert-butylphenol (Kamitori *et al.*, 1984), methyl palmitate, and methyl oleate (Sri Nurestri *et al.*, 2009) were found in *Pereskia grandifolia*. In this study, the TLC analysis showed presence of alkaloid, phenol, terpenoid and flavonoids while in LC-MS/MS analysis showed presence of fatty acid, flavonoids and phenolics compounds in *Pereskia bleo* extracts. The results showed not correlated with the previous research, therefore further confirmations of chemical compounds detected in *Pereskia bleo* extracts should be done in future. A bioassay guided fractionation may be

useful to isolate the bioactive compounds from the extract. It will be more definitive if identification of isolated compounds analysed by LC-MS/MS combined with Nuclear Magnetic Resonance Spectroscopy.

Oxidative stress can cause a various chronic disease such as diabetes, cardiovascular, cancer and other diseases (Simonian *et al.*, 1996). In diabetes, the pathogenesis of diabetic complication is associated with a higher oxidative stress (Baynes, 1991). Oxidative stress through the production of reactive oxygen species (ROS) leads to the damage to the pancreatic β cell, development of insulin resistance, impaired glucose tolerance and type 2 diabetes mellitus (Ceriello *et al.*, 2004; Wright-JR *et al.*, 2006). Antioxidant compounds such as polyphenol and flavones have ability to prevent or reduce the mechanism of molecules oxidation by removing free radicals and blocking others by oxidized themselves (Behnaz *et al.*, 2012). The previous studied also showed that the flavonoids and terpenes compounds isolated from medicinal plants found to stimulate secretion or possess an insulin like-effect (Marles *et al.*, 1995). Flavonoids are known to regenerate the damaged β cells in alloxan induced diabetic rats and acts as insulin secretagogues (Alagammal *et al.*, 2012). Besides, the flavonoids and phenolics compounds have been reported acts as scavengers of singlet oxygen and free radicals (Abu *et al.*, 2010; Aparna *et al.*, 2013). Thus, these compounds are important to protect the body damage caused by oxidative stress due to free radical release. In this study, the total phenolics content and flavonoids contents in *Pereskia bleo* extracts was revealed quantitatively. The phenolics contents were expressed as mg gallic acid equivalent (GAE) per gram of dry sample. The highest of phenolics contents in leaves *Pereskia bleo* extracts was found in methanol extract, 3.0$\pm$0.2 mg GAE/g. Stems extracts showed the highest phenolics

contents was from aqueous extract by 6.06± 0.1 mg GAE/g. Whereas in root extracts, the highest phenolics contents showed in chloroform extract by 3.64±4 mg GAE/g (Figure 4.13). The flavonoids contents were expressed as mg quarcetin equivalent (QUE) per gram of dry sample. Results showed that the highest flavonoids contents in leaves, stem and roots of *Pereskia bleo* extracts were from chloroform extract which were 5.2±0.7 mg QE/g, 11.07±0.2 mg QE/g, and 3.4±0.1 mg QE/g respectively (Figure 4.15). The finding are in agreement with the previous studies which *Pereskia bleo* extracts was also reported to have phenolics and flavonoids compound such as catechin, quarcetin, epicatechin, myricetin (Behnaz *et al.*, 2012), α-tocophrol and 2,4-di-tert-butylphenol (Sri Nurestri *et al.*, 2009).This plant also reported to contained high phenolics contents that contributed to antioxidant (Sim *et al.*, 2010).

Brine shrimp lethality bioassay method was performed according to Meyer method (Sirintorn *et al.*, 2004) and was carried out to investigated the toxicity of plant extracts. The method has been applied to plant extracts in order to facilitate the isolation of biologically active compound. The LC_{50} values of different *Pereskia bleo* aqueous extracts with 90% confident intervals are showed in Table 4.17. Low LC_{50} values indicated that the extracts are biologically active. The stems aqueous extract showed LC_{50} value was 3.55 µg/ml. Whereas both leaves and roots of *Pereskia bleo* aqueous extracts showed the LC_{50} values was 2.51 µg/ml. No mortality occurred in the control group. However, there is no comparison was made between the plant samples and other compounds. Further confirmations of toxicity level of *Pereskia bleo* extracts using other compound such as glipizide as a standard drug should be done in future.

Until now, there is no other scientific research was done on *Pereskia bleo* extracts as anti-hyperglyceamic agents. In this study, the anti-hyperglycaemic activity of *Pereskia bleo* extracts from leaves, stems and roots were evaluated through glycogen phosphorylase inhibition activity in Sprague dawley (SD) rats according to the method described by Essam, (2012) and Baginsky, (1974) with slightly modifications. The inhibition of this enzyme has been proposed in treating diabetes mellitus (Somsak *et al.*, 2003). This enzyme was important in glycogenolysis pathway by breakdown of glycogen by phosphorylase to glucose-1-phosphate and glucose (Essam *et al.*, 2012). Glucose-1-phosphate was then degraded by glycogen phosphorylase released phosphate and the absorbance of this substrate was measured at 655nm. The results showed the absorbance of solutions were increased proportional to the activity of this enzyme. In this study, result showed the leaves, stems and roots of *Pereskia bleo* extracts have inhibition of glycogen phosphorylase (GP) activity. The anti-hyperglycaemic activities of *Pereskia bleo* extracts were compared with glipizide as a standard reference drug. The percentage of GP inhibition in glipizide was 84.4%.At the concentration of 10mg/ml, the highest of GP inhibitions in leaves of *Pereskia bleo* extracts was from methanol extract with 82%, followed by aqueous extract 80%, chloroform extract 77%, hexane extract 38% and petroleum ether extract 14%. The highest of GP inhibitions in stems of *Pereskia bleo* extracts were from methanol extract with 78%, followed by aqueous extract 59%, chloroform 56%, hexane 16% and petroleum ether extract 16% respectively. Whereas, in root of *Pereskia bleo* extracts, the highest of GP inhibitions was also indicated from methanol extract which was 90%, followed by aqueous extract 79%, chloroform extract 78%, hexane extract 21% and petroleum ether extract 16% respectively.

The half maximal inhibitory concentration (IC_{50}) was evaluated in order to compare the effectiveness of plants extracts at a half of inhibitory activity. *Pereskia bleo* crude extracts were prepared at five different concentrations which were 20 mg/ml, 40mg/ml, 80mg/ml and 100mg/ml. Table 4.18 showed the IC_{50} of glipizide and *Pereskia bleo* crude extracts. The IC_{50} of Glipizide was 1.1µg/ml. While the IC_{50} of hexane and petroleum ether in leaves, stems and roots of *Pereskia bleo* crude extracts could not be determined due to the percentage of GP inhibition were below 50% even at dose of 100 mg/ml. In leaves of *Pereskia bleo* extracts, the highest of GP inhibition was from chloroform extract with IC_{50} 1.4 µg/ml, followed by methanol crude extract 1.6 µg/ml and aqueous crude extract 2µg/ml (Figure 4.18). In stem of *Pereskia bleo* crude extracts, the highest of GP inhibition was from methanol extract with IC_{50} 1.6 µg/ml, followed by aqueous crude extract 2.3 µg/ml and chloroforms crude extract3.2 µg/ml (Figure 4.19). While in roots of *Pereskia bleo* crude extracts, the highest of GP inhibition was from methanol extract with IC_{50} 1.2µg/ml, followed by aqueous crude extract 1.9µg/ml and chloroforms 2.9µg/ml (Figure 4.20).

The fractions of *Pereskia bleo* crude extracts were collected using column chromatography technique. Column chromatography was another useful technique in separation of bioactive compound. The principle of this method is the same as TLC technique, but the different is this technique enables separation in large quantities. In column chromatography, the stationary phase (silica gel) was always filled with mobile phase to avoid from dryness. Air bubbles trapped in dried stationary phase will cracked the phase and impairs the separation. The eluents were observed slowly travel through the column at different speeds, therefore an individual compounds were kept separately. The

combinations of toluene and ethyl acetate at ratio 30:70 (v/v) as a mobile phase was selected due to the higher amount of chemical compounds detected during TLC techniques. The results showed that the highest percentage of GP inhibitions and highest amount of chemical compounds in leaves, stems, and roots of *Pereskia bleo* crude extracts were from chloroform, methanol and aqueous extracts. The leaves of *Pereskia bleo* crude extracts showed the percentage of GP inhibition in these three extracts were 68.9%, 69.5% and 67% respectively (Figure 4.23 to Figure 4.25). In stems of *Pereskia bleo* crude extracts, the highest percentages of GP inhibitions were 50%, 51% and 56% respectively (Figure 4.28 to Figure 4.30). Whereas, in roots of *Pereskia bleo* crude extracts, the highest percentages of GP inhibitions were 55%, 67% and 49% respectively (Figure 4.33 to Figure4.35). Overall results of glycogen phosphorylase inhibitory bioassay in crude extracts and fractions collected from column chromatography indicated that chloroform, methanol and aqueous crude extracts were the best solvents to extract the anti-hyperglycaemic agents in *Pereskia bleo*. In previous TLC results revealed that *Pereskia bleo* crude extracts contains flavonoids, terpenoid and phenol compounds. Moreover, in total phenolics and flavonoids contents showed the chloroform, methanol and aqueous crude extracts contains higher amount of phenolics and flavonoids compounds. Previous study also showed that phenol, terpenoid and flavonoids played important roles in the treatment of diabetes (Chude *et al.*, 2001; Hassig *et al.*, 1999; Hsu *et al.*, 2000; Momoh *et al.*, 2011). Thus, probably the insulin-like activities of these compounds contained in *Pereskia bleo* extracts may responsible for these GP inhibitions activities.

The acute toxicity test is the first step on toxicological analysis of medicinal plants (Deciga-Campos *et al.*, 2007). This test was observed in non-diabetic rats administrated

with *Pereskia bleo* aqueous extracts from leaves, stems and roots. Non-toxicity test was experimental at doses 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight during 14 days study period. Table 4.19 showed the results of the acute toxicity of *Pereskia bleo* crude extracts. No toxic sign such as death, distress, fearfulness, urination or defecation, pain response and spontaneous activities were observed. These result revealed that *Pereskia bleo* crude extracts did not cause any acute toxicity and the LC_{50} values of *Pereskia bleo* crude extracts was more 1000mg/kg as no death were found during experiment. Pervious study also reported that *Pereskia bleo* crude extracts were assigned to class D (LC_{50} > 2000mg/kg), which is in a lowest toxicity class (Sim *et al.*, 2010).

A glucose tolerance test (OGTT) is a medicinal test to observe the fastest glucose taken to be cleared from blood. The change in blood glucose level in response to glucose load has been used in clinical for diabetes mellitus diagnosis and in research to evaluate the effectiveness of hypoglycaemic agents (Chutwadee *et al.*, 2009). The administration of *Pereskia bleo* crude extracts by oral gavages 30 minutes after administrations of glucose to normal rats in OGTT showed improved glucose tolerance. For the first 60 minutes, the blood glucose level of all groups showed a significantly ($p<0.05$) increased higher than at 0 minutes, but significantly ($p<0.05$) decreased from 60 to 120 minutes. The magnitude of effect also showed varied with the dose of administration. As showed in figure 4.36, at dose of 500mg/kg body weight of leaves, stems, and roots of *Pereskia bleo* crude extracts significantly produced a maximum fall of glucose level measured during 120 minutes compared to control group. The roots *Pereskia bleo* crude extracts (500mg/kg body weight) produced a highest significantly maximum fall of 21.5% at 120 minutes after glucose administration followed by roots aqueous crude extract (250mg/kg) 20%, stems

aqueous crude extract (500mg/kg) 14% and leaves aqueous crude extract (500mg/kg) 11%. While for stems aqueous crude extract (250mg/kg) and leaves aqueous crude extract (250mg/kg) showed a decreased in blood glucose levels with 9% and 3%. The glipizide as a standard drug showed a decreased in percentage of blood glucose level by 18%. These results showed that roots of *Pereskia bleo* aqueous extracts at both doses (250 mg/kg and 500 mg/kg) were more effective in reducing the blood glucose level as compared to glipizide as a reference drug. Thus, these results revealed that *Pereskia bleo* aqueous extracts might be effective hypoglycaemic agents as glipizide.

Hyperglyceamia is one of the important characteristics of diabetes mellitus. Alloxan is a toxic glucose analogue. It is a diabetogenic agent and widely used to induce diabetes in animal (Viana *et al.,* 2004). Alloxan used to induce diabetes in rats, leading to the pancreatic β cells damage without affecting other islet types (Goldener *et al.,* 1964; Solomon *et al*, 2012) resulting in decreased endogenous insulin release thus induces hyperglycaemia. The compounds are uptake selectively due to similarity of glucose structure. Diabetes then arises from the generation of cytotoxic oxygen free radicals which are targeting the DNA of pancreatic cell causing DNA fragmentation (Shankar *et al.*, 2007). In this study, the administration of *Pereskia bleo* aqueous extracts to alloxan-induced diabetic rat showed that *Pereskia bleo* aqueous extracts contained bioactive compounds that reduced the blood sugar level. There was a significantly elevation in blood glucose level in diabetic control group as compared to normal control group (Table 4.20). The *Pereskia bleo* aqueous extracts-treated group showed reduction of blood glucose level significantly as compared to diabetic control group starting at day 15 treatment. On day 25th, the treatment with glipizide as a standard drug showed decreased of blood glucose

level significantly by 72%.The leaves of *Pereskia bleo* at dose 250mg/kg and 500mg/kg showed the significantly rapid decreased in blood glucose level by 59% and 66%. Whereas the stem of *Pereskia bleo* at both doses showed a significantly decreased in blood glucose levels by 54% and 65%. While the roots of *Pereskia bleo* at both doses showed a significantly decreased in blood glucose levels by 53% and 58%. These results revealed that P*ereskia bleo* crude extracts have mechanism hyperglycaemia effects by either triggered the insulin secretion or by repairing the damage of pancreatic β cell.

Tissue proteins are known to contribute to the body weight (Rajkumar *et al.*, 1991). Decreases in body weight have been associated with diabetes mellitus due to excessive breakdown of tissue protein and could be due to catabolism of fats and protein (Hakim *et al.*, 1997; Kamalakkannan *et al.*, 2006). The breakdown of adipocytes and muscle tissues in alloxan-induce rat are due to the frequent urination and over conversion of glycogen to glucose (Lin *et al.*, 2006). In this study, the decreased in body weight were observed during the treatment of diabetic rats with *Pereskia bleo* crude extracts and glipizide as a standard drug. Results showed the alloxan-induced diabetic rats groups treated with leaves, stems and roots of *Pereskia bleo* aqueous extracts increased significantly in body weights as compared to diabetic control group during 25days (Figure 4.37). The percentage of body weight of normal group was increased significantly 59%. While in diabetic group treated with glipizide showed the body weight increased by 51% significantly. The diabetic rats treated with leaves of *Pereskia bleo* aqueous extract at dose 500mg/kg showed the highest increased in body weight by 35% significantly followed by stems and roots of *Pereskia bleo* at dose 250mgkg by 33%, leaves (250mg/kg) by 30%, stems (500mg/kg) by 27% and roots (500mg/kg) by 22% significantly increased in body weight. These results revealed

that *Pereskia bleo* extracts may have a positive anabolic effect by reducing the degeneration of adipocytes and muscle tissue through enhancing in glucose metabolism.

In this study, the change in concentration of hepatic enzymes such as alanine aminotransaminase (AST), serum aspartate aminotransaminase (AST), and alkaline phosphatase (ALP) was measured in normal and alloxan-induced diabetic rats during 25 days treatment. The hepatic enzymes worked as liver maker which are associated with various disorders that have an effect on liver tissue function and were studied to evaluate the hepatic function. The increase in activities of ALP during diabetes is indicator of tissue damage due to toxicant (Ravikumar *et al.,* 2010; Uboh *et al.,* 2010). The analysis of the enzymes activities in blood serum was used to observed the condition of liver tissue and any damage occur after being exposed to certain pharmacological agent such as alloxan (Eze *et al.,* 2012). In hepatocyte injury mechanism, ALP located in cytoplasm is release in the circulation after cellular damage due to alloxan administration. As a result, the injury of organelles such as mitochondria will triggered the released of soluble enzyme, ALT and AST (Muhammad *et al.,* 2008; Sallie *et al.,* 1991; Senthil *et al.,* 2003). In the present study, the best way to present the anti-hyperglycaemic effects of leaves, stems and roots *Pereskia bleo* aqueous extracts were by compared with diabetic control group. In the same time, the results obtained were also compared with standard range of SIEMENS Dimension Vista® System at Laboratory Medicine of University Malaya Medical Centre. Normal range for each tests in liver functions test were as below.

i. alanine aminotransaminase (Organization.) 30 – 65 IU/L
ii. serum aspartate aminotransaminase (AST) 15 – 47 IU/L
iii. alkaline phosphatase (ALP) < 136 IU/L

Table 4.21 showed the significant increased in these liver enzymes were observed in diabetic control rats as compared to normal control rats. The treatment of diabetic rats with leaves, stems and roots of *Pereskia bleo* extracts and glipizide as a reference drug showed a significantly decreased in the liver enzymes as compared to diabetic control. Treatment with *Pereskia bleo* extracts for leaves, stems and roots extracts showed the decreased in ALT and AST within the normal range except for stems extract at dose of 500 mg/kg showed higher than normal range in AST level. Whereas for ALP level, the result showed all groups were within normal range. In addition, there were no significant differences in ALT, ALP, and AST of the diabetic rats treated with *Pereskia bleo* groups as compared to groups of normal control and diabetic rats treated with glipizide. Therefore, these results revealed that the activities of these enzymes in diabetic rats treated with *Pereskia bleo* groups may equivalent with the activities of liver enzymes in the glipizide and normal control rats groups.

The measuring of creatinine and urea in blood serum is commonly used as renal dysfunction marker. The increase in creatinine and urea levels in blood serum indicates a kidney injury (Almdal *et al*., 1988). During renal diseases, glomerular filtration became weaken and lead to urea retention in blood. The creatinine is a waste product formed in muscle. It is transported through the bloodstream to the kidneys. The kidneys filter out and dispose the creatinine in urine. Thus, the abnormally high levels of creatinine in blood is the evidence of kidney injury (Alagammal *et al*., 2012). In the present study, the anti-hyperglycaemic effects of leaves, stems and roots of *Pereskia bleo* aqueous extracts were compared to diabetic control group. In the same time, the results obtained were also

compared with standard range of SIEMENS Dimension Vista® System at Laboratory Medicine of University Malaya Medical Centre. Normal range for renal function test as below:

i. Urea 2.5 – 6.5 mmol/L

ii. Creatinine 71 -106 µmol/L

Figure 4.23 showed the levels of serum urea and creatinine in diabetic rats was significantly increased as compared to normal control group. The alloxan-induced diabetic groups treated with leaves, stems and roots of *Pereskia bleo* aqueous extracts showed significantly ($p<0.05$) decreased in urea and creatinine level as compared to the diabetic control. The treatment with *Pereskia bleo* extracts and glipizide showed the decreased in urea level within the normal range. Whereas in creatinine levels, the diabetic rats treated with *Pereskia bleo* extracts groups and glipizide showed lower than normal range. The low level of creatinine in serum indicates the problems with renal function. In recent study, a lower in serum creatinine levels may associated with the increased risk of development of diabetes type 2 (Harita *et al.*, 2009). These results revealed that the treatment with *Pereskia bleo* aqueous extracts was effective in preventing the increasing in serum urea level in alloxan-induced diabetic rats. However, the low levels in serum creatinine revealed that the *Pereskia bleo* aqueous extracts may leads to renal dysfunction. Since the creatinine levels are vary according to muscle mass and body's size, a further test is needed to evaluate the renal function such as a glomerular filtration rate (GFR) and creatinine clearance rate (CrCl). GFR describes as the flow rate of filtered fluid through the kidney. Whereas the CrCl is referred as the rate of creatinine cleared in the body by the kidney.

Insulin is a potent inhibitor of lipolysis. When the level of insulin decreased in alloxan-induced diabetic rats due to β cell damage, the mobilization of free fatty acids from the peripheral fat deposit through the activity of hormone sensitive lipase was increase (Mbaka *et al*., 2012). Diabetes associated with alteration in plasma lipid, lipoprotein profile and increased the risk of coronary heart disease (Huang *et al*., 1988; Ime *et al.*, 2011). The level of lipid profile such as total cholesterol (TC), triglycerides (TG), LDL-cholesterol and HDL-cholesterol level are always use as indicator to observe any risk in cardiovascular disorder such as coronary heart disease. In this study, the anti-hyperglycaemic effects of alloxan-induced diabetic rats treated with *Pereskia bleo* aqueous extracts were compared with diabetic control group. The results obtained also were compared with standard range of SIEMENS Dimension Vista® System at Laboratory Medicine of University Malaya Medical Centre. The normal range of lipid profile as below:

i. Triglyceride < 0 mmol/L
ii. Total Cholesterol < 5.2 mmol/L
iii. LDL < 2.59 mmol/L
iv. HDL > 1.1 mmol/L

Table 4.22 showed the levels of plasma cholesterol, triglycerides and LDL-cholesterol in alloxan-induced diabetic rats treated with all *Pereskia bleo* extracts and glipizide as reference drug were significantly ($p<0.05$) decreased as compared to diabetic control. Whereas the HDL-cholesterol level in plasma showed significantly ($p<0.05$) increased as compared to diabetic control. All the results for diabetic groups treated with

Pereskia bleo extracts and glipizide showed within the normal range in lipid profile test. Thus, these results revealed that *Pereskia bleo* extracts may prevents the risk of cardiovascular disease developments as it is associated with diabetes mellitus.

Therefore, finding of the study revealed that alloxan-induced diabetic rats administrated with *Pereskia bleo* leaves, stems and roots aqueous extracts showed the significant results in blood glucose levels, body weights, lipid profiles, and renal functions tests. In this context, *Pereskia bleo* extracts was proven to contained anti-hyperglyceamic agents and has the potential to become as one of the medicinal plants in treatment for diabetic mellitus.

Samples of leaves, stems, and roots of *Pereskia bleo* was extracted with aqueous, hexane, petroleum ether, chloroform and methanol solvents. Preliminary phytochemical investigation using TLC methods has revealed the presence of flavonoids, phenols, alkaloid and terpenoid compounds in *Pereskia bleo* extracts. Further identifications of this chemical compounds using LCMS/MS method has confirmed the presence of phenolics acid and flavonoids compounds. Eight expected chemical compounds were detected in leaves and stem of *Pereskia bleo* aqueous extracts which were Apigenin glucoside, Apigenin rutinoside, Apigen based compounds, Chrysin, Jasmonic acid or dihydrocymellein, tryptophan, dimethyl ellagic acid and phenolics acid. Whereas 33 of unknown chemical compounds were detected in roots *Pereskia bleo* aqueous extracts. Further confirmation of these compounds can be done using reference standard or different analytical instruments.

The antidiabetic activity of *Pereskia bleo* were determined using two methods which were via *in vitro* by glycogen phosphorylase (GP) inhibitory bioassay and *in vivo* by administration of *Pereskia bleo* aqueous extracts to alloxan-induced diabetic rats. The bioassays showed the inhibitions of GP activity in both crude extracts and isolated compounds of *Pereskia bleo* aqueous extracts. The most potent inhibition of GP activity was determined in both chloroform extract of stems and roots with LC_{50} 3.2 µg/ml and 2.9 µg/ml, respectively. Followed by both aqueous extracts of stems and leaves with LC_{50} 2.3 µg/ml and 2.0 µg/ml, respectively.

Acute toxicity test of *Pereskia bleo* aqueous extracts has proven that these plants contain non-toxic substances due to the absences of toxic sign such as death, fearfulness,

urination, pain response and distresses. These results were in agreement with the previous study reported that this plant was classified in class D, which is in lowest toxicity class.

The administrations of *Pereskia bleo* aqueous extracts during anti-diabetic study showed that in glucose tolerance test (OGTT), the non-diabetic rats administrated with these plants showed a positive response in glucose blood level. The highest percentage of reduction in blood glucose level was detected in *Pereskia bleo* roots extracts at high doses (500mg/kg) which was 22% after 120 minutes glucose administrated. The percentage was higher than the percentage of reductions of glipizide as a reference drug which was 18%.

The treatment of alloxan-induced diabetic rats with leaves of *Pereskia bleo* aqueous extract at doses of 500 mg/kg showed the most potent in decreasing the blood glucose level, liver functions (ALT, AST, ALP), renal function levels (urea, creatinine), lipid profile test (Triglyceride, total cholesterol, LDL) and increased in HDL and body weight. The blood glucose level during 25 days of treatment with extract of *Pereskia bleo* was significantly decreased by 66%. In body weight, alloxan-induced diabetic rats treated with extract of *Pereskia bleo* showed significantly reductions by 35%. In liver function test, the significantly decreased in ALT, AST and ALP of alloxan-induced diabetic rats after treated with leaves *Pereskia bleo* extracts at dose 500 mg/kg, were 46%, 50% and 56%, respectively. In renal functions test; urea and creatinine showed significantly decreased at 59% and 66%, respectively. While in lipid profile, triglyceride level, total cholesterols and LDL were significantly decreased with 68%, 73% and 79%, respectively. Whereas, results showed the significantly increased in HDL level that was73% as compared to diabetic groups.

These finding suggests that the crude extracts of *Pereskia bleo* contains glycogen phosphorylase inhibitor as anti-diabetic agent. It is justified that the *Pereskia bleo* aqueous extracts had anti-glycaemia effect by reducing the blood glucose level which may trigger the insulin secretion. In addition, the results obtained from this study demonstrated the ability of *Pereskia bleo* extracts in preventing the loss of body weight, the damage of liver and kidney, and the risk of cardiovascular disease. Further research are needed to be carried out in order to have better understanding on the mechanism actions of the bioactive compounds in reducing the blood sugar levels in treatment of diabetes.

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Publication

Paper title "Antidiabetic activity of *Pereskia bleo* aqueous extracts in alloxan induced diabetic rats" has been submitted to Sains Malaysiana.

Seminar

Isolation And Characterization of Glycogen Phosphorylase Inhibitor As Anti-Diabetic Agent In *Pereskia bleo.*

Poster presented at Malaysia Natural Products International Seminar Silver Jubilee organized by University Malaysia Pahang on 23-24 November 2009.

Malaysian Natural Products International Seminar Silver Jubilee
"Natural Product R&D: *Leads from Nature*"

70. Isolation And Characterization Of Glycogen Phosphorylase Inhibitor As Anti-Diabetic Agent In *Pereskia Bleo*

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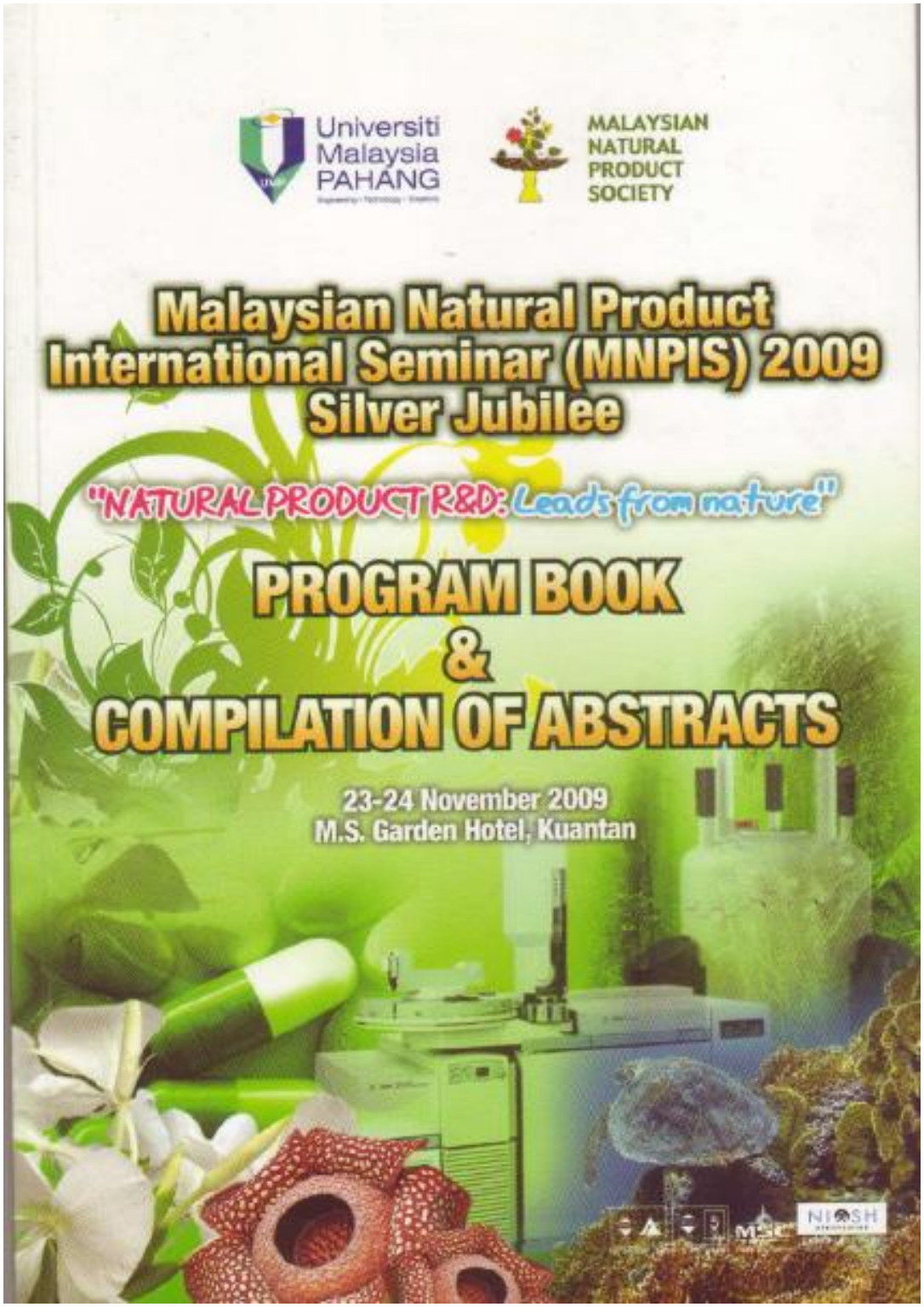
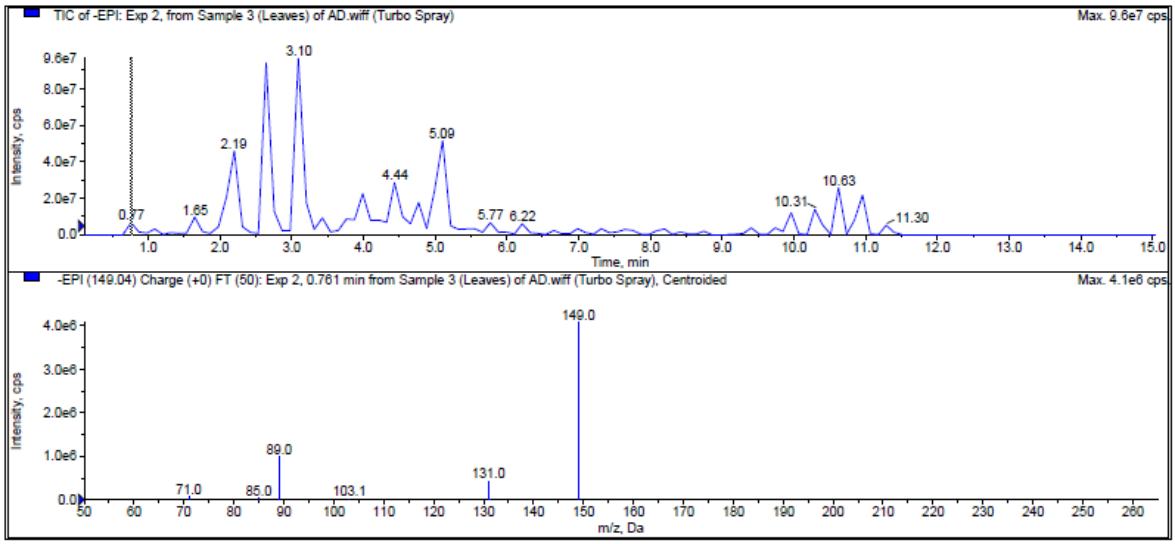
Abstract

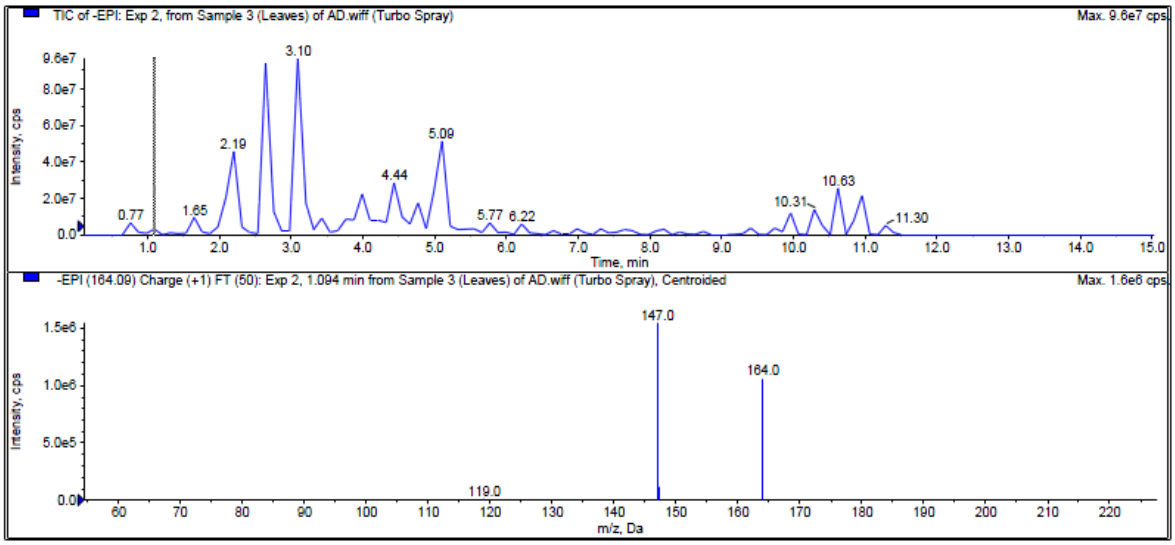
Pereskia bleo belongs to the botanical family Cactaceae and normally found in rainforest. It is commonly known in varies name such as 'jarum tujuh bilah' by locals Malaysian. This plant is believed to have anti-tumour, anti-rheumatic, anti-ulcer and anti-inflammatory activities. But for natives, this plant has been used traditionally in treatment of diabetes patients. However, there is no scientific research have been done about it. Since glycogen phosphorylase is involved as a key enzyme in regulation of blood glucose level, it is has been used to screen the bioactive compounds present in the medicinal plants for anti-diabetic agents. In this study 2 parts of *Pereskia bleo*; stem, and rhizomes were selected. A $10mgml^{-1}$ of standard concentration of crude extract was selected. The common extraction methods involved with hexane, petroleum ether, methanol, and chloroform solvent using soxhlet apparatus. The compounds were then separated by Thin Layer Chromatography (TLC) and detected with respective chemical reagents. In addition, screening compound was done in detecting of flavonoid, phenol, alkaloid, saponin and terpenoid qualitatively and quantitatively. The combinations activities of these compounds present in the plant acts as glycogen phosphorylase inhibitor in lowering blood glucose level. The anti-diabetic effects of petroleum ether and methanol from the rhizomes shows 90.61% and 91.33% respectively. While the hexane and chloroform of rhizome crude extract gives 22.21% and 10.84% inhibition. The hexane and petroleum ether of stem crude extract gives 16.47% and 16.04% inhibition. These results suggest that crude extract contains anti-diabetic active compounds for diabetes treatments. Further research is still in progress to evaluate for its anti-diabetic action by oral administration on rats model.

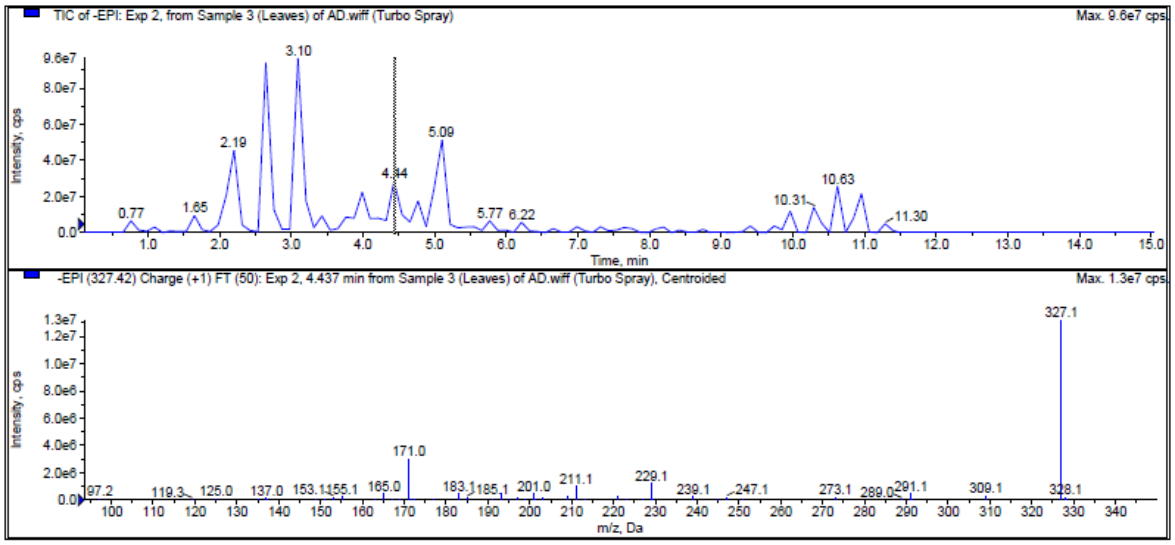

Universiti Malaysia PAHANG
MALAYSIAN NATURAL PRODUCT SOCIETY
Malaysian Natural Product International Seminar (MNPIS) 2009
Silver Jubilee
"NATURAL PRODUCT R&D: Leads from nature"
PROGRAM BOOK
&
COMPILATION OF ABSTRACTS
23-24 November 2009
M.S. Garden Hotel, Kuantan

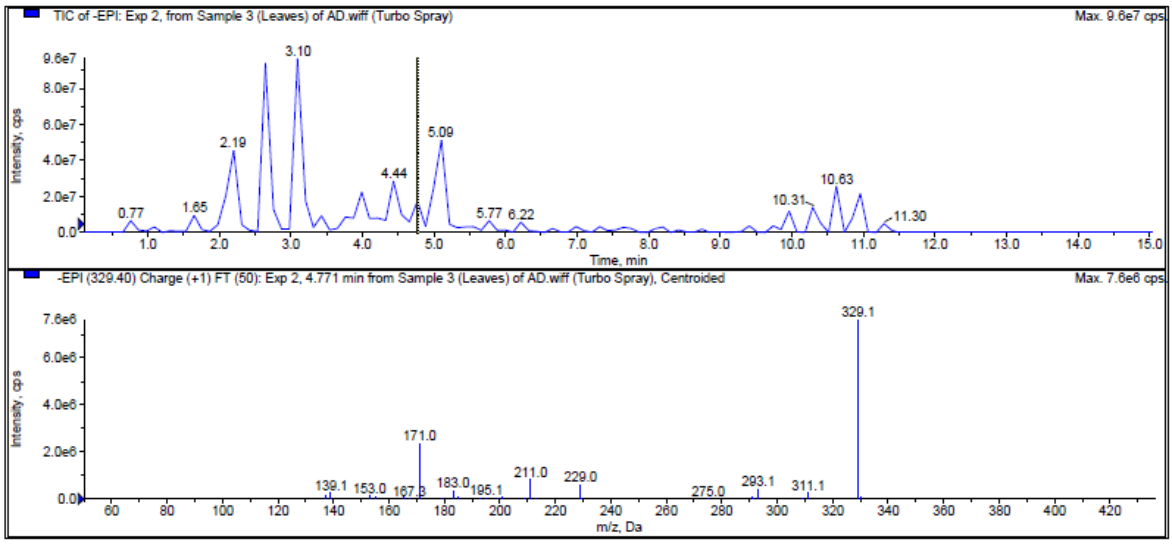
LCMS/MS Chromatogram

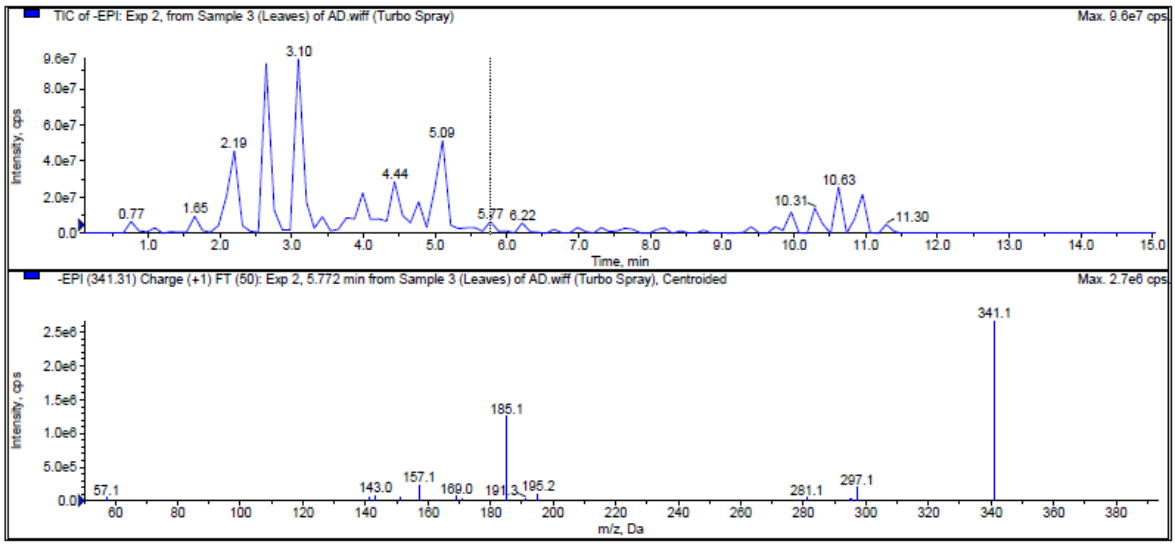
i. LCMS/MS chromatogram for unknown chemical compounds of *Pereskia bleo* water extracts on leaves aqueous extract.

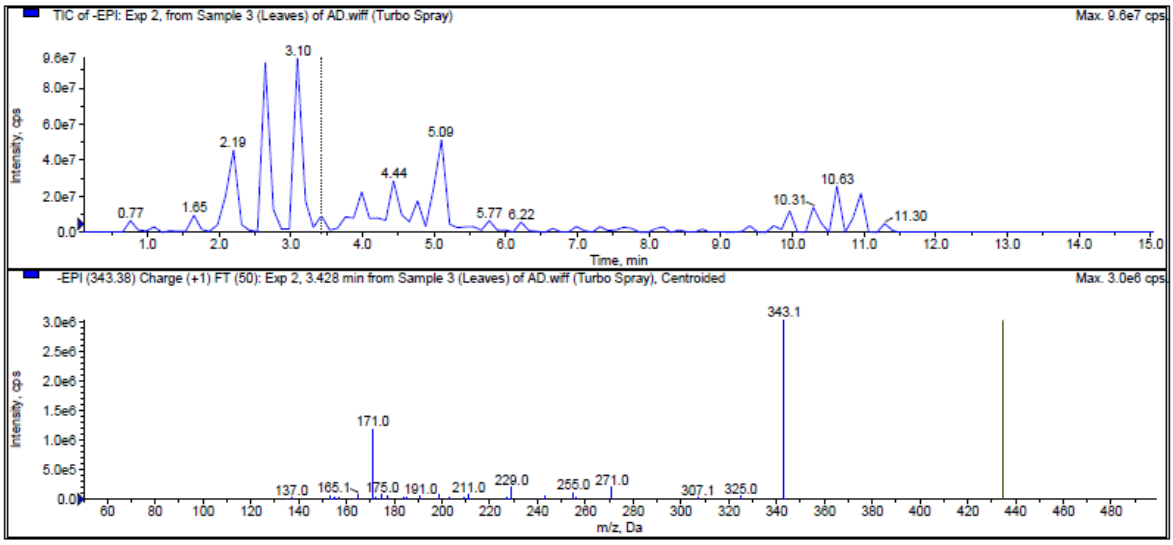


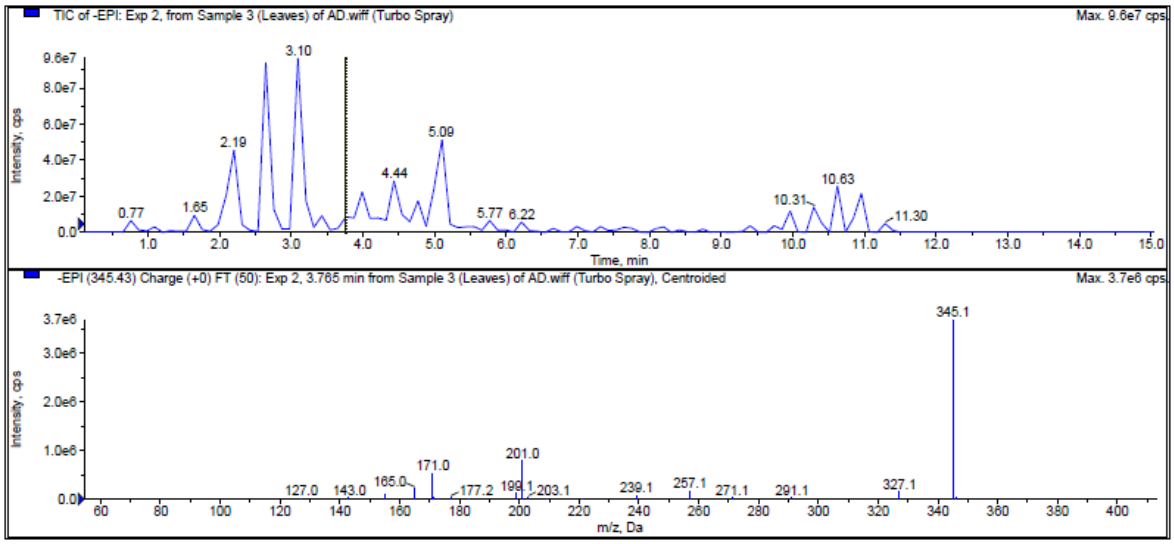


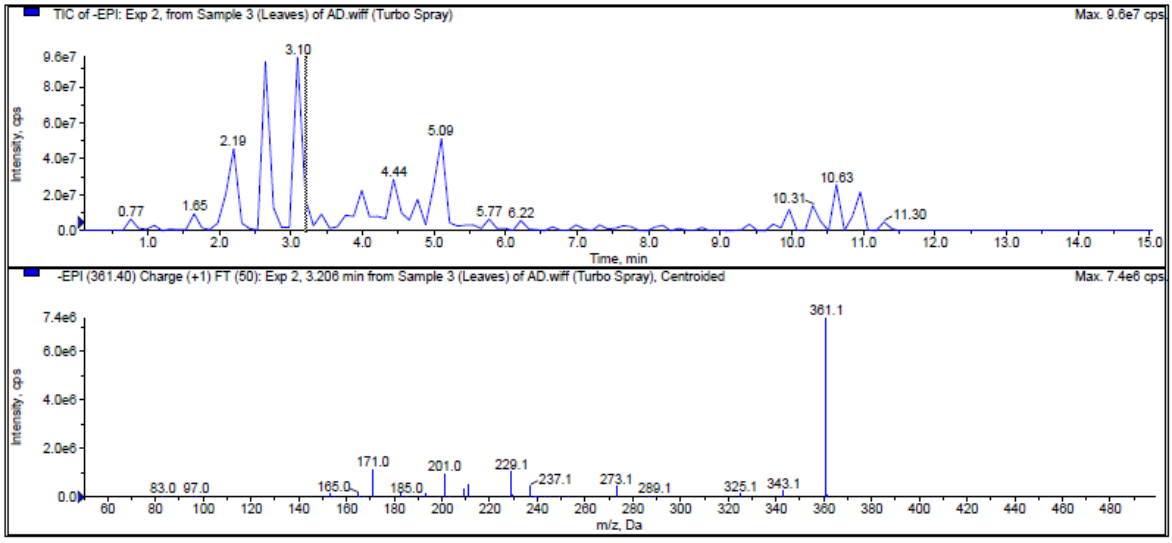

TIC of -EPI: Exp 2, from Sample 3 (Leaves) of AD.wiff (Turbo Spray)
Max. 9.6e7 cps.
Intensity, cps
Time, min
-EPI (327.42) Charge (+1) FT (50): Exp 2, 4.437 min from Sample 3 (Leaves) of AD.wiff (Turbo Spray), Centroided
Max. 1.3e7 cps.
m/z, Da


TIC of -EPI: Exp 2, from Sample 3 (Leaves) of AD.wiff (Turbo Spray)
Max. 9.6e7 cps.
Intensity, cps
Time, min
-EPI (329.40) Charge (+1) FT (50): Exp 2, 4.771 min from Sample 3 (Leaves) of AD.wiff (Turbo Spray), Centroided
Max. 7.6e6 cps.
m/z, Da


TIC of -EPI: Exp 2, from Sample 3 (Leaves) of AD.wiff (Turbo Spray)
Max. 9.6e7 cps.
Intensity, cps
Time, min
-EPI (341.31) Charge (+1) FT (50): Exp 2, 5.772 min from Sample 3 (Leaves) of AD.wiff (Turbo Spray), Centroided
Max. 2.7e6 cps.
m/z, Da


TIC of -EPI: Exp 2, from Sample 3 (Leaves) of AD.wiff (Turbo Spray)
Max. 9.6e7 cps.
Intensity, cps
Time, min
-EPI (343.38) Charge (+1) FT (50): Exp 2, 3.428 min from Sample 3 (Leaves) of AD.wiff (Turbo Spray), Centroided
Max. 3.0e6 cps.
343.1
171.0
m/z, Da


TIC of -EPI: Exp 2, from Sample 3 (Leaves) of AD.wiff (Turbo Spray)
Max. 9.6e7 cps.
Intensity, cps
Time, min
-EPI (345.43) Charge (+0) FT (50): Exp 2, 3.765 min from Sample 3 (Leaves) of AD.wiff (Turbo Spray), Centroided
Max. 3.7e6 cps.
345.1
171.0
201.0
m/z, Da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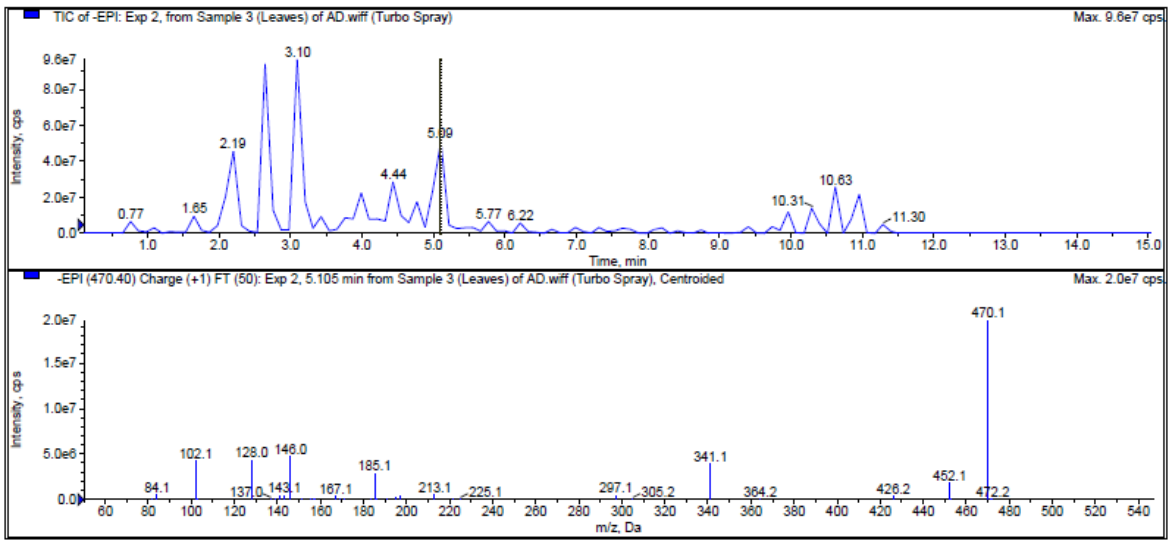
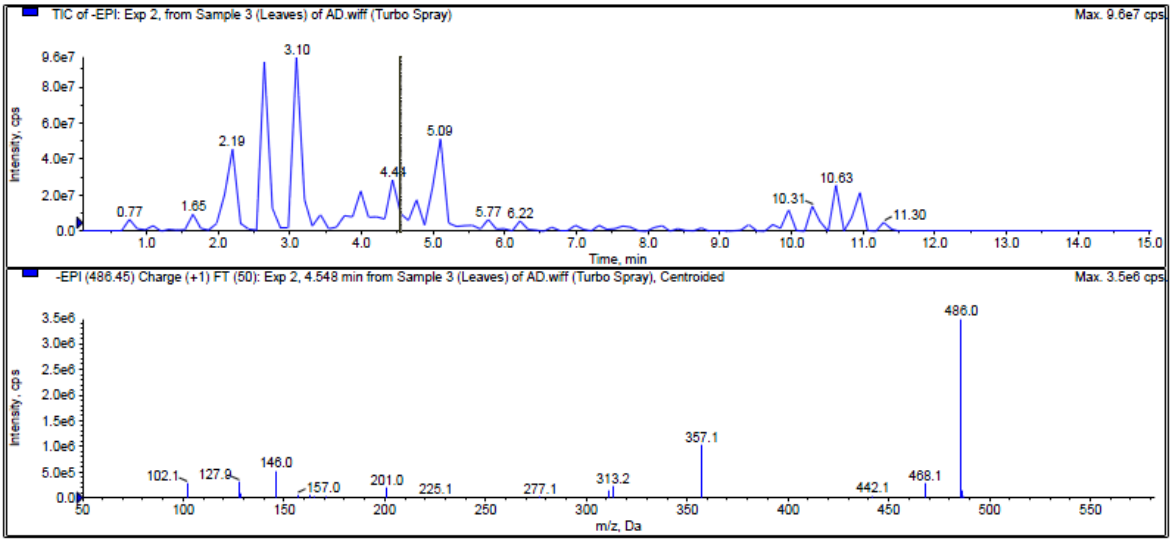
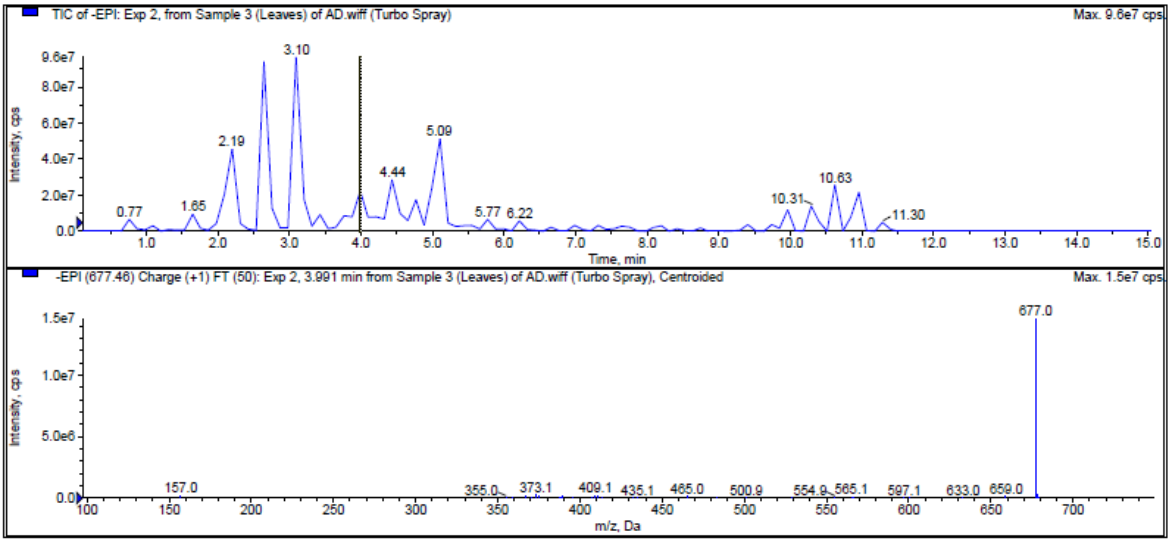
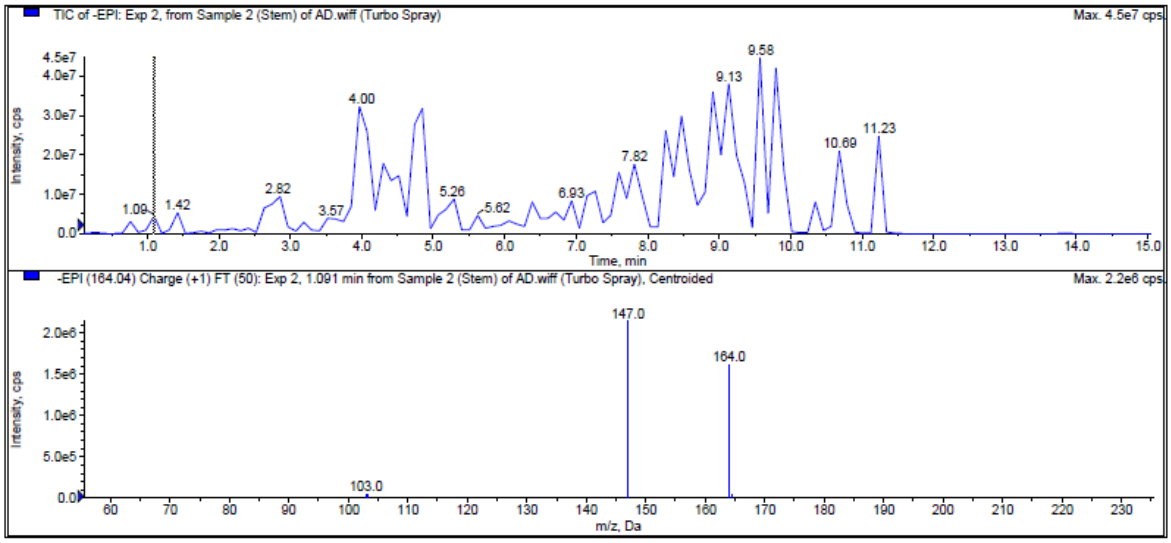
Max. 9.6e7 cps.
Intensity, cps
Time, min
-EPI (361.40) Charge (+1) FT (50): Exp 2, 3.206 min from Sample 3 (Leaves) of AD.wiff (Turbo Spray), Centroided
Max. 7.4e6 cps.
361.1
171.0
201.0
229.1
m/z, Da

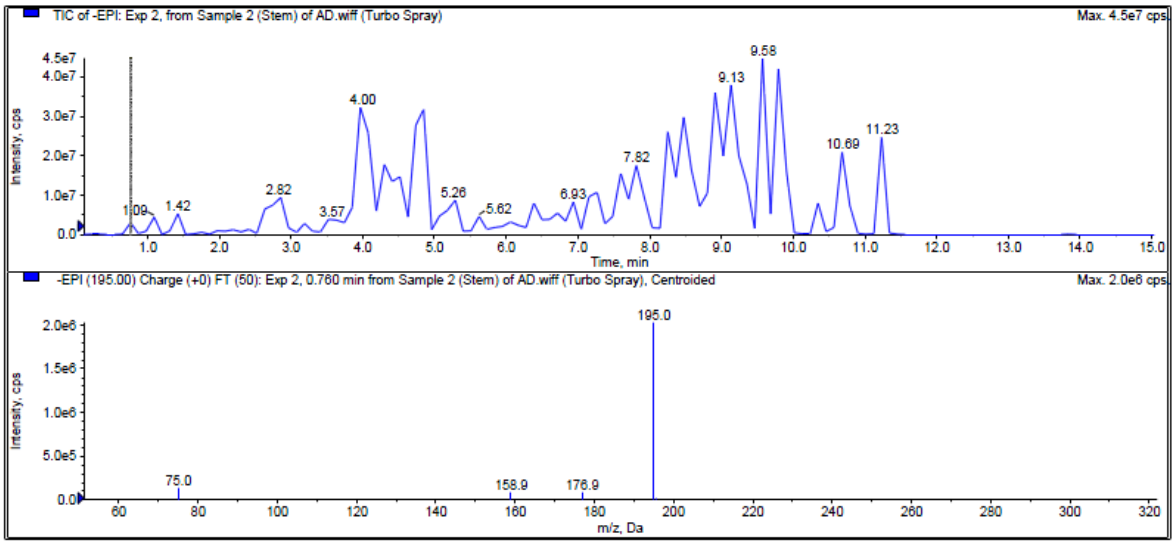

TIC of -EPI: Exp 2, from Sample 3 (Leaves) of AD.wiff (Turbo Spray)
Max. 9.6e7 cps.
Intensity, cps
Time, min
-EPI (470.40) Charge (+1) FT (50): Exp 2, 5.105 min from Sample 3 (Leaves) of AD.wiff (Turbo Spray), Centroided
Max. 2.0e7 cps.
m/z, Da

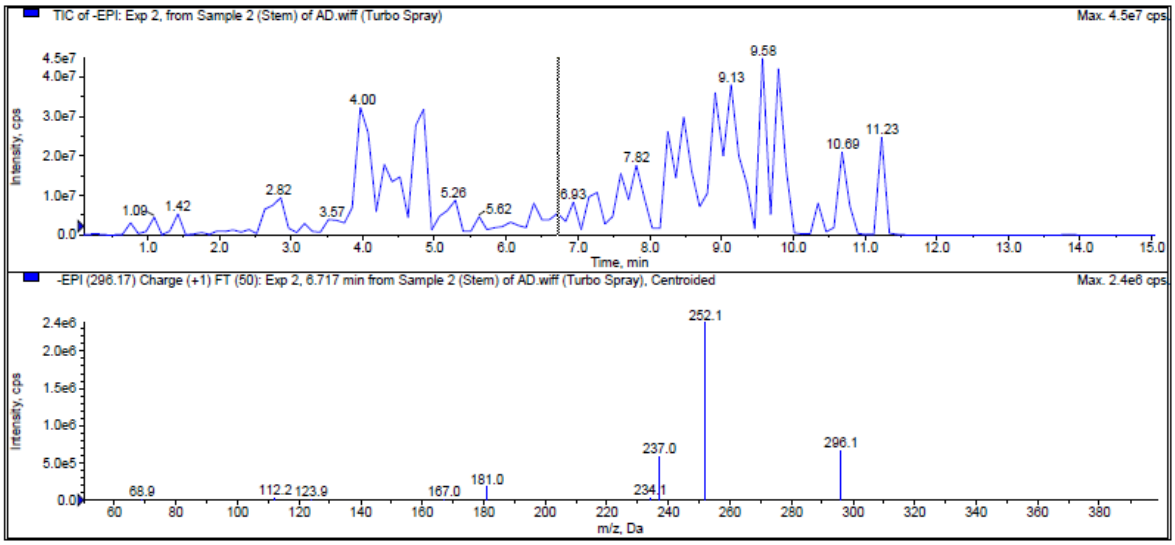

TIC of -EPI: Exp 2, from Sample 3 (Leaves) of AD.wiff (Turbo Spray)
Max. 9.6e7 cps.
Intensity, cps
Time, min
-EPI (486.45) Charge (+1) FT (50): Exp 2, 4.548 min from Sample 3 (Leaves) of AD.wiff (Turbo Spray), Centroided
Max. 3.5e6 cps.
m/z, Da

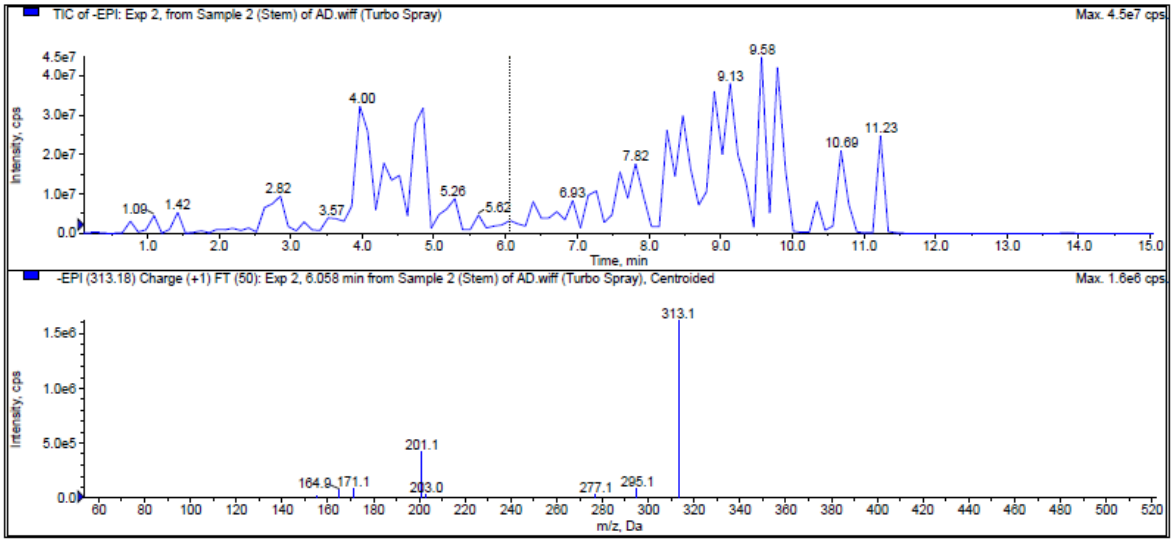

TIC of -EPI: Exp 2, from Sample 3 (Leaves) of AD.wiff (Turbo Spray)
Max. 9.6e7 cps.
Intensity, cps
Time, min
-EPI (677.46) Charge (+1) FT (50): Exp 2, 3.991 min from Sample 3 (Leaves) of AD.wiff (Turbo Spray), Centroided
Max. 1.5e7 cps.
m/z, Da

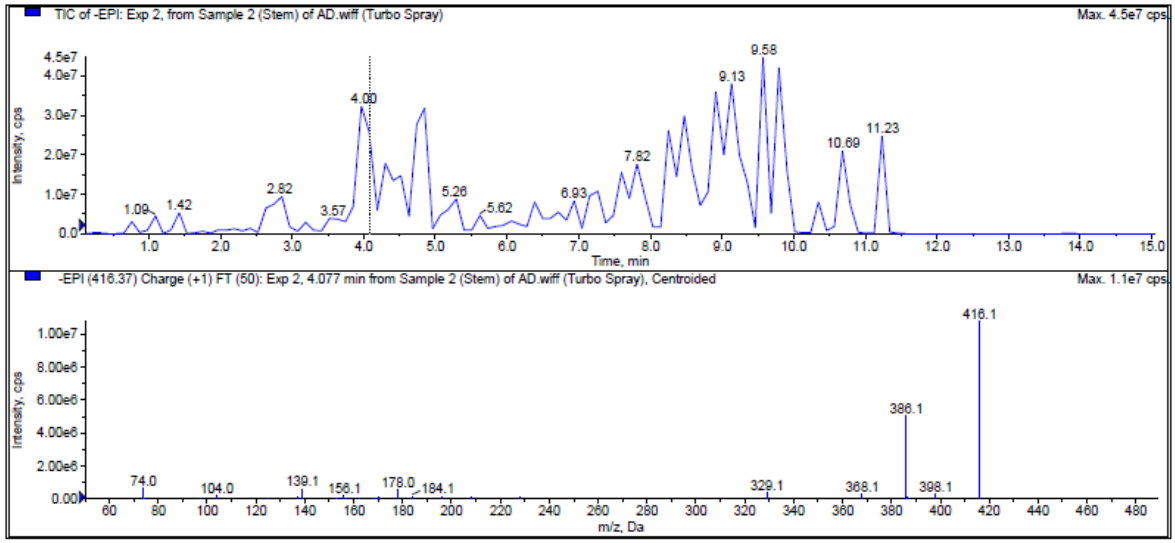
ii. LCMS/MS chromatogram for unknown chemical compounds of *Pereskia bleo* water extracts on stems aqueous extract.

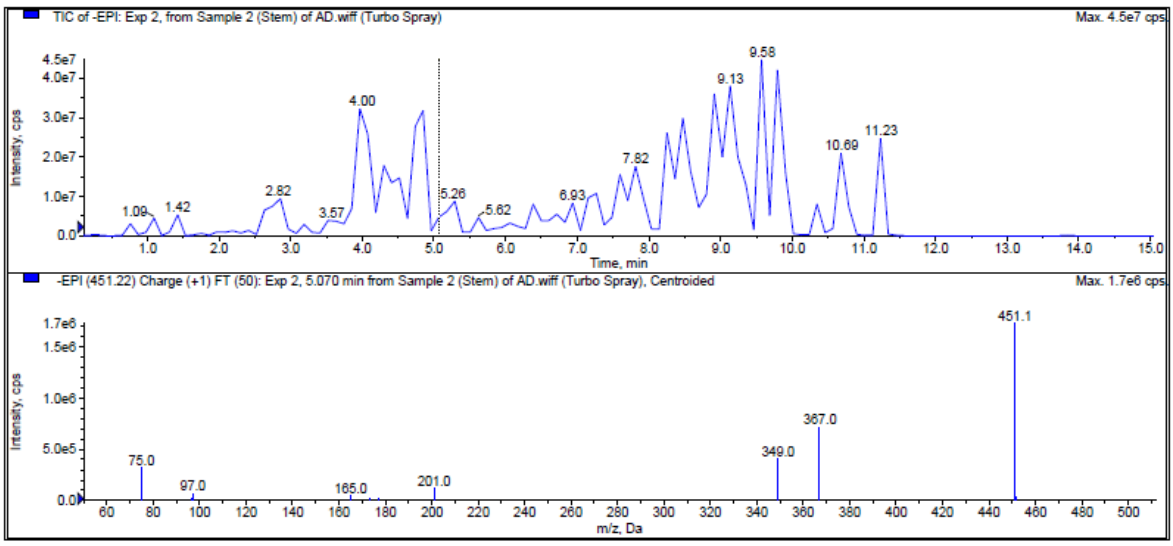


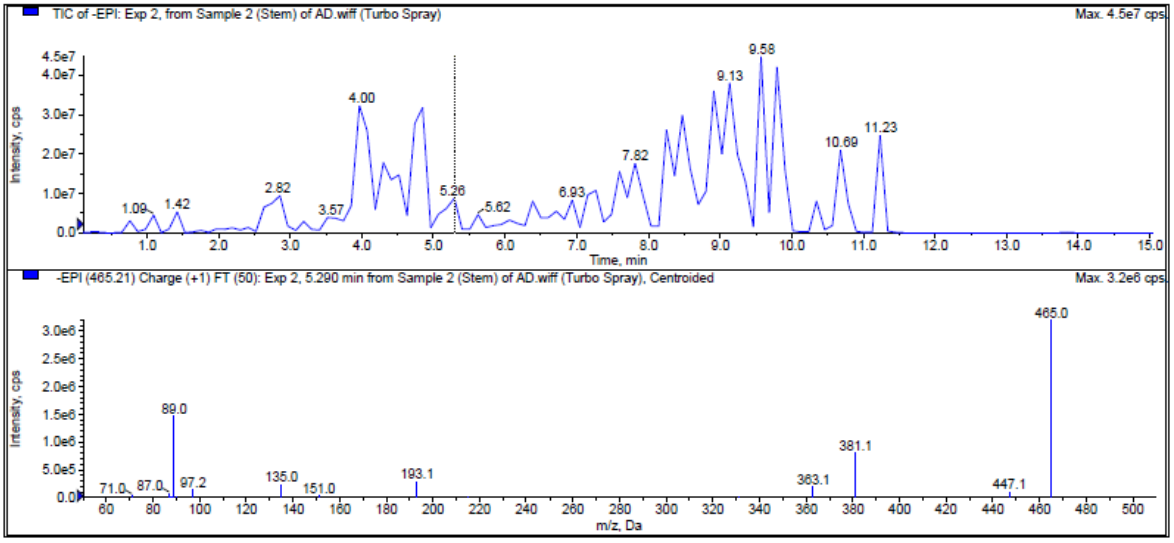


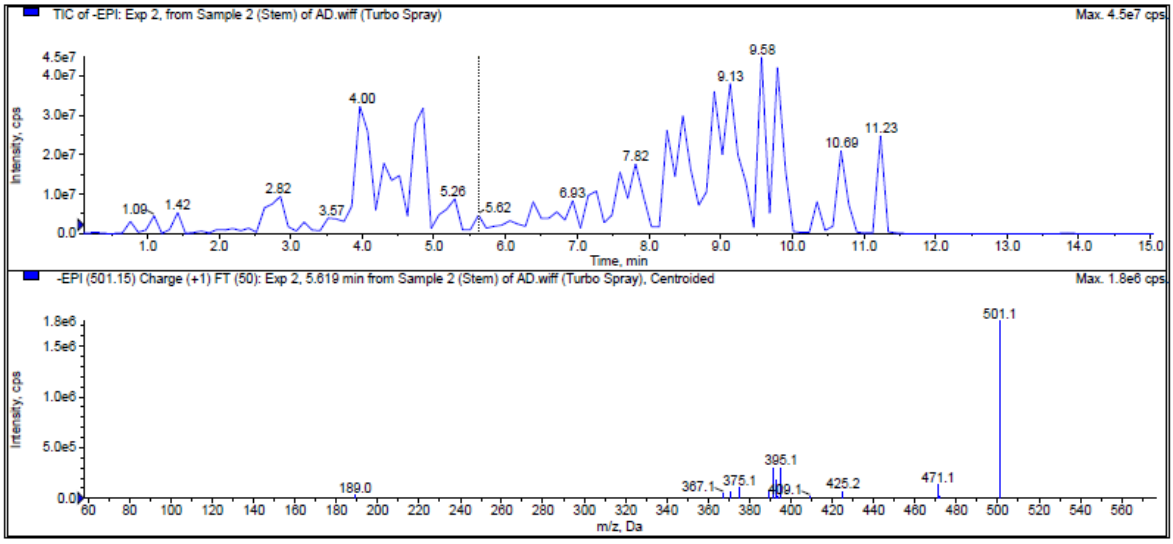

TIC of -EPI: Exp 2, from Sample 2 (Stem) of AD.wiff (Turbo Spray)
Max. 4.5e7 cps.
Intensity, cps
Time, min
-EPI (296.17) Charge (+1) FT (50): Exp 2, 6.717 min from Sample 2 (Stem) of AD.wiff (Turbo Spray), Centroided
Max. 2.4e6 cps.
m/z, Da

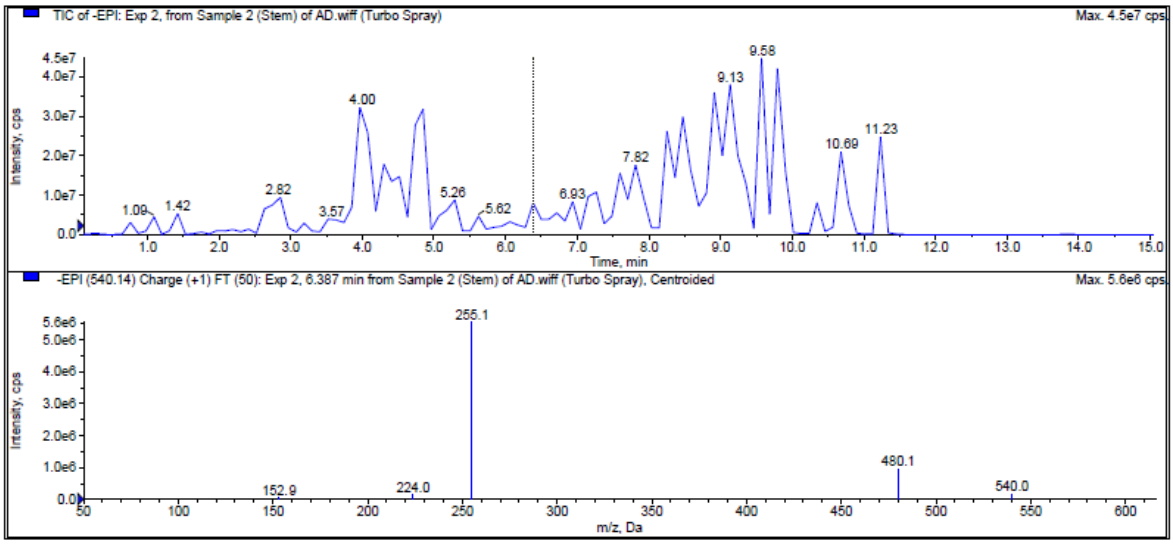

TIC of -EPI: Exp 2, from Sample 2 (Stem) of AD.wiff (Turbo Spray)
Max. 4.5e7 cps.
Intensity, cps
Time, min
-EPI (313.18) Charge (+1) FT (50): Exp 2, 6.058 min from Sample 2 (Stem) of AD.wiff (Turbo Spray), Centroided
Max. 1.6e6 cps.
m/z, Da

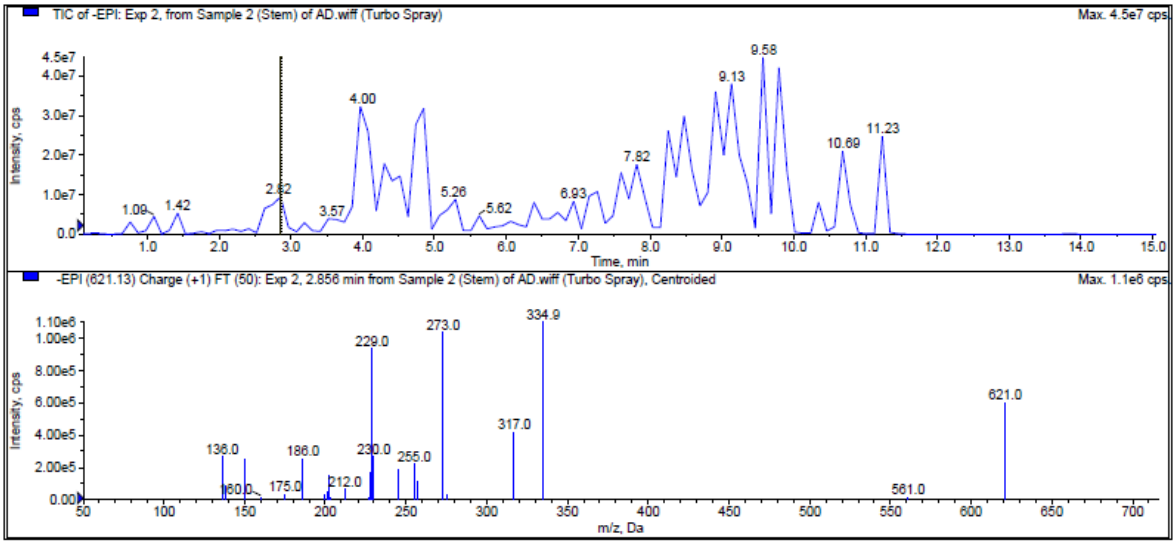

TIC of -EPI: Exp 2, from Sample 2 (Stem) of AD.wiff (Turbo Spray)
Max. 4.5e7 cps.
Intensity, cps
Time, min
-EPI (416.37) Charge (+1) FT (50): Exp 2, 4.077 min from Sample 2 (Stem) of AD.wiff (Turbo Spray), Centroided
Max. 1.1e7 cps.
m/z, Da


TIC of -EPI: Exp 2, from Sample 2 (Stem) of AD.wiff (Turbo Spray)
Max. 4.5e7 cps.
Intensity, cps
Time, min
-EPI (451.22) Charge (+1) FT (50): Exp 2, 5.070 min from Sample 2 (Stem) of AD.wiff (Turbo Spray), Centroided
Max. 1.7e6 cps.
m/z, Da


TIC of -EPI: Exp 2, from Sample 2 (Stem) of AD.wiff (Turbo Spray)
Max. 4.5e7 cps.
Intensity, cps
Time, min
-EPI (465.21) Charge (+1) FT (50): Exp 2, 5.290 min from Sample 2 (Stem) of AD.wiff (Turbo Spray), Centroided
Max. 3.2e6 cps.
m/z, Da


TIC of -EPI: Exp 2, from Sample 2 (Stem) of AD.wiff (Turbo Spray)
Max. 4.5e7 cps.
Intensity, cps
Time, min
-EPI (501.15) Charge (+1) FT (50): Exp 2, 5.619 min from Sample 2 (Stem) of AD.wiff (Turbo Spray), Centroided
Max. 1.8e6 cps.
m/z, Da


TIC of -EPI: Exp 2, from Sample 2 (Stem) of AD.wiff (Turbo Spray)
Max. 4.5e7 cps.
Intensity, cps
Time, min
-EPI (540.14) Charge (+1) FT (50): Exp 2, 6.387 min from Sample 2 (Stem) of AD.wiff (Turbo Spray), Centroided
Max. 5.6e6 cps.
m/z, Da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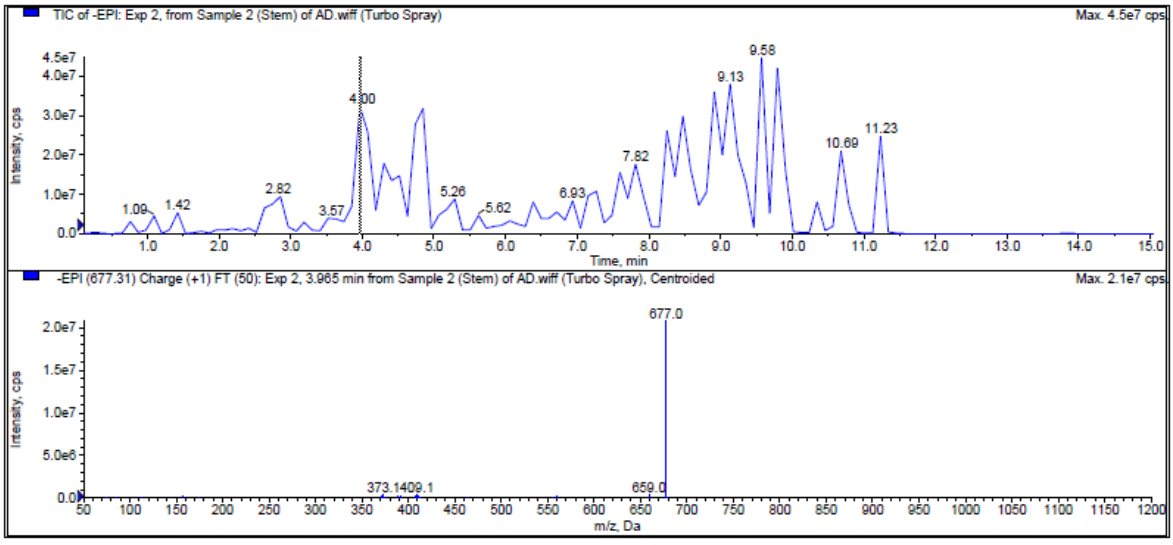
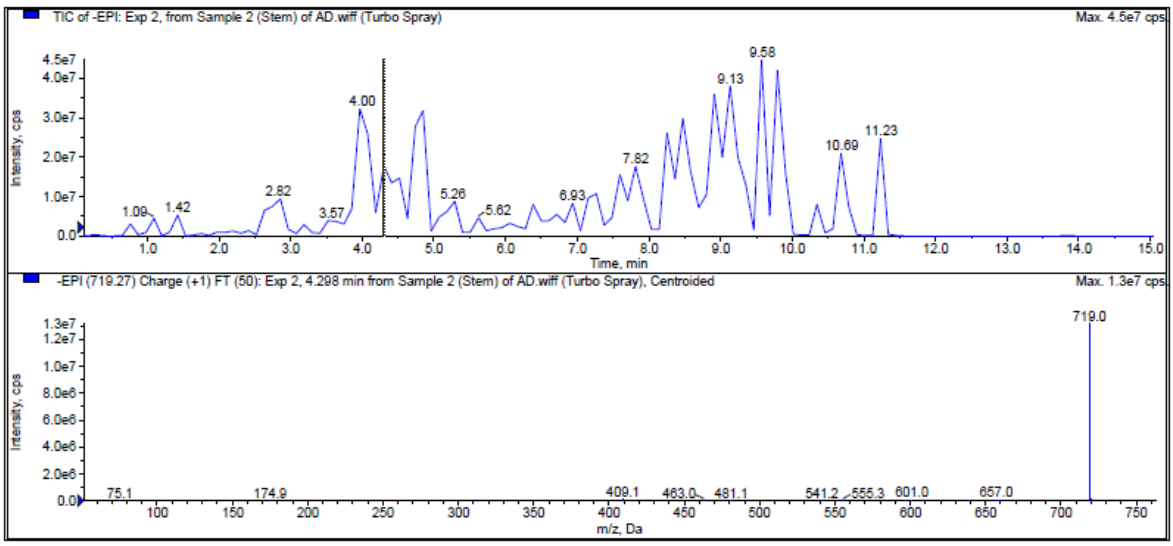
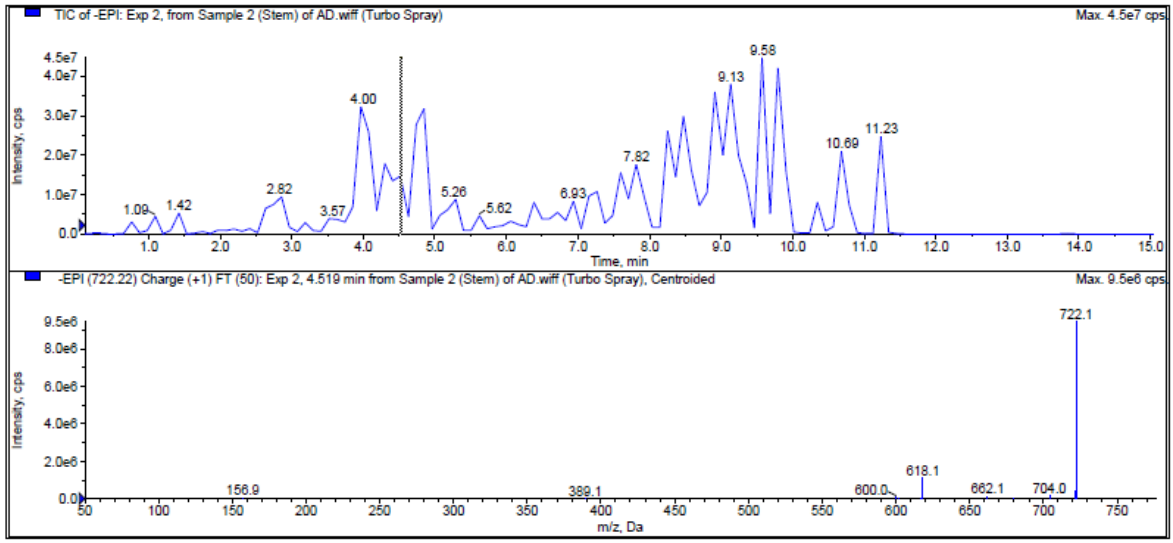
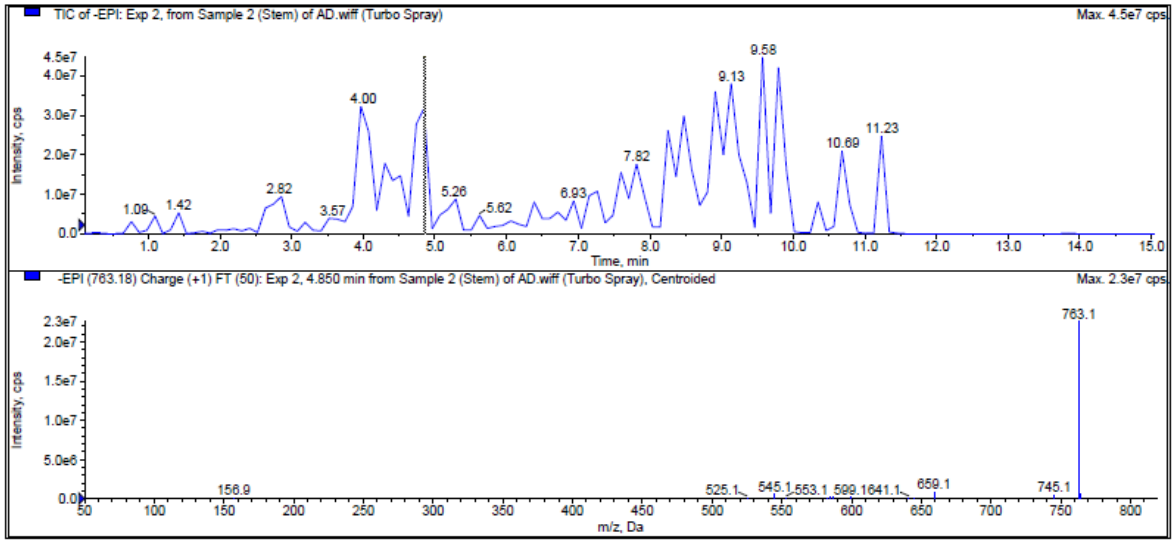
Max. 4.5e7 cps.
Intensity, cps
Time, min
-EPI (621.13) Charge (+1) FT (50): Exp 2, 2.856 min from Sample 2 (Stem) of AD.wiff (Turbo Spray), Centroided
Max. 1.1e6 cps.
m/z, Da

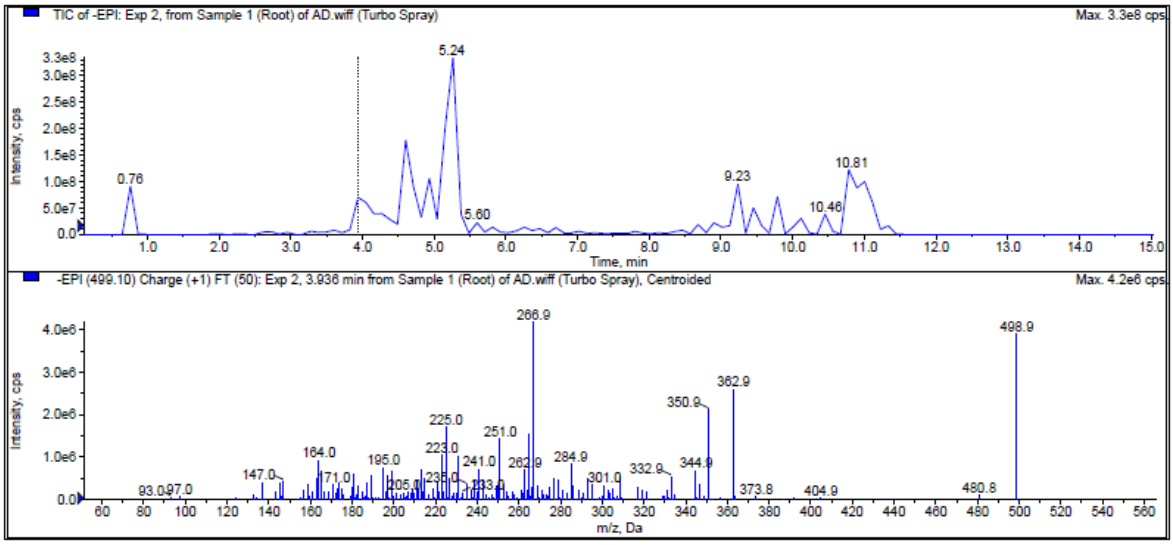

TIC of -EPI: Exp 2, from Sample 2 (Stem) of AD.wiff (Turbo Spray)
Max. 4.5e7 cps.
Intensity, cps
Time, min
-EPI (677.31) Charge (+1) FT (50): Exp 2, 3.965 min from Sample 2 (Stem) of AD.wiff (Turbo Spray), Centroided
Max. 2.1e7 cps.
m/z, Da

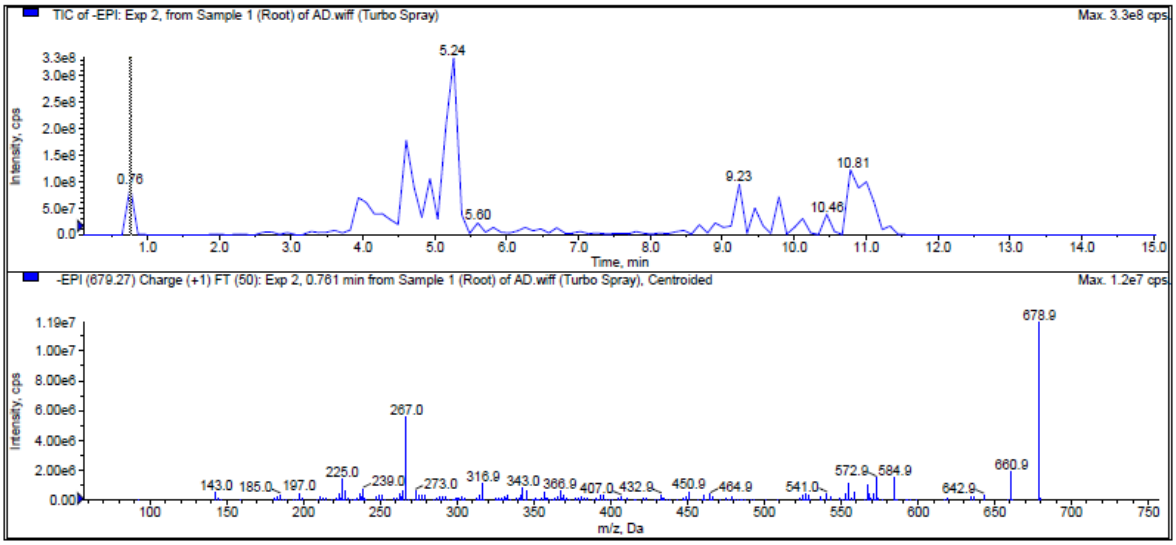

TIC of -EPI: Exp 2, from Sample 2 (Stem) of AD.wiff (Turbo Spray)
Max. 4.5e7 cps.
Intensity, cps
Time, min
-EPI (719.27) Charge (+1) FT (50): Exp 2, 4.298 min from Sample 2 (Stem) of AD.wiff (Turbo Spray), Centroided
Max. 1.3e7 cps.
719.0
m/z, Da

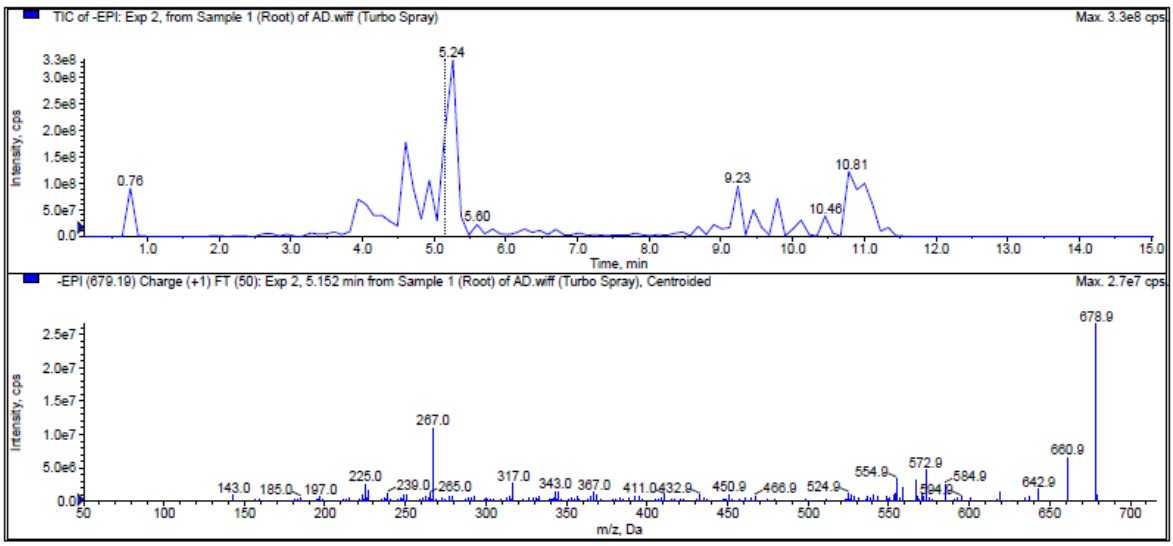

TIC of -EPI: Exp 2, from Sample 2 (Stem) of AD.wiff (Turbo Spray)
Max. 4.5e7 cps.
Intensity, cps
Time, min
-EPI (722.22) Charge (+1) FT (50): Exp 2, 4.519 min from Sample 2 (Stem) of AD.wiff (Turbo Spray), Centroided
Max. 9.5e6 cps.
722.1
m/z, Da

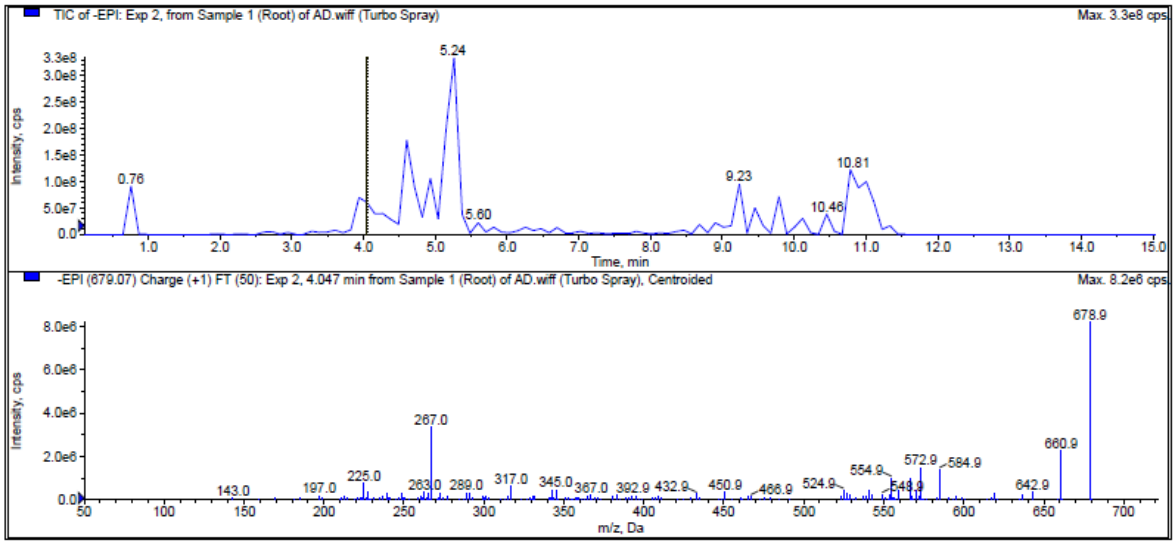

TIC of -EPI: Exp 2, from Sample 2 (Stem) of AD.wiff (Turbo Spray)
Max. 4.5e7 cps.
Intensity, cps
Time, min
-EPI (763.18) Charge (+1) FT (50): Exp 2, 4.850 min from Sample 2 (Stem) of AD.wiff (Turbo Spray), Centroided
Max. 2.3e7 cps.
763.1
m/z, Da

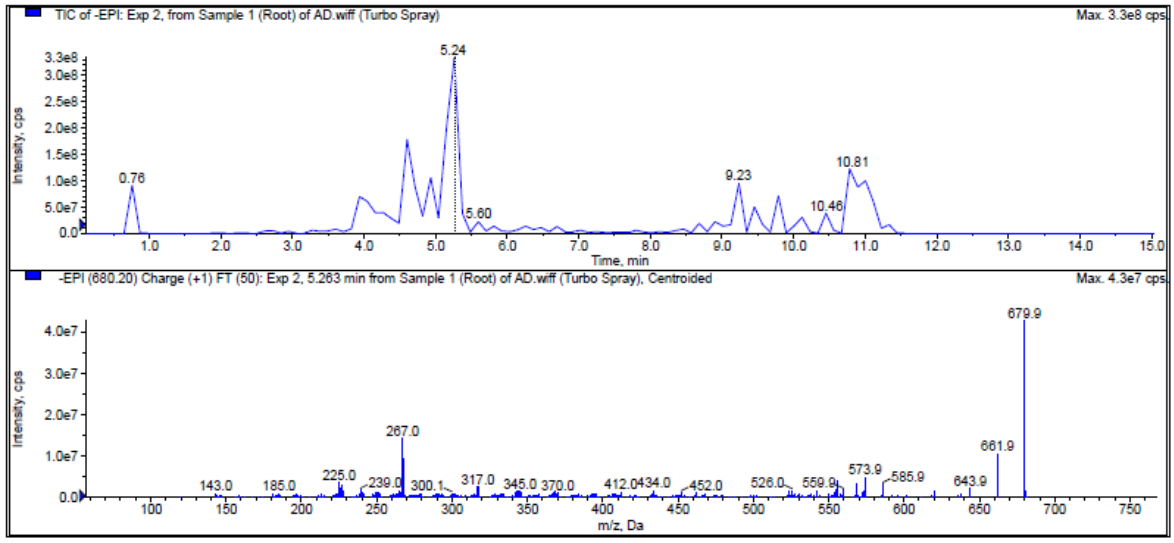
iii. LCMS/MS chromatogram for unknown chemical compounds of *Pereskia bleo* water extracts on roots aqueous extract.

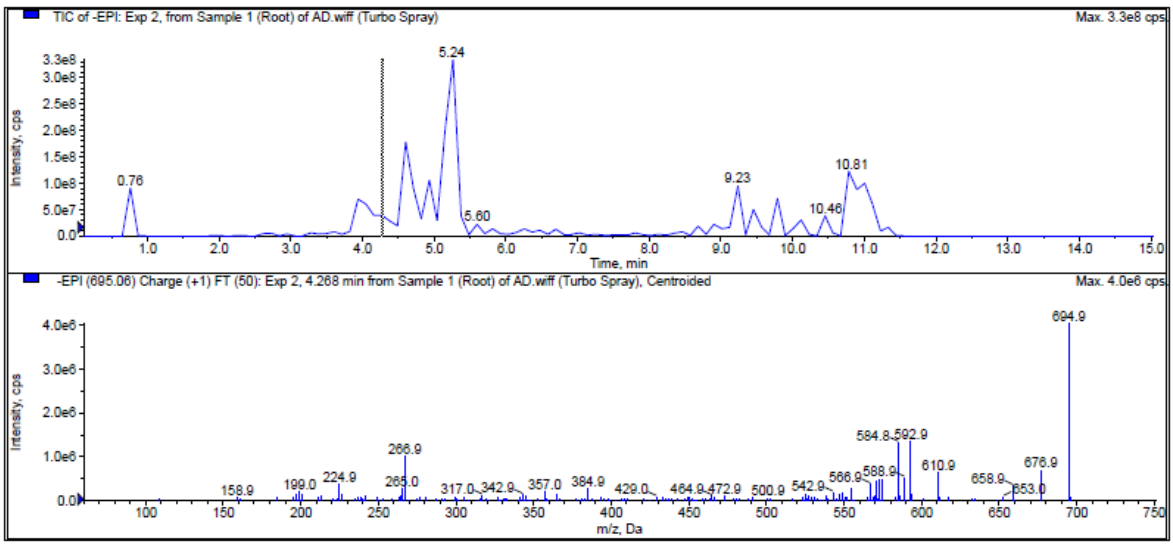


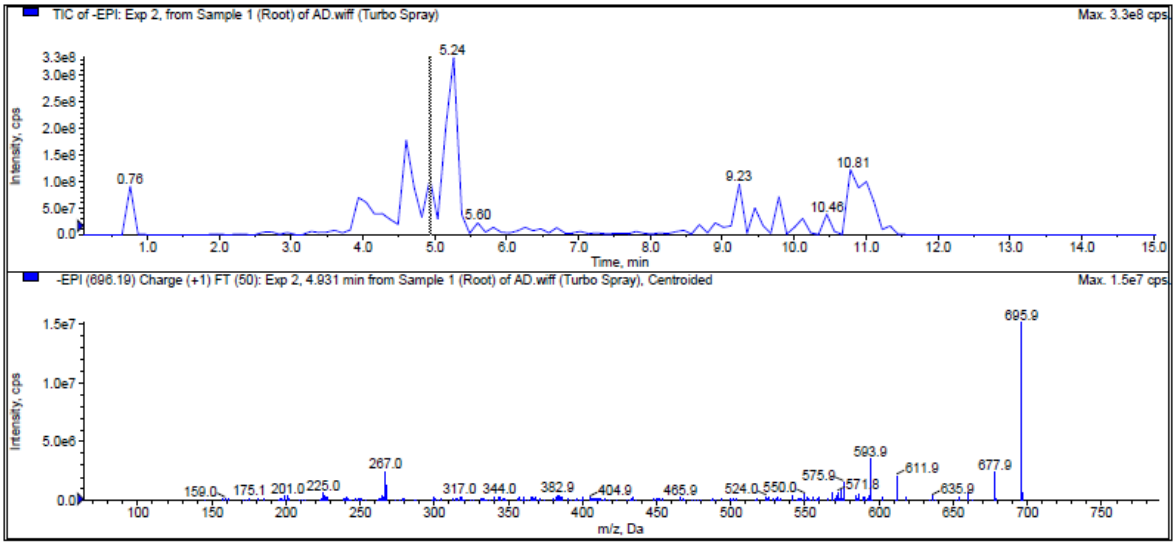


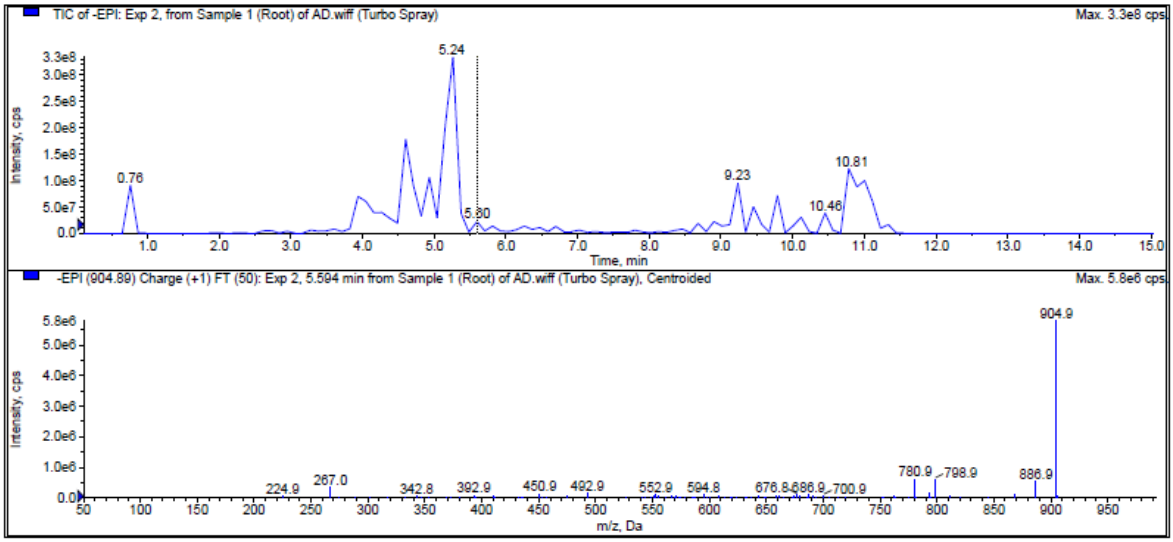

TIC of -EPI: Exp 2, from Sample 1 (Root) of AD.wiff (Turbo Spray)
Max. 3.3e8 cps.
Intensity, cps
Time, min
5.24
0.76
9.23
10.81
10.46
5.60
-EPI (679.19) Charge (+1) FT (50): Exp 2, 5.152 min from Sample 1 (Root) of AD.wiff (Turbo Spray), Centroided
Max. 2.7e7 cps.
m/z, Da
678.9
267.0
660.9
572.9
642.9


TIC of -EPI: Exp 2, from Sample 1 (Root) of AD.wiff (Turbo Spray)
Max. 3.3e8 cps.
Intensity, cps
Time, min
5.24
0.76
9.23
10.81
10.46
5.60
-EPI (679.07) Charge (+1) FT (50): Exp 2, 4.047 min from Sample 1 (Root) of AD.wiff (Turbo Spray), Centroided
Max. 8.2e6 cps.
m/z, Da
678.9
267.0
660.9
572.9
642.9


TIC of -EPI: Exp 2, from Sample 1 (Root) of AD.wiff (Turbo Spray)
Max. 3.3e8 cps.
Intensity, cps
Time, min
5.24
0.76
9.23
10.81
10.46
5.60
-EPI (680.20) Charge (+1) FT (50): Exp 2, 5.263 min from Sample 1 (Root) of AD.wiff (Turbo Spray), Centroided
Max. 4.3e7 cps.
m/z, Da
679.9
267.0
661.9
573.9
643.9


TIC of -EPI: Exp 2, from Sample 1 (Root) of AD.wiff (Turbo Spray)
Max. 3.3e8 cps.
Intensity, cps
Time, min
0.76
5.24
5.60
9.23
10.46
10.81
-EPI (695.06) Charge (+1) FT (50): Exp 2, 4.268 min from Sample 1 (Root) of AD.wiff (Turbo Spray), Centroided
Max. 4.0e6 cps.
m/z, Da
694.9
584.8
592.9
610.9
676.9
266.9


TIC of -EPI: Exp 2, from Sample 1 (Root) of AD.wiff (Turbo Spray)
Max. 3.3e8 cps.
Intensity, cps
Time, min
0.76
5.24
5.60
9.23
10.46
10.81
-EPI (696.19) Charge (+1) FT (50): Exp 2, 4.931 min from Sample 1 (Root) of AD.wiff (Turbo Spray), Centroided
Max. 1.5e7 cps.
m/z, Da
695.9
593.9
611.9
677.9
267.0


TIC of -EPI: Exp 2, from Sample 1 (Root) of AD.wiff (Turbo Spray)
Max. 3.3e8 cps.
Intensity, cps
Time, min
0.76
5.24
5.80
9.23
10.46
10.81
-EPI (904.89) Charge (+1) FT (50): Exp 2, 5.594 min from Sample 1 (Root) of AD.wiff (Turbo Spray), Centroided
Max. 5.8e6 cps.
m/z, Da
904.9
267.0
780.9
798.9
886.9

Glycogen Phosphorylase Inhibitory (GP) Bioassay

i. Absorbance of standard drug Glipizide on GP inhibition bioassay.

Sample	Absorbance			Averages	% of GP Inhibition	STDV
	1	2	3			
Std Glipizide (mg/ml)						
0	0.869	0.865	0.881	0.872	0.00	0.008
2	0.155	0.138	0.154	0.149	82.79	0.010
4	0.144	0.155	0.153	0.151	82.60	0.006
6	0.140	0.160	0.155	0.152	82.49	0.010
8	0.140	0.133	0.138	0.137	84.18	0.004
10.0	0.136	0.133	0.136	0.135	84.41	0.002

ii. Absorbance of *Pereskia bleo* leaves extracts at five difference concentrations and solvents on GP inhibition bioassay.

Leaves [mgml-1]	Absorbance			Averages	% GP Inhibition	STDV
	1	2	3			
Hexane						
0	0.693	0.706	0.681	0.693	0.00	0.013
2	0.511	0.538	0.540	0.530	23.46	0.016
4	0.492	0.524	0.525	0.514	25.77	0.019
6	0.501	0.473	0.402	0.459	33.72	0.051
8	0.478	0.411	0.460	0.450	35.02	0.035
10.0	0.442	0.422	0.447	0.437	36.85	0.013
Pet ether						
0	0.693	0.706	0.681	0.693	0.00	0.013
2	0.615	0.642	0.644	0.634	8.43	0.016
4	0.622	0.593	0.620	0.612	11.61	0.016
6	0.612	0.585	0.614	0.604	12.76	0.016
8	0.589	0.615	0.602	0.602	13.01	0.013
10.0	0.587	0.601	0.594	0.594	14.16	0.007
Chloroform						
0	0.693	0.706	0.681	0.693	0.00	0.013
2	0.214	0.231	0.238	0.228	67.10	0.012
4	0.194	0.176	0.193	0.188	72.88	0.010
6	0.183	0.172	0.175	0.177	74.47	0.006
8	0.150	0.166	0.163	0.160	76.93	0.009
10.0	0.124	0.122	0.131	0.126	81.84	0.005

‘Continued’

Leaves	**Absorbance**			**Averages**	**% GP**	**STDV**
[mgml-1]	**1**	**2**	**3**		**Inhibition**	
Methanol						
0	0.693	0.706	0.681	0.693	0.00	0.013
2	0.284	0.284	0.253	0.274	60.45	0.018
4	0.204	0.231	0.225	0.220	68.21	0.014
6	0.173	0.178	0.182	0.178	74.33	0.005
8	0.142	0.146	0.146	0.145	79.09	0.002
10.0	0.149	0.132	0.141	0.141	79.67	0.009
Water						
0	0.693	0.706	0.681	0.693	0.00	0.013
2	0.321	0.350	0.349	0.340	50.87	0.016
4	0.230	0.231	0.199	0.220	68.21	0.018
6	0.174	0.176	0.177	0.176	74.61	0.002
8	0.163	0.165	0.167	0.165	76.16	0.002
10.0	0.101	0.170	0.140	0.137	80.20	0.035

iii. Absorbance of *Pereskia bleo* stems extracts at five difference concentrations and solvents on GP inhibition bioassay.

Stem	Absorbance				% GP	STDV
[mgml-1]	1	2	3	Averages	Inhibition	
Hexane						
0	0.693	0.706	0.681	0.693	0.00	0.013
2	0.604	0.604	0.619	0.609	11.99	0.009
4	0.586	0.585	0.588	0.586	15.27	0.002
6	0.579	0.590	0.589	0.586	15.32	0.006
8	0.578	0.580	0.581	0.580	16.23	0.002
10	0.573	0.586	0.570	0.576	16.71	0.009
Pet ether						
0	0.693	0.706	0.681	0.693	0.00	0.013
2	0.640	0.662	0.663	0.655	5.35	0.013
4	0.620	0.647	0.649	0.639	7.71	0.016
6	0.634	0.623	0.630	0.629	9.10	0.006
8	0.605	0.580	0.597	0.594	14.16	0.013
10	0.619	0.554	0.570	0.581	16.04	0.034

'Continued'

Stem [mgml-1]	Absorbance 1	2	3	Averages	% GP Inhibition	STDV
Chloroform						
0	0.693	0.706	0.681	0.693	0.00	0.013
2	0.402	0.434	0.383	0.406	41.28	0.026
4	0.326	0.349	0.345	0.340	50.87	0.012
6	0.386	0.327	0.335	0.349	49.52	0.032
8	0.384	0.312	0.283	0.326	52.84	0.052
10	0.321	0.288	0.315	0.308	55.49	0.018
Methanol						
0	0.693	0.706	0.681	0.693	0.00	0.013
2	0.288	0.281	0.280	0.283	59.10	0.004
4	0.227	0.259	0.231	0.239	65.46	0.017
6	0.226	0.231	0.220	0.226	67.39	0.006
8	0.194	0.177	0.180	0.184	73.46	0.009
10	0.160	0.151	0.139	0.150	78.32	0.011
Water						
0	0.693	0.706	0.681	0.693	0.00	0.013
2	0.378	0.361	0.374	0.371	46.39	0.009
4	0.351	0.367	0.362	0.360	47.98	0.008
6	0.312	0.351	0.342	0.335	51.59	0.020
8	0.291	0.278	0.279	0.283	59.15	0.007
10.0	0.274	0.282	0.289	0.282	59.30	0.008

iv. Absorbance of *Pereskia bleo* roots extracts at five difference concentrations and solvents on GP inhibition bioassay.

Root [mgml-1]	Absorbance 1	2	3	Average	% Inhibition	STDV
Hexane						
0	0.693	0.706	0.681	0.693	0.00	0.013
2	0.698	0.626	0.603	0.642	7.18	0.050
4	0.533	0.639	0.555	0.576	16.81	0.056
6	0.509	0.567	0.568	0.548	20.81	0.034
8	0.549	0.573	0.509	0.544	21.44	0.032
10	0.575	0.509	0.546	0.543	21.48	0.033

‘Continued’

Root		Absorbance		Average	%	STDV
[mgml-1]	1	2	3		Inhibition	
Pet ether						
0	0.693	0.706	0.681	0.693	0.00	0.013
2	0.642	0.568	0.601	0.604	12.76	0.037
4	0.637	0.604	0.601	0.614	11.27	0.020
6	0.605	0.671	0.558	0.611	11.66	0.057
8	0.606	0.634	0.580	0.607	12.33	0.027
10	0.594	0.580	0.579	0.584	15.56	0.008
Chloroform						
0	0.693	0.706	0.681	0.693	0.00	0.013
2	0.462	0.466	0.470	0.466	32.66	0.004
4	0.204	0.207	0.210	0.207	70.09	0.003
6	0.164	0.199	0.135	0.166	76.01	0.032
8	0.174	0.183	0.131	0.163	76.49	0.028
10	0.161	0.166	0.134	0.154	77.79	0.017
Methanol						
0	0.693	0.706	0.681	0.693	0.00	0.013
2	0.065	0.067	0.077	0.070	89.93	0.006
4	0.081	0.079	0.074	0.078	88.73	0.004
6	0.059	0.056	0.065	0.060	91.33	0.005
8	0.068	0.066	0.066	0.067	90.37	0.001
10	0.063	0.074	0.073	0.070	89.88	0.006
Water						
0	0.693	0.706	0.681	0.693	0.00	0.013
2	0.321	0.349	0.350	0.340	50.87	0.016
4	0.204	0.231	0.225	0.220	68.21	0.014
6	0.173	0.176	0.176	0.175	74.71	0.002
8	0.163	0.173	0.159	0.165	76.16	0.007
10.0	0.132	0.121	0.174	0.142	79.43	0.028

Antidiabetic Effects of *Pereskia bleo* extracts on Sprague dawley (SD) rats.

i. Effects of *Pereskia bleo* water extracts on blood glucose levels

GROUP	NO		Blood Glucose Leven [mg/dl)				
		0	5	10	15	20	25
Normal Control	1	87.1	78.6	73.0	66.6	68.4	71.8
	2	75.8	89.7	65.4	99.0	89.9	94.4
	3	85.6	65.4	83.9	68.4	63.6	68.3
	4	68.0	85.5	85.6	75.6	79.9	87.1
	5	56.4	88.9	61.9	64.8	62.4	70.5
	6	79.3	60.7	77.0	86.4	81.1	78.0
	Average	75.4	78.1	74.5	76.8	74.2	78.4
	SD	11.6	12.4	9.6	13.5	11.1	10.4
Diabetic Control	1	292.0	326.3	362.0	381.5	395.9	388.9
	2	286.0	311.8	359.1	382.4	408.0	400.4
	3	269.0	297.4	356.3	363.6	369.2	367.2
	4	289.3	317.1	352.8	380.8	393.3	384.6
	5	265.4	293.9	344.4	361.7	367.2	360.1
	6	285.2	330.5	379.6	379.1	380.1	381.7
	Average	281.2	312.8	359.0	374.9	385.6	380.5
	SD	11.1	14.9	11.8	9.5	16.2	14.7
Glipizide 3mg/kg	1	296.9	301.6	270.0	279.2	265.8	263.6
	2	291.3	292.7	277.4	284.4	271.4	269.7
	3	295.1	292.7	280.1	289.9	279.2	271.4
	4	283.5	277.1	260.7	272.4	272.9	269.2
	5	272.0	254.8	234.5	246.1	246.9	236.7
	6	271.3	260.2	240.6	245.5	251.3	249.6
	Average	285.0	279.9	260.6	269.6	264.6	260.0
	SD	11.3	19.1	19.1	19.3	12.8	13.9
Leaf 250mg/kg	1	299.7	241.9	201.0	148.0	136.9	122.4
	2	292.8	249.0	196.4	139.9	129.4	127.0
	3	293.5	234.5	185.3	123.8	119.1	114.7
	4	288.3	228.2	165.8	131.4	117.0	117.2
	5	272.6	231.4	181.1	125.1	109.2	108.8
	6	258.1	200.4	178.0	100.2	100.2	97.9
	Average	284.2	230.9	184.6	128.1	118.6	114.7
	SD	15.7	16.7	12.8	16.4	13.3	10.3

‘Continued’

GROUP	NO	Blood Glucose Leven [mg/dl]					
		0	**5**	**10**	**15**	**20**	**25**
Leaf 500mg/kg	1	298.2	279.9	225.1	153.2	102.5	99
	2	293.0	271.0	201.3	178.2	114.9	112
	3	289.0	261.1	228.5	149.0	98.0	98
	4	286.4	241.3	194.5	174.4	104.8	95
	5	279.7	253.2	189.7	160.1	85.4	96
	6	260.9	254.7	190.2	145.0	89.3	80
	Average	284.5	260.2	204.9	160.0	99.2	96.7
	SD	13.1	13.7	17.5	13.6	10.8	10.2
Stem 250mg/kg	1	282.9	200.0	211.1	204.4	170.6	129.1
	2	284.7	184.0	199.8	198.0	175.0	138.0
	3	276.6	178.0	181.0	173.8	159.0	126.0
	4	274.0	173.0	195.3	195.5	155.3	136.3
	5	258.9	169.0	189.0	173.7	142.0	109.8
	6	259.2	162.0	182.9	175.4	155.5	133.0
	Average	272.7	177.7	193.2	186.8	159.6	128.7
	SD	11.3	13.3	11.3	14.0	11.9	10.3
Stem 500mg/kg	**1**	297.0	201.1	187.4	153.8	96.0	99
	2	296.9	207.3	185.1	158.0	144.0	119
	3	289.2	181.7	175.8	153.9	132.0	105
	4	278.6	172.0	162.0	129.0	99.0	84
	5	270.7	170.7	164.2	152.6	129.0	97
	6	268.8	159.0	161.0	149.3	111.0	92
	Average	283.5	182.0	172.6	149.4	118.5	99.3
	SD	12.6	18.8	11.9	10.4	19.4	11.9

‘Continued’

GROUP	NO	Blood Glucose Leven [mg/dl)					
		0	**5**	**10**	**15**	**20**	**25**
Root 250mg/kg	**1**	294.0	231.8	204.4	195.9	152.3	148.8
	2	294.2	228.5	195.9	193.1	160.9	141.7
	3	289.4	201.9	198.0	183.8	146.1	139.6
	4	279.2	186.0	185.3	194.0	139.2	131.2
	5	273.4	199.4	179.7	161.5	135.0	108.8
	6	265.2	196.6	174.0	154.9	140.5	113.0
	Average	282.6	207.4	189.6	180.5	145.7	130.5
	SD	11.9	18.5	11.7	17.9	9.6	16.3
Root 500mg/kg	1	299.7	217.1	137.3	119.8	129.3	136.8
	2	297.1	211.9	140.2	129.5	123.0	135.3
	3	294.0	203.4	137.1	125.0	130.8	130.5
	4	285.9	185.0	130.8	119.1	122.2	124.1
	5	273.3	178.2	115.4	96.2	107.7	96.3
	6	260.4	175.5	99.0	89.4	107.1	94.7
	Average	285.1	195.2	126.6	113.2	120.0	119.6
	SD	15.4	17.9	16.2	16.4	10.3	19.2

ii. Effects of *Pereskia bleo* water extracts on body weight change.

GROUP	NO	Body weight (g)					
		0	**5**	**10**	**15**	**20**	**25**
Normal Control	**1**	128	138	149	167	189	191
	2	127	137	147	154	173	197
	3	121	136	153	169	188	201
	4	115	137	148	165	170	166
	5	121	141	157	163	173	194
	6	124	144	166	175	179	219
	Average	123	139	153	166	179	195
	SD	5	3	7	7	8	17
Diabetic Control	**1**	116	123	121	106	97	86
	2	121	130	142	139	121	110
	3	110	117	116	123	114	111
	4	127	128	113	104	96	99
	5	114	118	111	105	104	97
	6	103	107	109	112	89	79
	Average	115	121	119	115	104	97
	SD	8	8	12	14	12	13
Glipizide 3mg/kg	**1**	135	158	173	174	180	182
	2	120	131	140	147	148	179
	3	125	135	149	163	172	177
	4	116	170	191	201	212	211
	5	118	125	147	158	163	178
	6	151	167	198	216	228	231
	Average	127	148	166	176	184	193
	SD	13	19	24	27	30	23
Leaf 250mg/kg	1	125	121	112	125	137	141
	2	100	135	146	149	158	163
	3	136	161	173	180	186	189
	4	131	144	155	163	169	166
	5	119	123	136	142	151	156
	6	129	126	132	136	141	144
	Average	123	135	142	149	157	160
	SD	13	15	21	20	18	17

‘Continued’

GROUP	NO	Body weight (g)					
		0	**5**	**10**	**15**	**20**	**25**
Leaf 500mg/kg	**1**	129	154	163	170	178	174
	2	125	126	139	144	155	166
	3	138	159	163	176	179	174
	4	106	122	136	144	151	154
	5	128	134	139	143	156	162
	6	100	117	124	139	147	152
	Average	121	135	144	153	161	164
	SD	15	17	16	16	14	10
Stem 250mg/kg	1	126	138	149	149	153	158
	2	120	123	129	135	141	148
	3	100	102	108	118	126	132
	4	108	128	134	139	145	152
	5	96	118	128	132	136	144
	6	121	129	135	149	153	156
	Average	112	123	131	137	142	148
	SD	12	12	13	12	10	10
Stem 500mg/kg	1	121	125	133	139	145	149
	2	120	123	146	151	161	164
	3	116	117	125	131	135	140
	4	135	141	153	158	166	165
	5	111	123	137	139	142	148
	6	109	116	124	129	128	137
	Average	119	124	136	141	146	151
	SD	9	9	12	11	15	12
Root 250mg/kg	**1**	123	129	134	139	141	146
	2	110	127	135	143	148	154
	3	108	114	128	134	144	149
	4	109	134	143	139	140	148
	5	116	127	131	148	155	161
	6	128	131	149	151	156	165
	Average	116	127	137	142	147	154
	SD	8	7	8	6	7	8

‘Continued’

GROUP	NO	Body weight (g)					
		0	5	10	15	20	25
Root 500mg/kg	1	154	143	141	134	128	136
	2	121	134	139	145	148	152
	3	127	133	141	147	151	157
	4	111	139	159	161	167	174
	5	124	136	139	146	151	159
	6	125	136	149	151	159	155
	Average	127	137	145	147	151	156
	SD	14	4	8	9	13	12

iii. Effects of *Pereskia bleo* water extracts on lipid profile test

GROUP	NO	Liver Func. Test (U/L)			Lipid Profile Test (mmol/L)				Urea (mmol/L)	Creatinine (µmol/L)
		ALT	AST	ALP	TRIG	T.CHOL	HDL	LDL		
Normal Control	**1**	64	42	45	0.717	1.600	1.200	0.410	3.90	34
	2	62	43	40	0.760	1.600	1.270	0.524	3.80	41
	3	58	39	40	0.690	1.100	1.320	0.480	4.90	36
	4	49	42	42	0.772	1.600	1.460	0.650	3.80	37
	5	45	38	31	0.752	1.600	1.210	0.440	3.10	37
	6	46	39	36	0.820	1.600	1.554	0.730	2.50	37
	Average	54	40	39	0.752	1.517	1.336	0.539	3.67	37
	SD	8	2	5	0.05	0.2	0.1	0.1	0.8	2.0
Diabetic Control	1	109	93	86	1.700	2.500	0.270	1.220	12.10	100
	2	105	99	86	1.620	2.300	0.590	1.321	9.60	97
	3	96	96	89	2.690	2.200	0.590	1.280	6.90	110
	4	95	91	95	1.512	2.400	0.550	1.000	6.00	98
	5	90	94	88	2.052	2.200	0.560	1.253	11.90	90
	6	88	88	87	0.820	2.300	0.520	1.010	10.70	101
	Average	97	94	89	1.732	2.317	0.513	1.181	9.5	99
	SD	8	4	3	0.6	0.1	0.1	0.1	2.6	6.5

‘Continued’

GROUP	NO	Liver Func. Test (U/L)			Lipid Profile Test (mmol/L)				Urea (mmol/L)	Creatinine (µmol/L)
		ALT	AST	ALP	TRIG	T.CHOL	HDL	LDL		
Glipizide 3mg/kg	**1**	60	44	38	0.400	1.900	1.620	0.333	2.40	20
	2	64	45	33	0.520	1.700	1.520	0.123	3.20	32
	3	59	51	36	0.490	1.500	1.280	0.180	3.60	24
	4	55	46	38	0.460	1.500	1.610	0.321	3.10	34
	5	59	44	37	0.580	1.400	1.520	0.432	2.90	31
	6	59	36	39	0.560	1.500	1.670	0.255	4.50	34
	Average	59	44	37	0.502	1.583	1.537	0.274	3.28	29
	SD	3	5	2	0.07	0.2	0.1	0.1	0.7	5.8
Leaf 250mg/kg	**1**	49	48	37	0.470	1.500	1.900	0.710	4.60	37
	2	51	44	46	0.690	1.200	2.020	0.880	4.10	41
	3	54	51	39	0.680	1.900	2.100	0.530	3.60	46
	4	59	42	41	0.540	1.800	1.780	0.460	3.90	43
	5	48	49	35	0.740	1.900	1.840	0.710	3.40	39
	6	53	49	36	0.550	1.900	1.990	0.700	3.60	41
	Average	52	47	39	0.612	1.700	1.938	0.665	3.867	41
	SD	3	3	4	0.1	0.3	0.1	0.1	0.4	3

'Continued'

GROUP	NO	Liver Func. Test (U/L)			Lipid Profile Test (mmol/L)				Urea (mmol/L)	Creatinine (µmol/L)
		ALT	AST	ALP	TRIG	T.CHOL	HDL	LDL		
Leaf 500mg/kg	**1**	66	32	42	0.480	1.400	1.452	0.555	4.20	34
	2	50	42	36	0.420	1.700	1.300	0.690	3.70	34
	3	58	48	30	0.400	1.500	1.350	0.470	2.80	35
	4	64	45	24	0.440	1.500	0.900	0.524	2.70	35
	5	58	37	32	0.440	1.700	1.330	0.440	3.00	29
	6	60	40	34	0.300	1.600	0.960	0.553	3.30	35
	Average	59	41	34	0.413	1.567	1.215	0.539	3.28	34
	SD	6	6	6	0.06	0.1	0.2	0.09	0.6	2
Stem 250mg/kg	**1**	67	40	43	0.890	1.900	2.000	0.510	3.50	45
	2	63	42	38	0.660	1.600	1.945	0.630	3.90	39
	3	67	50	35	0.550	1.300	1.990	0.770	4.10	41
	4	57	49	39	0.580	1.900	2.000	0.690	4.80	43
	5	57	45	29	0.580	1.900	1.699	0.647	5.00	47
	6	63	39	32	0.590	1.400	1.921	0.642	3.50	39
	Average	62	44	36	0.642	1.667	1.926	0.648	4.13	42
	SD	5	5	5	0.1	0.3	0.1	0.09	0.6	3

'Continued'

GROUP	NO	Liver Func. Test (U/L)			Lipid Profile Test (mmol/L)				Urea (mmol/L)	Creatinine (µmol/L)
		ALT	AST	ALP	TRIG	T.CHOL	HDL	LDL		
Stem 500mg/kg	**1**	55	53	35	0.550	1.700	0.930	0.570	4.10	37
	2	38	51	36	0.530	1.500	1.000	0.540	3.60	40
	3	61	49	31	0.560	1.500	1.400	0.250	4.40	44
	4	62	49	41	0.750	1.500	0.900	0.580	3.30	28
	5	50	44	39	0.760	1.700	1.350	0.530	3.20	33
	6	58	48	35	0.560	1.700	1.100	0.340	2.80	42
	Average	54	49	36	0.618	1.600	1.113	0.468	3.567	37
	SD	9	3	3	0.1	0.1	0.2	0.1	0.6	6
Root 250mg/kg	**1**	51	44	27	0.883	1.800	1.741	0.620	5.90	36
	2	60	39	25	0.690	1.800	1.725	0.628	4.60	26
	3	59	42	33	0.662	1.500	1.723	0.764	3.90	40
	4	61	53	35	0.600	1.900	1.811	0.782	4.90	34
	5	60	52	41	0.650	1.800	1.711	0.540	4.10	28
	6	55	46	35	0.869	1.900	1.883	0.750	5.20	36
	Average	58	46	33	0.726	1.783	1.766	0.681	4.767	33
	SD	4	6	6	0.1	0.1	0.07	0.1	0.7	5

‘Continued’

GROUP	NO	Liver Func. Test (U/L)			Lipid Profile Test (mmol/L)				Urea (mmol/L)	Creatinine (µmol/L)
		ALT	AST	ALP	TRIG	T.CHOL	HDL	LDL		
Root 500mg/kg	**1**	51	45	33	0.380	1.300	0.900	0.560	4.30	35
	2	57	44	33	0.430	1.400	0.795	0.590	5.20	37
	3	63	42	35	0.480	1.400	0.855	0.550	5.10	41
	4	58	44	30	0.720	1.600	0.838	0.600	4.40	26
	5	69	37	27	0.540	1.500	0.752	0.630	3.60	29
	6	64	39	34	0.780	1.600	0.999	0.580	4.60	25
	Average	60	42	32	0.555	1.467	0.856	0.585	4.533	32
	SD	6	3	3	0.2	0.1	0.09	0.03	0.6	6