CHAPTER ONE

1.1 INTRODUCTION

Diabetes mellitus is a diverse metabolic disorder which causes modification in the lipid carbohydrate, and protein metabolism (Mutalik *et al*., 2003). The disorder is caused by the shortage, lack of production of insulin secretion or reduced sensitivity of tissue to insulin, leading to overt hyperglycemia. Hyperglycemia-induced oxidative stress is a crucial etiological factor implicated in Diabetes mellitus (Pong, 2003). *In vitro* method have been employed for a long time for testing toxicity, However, *in vitro* toxicity tests became plausible due to the advancement in technology and high throughput screening. *In silico* toxicology is one type of toxicity assessment that uses computational resources to predict toxicity of chemicals and also in the organization, analysis, modelling, simulation and visualization of compounds. (Deeb and Goodarzi, 2012). *In silico* pharmacology uses the information obtained from computerized tools to analyze the beneficial or adverse effects of a drug or component for therapeutic purposes (Valerio, 2009). Evaluation of plant components in the treatment of diabetes mellitus has grown more interest, as the components contains many bioactive substances with therapeutic potential, Apart from the currently available therapeutic options for the treatment of diabetes such as oral hypoglycemic agents and insulin, which have some limitations, many herbal medicines have undergone several research and they are recommended for the treatment of diabetes mellitus (Pund *et al*., 2012). A broad number of ingredients found in medicinal plants act on different drug targets by various modes and mechanisms, they also have the potential to impart therapeutic effect in complicated disorders like diabetes and its complication (Hemalakshmi *et al*., 2012).

1.2 STATEMENT OF PROBLEM

Various components of medicinal plants have undergone several research over the years in the treatment of diabetes mellitus, but there seem to be some toxicological effects of this plants providing a short term or long term side effect. Patient with diabetes are administered drugs very often and the consumption of drugs with greater side effects often affect the patient over a long period of time. This study evaluates the effect of some toxic chemical components of *Costus spicatus* flower in the treatment of diabetes.

1.3 JUSTIFICATION OF THE STUDY

The use of medicinal plant have been understudied over time for the treatment of diabetes, *In vitro* method is the most used in the evaluation of toxicity. This research study shows how *In silico* method can be used to evaluate toxicity by the identification of the *Costus spicatus* antidiabetic molecules.

1.4 AIM AND SPECIFIC OBJECTIVES OF THE STUDY

To carry out the phytochemical analysis and *In silico* toxicological evaluation of the antidiabetic molecules in *Costus spicatus* flower .

CHAPTER TWO

2.0 LITRATURE REVIEW

2.1 DIABETES MELLITUS

Diabetes mellitus (DM) is a disease caused by hyperglycemia which results to insulin resistance and insulin secondary deficiency caused by the failure of beta- (β-) pancreatic cells. Diabetes mellitus is an endocrine disorder, which is as a result of increase in obesity, changing lifestyles, and an ageing population, and it is growing globally (Machado *et al*., 2007). Diabetes Mellitus is associated with chronic complications including microvascular, macrovascular, and neuropathic disorders. Hyperglycaemia can result from defects in insulin secretion, insulin action or both (Scobie,2007; Saqf el Hait *et al*., 2013). Chronic hyperglycaemia in diabetes is associated with damage to or failure of the blood vessels ,the eyes, kidneys, nervous system and heart (Saqf el Hait *et al.*, 2013 ; Curtis *et al*., 2008).The estimation according to the World Health Organization (WHO) shows that around 173 million adults have diabetes, and about two-thirds of these patients live in developing countries (Wild *et al.*, 2004). Despite these alarming statistics, there is no specific and definite therapy currently for diabetes. However, many chemotherapeutic drugs have been produced and are in the drug market to manage the diabetes mellitus ever since the accidental discovery of sulfonamides which possesses hypoglycemic action (Robinson and Johnston ,1997).

2.2 SYMPTOMS OF DIABETES MELLITUS

Few studies have shown the prevalence and duration of symptoms and signs of diabetes diagnosis (Colagiuri *et al*., 2002) ,the similarities between a typical diabetic patient and

symptoms of glycaemic level are blood pressure (BP) and weight (Bulpitt *et al*., 1998). Signs symptoms of diabetes mellitus can be divided into three main categories namely:

(i) those related to expression of physical symptoms e.g. polydipsia (increased thirst and consequent increased fluid intake), polyuria (Frequent urination) glycosuria (glucose in urine), polyphagia, the (extreme hunger or increased appetite), unexplained into the weight loss despite normal or increased eating, decreased of skin turgor (very dry skin), unexplained tiredness , irreducible fatigue, marked deceased in level of consciousness or dizziness, nocturia, tachycardia (fast heart rate), dehydration and dry mouth or hyposalivation. Most people experience what is generally referred to as the classical triad of diabetes mellitus symptoms i.e. polyuria, polydipsia and polyphagia or the 3P's of diabetes mellitus symptoms.

(ii) those arising from specific long term lesions of diabetes mellitus e.g. microangiopathy particularly in the eye known as retinopathy (sudden vision changes), in the kidney referred to as nephropathy and in the nerves which shows as a tingling sensation or numbness in the hands or feet a condition called neuropathy

(iii) those resulting from acceleration of increased predisposition to disease processes, e.g. atherosclerosis, frequent or recurrent skin and urinary tract infection.

2.3 TYPES OF DIABETES MELLITUS

The various types of diabetes mellitus extend across a clinical continuum of hyperglycaemia and insulin requirements (Puavilai *et al*., 1999). The classification system identifies two major types of diabetes mellitus which are : (i) Type-1- diabetes mellitus, (ii)Type-2-diabetes mellitus

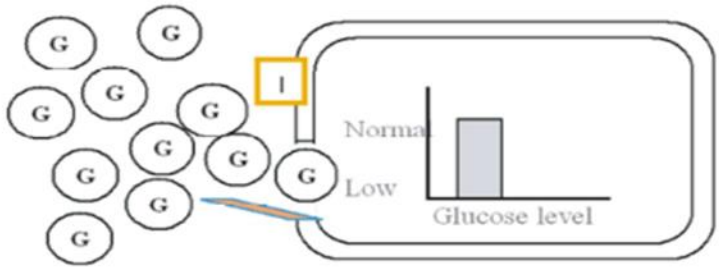

Figure 1: This illustrates a person without diabetes mellitus, the insulin that is released from the pancreas acts as a key to open the cell "door" for glucose from the blood to enter. Implicitly glucose level is low ,(I represents Insulin) and (G represents Glucose).

Source: Akeenobumatsu. (2008).

2.3.1. Type -1- diabetes mellitus

Type -1- diabetes mellitus is associated with the loss of the insulin-producing beta cells of the islets of Langerhans located in the pancreas, leading to the deficiency of insulin. This type can be further classified as immunemediated or idiopathic. Most of type 1 diabetes cases are immune-mediated, in which beta cell loss is a T-cell-mediated autoimmune attack (Rother, 2007) . Type 1 diabetes is known to affect children or adults, but was orriginally termed "juvenile diabetes" because a majority of these diabetes cases were in children (Merck Manual Professional, 2010).

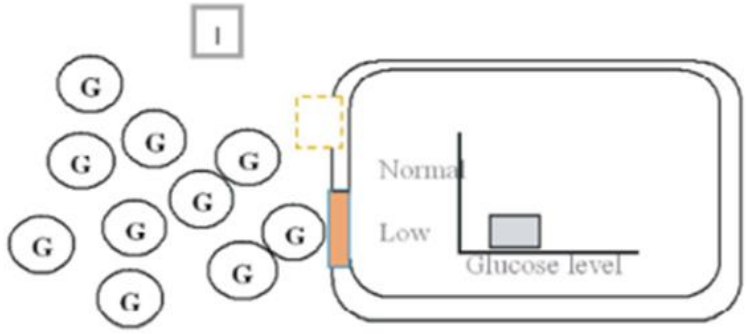

Figure 2: This illustrates type 1 diabetes mellitus, insulin, which serves a key to open cell "doors", is not present, so glucose cannot enter into the cells thus glucose level is low.

Source: Akeenobumatsu (2008).

2.3.2. Type-2- diabetes mellitus

Type-2- diabetes mellitus is insulin resistant, which can be closely associated with reduced insulin secretion in the pancreas (Gardner *et al*., 2011) The defective response of body tissues to insulin is known to involve the insulin receptor. Type 2 diabetes is the most common type, In the early stage of type 2 DM, the predominant abnormality is reduced insulin sensitivity. At this stage, hyperglycemia can be reverted by a variety of measures and drug administration that will help improve insulin sensitivity or reduce the glucose production by the liver.

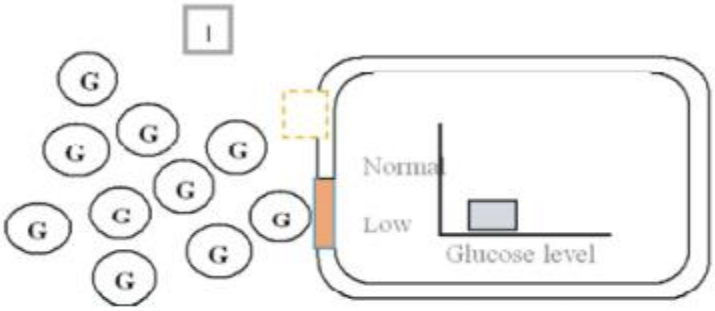

Figure 3: This illustrates that in type-2-diabetes mellitus, insulin serves as a key to open cell "doors", is present, but the receptors are unresponsive or less sensitive to it, therefore the glucose still has difficulty finding its into the cell which makes the glucose level is also low.

Source : Akeenobumatsu (2008).

2.4. TREATMENT AND MANAGEMENT OF DIABETES MELLITUS

Patients with type I diabetes mellitus requires a direct intravenous injection of insulin because the body cannot produce enough or even any insulin. Weight gain and hypoglycemia are common side effects of insulin therapy (Fowlar, 2007; Jeon *et al*., 2007). Vigorous insulin treatment also increases the risk of atherogenesis (Umpierre *et al*., 2011). In the case of type II diabetes, diabetic management requires any achievable combination depending on the patient like exercises, a combination of diet and weight loss, or Patients who can not manage or have poor diabetic control after lifestyle modifications are usually placed on oral hypoglycemic. Some type II diabetes patients who fail to respond to these measures and must then proceed to insulin therapy (Olokoba *et al*., 2012; Mohan *et al*., 2007). Type 2 diabetes cannot be completely cured but its severity and symptoms can be managed by the use of drugs and lifestyle modifications. Some of the most commonly used pharmacological agents for the treatment of type 2 diabetes include drugs from different classes such as Biguanides (Klip and Leiter, 1990), Sulfonylureas (Aquilante, 2010) , Meglitinides (ex. repaglinide and nateglinides) and thiazolidinediones (Greenfield and Chisholm, 2004). The drugs belonging to these classes are administered as the first line of defense to prevent deterioration of the diabetic state.

2.4.1 Metformin

Biguanides is an anti-diabetic agent, of which metformin is the most commonly used. Metformin suppresses hepatic production of glucose and also increases insulin sensitivity. It enhances glucose uptake by phosphorylating glucose transporter (GLUT) enhancer factor, elevating fatty acid oxidation, and reducing the absorption of glucose from the gastrointestinal tract (Olokoba *et al*., 2012). Metformin is the first line of treatment for type 2 diabetes. It is an oral antidiabetic agent that is used in the treatment of type 2 diabetes and it deals with insulin

resistance (Tripathi and Srivastava, 2006). Metformin has few side effects which include lactic acidiosis, gastrointestinal symptoms (nauseas and vomiting) (Wang *et al*., 2017).

2.4.1.1. Mechanism of action

Metformin possesses it action by decreasing level of glucose absorbed by the intestine , improvement of the peripheral glucose uptake, increase the insulin sensitivity and also lowers the fasting plasma insulin levels, which gives rise to the reduction of blood glucose concentrations thereby not causing hypoglycemia (Wang *et al*., 2017) Metformin action is initiated by the activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK), which leads to termination of glucose production through gluconeogenesis and elevated level of peripheral glucose uptake. Its glucose reducing effect is as a result of decreased hepatic glucose output (gluconeogenesis and glycogenolysis) and increased insulin induced glucose uptake and glycogenesis in skeletal muscle (Tripathi and Srivastava, 2006).

2.4.2 Repaglinide

Repaglinide is commonly available with the name of Prandin and nateglinide, which are called Starlix, it is a short-acting substances which promote the secretion of insulin and were recently approved for the management of type 2 diabetes (Rosenstock *et al*., 2004; Raskin *et* al., 2003). These drugs are shortly effective before meals in the stimulation of rapid release of insulin.

2.4.2.1 Mechanism of action

The mechanism of these classes of drugs was related to the that of sulfonylureas; repaglinide exert its efficacy by inhibiting ATP-sensitive potassium channels (KATP channel) in pancreatic β-cells, thus inducing depolarization of β-cell membranes and inflow of Ca2+ ions into the cells to stimulate insulin secretion (Gromada *et al*., 1995; Hansen *et al*.,

2002). Repaglinide is rapidly absorbed after meals, produces peaks of insulin concentrations within 1 h (t½=0.6 h). It is metabolized in the liver and excreted mainly through the fecal matter and urine (Culy and Jarvis, 2001). In recent clinical trials, rapaglinide increases insulin response to postprandial glucose, resulting in reductions of HbA1c and fasting plasma glucose (FPG) levels (Hansen *et al*., 2002).

2.5 MEDICINAL PLANTS

Medicinal plants have been used extensively in the treatment and prevention of the disease in the population in recent years, the medicinal value of plants depends on some active chemical substances that produce defined physiological or therapeutic actions in the human body (Yadav et al., 2017). Plants are considered to be a rich source of various bioactive chemicals (Narendiran et al., 2016). Secondary metabolites also known as phytochemicals from plants materials have various pharmacological activities can be antibiotic, anti-carcinogenic, anti-oxidative, antiallergic and hypoglycaemic. These secondary metabolites prevent the cells from any damages that can be caused by unstable molecules also known as free radicals (Harini and Nithyalakshmi, 2017). There have now been a fast growing interests in the use of natural antimicrobial compounds such as extracts from plants, for the preservation of foods. There is therefore the need to research on plants medicinal values (Chavan, 2017).

2.5.1 EXAMPLES OF MEDICINAL PLANTS USED IN THE TREATMENT OF DIABETES MELLITUS

2.5.1.1 *Panax ginseng*

They belong to the Araliaceae family, they are perennial plants which are known to be slow-growing with fleshy roots. The root of ginseng has been used for over 2,000 years in the Far East for its health-promoting properties (Aswar *et al*., 2008). It is found to contain secondary

metaboilites such as saponins, triterpene or glycosides. Commonly referred to as ginsenosides, polysaccharides, peptides, polyacetylenic alcohol, and fatty acids (Mishra *et al*., 2011). Ginseng polypeptide, can be isolated from the roots of *Panax ginseng*, it then decreases the level of blood sugar and liver glycogen when administered intravenously. The aqueous extract of root of *Panax ginseng* showes a remarkable hypoglycemic activity on administration to mice (Tripathi *et al*., 2011). It increases insulin production, reduces death of pancreatic β-cells and insulin resistance, improves postprandial glycemia in diabetic patients (Ranjbar *et al*., 2011).

2.5.1.2 *Momordica charantia*

They belong to the Cucurbitaceae family, commonly known as bitter gourd, bitter melon or karela, is grown in tropical countries of Asia, Africa and South America. It is a very common in folk medicine ,it is remedy for diabetes and helps in blood sugar-lowering actions. The ripe juice or unripe fruit have been ued in animal experimental models as well as human clinical trials (Sharma and Arya, 2011).The phytochemicals isolated from the plant are identified to be hypoglycemic agents ,compounds like Charantin, polypeptide-P and vicin (Chauhan *et al*., 2010).Various studies have shown hypoglycemic effect in various animal models using extract of fruit pulp, seed, leaves and whole plant of Momordica charantia (Mishra *et al*., 2011 ; Bnouham *et al*., 2006). The alcohol extracted charantin from Momordica charantia and it consists of mixture of steroids and it is found to be more potent compered to the oral hypoglycemic agent tolbutamide in an animal study. Bitter melon also contains polypeptide, polypeptide- P which looks like insulin, and have a similar structure with bovine insulin. It was found to decrease blood sugar levels when administered subcutaneously into type 1 diabetic patients and appears to inhibit gluconeogenesis and is believed to improve glucose tolerance in Type II diabetes (Kumar *et al*., 2011).

2.5.1.3.*Eugenia jambolana*

It belongs to the Myrtaceae family, it is commonly known as Jamun or black plum, is being widely used to treat diabetes by the traditional practitioners over many centuries. It is a large evergreen tree growing up to 30 m high (Kokate *et al*.,2001). Preliminary studies on seeds and decoction of dry leaves of E. jambolana have shown anti-hyperglycemic activity (Romila *et al*., 2010).The oral administration of the pulp extract of the fruit resulted in the enhancement of insulinemia in normoglycaemic and diabetic rats. The incubation of isolated pancreatic islet cells of normal and diabetic animals with this plant extracts resulted in increased insulin secretion. In addition, the extract inhibited insulinase activity from liver and kidney (Kumar *et al*., 2011).Oral administration of dried alcoholic extract of the seeds caused hypoglycemia and reduced glycosuria. In addition, the treatment also restores the altered hepatic and skeletal muscle glycogen content and hepatic hexokinase, phosphofructokinase, glucose-6-phosphate and glucokinase levels (Kavimani *et al*., 2011).

2.6 *Costus spicatus*

2.6.1 Classification of *Costus spicatus*

Costus spicatus (Jacq) Swis a herbaceous species with neotropical distribution belonging to the family Zingiberaceae (Specht and Stevenson., 2006). Zingiberaceae is a family with about fifty two genera and more than 1,300 species distributed across tropical Africa, Asia, and the Americas (Abou and EL-far, 2013), they are sometimes branched with aerial shots that have a characteristic monistichous (one-sided) spiral phyllotaxy (Kirchoff and Rutishauser, 1990),

Costus spicatus was formerlyin the family of Costaceae was placed as a subfamily within the larger Zingiberaceae family due to broad similarities of inflorescence and floral characters (Specht *et al* ,2006). *Costus Spicatus* is known popularly as "cana-do-brejo," in Brazil , the common names in english are spiked spiralflag ginger , cockscrew ginger and Indian head ginger (Specht *et al.,* 2006).

Table 1: Taxonomic classification *Costus spicatus*

Source: Singh *et al* (2011).

ITEM	NAME
Kingdom	*Plantae*
Subkingdom	*Tracheobionta*
Super division	*Spermatophyta*
Division	*Magnoliophyta*
Class	*Liliopsida*
Sub class	*Zingiberidae*
Order	*Zingiberales*
Family	*Costaceae*
Genus	*Costus*
Species	*Spicatus*
Other scientific names	
Alpina spicata Jacq.	
Amomum petiolatum Lam.	
Costus cylindricus Jacq.	
Coustus micranthus Gagnep.	

2.6.2 CULTIVATION

Costus spicatus is mostly cultivated during the rainy seasons, they are also known to grow well on rich moist soil like clayey and loam soil in a shady area (Muniyandi *et al*., 2013).The plant grows in the climate with high humidity and low temperature. *Costus spicatus* propagation is in

different methods like, vegetative methods using rhizome pieces (Srivastava, 2011), division of culms, stem cuttings or via seeds dispersed by birds.

2.6.3 DISTRIBUTION

The cane of the swamp (*Costus spicatus* Jacq.) Zingiberaceae family species, also known as cane of the bush or cane monkey (Silva, 2003), is a perennial plant whose aerial part can reach 1.0 to 2.0 meters in height, native throughout most of Brazil, Dominican republic and mainly in the Atlantic Forest and Amazon region (Sarin *et al*., 1974),and can be found also in countries like Haiti ,Mexico, Guadleloupe ,Trinidad and Tobago (Wabale *et al*., 2011).

2.6.4 MORPHOLOGY

It is characterized for being a perennial, rhizomatous, unbranched, erect, which can reach 1.0 to 2.0 meters in height. The leaves are spirally arranged with invaginating the extension base with terminal inflorescence (Petersen, 1990). The plant can grow up to 1.2m height in frost free areas but they are usually found to grow up to about 1.8 m tall in cooler areas where its roots get dried up and dies back during the dry season. The plant flowers during the raining seasons , the aerial parts withering away during the dry season (Nehete *et al*., 2010), the flower is shaped in a red upright cone, thus the common name “Red head ginger” ,the flower is up to 2 to 3 cm long bracts spiral dense, intertwined, glabra, and red (Petersen, 1990).

2.6.5 TRADITIONAL USAGE OF *Costus spicatus*

Costus spicatus is used because of its diuretic and purifying action, to expel kidney stones and for the relief of urinary infections (Lorenzi and Matos, 2002), the plant can also be used in the treatment of sore throat, colds and dysentery. The rhizome of the plant is used in the treatment of

complaints of the bladder and urethra and to expel kidney stones. Their use in traditional medicine includes the use of leaves, stems and rhizomes as a diuretic and tonic (Lorenzi and Matos, 2007). In Brazilian folk medicine, *Costus spicatus* tea is used with depurative purposes (Borrás, 2003). (Boorhem *et al*., 1999) describe the decoction of the vegetative plant parts of the species active in the treatment of vaginal irritation, leucorrhea and ulcers. The juice of fresh stem dilute is effective in the treatment of gonorrhea, syphilis, nephritis, insect bites, bladder problems and diabetes (Borrás, 2003). The sheets can be combined with Bonamia ferruginea "vine-Tuira" (Choisy) Hallier in the form of a potion in the treatment of malaria and hepatitis (Silva, 1998).

2.7 PHARMACOLOGICAL ACTIVITY OF *Costus spicatus*

Pharmacological research shows that *Costus spicatus* leaf extract of decoctions, as well as aqueous or alcoholic infusions, are commonly used for the treatment of gonorrhea, renal calculi, infections, cutaneous ulcers, leucorrhoea, urethritis, and inflammation (Carriconde *et al*., 1996). A large number of anti-diabetic medicines are now available in the drug market for the treatment diabetes and their related complications. However, due to unwanted side effects of this drugs, the efficacies of these compounds are questionable and there there is a need the development of new compounds for the treatment of diabetes (Moller, 2001; Oubre *et al*., 1997).

2.7.1 Anti-inflammatory activity

Inflammation is a physiopathological response of tissues injuries that is closely related to the pathological process of various inflammatory diseases (Vazquez *et al*., 2011), Regarding the adverse side effects of synthetic and chemical drugs, several medicinal plants were used as an alternative source with little side effects, *Costus spicatus* have been shown to exhibit potent anti-inflammatory effects (Gomase *et al*., 2011; Srivastava *et al*., 2013). The anti-inflammatory activity of methanol extract of *Costus spicatus* was evaluated using carrageenan-induced paw

oedema test, Results revealed that methanol extracts of *Costus spicatus* has significant anti-inflammatory, analgesic and antipyretic activities. the rhizome of *Costus spicatus* has been known traditionally in treatment inflammatory and painful conditions, The ethanolic extract of the rhizome of *Costus spicatus* possesses antipyretic properties and anti-inflammatory (Binny *et al*., 2010).

2.7.2 Antioxidant activity

Antioxidants are a group of substances that marks the stop of an oxidation processes by scavenging free radicals and also the induction of cellular antioxidant enzymes (Daisy *et* al., 2016). Oxygen tends to accept their electrons one at a time, leading to the development of reactive oxygen species (ROS) (Navrot *et al*., 2016). ROS molecules plays a role in oxidative stress involved in atherosclerosis, cancer, cirrhosis, and diabetes (Nehete *et al*., 2010). Flavonoids are present in *Costus spicatus* plant, which could provide protection for living organisms against ROS hazards due to their redox properties including free-radical scavenging and strong metal ions chelation (Govindarajan *et al*., 2005).

2.8 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

GC-MS is an analytical technique that combines the separation properties of gas-liquid chromatography with the detection characteristics of mass spectrometry to identify various substances or components within a test sample (Chauhan *et al*., 2014). Gas chromatography can separate both volatile and semi-volatile compounds with large resolution, but it cannot identify them while mass spectrometry can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot, readily separate them (Hussain and Maqbool, 2014). It is used to determine drugs and their metabolites in the pharmaceutical area, and molecular weights and elemental composition in complex mixtures. It is used in the

determination of volatile and semi- volatile organic compounds in mixture (Sneddon *et al*., 2007).

2.8.1 Principle of GC-MS

GC/MS works by combining two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS) in the analysis of complex organic and biochemical mixtures. The GC-MS instrument consists mainly of two main parts. The gas chromatography portion is known to separate different compounds in the sample into forms of pure chemicals based on their volatility by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column. Spectra of compounds are collected as they exit a chromatographic column by the mass spectrometer. The separation of the phase ions is achieved within the mass spectrometer by using an electrical and magnetic fields to distinguish the ions. The mass spectrometer identifies and then quantifies the various chemicals according to their various mass-to-charge ratio (m/z). These spectra can be stored and then computerized for analysis (Hussain and Maqbool, 2014). A plot of this signal as a function of time generates a series of symmetrical peaks in a chromatogram, which provides some information on the sample composition. The time of retention of the peaks can help to identify the sample constitutes by comparing it to the retention time of various standards, The heights of the peaks or the area under the peaks provides a quantitative measure of the amount of each component (Hussain and Maqbool, 2014).

2.9 UV-SPECTROSCOPY

Ultraviolet (UV) spectroscopy is a physical which uses techniques like optical spectroscopy that uses light in the visible, ultraviolet, and near infrared ranges (Pavia *et al*., 2001). The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/VIS spectroscopy can be used to determine the concentration of the absorber in a solution. It is necessary to know how rapidly the absorbance changes with concentration (Skoog *et al*., 2004). Ultraviolet and visible spectrometers have been used generally for the last 35 years and over time it has become the most important analytical tool or instrument in the modern day research laboratory (Sharma, 2004).In various applications other techniques could have been employed but none is more efficient compered to UV-Visible spectrometry because of its simplicity, versatility, speed, accuracy and cost-effectiveness.

2.9.1 Principle of UV-Spectroscopy

A molecules will exhibit absorption in visible or ultraviolet region when radiation causes an electronic transition within its structure. Thus, the absorption of light by a sample in the ultraviolet or visible region co exists together by a change in the electronic state of the molecules in the sample. Light supplies an energy which excites the electrons from their ground state orbital to a greater energy, excited state orbital or anti-bonding orbital. Potentially, three types of ground state orbitals may be involved, which are :σ (Bonding) molecular orbital, π (Bonding) molecular orbital, n (non-Bonding) atomic orbital. In addition, two types of anti-bonding orbitals may be involved in the transition which are :σ* (sigma star) orbital, π* (pi star) orbital (Donald *et al*., 1994). The wavelength of maximum absorption and the intensity of absorption are determined by molecular structure. Transitions to π* antibonding orbitals which occur in the ultraviolet region for a particular molecule may well take place in the visible region if the

molecular structure is modified. Many inorganic substances in a giving solution shows the absorption in visible region. These includes various elements like salt which do not have complete electrons in their innermost shell (mainly transition metals) whose ions are joined together by hydration e.g. [Cu(H204)]2+. The absorption emerge from a transfed charge process, where electrons can move from one part of the system to another by the energy given of by the visible light (Skoog *et al*., 2004).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1 Collection of Plant Material

Fresh flowers of *Costus Spicatus* plant were collected at the chapel, library and hostel area of Mountain Top University. The plant was identified at the Botany Department of the University of Lagos, where a voucher specimen (Number 8571) was prepared and deposited.

3.2. METHODOLOGY

3.2.1. Preperation of Aqueous Extract

The identified sample were toughly rinsed under running water to remove contaminants, oven dried at 50 degree Celsius when it attains a constant weight it was pulverized using an electric blender . The Pulverized flower (350g) was weighed and 2600ml of distilled water, kept in a container with a lid and placed in a cupboard for 48hours. It is then sieved with a cheesecloth the extract was poured in a container, wathman's filter paper and funnels where then used to further separate concentrate from the residue .The residue was collected in beakers and placed in the

oven to concentrate the extract which yielded 47.57g (15.85% yield). The concentrates was then stored in a refrigerator at −4°C.

3.2.2 Qualitative Phytochemical analysis

The aqueous flower extract was tested for the presence of bioactive compounds using standard methods as described by Trease and Evans (1989) with slight modification.

Test for alkaloids (Mayer's test)

0.5 g of methanol leaf extract of *V. amygdalina* (crude extract) was dissolved in 5mls of distilled water. 2 ml of 1% hydrochloride (HCl) was added and heated gently. 3 ml of Mayer's reagent was

added to the mixture. Turbidity of the resulting precipitate indicated the presence of alkaloids

Test for carbohydrates (Molisch's test)

0.5 g of crude extract was dissolved in 5 ml of distilled water. 2mls of Molisch reagent was added and the mixture was shaken properly. 2 ml of conc. sulphuric acid (H_2SO_4) was poured carefully along the side of the test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrate.

Test for saponin (Froth test)

0.5 g of crude extract was diluted with distilled water to 20 ml and was shaken in a graduated cylinder for 5 mins. Formation of foam indicated the presence of saponin.

Test for terpenoids (Salkowski's test)

0.5 g of crude extract was dissolved in 5 ml of distilled water. 2 ml of chloroform was added and 3 ml of conc. H_2SO_4 was carefully added to form a layer. The appearance of reddish brown coloration at the interphase indicated the presence of terpernoids.

Test for phenol (Ferric chloride test)

0.5 g of crude extract was dissolved in 5 ml of distilled water and 4 drops of ferric chloride ($FeCl_3$) solution was added. The formation of bluish black color indicated the presence of phenol.

Test for tannin

0.5 g of crude extract was dissolved in 5 ml of distilled water and 2ml of 2% $FeCl_3$ solution was added. The formation of blue-green coloration indicated the presence of tannin.

Test for protein (Ninhydrin test)

0.5 g of crude extract was dissolved in 5 ml of distilled water. 2 ml of 0.2% Ninhydrin reagent was added and the mixture was boiled for 5 mins. The formation of violet/blue color indicated the presence of amino acids.

3.2.3 GC-MS ANALYSIS

The GC-MS analysis was carried out using a Hewlett Packard Gas Chromatograph (Model 6890 series) equipped with a flame ionization detector and Hewlett Packard 7683 series injector, MS transfer line temperature of 250°C. The GC was equipped with a fused silica capillary column-HP-5MS (30 x 0.25 mm), film thickness 1.0 μm. The oven temperature was held at 50°C for 5 min holding time and raised from 50 to 250°C at a rate of 2°C /min, employing helium gas (99.999%) as a carrier gas at a constant flow rate of 22 cm/s. 1.0 micron of extract (1 mg dissolved in 1 ml absolute alcohol), at a split ratio of 1:30 was injected. MS analysis was carried out on Agilent Technology Network Mass Spectrometer (Model 5973 series) coupled to Hewlett

Packard Gas Chromatograph (Model 6890 series) equipped with NIST08 Library software database. Mass spectra were taken at 70 eV/200°C, scanning rate of 1 scan/s. Identification of compounds was conducted using the database of NIST08 Library. Mass spectrum of individual unknown compound was compared with the known compounds stored in the software database Library (Ajayi *et al.*, 2011).

3.2.4 UV-visible spectrophotometry

The absorbance and wavelength of the peaks were determined for the aqueous flower extract by a wavelength scan between 200 and 227 nm (Rice-Evans and Miller, 1996). The UV-visible spectra were recorded on a (Lamotte SMART Spectro 2, China) UV-Vis spectrophotometer.

3.2.5 Molecular docking study on selected anti-diabetic phytomolecules

3.2.5.1 Molecular Docking

Docking of the ligands to various protein targets and determination of binding affinities was carried out using Vina (Trott & Olson, 2010). Pdbqt format of the receptors, as well as those of the ligands, was dragged into their respective columns and the software was run. The binding affinities of compounds for the three protein targets were recorded. The compounds were then ranked by their affinity scores. Molecular interactions between the receptors and compounds with most remarkable binding affinities were viewed with Discovery Studio Visualizer, BIOVIA, 2020.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Analysis

The result of the phytochemical analysis carried out on the aqueous extract of *C. spicatus* flower revealed the presence of some important bioactive components which is shown in Table 2.

Table 2: Qualitative analysis of aqueous extract of *Costus spicatus* flowers

SECONDARY METABOLITES	FLOWER
Carbohydrate	++
Tannins	- -
Alkaloids	++
Phenols	- -
Flavonoids	++
Proteins	++
Terpenoids	- -
Anthraquinone	- -

Present ++; Absent –

4.2 UV-spectroscopy analysis of aqueous extract of *C. spicatus* flower

The Ultra violet -Visible spectroscopy of aqueous extract *Costus spicatus* flower revealed the varying absorbance of the flower extract at different wavelengths as shown in Figure 4.

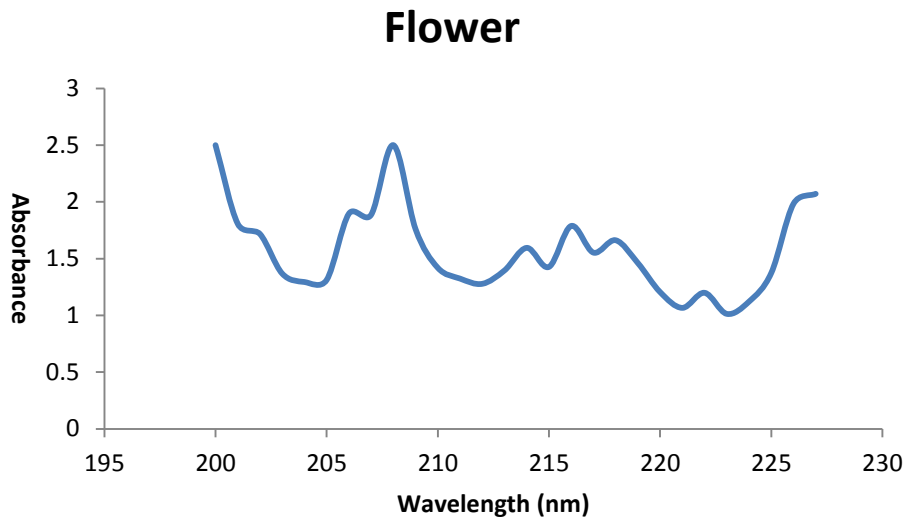

Figure 4: Ultra violet -Visible spectroscopy of Costus spicatus flower

4.3 Gas chromatography-Mass spectrometry (GC-MS) analysis

4.3.1 Chromatogram of aqueous extract of *C. spicatus* flower

Figure 2 shows the GC-MS chromatogram of aqueous extract of *C. spicatus* flower. Peak 1 with the retention time of 15.279 was identified as Hexadecanoic acid, methyl ester and as the major phyto-component of *C. spicatus* while the other peaks were of other phyto-components present in the plant.

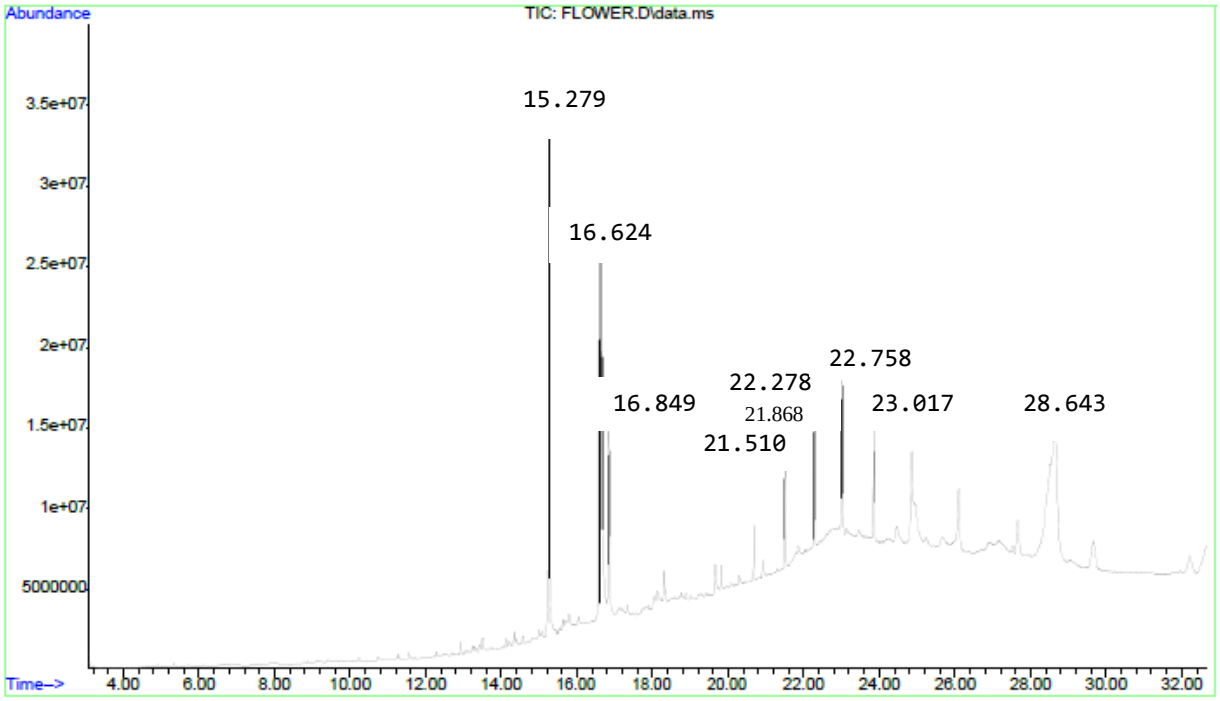

Figure 5: GC-MS chromatogram of aqueous extract of *C. spicatus* flower

4.3.2 Phyto-component of aqueous extract of *C. spicatus* flower

Other phyto-components of aqueous extract of *C. spicatus* flower other than Hexadecanoic acid, methyl ester were identified. The phytochemical components identified in the aqueous extract of *C. spicatus* flower by GC-MS showing their peak, retention time, library ID, % of total and chemical formula is shown in Table 3.

Table 3: Phytochemical components identified by GC-MS in aqueous extract of *C. spicatus* flower showing the peak, retention time, library ID, % of total and chemical formula

Peak	Retention time	Library ID	Area %	Chemical formula
1	15.279	Hexadecanoic acid, methyl Ester	11.34	$C_{17}H_{34}O_2$
2	16.624	9,12-Octadecadienoic acid, methyl ester, (E,E)-	13.21	$C_{19}H_{34}O_2$
3	21.510	Cyclononasiloxane, octadecamethyl	1.49	$C1_8H_{54}O_9Si_9$
4	21.868	Carbonic acid, but-3-en-1-yl penta decyl ester	1.62	$C_{20}H_{38}O_3$
5	22.278	Cyclononasiloxane, octadecamethyl	2.11	$C_{18}H_{54}O_9Si_9$
6	22.758	[1,2,4]Triazolo[1,5-a]pyrimidine-6 -carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester	2.47	$C_8H_9N_5O_2$
7	23.017	Cyclononasiloxane, octadecamethyl-	3.28	$C_{18}H_{54}O_9Si_9$
8	28.643	Cyclohexane, 1,1'-(2-methyl-1,3 propanediyl)bis-	18.66	$C_{16}H_{30}$

4.4 Adsorption, Distribution, Metabolism, Excretion and Toxicological properties of potential inhibitors of selected compounds of Aqueous Extract of *Costus spicatus* flower

The Adsorption, Distribution, Metabolism, Excretion and Toxicological (ADMET) properties of selected compounds in Aqueous Extract of *Costus spicatus* flower and reference drugs, Metformin and Repaglinide, on potential inhibitors of antidiabetic targets are shown in Table 4.

Table 4: ADMET properties of potential inhibitors of antidiabetic targets

		ABSORPTION	

		Intestinal absorption (%)	Water solubility (log mol/L)	Skin Permeability (log Kp)	P-glycoprotein substrate	P-glycoprotein I inhibitor	P-glycoprotein II inhibitor
R	Metformin	59.40	-2.657	-2.74	YES	NO	NO
R	Repaglinide	99.12	-3.69	-2.74	YES	NO	NO
1	Methyl 6-O-[1-methylpropyl]-beta-d-galactopyranoside	74.19	-0.83	-3.25	NO	NO	NO
2	d-Lyxo-d-manno-nononic-1,4-lactone	22.72	-1.15	-0.33	YES	NO	NO
3	Galacto-heptulose	21.88	-1.32	-2.94	NO	NO	NO
4	Oleic acid	91.77	-5.69	-2.52	NO	NO	NO
5	β-sitosterol	94.44	-6.93	-2.76	NO	YES	YES
6	Ethyl iso-allocholate	96.84	-5.19	-4.18	YES	YES	YES
7	Stigmasterol	94.41	-6.93	-2.76	NO	NO	YES
8	Vitamin E	90.97	-7.72	-2.69	NO	NO	YES
		DISTRIBUTION					

		VDss (human) (log L/kg)	Fraction unbound (human)	BBB permeability	CNS permeability		
R	Metformin	-0.33	0.88	-0.80	-4.24		
R	Repaglinide	-1.52	0	-0.027	-2.28		
1	Methyl 6-O-[1-methylpropyl]-beta-d-galactopyranoside	-0.41	0.78	-1.21	-3.89		
2	d-Lyxo-d-manno-nononic-1,4-lactone	-0.03	0.85	-1.3	-4.31		
3	Galacto-heptulose	-0.37	0.91	-1.23	-3.84		
4	Oleic acid	-0.57	0.046	-0.18	-1.65		
5	β-sitosterol	0.12	0	0.79	-1.61		
6	Ethyl iso-allocholate	-0.16	0.065	-0.81	-2.29		
7	Stigmasterol	0.14	0	0.79	-1.61		
8	Vitamin E	0.99	0	0.97	-1.60		
		METABOLISM					

		CYP2D6 substrate	CYP3A4 substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP3A4 inhibitor
R	Metformin	NO	NO	NO	NO	NO	NO
R	Repaglinide	NO	NO	NO	NO	YES	NO
1	Methyl 6-O-[1-methylpropyl]-beta-d-galactopyranoside	NO	NO	NO	NO	NO	NO
2	d-Lyxo-d-manno-nononic-1,4-lactone	NO	NO	NO	NO	NO	NO
3	Galacto-heptulose	NO	NO	NO	NO	NO	NO
4	Oleic acid	YES	YES	YES	NO	NO	NO
5	β-sitosterol	NO	YES	NO	NO	NO	NO
6	Ethyl iso-allocholate	NO	YES	NO	NO	NO	NO
7	Stigmasterol	NO	YES	NO	NO	NO	NO
8	Vitamin E	NO	YES	NO	NO	NO	NO
		EXCRETION		**TOXICITY**			

		Total Clearance (log ml/min /kg)	Renal OCT2 substrate	Max. tolerated dose (log mg/kg/day)	AMES toxicity	Hepatotoxicity	Skin sensitization
R	Metformin	0.1	NO	0.811	YES	NO	YES
R	Repaglinide	0.81	NO	0.325	NO	YES	NO
1	Methyl 6-O-[1-methylpropyl]-beta-d-galactopyranoside	1.58	NO	1.56	NO	NO	NO
2	d-Lyxo-d-manno-nononic-1,4-lactone	0.82	NO	1.92	NO	NO	NO
3	Galacto-heptulose	0.95	NO	1.81	NO	NO	NO
4	Oleic acid	1.88	NO	-0.94	NO	YES	YES
5	β-sitosterol	0.63	NO	-0.50	NO	NO	NO
6	Ethyl iso-allocholate	0.75	NO	-0.93	NO	NO	NO
7	Stigmasterol	0.62	NO	-0.54	NO	NO	NO
8	Vitamin E	0.80	NO	1.09	NO	NO	NO

VDss: Steady-state volume of distribution, BBB: Blood-brain barrier, CNS: Central nervous System, OCT2: Organic cation transporter 2, AMES: *Salmonella typhimurium* reverse mutation assay, MTD: Maximum tolerated dose in human

CHAPTER FIVE

5.0 Discussion, conclusion and recommendation

5.1 Discussion

The phytochemical identification of plant is of paramount importance in justifying their acceptability in the modern system of medicine. *Costus spicatus* has many medicinal and Therapeutic action and scientifically validated documents. The phytochemical characters of the *Costus spicatus* flower were investigated, The qualitative phytochemical analysis of aqueous extract of *Costus spicatus* flower extract contains alkaloids, flavonoids, carbohydrate, proteins which important in disease prevention and health preservation. The GC-MS chromatogram of

aqueous extracts of *Costus spicatus* is shown in Figure 5. A total of eight compounds identified from the aqueous extract of the *Costus spicatus* flower are shown in table 3.All these compounds are of pharmacological importance as they possess the properties such as anti-diabetic, analgesic, antibacterial, and antifungal activity.

The qualitative UV-spectrophotometry profile of aqueous extract of *Costus spicatus* flower shown in figure 4 was taken at the wavelength of 200nm to 227nm due to the sharpness of the peaks,the profile shows the peaks at 200,206,214,216,218,218,222 and 227nm with the absorption 2.5,1.9,2.5,1.7,1.6,1.5,1.2 and 2.2 respectively ,the appearance of more than one peck indicates the presence of unsaturated groups. The spectrum shows the highest peaks at 200nm and 214nm which shows the presence of organic chromospheres within the *Costus spicatus* extract.

The ADMET properties of two reference antidiabetic drug and eight phytomolecules identified by this study are shown in Table 3. Molecules with less than 30% absorbance are considered to be poorly absorbed in the intestine (Pires et al., 2015). Consequently, d-Lyxo-d-manno-nononic-1,4-lactone and galactoheptulose are poorly absorbed (<30%). On the contrary, Methyl 6-O-[1-methylpropyl]-beta-d-galactopyranoside and oleic acid possesses good absorption properties with a 74 and 93% value. However, all lead compounds identified in the flower possessed good intestinal absorptive property with over 90% rate of absorption. The high percentage absorption observed for compounds isolated from the flower may be as a result of the inhibition of P-glycoprotein I and II (Table 3). P-glycoprotein forms an aqueous transmembrane pore through which drugs are actively transported from the cytosol to the extracellular media (Lin & Yamazaki, 2003). Therefore, the ability of compounds to inhibit this protein is crucial to the bioavailability of drugs as increased intestinal expression of P-glycoprotein could reduce the

absorption of compounds that are P-glycoprotein as observed in this study. Of all compounds, Methyl 6-O-[1-methylpropyl]-beta-d-galactopyranoside, d-Lyxo-d-manno-nononic-1,4-lactone, and galactoheptulose are not BBB and CNS permeants due to their low lipophilicity as compounds with log BB less than -1.0 and log PS less than -3.0 are considered to be poorly distributed in the brain and central nervous system respectively. This suggests that the compounds do not possess neuromodulatory ability and may effectively get to other targets.

The volume of distribution at steady state (VDss) is the volume of blood necessary to hold the compound present in the body at the concentration observed in the vascular compartment (Yates & Arundel, 2008). The higher the VDss value the more likely the drug is distributed in the tissue rather than plasma, results obtained showed that four lead compounds from the flower had lower theoretical dose required for uniform distribution in the plasma.

Cytochrome P450 (CYP) enzymes are responsible for the metabolism of most clinically used drugs (Ota et al., 2015). Out of over 50 known CYP enzymes, CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 enzymes metabolize about 90% of drugs (Lynch & Price, 2007). This study shows that the eight hit compounds may not inhibit most of the cytochrome enzymes. This suggests that the compounds may not cause drug to drug interaction associated with the inhibition of CYP enzymes. Several drugs have been withdrawn from the market because metabolic inhibition by other drugs led to life-threatening conditions (Dresser et al., 2000).

None of the eight hit compounds are substrate for renal OCT2 substrate, a poly-specific, bi-directional, facilitative diffusional transporter predominantly expressed on the basolateral membrane of kidney proximal tubule. Consequently, the predictive mode of elimination of the compounds is via sweat, bile, and faeces.

The maximum tolerated dose provides an estimate of the toxic dose threshold of drugs in humans. As a standard, a maximum tolerated dose lower than or equal to 0.477 (log mg/kg/day) is considered low and high if more than 0.477 (log mg/kg/day). Consequently, the eight compounds identified in this study may only be administered at low doses due to their low maximum tolerated dose. This property is normal for potent drugs as ideal drug candidates are effective at low doses. AMES toxicity is widely employed to assess the mutagenic potential of compounds using bacteria. Results obtained from the AMES study suggests that the compounds may not be mutagenic and therefore may not act as carcinogens. Also, seven of the eight compounds may not be hepatotoxic and may not be sensitive to the skin as shown in the predictive toxicity test while oleic acid may be hepatotoxic and sensitive to the skin.In all, the seven compounds possessed reasonable pharmacokinetic properties expected of promising drugs that could further be explored using other models prior to their enlistment as antidiabetic agents.

5.2 Conclusion

The field of *in silico* toxicology has been in a continuous usage through and new methods have been introduced to improve of the existing ones. *In silico* research tools can be very effective in assessing chemicals toxicity. From the results of this study, it is speculated that the aqueous extract of *Costus spicatus* flower has a potential hypoglycemic action could be related to the synergic action of molecules with antioxidant profile which can enhance the secretion of insulin

by the pancreas and the increase in absorption of tissue glucose level and enhancing peripheral glucose utilization and *Costus spicatus* also has reduced toxicological effects.

5.3 Recommendation

This research supports various potentials of *Costus spicatus* and its therapeutic role in the treatment of diabetes, this research also opens new research areas. Furthermore, it covers new ways to explore the compounds responsible for these therapeutic effects. However, these data must be further studied on the molecular basis. Therefore, I recommend the following research studies:

• *Costus spicatus* safety assessments of different extracts of various plant parts.

• Therapeutic possibilities of different solvents of *Costus spicatus* extract such as hexane extract, methanol extract, ethanol extract, and chloroform extract, and the active components either alone or in combination with drugs.

• Mechanism of action of *Costus spicatus* and its active constitutes in the production of drugs for treatment of diabetes mellitus.

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