TOXICOLOGICAL AND SPECTROSCOPIC EVALUATION OF SELECTED HERBAL PREPARATIONS (PAXHERBAL DIARTH AND ANTI DIABETES) SOLD IN MINNA AND SURBURBS

BY

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MTech/SLS/2018/8126

THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGER-STATE, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY IN BIOCHEMISTRY

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DECLARATION

I hereby declare that this thesis title: **"Toxicological and Spectroscopic Evaluation of Selected Herbal Preparations (Paxherbal Diarth and Anti Diabetes) sold in Minna and Surburbs"** is collection of my original research work and has not been submitted or presented in part or full for any other diploma or degree of this or any other university. Information from other sources (published or unpublished) has been duly acknowledged.

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CERTIFICATION

This thesis titled: **"Toxicological and Spectroscopic Evaluation of Selected Herbal Preparations (Paxherbal diarth and Anti Diabetes) Sold in Minna and Surburbs"** by ELIMIAN Isaac (MTech/SLS/2018/8126) meets the regulations governing the award of M.Tech of the Federal University, Minna and it is approved for its contribution to scientific knowledge and literary presentation.

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ABSTRACT

Traditional medicine refers to health practices, approaches, knowledge and believes incorporating plant and mineral based medicines, spiritual therapies to treat, diagnose and prevent illness. Hence, traditional medicinal preparations having antidiabetic properties can provide useful sources for the discovery of safer hypoglycaemic agents.The two sample preparations (paxherbal diarth and antidiabetes) were analyzed for phytochemical content, as well as their hyperglycaemic and toxicity study.Qualitative and quantitative screening were carried out according to standard procedures, acute and sub-chronic toxity study were carried out according to standard procedures, GC-MS and FTIR were carried out using mass spec analyzer and FTIR spectrometer respectively, histopathology were carried out through microscopic staining according to standard procedures. Lorke's method was used for the acute toxicity testing, GC-MS and FTIR were used to ascertain the bioactive compounds present in the two liquid preparations. The qualitative and quantitative analysis confirmed the presence of various phytochemicals viz: tannins, flavonoids and saponins which had the ability to protect diabetes from oxidative damage. Acute toxicity evaluation indicates there was no mortality even at higher dose of 5000 mg/kg bodyweight for both liquid preparations. For sub-chronic toxicity, there was no mortality and there was no significance ($p>0.05$) difference in the organ weight while there was a significance ($p<0.05$) increase in body weight (g). There was no significance ($p>0.05$) difference in the liver function parameter; total protein (g/L), total bilirubin (mg/dL), direct bilirubin (μmol/L), urea (mg/dL) and creatinine (mg/dL) of the albino rat which indicates that the liquid preparations were not toxic. GC-MS analysis confirmed the presence of different bioactive compounds in the various sample liquid preparations. Sample A (Paxherbal Diart) showed the presence of ethanamine, 2, 2-diethoxy, pentane, 3-ethyl-2,2-dimethyl, propanenitrile, 2- hydroxyl and oleic acid. Sample B (Anti diabetes) showed the presence of Oleic acid, 1,5- Methano-1H, 7,11H-furo[3,4-g]pyranol[3,2-b]xanthenes-7,15-dione,3,3a,4,5-tetrmethyl-1-13-bis(3-methyl-2-buten-1-yl)-, (1R,3As,5S,14As) and 4-tert-Butyl-O-phenylenediacetate. The diabetic rats induced with alloxan treated with sample A and sample B preparations resulted in significant ($p<0.05$) decrease in the blood glucose level (sample A in group 3 shows from the week 1 to final week there was a decrease from 118.03±2.036 to 114.00±0.000 and the sample B in group 3 shows from the week 1 to final week there was a decrease from 110.57±0.003 to 101.37±0.003) when compared with the negative control group that were induced with alloxan without treatment (a rise from 121.03±0.003 to 158.38±0.003) . There were no significant differences ($p>0.05$) in the blood glucose level of the treatment groups (Alloxan + sample A and B preparations) when compared with the metformin treated group of albino rats (from 112.31±0.003 to 114.31±0.000. This study revealed that both samples A and B preparation were not toxic and are found to be efficacious against hyperglycaemia due to the presence of phytochemicals present in both herbal preparation which can be use in the prevention and treatment of oxidative stress related illness like diabetes mellitus.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Diabetes mellitus is a disorder in which the body does not produce enough or respond normally to insulin, causing blood glucose levels to be abnormally high. A weak or insufficient insulin secretory response is a defining feature of diabetes mellitus, which causes impaired glucose utilization and consequent hyperglycemias (Nishita *et al.,* 2016). Diabetes mellitus (DM) is commonly referred to as "sugar" and it is the most common endocrine disorder usually occurs when there is deficiency or absence of insulin or rarely, impairment of insulin activity (insulin resistance). Both the hormones insulin and glucagon are released by the pancreas. Insulin is secreted by the beta (ß) cells in the islets of langerhans, and the alpha (α) cells in those same organelles secrete glucagon. Insulin decreases blood sugar levels by promoting glycogenesis and supplying glucose to the muscles, liver, and adipose tissue (Svendsen *et al*., 2018). While alpha (α) cells are important for controlling blood sugar by producing glucagon and increasing blood sugar level by speeding erythrocytes, brain tissue, and glycogenolysis don't need insulin for glucose use. In addition to increased risk of obesity, metabolic and cardiovascular disorders, and malignancy in future life of fetus after delivery (Shemer *et al*., 2015). 80% to 90% of all instances of diabetes mellitus are caused by type II diabetes. Geographical variation can affect the severity of the issues as well as overall morbidity and mortality rates (Varlamis *et al.,* 2017). Additionally, those with diabetes who engage in modest physical exercise have a negligibly decreased chance of passing away than inactive people. The growing prevalence of diabetes and other non-communicable diseases is one of the greatest health threats to economic developments afflicting WHO. Diabetes is

characterized by an abnormality in the production or insulin secretion, as is the case with Type 1 diabetes mellitus (T1DM) and pancreatic duct stenosis, or by the emergence of insulin resistance or abnormal insulin production, as seen in Type 2 diabetes (T2DM) and some secondary diabetes (Azeez *et al*., 2022).

1.2 Statement of the Research Problem

Diabetes mellitus (DM) is a disorder that affect human health in the 21st century and the number of diabetes has risen globally. In 2019, Diabetes accounted for 463 million adults aged 20 to 79 worldwide, this amount is anticipated to increase to 578 million by the year 2030. Diseases resulting from diabetes include, heart disease, stroke, kidney disease, eye problems, dental disease, nerve damage, feet problems and loss of limbs. As stated by a study from world health organization, the number of diabetic patient from 171 million in 2000 to 366 million, according to projections or more by 2030. In 2017, Africa's total diabetes prevalence was 3.3 percent, with Nigeria reporting the highest number of diabetes, with an estimated 3.9 million adults aged 20 to 79 suffering from the disease (WHO, 2017). Many people whose body parts have been amputed as a result of this disorder (Diabetes mellitus) is a concern today for human health. Clinical available anti-diabetic drugs have shown a vast array of side effects and toxicity coupled with public health and economic burden. Many people in developing countries depend on herbal traditional medicine for the treatment of metabolic diseases such as diabetes mellitus because of the presence of wide range of bioactive phytochemical compounds in plants, despite their widespread use, natural plant products have some drawbacks including the presence of potential carcinogenic agents, toxicity and are also associated with organ damage. Diabetes mellitus has been linked to oxidative stress which arises from the excessive production of free radicals in the mitochondrial electron transport chain.

1.3 Justification for the Study

Phytochemicals, including polyphenols and flavonoids have antioxidant properties and can scavenge free radicals to reduce oxidative stress and lead to treatment of diabetes mellitus. The high use of herbal preparations in the treatment of diabetes though may be potent but some are toxic to the body. Proper knowledge and documentation of toxicity, presence of potential carcinogenic agents in some of these plants and complexity of the intrinsic metabolites making them unsuitable for therapeutic application is needed. The two herbal preparations that will be use during the course of the study as claimed by the manufacturers contain some phytochemicals (alkaloids, flavonoids, tannins, saponin) and can often have anti-diabetic effects and compounds which help in the treatment of diabetes, that is why a proper screening and analysis is to be carried out. Also, traditional healers do not possess adequate knowledge to understand the active components of the plant extracts and their mechanism of action. As such, it is important to understand and document the role of plant-derived bioactive phytochemicals in regulating blood glucose levels and diabetes mellitus.

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1.4 Aim and Objectives of the Study

1.4.1 Aim

The aim of the study was the toxicological and spectroscopic evaluation of selected herbal preparations (paxherbal and antidiabetes preparation) sold in minna and surburbs.

1.4.2 Objectives

The objectives of the study are to determine;

i. the qualitative and quantitative evaluation of phytochemical contents of Sample A and B preparations (paxherbal and antidiabetes preparations).
ii. the acute and sub-chronic toxicity testing of the two liquid preparations (paxherbal and antidiabetes preparation).
iii. compounds present in the two liquid preparations (paxherbal and antidiabetes preparation) using GC-MS and FTIR.
iv. the effect of the liquid preparations (paxherbal and antidiabetes preparation) on liver and kidney function and oxidative stress indices in albino rat.
v. the effects of the two liquid preparation on haematological parameters of the albino rats.
vi. histological investigation of the vital organ (kidney and liver).

CHAPTER TWO

2.0 LITERATURE REVIEW

DM (Diabetes mellitus) is a metabolic disorder of numerous causes characterized by hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism arising from deficiency in insulin secretion or insulin action. Numerous organs can become permanently damaged, dysfunctional, or fail as a result of diabetes mellitus. Three basic kinds of diabetes mellitus are distinguished, they include the following type 1 diabetes, type 2 diabetes and gestational diabetes mellitus (American Diabetes Association, 2019).

Type 1 Diabetes (insulin-dependent diabetes mellitus) is an autoimmune disorder that occurs when insulin-producing cells of the pancreas in the body have been damaged and the pancreas produces insufficient insulin. A type 1 diabetic patient must take insulin daily, and it occurs in children and young adults.

Type 2 diabetes (Insulin-independent diabetes mellitus) which is responsible for 90% of diagnosed cases of diabetes mellitus in adults. It occurs when the pancreas produces enough insulin but the body cannot use the insulin efficiently.

Gestational diabetes mellitus (GDM) involves a type of glucose intolerance that manifests itself during pregnancy second or third trimester. Gestational diabetes mellitus (GDM) is brought on by the hormonal changes during pregnancy or a shortage of insulin (American Diabetes Association, 2019).

Renal, nerve, heart, and eye damage, likewise blood vessels is a result of hyperglycemia (Alqathama *et al*., 2020). In 2019, Diabetes accounted for 463 million adults aged 20 to 79 worldwide; this amount is anticipated to increase to 578 million by the year 2030, based on the IDF Diabetes Atlas' ninth edition, published by the International Diabetes

Federation published from the IDF (International Diabetes Federation, 2019). Every six seconds, someone with diabetes mellitus dies; this rate is higher than the sum mortality of HIV (1.5 million), TB (1.5 million), and malaria rates (0.6 million) (Alqathama *et al*., 2020). Observing the International Diabetes Federation's 2019 statistics (International Diabetes Federation, 2019). According to estimates, 7.5% of the adult population, or 373.9 million persons globally between the ages of 20 and 79, have impaired glucose tolerance. Adults under 50 make up the majority of those with impaired glucose tolerance (180.0 million–48.1%).Men aged 20 to 79 are predicted to have a slightly greater prevalence of diabetes than women (9.6% versus 9.0%). Between 2019 and 2030 and 2045, there will likely be a rise in the likelihood of having diabetes in both women and men. China, the United States, Brazil, Mexico, Japan, Pakistan, Thailand, Nigeria, Indonesia, and India are among the top 10 countries in the world by population. In spite of the hazards of GDM in pregnant women have plainly been identified, it is unclear if the risks may be decreased or not by the treatment that lowers and manages pregnant women's blood glucose levels. GDM also raises the possibility of developing type 2 diabetes after delivering delivery. Diabetes mellitus is on the rise nowadays due to significant cultural and social changes, including population aging, decreased physical activity, dietary changes, and others. Diabetes comes with a price tag that includes rising medical expenses and a financial strain.

2.1 Factors that Contribute to Diabetes

Chronically elevated glucose levels are a symptom of diabetes (hyperglycaemia). Many patients, particularly those who have type 2 diabetes, also have dangerous levels of cholesterol and other blood lipids, chronically high insulin levels, and raised blood pressure (hypertension). The long-term consequences of diabetes are caused by all of these causes, which include:

Diabetes-related angiopathy, atherosclerosis, cardiovascular diseases, and stroke: The primary factor in death for diabetics is these cardiovascular diseases (American Diabetes Association, 2019).

i. End-stage renal disease, also known as diabetic nephropathy, is a kidney condition that needs kidney transplantation or dialysis for treatment.

ii. Eye conditions: These consist of cataracts, glaucoma, and diabetes retinopathy. Blindness and visual impairment are frequently brought on by diabetes.

iii. Nerve damage (diabetic neuropathy): This includes autoimmune neuropathy, which could impair sexual dysfunction and incontinence are exacerbated by poor digestion (gastroparesis), as well as neuropathy in the limbs, which frequently causes the limbs to hurt or become numb. Hearing and other senses may also be compromised by neuropathy.

iv. Infections and wounds: Diabetes is the main factor in nontraumatic foot and limb amputations, and this is due to foot problems and skin issues such as ulcers. Illnesses like yeast infections, thrush, infections of the urinary tract, and periodontal disease are also more prevalent among diabetes.

v. Musculoskeletal problems: Diabetic individuals experience more musculoskeletal disorders than nondiabetic patients, including gout, osteoporosis, restless legs syndrome, and myofascial pain syndrome.

vi. Complications of pregnancy: Preelampsia, miscarriage, stillbirth, and birth abnormalities are all made more likely by diabetes.

vii. Seventh: Emotional challenges: However, not all research examining the connection between diabetes and mental disease have discovered higher incidences of anxiety, despair, and other mental illnesses among people with diabetes. Diabetic individuals may also have acute episodes of hyperglycemia and

hypoglycemia in addition to chronic hyperglycemia (low glucose). Seizures, harm to the brain, and a possibly dangerous diabetes coma can all result from severe cases. Among the acute glucose crises:

i. Insulin shock: This severe form of hypoglycaemia is generally brought on by taking too much insulin or some antidiabetic medications.
ii. Diabetic ketoneuria: A deficiency using insulin make the body aches fat for fuel rather than glucose. Ketones, a hazardous byproduct, together with severe hyperglycaemia are the end results.
iii. Hyperosmolar hyperglycaemic nonketotic state: this condition is characterized by extreme hyperglycaemia and dehydration.

Although they can occur in people who do not know they have diabetes, these hazardous glucose complications are most common in people with uncontrolled diabetes. As stated by the US centers for disease prevention and control, almost thirty percent of the estimated 20.8 million Americans who have diabetes have not yet received a diagnosis (American Diabetes Association, 2019).

2.1.1 Diabetes symptoms and signs

Because symptoms of diabetes can have many different origins and because some individuals have no symptoms or ignore warning indications, diabetes frequently goes undiagnosed.

Possible diabetes warning signs include:

1. Abnormal thirst (polydipsia)
2. Dehydration and excessive urination (polyuria)
3. An excessive appetite or hunger (polyphagia)
4. Unaccounted-for weight loss

5. Nearsightedness, blurred vision, or another eye condition
6. Recurrent infections such as skin infections, yeast infections, and urinary tract infections, and others

Although the signs and of type 1 diabetes can be confused with those of the flu or other common ailments, it can develop quickly and frequently follows a sickness. It might take years for type 2 diabetes to develop, and it sometimes only becomes clear when long-term problems including erectile dysfunction, diabetic neuropathy-related leg discomfort, or claudication arise.

Some individuals, especially those who are young and have type 1 diabetes, do not receive a diagnosis for their emergency condition known as diabetic ketoacidosis until they are taken to the hospital. Indications of diabetic ketoacidosis include disorientation, heavy labourious breathing, and breath that smells sweetly fruity or wine-like. Patients occasionally experience more severe consequences such as hyperosmolar hyperglycaemic nonketotic syndrome, insulin shock, or diabetic coma before being diagnosed with diabetes.

People are urged to have routine glucose tests for diabetes screening in order to help prevent such problems, especially if they have risk factors (American Diabetes Association, 2019).

2.2 Diabetes Causes and Risk Factors

The causes of diabetes are complex and only few are understood. This illness is typically regarded as complex since it involves a number of risk factors and predisposing variables. In many circumstances, a person's diabetes may be influenced by their environment, lifestyle (smoking, much intake of alcohol) and heredity.

Contradictory risk factors for the various disease manifestations can further complicate matters. As an example, while diabetes mellitus (type 2 and gestational diabetes) are more prevalent in individuals of various racial and ethnic groups, autoimmune diabetes (type 1 and adult-onset latent autoimmune diabetes, LADA) is more prevalent among the white race. Typically, diagnosis of type 1 occurs in youngsters, although gestational diabetes and type 2 are risk factors as people get older (American Diabetes Association, 2019).

Type 2 diabetes has several risk factors, including metabolic syndrome, and insulin resistance prediabetes. Other causes and danger elements for diabetes include (American Diabetes Association, 2019).

1. Family and genetic history: Wolfram syndrome as well as adult-onset diabetes in the youth (MODY) are known to be caused by certain genes. Additionally, types 1 and 2 diabetes are caused by genes.
2. To varied degrees, the medical history of the family is also important: The American Diabetes Association claims, a person has a 10 to 25% probability of having type 1 diabetes if both of their parents have the condition, and a 50% chance of developing type 2 diabetes if both of their parents have the condition.
3. Body type and weight: Type 2 diabetes and gestational are both heavily influenced by weight gain and overweight. Resistance to insulin and the metabolic syndrome are facilitated by excess body fat, particularly about the stomach (central obesity). The majority of autoimmune diabetes patients (those with type 1 and LADA) are of normal weight, and excess weight has not historically been associated with these diseases. Recent studies, however, suggest that type 1 diabetes may be accelerated by obesity and that the rising incidence there may be at least a 10% chance partially attributed to the increase in adolescent obesity. Additionally,

autoimmune diabetes, people who put on weight run the risk of developing insulin resistance and double diabetes.

4. Sex: According to the National Institute of Health, males represent 53% of adult diabetes cases, despite less than 49% of the adult population in the United States (NIH). Prior until 1999, when a gap started, diabetes was more common among American men and women than comparable, according to a statistical analysis done by the US centers for disease prevention and control (CDC). There has been little or no study done to clarify this pattern. The observed rise in male hypogonadism (low amount of testosterone), which researchers have connected regarding insulin resistance, in recent years may be one contributing cause.
5. Physical activity level: Regarding insulin resistance are largely attributed to a lack of regular exercise (American Diabetes Association, 2019).
6. Nutrition: There is debate regarding the role that diet plays in the onset of diabetes. According to some research, consuming large amount of beverages and other simple carbohydrate increases your risk of developing diabetes mellitus, whereas whole grains and other foods with a minimal glycaemic index lowers your risk. However, the ADA asserts that consuming sugar-containing foods does not cause the illness (American Diabetes Association, 2019). According to the ADA, the real problem is weight gain brought on by sedentary behavior and excessive calorie consumption. The possibility of a connection between type 1 diabetes and early cow's milk consumption is the subject of another debate. There may be a correlation, but not all academics have noticed it. There will probably be further scientific study on this subject.
7. Hormones: These chemical messengers can have a variety of effects on diabetes. For instance, stress hormones like cortisol, have connected to varying levels of

glucose in type 2 diabetes, as well as stress hormones in pregnant women. In the past, women were connected to a child's threat of developing diabetes type 1. Some adolescents may be more vulnerable to developing diabetes due to teenage years see the release of growth and sex hormones. Secondary diabetes has been associated to a variety of hormonal treatments using estrogens, growth hormone, and anabolic steroids, injectable contraception and prostate cancer treatment using androgen deprivation therapy, as well as corticosteroids.

8. Medical interventions: Medications such as beta blockers and diuretics (another category of antihypertensive), Secondary diabetes risk has been associated with immunosuppressive, antiretrovirals (AIDS/HIV medications), antipsychotics, lithium, and several antidepressants, anticonvulsants, and chemotherapy medications in addition to hormonal therapies. Secondary diabetes may also arise from radiation therapy and pancreatectomy. Type 1 diabetes has been connected to medications such as L-asparaginase and the pneumonia drug pentamidine (used to treat leukemia) (American Diabetes Association, 2019).
9. Viruses: After contracting a virus, some people develop type 1 diabetes. Viruses like the mumps, rubella, and coxsackie virus are thought to be connected to type 1 diabetes (connected to the viral family that causes hepatitis and polio).
10. Cigarette : Smoking increases the risk of developing type 2 diabetes and perhaps more types is of diabetes.
11. Alcohol: Regular alcohol consumption increases the chance of developing diabetes. For instance, it might result in pancreatitis. Light drinking, however, has been connected to a decree sed risk of developing diabetes, according to certain studies. The majority they pose a risk, variables can be categorized either as uncontrollable (because of genetics and aging) or as manageable (exercise and

diet), depending on the situation. Some obesity, may be influenced by both heredity and way of life decisions. Nobody can change their uncontrolled danger signs, although they can reduce their controllable risk variables by better health practices, which will lessen their risk of acquiring diabetes (Azeez *et al*., 2022).

2.3 Epidemology of Diabetes

366 million people were predicted to have diabetes in 2011, and 552 million were projected to have it by 2030. Type 2 diabetes is becoming more prevalent worldwide, with 80 percent of individuals affected residing in low- and middle-income countries. In 2011, diabetes mellitus contributed in 4.6 million fatalities. The year 2030 is predicted that 439 million people worldwide would suffer from type 2 diabetes. Due to the prevalence of type 2 Diabetes mellitus varies significantly by location and is influenced by environmental and behavioural risk factors. Research into the writing has showed that there is little information about the occurrence in environments that are both rural and urban, and in both sexes are equally affected. Less than 10% of Diabetes mellitus instances of type 1 diabetes seem to be prevalent in Africa, which appears to be the disease's most prevalent type. As stated by a 2011 about 25.8 million Americans (7.8% of the population), according to a report from the Centers for Disease Control and Prevention (CDC) have diabetes mellitus, 2010 saw 90% to 95% of those individuals have type 2 diabetes (Department of Health and Human service, 2011).

20 years from now, it's anticipated because of its widespread in adults with diabetes, particularly type 2 diabetes, which is on the rise, will ascend. Almost all of this increase is expected to occur in developing nations, where the most of the patients are adults ages 45 to 64 (Wild, 2004).

2.3.1 Pathophysiology

Insulin insensitivity a characteristic of type 2 diabetes mellitus, which is caused by insulin resistance, dwindling insulin output, and finally failing beta-cells in the pancreas. Less glucose is consequently sent to the liver, muscles, and fat cells. There is an increase in the breakdown of fat with hyperglycaemia. Recent research has shown types 2 diabetes mellitus pathogenesis includes reduced alpha-cell activity (Alam *et al*., 2019).

This flaw prevents meals from inhibiting the glucagon and liver levels that rise while fasting. Low insulin levels and increased insulin resistance lead to hyperglycaemia. In the gut, the incretins are crucial in modulating the release of insulin and, in the case of glucagon inhibition by GLP-1. Regardless of the fact that type 2 diabetes mellitus sufferers have impaired GIP activity, the insulinogenic properties of GLP-1 are still intact, it becomes a potentially useful therapeutic substitute. Although GLP-1is quickly via DPP-IV in vivo inactivation, just as GIP.

This issue has been addressed by two different therapeutic philosophies. Increased half-lives of GLP-1 mimics and inhibitors of DPP-IV halt the oxidation of both endogenous GLP-1 and GIP. Both classes of medications have shown promise and have the potential to improve beta-cell mass and function, normalize fasting and postprandial glucose levels, and normalize insulin sensitivity. Studies on the involvement of the malfunction in the mitochondria genesis of type 2 diabetes, both the development of insulin resistance are still being conducted (Alam *et al*., 2019). Additionally important is adipose tissue since it may operate as an endocrine organ (secretion of various adipocytokines, i.e., leptin,TNF-alpha, resistin, and adiponectin implicated in insulin resistance and possibly beta-cell dysfunction).

Most persons with type 2 diabetes mellitus are obese, and their bodies are covered in visceral fat. Consequently, the development of type 2 Diabetes mellitus is heavily reliant on fat tissue. Although the portal/visceral hypothesis, which places a major emphasis on increased non-esterified fatty acid concentrations, is the most often utilized explanation to explain this connection, two additional theories are emerging (accumulation of triglycerides in pancreatic, liver, and muscle cells).

Research on the relationships between type 2 diabetes mellitus and these two hypotheses is based on these two premises, our obesogenic environment, and the possibility of developing diabetes mellitus in the coming ten years.

Insulin is the primary hormone that regulates the uptake of glucose from the blood into the majority of body cells, including the liver, adipose tissue, and muscle, with the exception of smooth muscle, where insulin operates via the IGF-. Therefore, a lack of insulin or an insensitivity to its receptors are the primary causes of all forms of diabetes mellitus (America Diabetes Association, 2019).

The three primary sources of glucose for the body are digestion of food, the breakdown of glycogen (glycogenolysis), the liver's form of glucose storage, and gluconeogenesis, the body's method of generating glucose from non-carbohydrate substrates. Insulin is necessary to regulate the body's glucose levels. Gluconeogenesis and the transport of glucose into fat and muscle cells can both be accelerated by insulin. Additionally, it can promote the storage of glucose as glycogen.

The pancreatic islets of Langerhans contain beta cells, often referred to as - cells, which secrete insulin into the body in response to increased blood glucose levels, typically after meals. Insulin is used by almost two-thirds of the body's cells to absorb glucose out of the blood and use it as fuel, transform it into other molecules they need, or store it. When

blood glucose levels are low, the beta cells produce less insulin and glycogen breaks down into glucose. The biologically active hormone glucagon is different from insulin and is primarily responsible for controlling this procedure.

The body's cells that require glucose are unable to efficiently absorb it, and it cannot be stored in the liver and muscles if not enough insulin is present, cells do not respond well to the effects of insulin (insulin resistance), or the insulin is defective. In cases of total insulin shortage, consistently high blood sugar levels, poor protein synthesis, and other metabolic acidosis are the end results. The kidneys approach a reabsorption threshold and the body excretes glucose in the urine when blood sugar levels are continuously high (glucosuria). As a result of the kidneys inability to reabsorb water, there is an increase in the osmotic pressure of the urine, increased polyuria, and more fluid loss. The osmotically replenished lost blood volume—which is made up of water in body cells and other compartments—leads to dehydration and increased thirst (polydipsia). Low intracellular glucose also makes people more hungry, which leads to overeating (polyphagia) (Mogotlane, 2013).

2.4 Screening and Diagnosis

Tests for blood glucose levels are used to diagnose diabetes mellitus, which might manifest in whichever of the ways listed below.

i. Level of fasting plasma glucose: 126 mg/dL or below (7.0 mmol/L). When the patient has had enough time to fast overnight, or in the morning before breakfast, blood is collected for this test.
ii. Plasma glucose was 11.1 mmol/L (200 mg/dL) two hours after a 75 gram oral glucose load, as in a glucose tolerance test (OGTT).

iii. Symptoms of high blood sugar and blood sugar levels of 200 mg/dL or less, whether or not the patient is fasting.

iv. A1C (HbA1C) glycated hemoglobin 48 mmol/mol (6.5 DCCT%) (Toumpanakis *et al*., 2018).

2.5 Pharmaceutical Substances

2.5.1 Biguanides

In patients who are overweight or obese, biguanides—of which metformin is the most frequently prescribed—suppress GLUT-enhancer factor is phosphorylated to increase hepatic glucose production, insulin sensitivity, and glucose uptake, reduced gastric glucose absorption and fatty acid oxidation. Type 2 diabetes mellitus (T2DM) is characterized by higher hepatic glucose production, which is mostly connected to a rise in gluconeogenesis, insulin resistance, and cell dysfunction. Since more than 70 years ago, biguanides used to treat type 2 diabetes mellitus (T2DM) is metformin, which is the most commonly prescribed oral anti-diabetic medication in the world (Khan *et al*., 2020).

2.5.2 Sulfonylureas

These are often well tolerated but provide a risk of hypoglycaemia because they enhance endogenous insulin secretion. Sulfonylurea therapy for elderly diabetes mellitus patients increases their risk of hypoglycaemia by 36% as compared to younger people. Sulfonylureas or biguanides are the most popular and acknowledged as the most economical treatments for diabetes mellitus among all oral drugs. At the 10-year follow-up, obese patients with intensive glycemic management using sulfonylureas or insulin and biguanides (such as metformin) saw considerably better health outcomes., despite the fact that the 20-years all-cause mortality rate in the UK prospective diabetes trial had a

relative risk of 0.87 and that this benefit was only seen in the study's overweight participants (Jonas *et al*., 2021).

2.5.3 Meglitinides

Although the binding location is different, the non-sulfonylurea secretagogues repaglinide and nateglinide work on the pancreatic beta-cells ATP-dependent K-channel to stimulate the beta cells secretion of insulin. Meglitinides lower the risk of hypoglycaemia due to their rapid onset and short half-life (4-6 hrs). The meglitinides administered prior to meals to control postprandial blood sugar. When a meal is missed, flexibility is possible without running the danger of hypoglycaemia. With the exception of patients with end-stage renal illness, dosage modification is not indicated due to the fact that repaglinide is mostly metabolized in the liver and eliminated by the kidneys in relatively tiny amount in individuals with renal failure (Khan *et al*., 2020).

2.5.4 Thiazolidinediones

A specific ligand for the transcription factor Peroxisome Proliferator-Activated Gamma, thiazolidinedione is more sensitive to insulin. They are the first drugs to treat the essential issue in insulin resistance in people with diabetes type 2, and in light of the food and drug administration (FDA) most recent recommendation, the majority of the class now consists of pioglitazone against using rosiglitazone due to an elevated risk of cardiovascular events. Pioglitazone can be utilized when there is renal dysfunction, and it is well tolerated by older persons because it is not linked to hypoglycaemia. However, due to worries about fluid retention and women's fracture risk, and peripheral edema, its usage may be restricted in elderly people with diabetes. Pioglitazone is not recommended in people with congestive heart failure in elderly persons who have class III-IV heart failure and should be avoided (Yoon *et al*., 2006).

2.5.5 Glucosidase-alpha inhibitors

Although not frequently used to treat people with type 2 diabetes, acarbose, voglibose, and miglitol are probably safe and effective. These medications should not be used in patients with severe renal impairment because they are most useful for treating postprandial hyperglycaemia. Given the high frequency of adverse impacts like diarrhea and flatulence, their usage is typically restricted. Most recent medication, In a study, voglibose was found to considerably improve glucose tolerance, slow the progression of the illness, and the proportion in terms of patients who develop normoglycemia (Chiniwala and Jabbour, 2011).

2.5.6 Therapies based on incretins

A key component of incretin-based medicines, which seek to address this hitherto unrecognized element of Diabetes mellitus pathogenesis, is GLP-1, or glucagon-like peptide (GLP-1) analogues. These medications lead to persistent benefits in body weight management and glycemic control (Stonehouse *et al*., 2011). They are available as an aid in individuals a type 2 diabetes patient as monotherapy, as an adjunct in addition to oral hypoglycaemic medicines, to diet and exercise, or both. Exenatide, an incretin mimic, are two examples of medications (Chinawala and Jabbour, 2011).

Application of GLP-1 therapies carries no risk of low blood sugar (unless combined with insulin secretagogues). Additionally, latest study indicates it is incretin-based treatments may be advantageous to the central nervous system, sleep, inflammation, cardiovascular health, and hepatic health (Stonehouse *et al*., 2011).

2.6 Plant Bioactive Substances with Type 2 Antidiabetic Activity

Numerous plants utilized as traditional antidiabetic treatments have been shown to have hypoglycaemia and antidiabetic properties, and the processes behind their hypoglycaemic activity have been thoroughly explored (Kayarohanam and Kavimani, 2015; Sidhu and Sharma, 2013). The mechanisms of conventional herbal and organic diabetic treatments derived from traditional medicinal botanicals are the main topic of this review.

2.6.1 M*ormodica Charantia* (Bitter Melon)

Among the most popular veggies grown in tropical climates is *Mormodica charantia* (MC), especially in Central and South America, East Africa, South-North Asia, Vietnam, India, and China (Li *et al*., 2004; Rathi *et al*., 2002). It is referred to as bitter gourd and belongs to the family Cucurbitaceae. In addition to being consumed as a vegetable, MC is also thought to be a herbal remedy. Its bioactivities, particularly its anti-diabetic activities include anti-inflammatory, antioxidant, antiviral, anti-cancer, and antibacterial actions, etc (Saeed *et al*., 2018).

a b

Figure 2.1: Momordica charantia's (MC) morphological traits are depicted in images: both the (a) entire plant and the (b) immature fruit.

2.6.2 Phytochemstry of M*omordica charantia*

There have been numerous studies that have published bitter melon active ingredients that aid with managing type 2 diabetes. The following substances are included in steroidal saponins: steroids, momordicosides (A, B, C, D, E, F1, F2, I, K, L), acyl glucosyl sterols, fatty acids, amino acids, alkaloids, phenolic compounds, and vitamins, carbohydrates, and minerals are among the significant phytochemicals found in plants (Tan *et al*., 2014).

2.6.3 Antidiabetic activity of M*omordica charantia*

When examined in tests with rats, gerbils, langurs, and humans, M*omordica charantia's* fruits, seeds, and callus all contained proteins that resemble insulin that are similar and human insulin exhibited a regular hypoglycaemic impact. M*omordica charantia* has long been regarded as a remedy for diabetes mellitus in China and India. These days, numerous studies concentrating on its anti-hyperglycaemic properties have been conducted by specialists. Indeed, numerous studies have demonstrated it's bioactivities considerably decrease blood sugar amounts. These studies also on bitter melon showed it is capable of improving tolerance to glucose in humans, as well as in normal and diabetic rats (Jiang *et al*., 2016). Numerous studies have demonstrated that M*omordica charantia* bioactive components have strong anti-diabetic effects.

Figure 2.2: The process by which M. *charantia* lowers blood glucose levels.

2.6.4 Ginseng Panax A. C. Meyer

The most well-known traditional herb in Korea that had been utilized in folk remedy for a very lengthy time is ginseng. Ginseng is a member of the Araliaceae family's Panax genus (Attele *et al*., 2002). It typically spreads in Eastern Asia's milder temperate regions, including Northeast China, Korea, Eastern Siberia, and North America.

Figure 2.3: Ginseng Panax Meyer, C. A.

2.6.5 Antidiabetic activity of *Allium cepa linn*

Allium cepa Linn's hypoglycaemic action. Reports of extracts are available. S-methyl cysteine sulfoxide, which is present in the bulb section, has been shown to have anti-diabetic properties. These substances have strong antioxidant properties that may explain their ability to reduce blood sugar levels. S-methylcysteine sulfoxide exerts its anti-diabetic effects in three different ways: (1) by promoting the body's generation of insulin and increasing pancreatic secretion; (2) by impeding the absorption of dietary glucose; and (3) by helping the body use insulin efficiently (Kumari and Augusti, 2002).

2.6.6 The onion sativum (Allium)

The onion sativum Linn, a common herb often known as garlic, is a member of the Allium family. There were instances of it in Asia, Africa, and Europe. Originally from Asia, it is now extensively farmed and used as a condiment, particularly in Asian cuisine (Singh and Kumar, 2017).

Their effects demonstrate that garlic has been used for more than a thousand years as a potent medicinal plant to cure a variety of illnesses. Numerous scientific investigations have revealed that garlic contains a variety of biological properties, including tumour-fighting capability, antibiotic properties using antibiotics properties, and particularly anti-hyperglycaemic action (El-Saber Batiha *et al*., 2020).

2.6.7 Antidiabetic activity of T*inospora cordifolia*

T. *cordifolia* has a polysaccharide that was isolated from this plant, and this polysaccharide revealed the -cell regeneration characteristics that could be used to develop an anti-diabetic drug with minimal adverse effects (Sangeetha *et al*., 2011). With two weeks of oral administration of T. cordifolia roots extract, researchers found that they could enhance the body's capacity to control blood sugar levels by increasing insulin secretion and decreasing the process of glucosgenolysis (Patel and Mishra, 2011). Patel studied the alkaloidal portion of T. cordiforlia extract's hypoglycaemic properties in 2011. This study proved T. cordilia's antidiabetic effect by increasing insulin release, enhancing insulin sensitivity, and blocking the gluconeogenesis process (Ahmad *et al*., 2016).

Figure 2.4: (a) Tinospora crispa leaves and (b) the entire plant.

2.6.8 Anti-diabetic effects of C*ostus pictus*

So far, numerous studies have been conducted on an insulin plant to ascertain its antidiabetic effectiveness (Sidhu *et al*., 2014). To learn more about the impacts of C. *pictus*, an in vitro investigation was conducted. The findings of that experiment demonstrated that the Costus pictus has -amylase and -glucosidase inhibitory action is a crucial mechanism of its antidiabetic effect. In additional studies, rats that had been caused by streptozotocin and alloxan showed an important drop in blood glucose levels after receiving oral administration of C. pictus ethanolic and methanolic extracts. These extracts may also boost insulin levels. The antidiabetic property is stimulated together along with detection of trace components including K, Ca, Cr, Mn, C, and Zn. The other research likewise revealed that C. *pictus* in vitro pancreatic islet cultures and streptozotocin-induced diabetic rats both exhibited antidiabetic properties. Results were also favorable when the activity of enzymes that hydrolyze carbohydrates, such as amylase and -glucosidase, was inhibited (Ramu and Kumar, 2016). In order to assess the insulin secretion and antihyperglycemic efficacy in diabetic rats (Gireesh *et al*., 2009) conducted a test in living organisms with C. pictus leaves in 2009. Their findings recommended that a plant that produces insulin improve glucose utilization while also

lowering blood glucose levels and secreting more insulin (Gireesh *et al*., 2009). In vitro research conducted in 2010 revealed C*ostus pictus*, extract could increase insulin release from islets of Langerhans –cells (Al-Romaiyan *et al*., 2010).

2.6.9 Antidiabetic activity of P. *vulgaris*

Previous research has demonstrated that alpha-amylase is present in kidney bean seeds inhibitors that block animal and insect alpha-amylase activity. This is connected to the control of type 2 diabetes mellitus by inhibiting DPP-IV and phytohaemoagglutinin are lectins may be able to regulate the function of GLP-1, a glucagon-like peptide (glucagon-like peptide-1) (Ngoh and Gan, 2018; Nolan *et al*., 2020). Three variants of the phaseolamin -amylase inhibitor, -AI1, both -AI2 and -AIL, have been discovered and evaluated in several clinical investigations against the action of -amylase in the common bean P. vulgaris. Depending on how these enzyme inhibitors work, they could decrease absorption of carbohydrates by preventing the behaviour of mammalian and insect –amylases (Barrettt and Udani, 2011). The lowering of the glycaemic index could also reduce the chances of developing insulin sensitivity in people with diabetic nephropathy, hence reducing the major side effects of the condition. Recently, a different experiment is still being conducted to establish whether P.vulgaris extract inhibits -amylase (Micheli *et al*., 2019). The current study also showed that -amylase inhibitor considerably reduced -amylase activity and that long-term use of P. vulgaris extract could lessen a number of diabetes-related issues.

2.6.10 Euphorbia hirta's anti-diabetic properties

The ethanolic extract of Euphorbic hirta was shown to have the ability to lower blood sugar levels (hypoglycaemic action). -glucosidase activity is inhibited by extract of ethanol and fractions of ethyl acetate, according to prior in vitro studies. The similar

outcome was also seen in in vivo experiments (Sivaji, 2014; Widharna *et al*., 2010; Kumar *et al*., 2010). The ability to treat diabetes by inhibiting enzymes involved during the breakdown of saccharides like -amylase was demonstrated by an occurrence of numerous bioactive substances, particularly in ethyl acetate and chloroform extracts (Tran *et al*., 2020). In vivo as well as in vitro experiments, polyphenolic substances have been widely shown to have inhibitory effect against -amylase. E. hirta contains phenolics and flavonoids such as quercetin, quercitin, and rutin that have shown to be potent mammalian -amylase inhibitors. It also demonstrated that E. hirta had modest inhibition of -amylase, despite the fact that the inhibitory powder of E. hirta is not as potent like acarbose, a common medicine in the treatment of diabetes. In mice with diabetes caused by streptozocin (Kumar *et al*., 2010). Euphorbia hirta's ethanolic extract was found to have hypoglycaemic effects on alloxan-induced diabetic mice in 2014, lowering blood glucose levels. -glucosidase activity was decreased by ethanol extract and ethyl acetate fractions in other in vitro tests. The similar outcome was also seen in in vivo experiments (Sivaji, 2014). In 2015, Manjur Ali Sheliya and his collaborators investigated how novel prenylated flavonoids from E. hirta inhibited –glucosidase (Sheliya *et al*., 2016) extracted from the methanolic extract's ethyl acetate fraction. They extracted four active chemicals, including quercetrin, successfully utilizing medium pressure liquid chromatography techniques.

i. Dimethyoxy quercetin

ii. Hirta coumaroflavonoside (7-O-(P-coumaryl) 5',7',4'-trihydroxy-6' (3,3-dimethylallyl)

The flavonoid (3-O-D-glucopyranosyl-2'-1'-O-L-rhamnopyranoside)

iii. The compound 5,7,3'4, 6-trihydroxy-6-hirtaflavonoside (3,3-dimthylallyl)

-8-(iso-butenyl-flavonol-3-C-β-D-glucopyranoside)

The blockade capacity of single compounds against -glucosidase was assessed and contrasted with that of the reference medication acarbose. These flavonoids boosted -glucosidase inhibition, according to the results. That investigation lends support to the ethanomedicinal usage with E. hirta in the folk medical treatment from diabetes. Extracts of E. hirta that are aqueous, hydroalcoholic, and alcoholic were investigated by Manjur Ali Sheliya and his team in 2016 for their capacity to inhibit in vitro -glucosidase and –amylase (Sheliya *et al*., 2016). The results of their investigation unmistakably shown that the methanolic extract of E. hirta had potent inhibitory activity against -glucosidase and very weak inhibitory action against -amylase, two proteins intimately connected to diabetes mellitus. E. hirta extract can be utilized to reduce postprandial hyperglycaemia with fewer adverse effects, and it offers useful data for future research on the management of type 2 diabetes mellitus.

In a 2013 in Dr. Ly Le and her silico research team used molecular docking and pharmacophore analysis to examine the anti-diabetic properties with bioactive chemicals during Euphorbia hirta Linn.

(Trinh and Le, 2014). By determining whether or not a connection exists between different bioactive substances in E. *hirta* and the specific protein pertaining type 2 diabetes in humans by figuring out the bond vigor and pharmacopluse using modeling, eight interesting substances from E. *hirta* were discovered, including cyandi myricitrin and 3,5-O-diglucose, and pelargonium. This study showed the impact of bioactive chemicals from E. *hirta* on a few diabetes-related proteins. The investigation's findings suggested that those bioactive substances could be used to create type 2 diabetes mellitus medications. To ascertain more precise the relationship between the durability of encounters between ligands and proteins, however, the molecular dynamic methodology

must be used in conjunction with other methods. As a result, in vitro and in silico research shows this plant possesses the capacity to block two enzymes.

Bioactive substances, the like of organic acids like oxalic acid and tartaric acid, promote the consumption of glucose (Liu *et al*., 2020).

2.6.11 Antidiabetic activity *of Zingiber officinale rosc*

According to Sharma and Shukla, both normal fasting animals and alloxan diabetic animals can drop their blood glucose concentrations by drinking ginger juice. Because it can thicken digestive fluids, delay gastric emptying, and function as a diffusion barrier, the process of reducing the glucose effect was described. Other research exhibited that the traditional ginger can be used as a medicinal plant to reduce the amount of glucose in tissue of diabetic rats by enhancing peripheral glucose utilization and restoring the liver's damaged function (Abdul-Razzak *et al*., 2012).

Additionally, *Zingiber officinale Rosc*'s rhizome demonstrated the potential for its bioactive components can improve glucose absorption in L6 myotube culture (Li *et al*., 2012). According to that research, the primary active ingredient promoting glucose absorption was phenolic gingerol. Other research revealed that the fresh ginger sample's solution inhibited the activity of the enzymes alpha-glucosidase and alpha-amlase as well as the angiotensin-converting enzyme (Oboh *et al*., 2012; Akinyemi *et al*., 2014).

Additionally, ginger powder can lower glucose levels and stimulate inflammatory processes that can result in the emergence of insulin resistance (Sahardi and Makpol, 2019).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.1 Materials

Sample Collection: Two samples (antidiabetic liquid preparations) were purchased from Minna and surburbs. Coded as sample A and sample B

Plate 3.1: Sample A

Plate 3.2: Sample B

3.1.2 Reagents

All the chemicals used were procured from Simbest Scientific and Chemicals, Minna. The chemicals were hydrochloric acid (HCl), tetraoxosulphate (vi) acid (H_2SO_4), ferric chloride ($Fecl_3$), allium chloride ($AlCl_3$)

3.1.3 Equipment and materials

Electronic weighing balance (model number:12051, manufacturer: Sri Krishna traders), water bath (model number: DK-420, manufacturer:Chinese supplier wuhan), uv vis spectrophotometer (model number: 3092, manufacturer: Labinda India).

3.2 Qualitative Phytochemical Screening

3.2.1 Test for flavonoids

A 5 ml of the liquid preparations for the different preparation was heated with 10 ml of ethyl acetate in a test inside a water bath for 3minutes. The mixture was filtered and 4 ml of the filterate was shaken with 1 ml of dilute ammonia solution. Observation of a yellow colouration indicate the presence of flavonoid (Harborne, 1973 Sofowara, 1993).

3.2.2 Test for tannins

A 0.5 ml of the liquid preparation for the two different sample preparation was boiled in 20 ml distilled water in a test tube and filtered. 0.1% ferric chloride ($Fecl_3$) solution was then added to the filterate. The presence of a brownish green colouration indicate the presence of tannin (Harborne, 1973 Sofowara, 1993).

3.2.3 Test for saponins

A 0.5 ml of the liquid preparation for the two different preparation was boiled in 20 ml of distilled water in a test tube in a boiling water bath and filtered. 10 ml of the filterate was mixed with 5 ml of distilled water and was shaken vigorously for the formation of stable persistent froth. The frothing was mixed with drops of olive oil and shaken vigorously for the formation of emulsion which indicate the presence of saponins (Harborne, 1973; 1993).

3.2.4 Test for alkaloid

A 0.5 ml of the liquid preparation for the two different sample preparation was stirred with 5 cm^3 of 1 % aqueous HCl on a steam bath, few drops of picric acid solution was added to 2 cm^3 of the different preparation. The formation of reddish brown precipitate indicates the presence of alkaloids (Trease and Evans 1989; Harborne, 1976).

3.2.5 Test for reducing sugar (Benedict test)

A 0.5 ml of the liquid preparation for the different sample preparation was mixed thoroughly with 3 cm^3 of distilled water and filtered, 3 drops of the filterate was added to 3 cm^3 of benedict reagents and placed in a boiling water for 5 mins. The formation of a brick red precipitate indicate the presence of reducing sugar (Harborne, 1976).

3.3 Quantitative Phytochemical Screening

3.3.1 Determination of total flavonoids

Total flavonoid was determined using aluminium chloride colorimetric method (Chang *et al*., 2002). Quercetin was used to establish the calibration curve. Exactly 2 ml of the liquid preparation of the two different sample was added into test tubes containing 1.5 ml of methanol. 0.1 ml of 10% $AlCl_3$ solution and 0.1 ml sodium acetate ($NaCH_3C00^-$) was added, followed by 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. The amount of 10 % $AlCl_3$ was substituted by the same amount of distilled water in blank.

Formula for calculating flavonoid

$C = {}_CV/m$

C = total flavonoid content mg

c = concentration of quercetin obtained from calibration curve in mg/ml

V = volume of extract in ml

m = mass of extract in gram.

3.3.2 Determination of total alkaloids

The two sample preparation of 2 ml differently were added differently into 96% ethanol-20% H_2SO_4 (1:1). 1 ml of the filterate was added to 5 ml of 60% tetraoxosulphate (vi) acid and allowed to stand for 5 min, then 5 ml of 0.5% formaldehyde was then added and allowed to stand for 3 hours . The reading was done using absorbance at 565nm. As a reference alkaloid, vincristine's E296's extinction coefficient (ethanol [ETOH] = 15136M-1cm^{-1}) was employed (Harborne, 1976).

The formula for calculating total alkaloids is shown below

$Y = aX + b$

Y = absorbance, X = concentration

3.3.3 Determination of saponins

The two sample preparation of 2 ml differently were added differently to 20 ml of 1N HCl and was boiled for 4 hours. After cooling it was filtered and 50 ml of petroleum ether was added to the filterate for ether layer and evapourated to dryness. 5 ml of acetone was added to the residue. 0.4 ml of each was taken into 3 different test tubes. 6ml of ferrous sulphate reagent, was added into them followed by 2 ml of concentrated H_2SO_4. It was thoroughly mixed after 10 min and the absorbance was taken at 490nm. Standard saponin was used to establish the calibration curve (Oloyed, 2005).

Formula for calculating saponins

W = v X Cm

Where V indicates the volume of extraction solvent (ml)

C represents the concentration obtained from diogenin standard curve (mg/ml)

W indicates the dry weight of the sample (g) used for extraction.

3.3.4 Determination of tannins

Each of the sample preparation made up of 2 ml were weighed differently and put differently into a 50 ml beaker. 20 ml of 50% methanol was added, parafilm was placed on top, and incubated for one hour at 77–80^{O}C in a water bath. To make sure the mixture was homogeneous, it was vigorously shaken. The extract was accurately filtered through two layers of Whatman No. 41 filter paper, 20 ml of water, 2.5 ml of Folin-Denis reagent, and 10 ml of Na_2CO_3 were added to the 100 ml volumetric flask and thoroughly mixed. The correct amount of water was added to the concoction, and it was thoroughly mixed, a bluish-green colour after 20 minutes of standing. Colour development is completed, and a UV spectrophotometer model 752 reading of the absorbance of the samples and standard solutions of tannic acid was made at a wavelength of 760 nm (AOAC, 1984).

3.4 Acute Toxicity Study

The oral acute toxicity of the two liquid preparation was carried out using Lorke's method in three-hour-fasted albino rats. Briefly, in the phase I of this method, the albino rats were distributed among three groupings of three each albino rat for each of the liquid preparation. The albino rats were given 10, 100, and 1000 mg/kg dosages of each of the liquid preparation and observed for twenty-four hours. In the absence of mortality and toxicological signs, phase II of this method was conducted. In the phase II, three groups of one albino rat each were formed from the albino rat each for each of the liquid preparation, and administered higher doses of 1900, between 2900 and 5000 mg/kg of each of the liquid preparation and noticed for mortality and/or any signs of toxicity for twenty-four hours.

3.5 Sub-Chronic Toxicity Study

For sub-chronic toxicity study, fourteen (14) albino rats were used. There were six groups formed (3 groups for sample A and 3 groups for sample B) and a control group. Group 1 received 1% dimethyl sulfoxide (DMSO) orally as the oversight group. Group 2, 3, and 4 groups of albino rats received oral administration of 100mg/kg, 300mg/kg and 1000mg/kg respectively daily for a period of 28 days (Osano *et al*., 2016).

3.5.1 Weight changes in the body and mortality

During the entire dosing period, the albino rats were observed daily for toxicity signs and mortality, the albino rats were weighed prior to dosing, after every 7 days, and before sacrifice on the last day. Weight of each albino rat was recorded separately. After administration of the liquid sample preparation for twenty eight days, the albino rats were anaesthetized in airtight dissection jar containing cotton soaked in diethyl ether. Each anaesthetized albino rat was laid on a dissecting board and a pair of scissors used to open

the albino rat by cutting through vertical mid-line from neck to peritoneum (Osano *et al*., 2016)

3.5.2 Blood sample collection

Blood samples were collected through cardiac puncture. After anaesthesia with diethyl ether, a 22 guage needle attached to 3ml syringe was inserted to the notch at the caudal aspect of the sternum and directed to the heart. The blood collected were divided into two portions, one for haematological analysis, and the other for biochemical analysis.

3.5.3 Haemological tests

Blood for haematological test was collected through cardiac puncture. The blood from experimental albino rats was collected in EDTA containing tubes for haematological assay. Parameters including white blood cells differential counts (WBC) and red blood cells (RBC) counts, red blood cells distribution width (RDW), haemoglobin concentration (HB), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC), mean cell volume (MCV), platelets counts and platelet distribution width (PDW).

3.5.4 Determination of biochemical parameters

The biochemical parameters determined included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, protein, albumin, urea, and creatinine.

3.6 Management of Experimental Animals for Blood Glucose Level

Forty five (45) albino rats weighing 100 -130g was purchased from Ilorin and was housed in wire meshed cages. The albino rats was fed with a standard rat diet for 14 days for acclimatization. The eight groups of albino rats were separated into 5 albino rat each and

was allocated different treatment. Treatment with the two liquid preparation last for twenty one (21) days. Metformine (100 mg/kg bw), a standard diabetic medication was given orally to the diabetic control group. All groups were allowed access to food and water.

3.6.1 Experimental grouping

Sample A

Group 1: Induced with 0.18ml of alloxan but administered 100 mg/kg of Sample A

Group 2: Induced with 0.18ml of alloxan but administered 300 mg/kg of Sample A

Group 3: Induced with 0.18ml of alloxan but administered 1000 mg/kg of Sample A

Sample B

Group 1: Induced with 0.18ml of alloxan but administered 100 mg/kg of Sample B

Group 2: Induced with 0.18ml of alloxan but administered 300 mg/kg of Sample B

Group 3: Induced with 0.18ml of alloxan but administered 1000 mg/kg of Sample B

Group 4: Albino rat induced with 0.18ml of alloxan (The control)

Group 5: Albino rat induced with 0.18ml of alloxan and treated with standard drug metformin.

3.7.1 Blood sample collection

After an overnight fast, rats were anesthetized with diethylether vapor on the final day of therapy, and then they were sacrificed. The serum was attained by centrifuging the blood samples at 3000 rpm for 15 minutes after the blood had been drawn via carotid puncture

and allowed to clot for fifteen minutes. For further investigation, the serum was kept in the refrigerator at -20^0 C.

3.7.2 Biochemical parameters

Randox biochemical kit was purchased from sim best scientific and chemical Minna, the kits were used for the biochemical parameters.

3.7.2.1 *Liver marker enzyme estimation*

The alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and total protein (TP) activities were evaluated using commercially available kit (Randox kit). Detailed procedures for the above measurements was performed according to the kit manufacturer's instructions.

3.7.2.1.1 *Determination of aspartate amino transferase (AST)*

Principle: L-glutamate and oxaloacetate are produced when α-ketoglutarate and L-aspartate react in the presence of AST. Oxaloacetate is used in the indicator reaction to kinetically measure the αconsumption of NADH.

α-Ketoglutarate + L-aspartate $\xrightarrow{\text{AST}}$ Oxalacetate + L-glutamate

NADH + Oxalacetate $\xrightarrow{\text{MDH}}$ sNAD + Malate

Method: While solution B included 2, 4 dinitrophenylhydrazine, L-aspartate, oxoglutarate, and phosphate buffer were all present in solution A. 0.25 ml of solution A and 0.05 ml of sample were combined. It was combined and 30 minutes of incubation at 37 °C. After that, 0.25 cc of solution B was added, stirred, and allowed to stand at 25 °C for 20 minutes. The mixture was then well blended with 0.25 cc of 0.1M NaOH. The same method used for the sample was repeated after adding 0.05 milliliters of distilled water was added to the test tube. After five few minutes, the absorbance was measured at

546 nm. The standard curve accustomed to read the sample's AST activity. This was done using a modified version of (Reitman and Frankel's 1957) approach developed by (Schmidt *et al*., 1963).

3.7.2.1.2 ***Determination of alanine amino transferase (ALT)***

Principle: L-glutamate and pyruvate are produced when α-ketoglutarate interacts with L-alanine when ALT is present. The indicator process uses pyruvate to kinetically measure the consumption of the nicotinamide adenine dinucleotide reduced form (NADH).

α-Ketoglutarate with L-alanine $\xrightarrow{\text{ALT}}$ L-glutamate + Pyruvate

NADH (H+) + Pyruvate $\xrightarrow{\text{LDH}}$ NAD^+ + Lactate

Method: While solution B included 2.4-dinitrophenylhydrazine was present in solution A of buffer L-alanine, (phosphate buffer), and α-oxoglutarate. 0.25 ml of solution A and the sample test tube received 0.05 ml of sample by pipette. It was combined and 30 minutes of incubation at 37 °C. Then Solution B was included in 0.25 cc., the mixture was again stirred, and the mixture was standing at 25 °C for 20 minutes. The mixture was back then given 0.25 cc of 0.1M NaOH. It was combined, and the blank sample was prepared making use of 0.05 ml of distilled water instead among the sample. After 5 minutes, at 540 nm, the absorbance was measured. The standard curve was used to read the sample's ALT activity. This was done in accordance with (Reitman and Frankel's 1957).

3.7.2.1.3 ***Determination of alkaline phosphatase (ALP)***

Principle: the response between serum alkaline phosphatase as well as the colourless Substrate for phenolphthalein monophosphate serves as the foundation for this technique. Phenolphthalein monophosphate is hydrolyzed by the ALP into phosphoric acid and

phenolphthalein. At pH 9.8, the mixture turned pink, and ALP was detected using spectrophotometry at 540 nm.

$$\text{P-nitrophenylphosphate} + H_2O \xrightarrow{\text{ALP}} PO_4 + \text{P-nitrophenol (pink, pH= 9.8)}$$

Method: 0.05 milliliters each of the sample and distilled water were added to the appropriate test tubes with labels. Each test tube received 3.0 ml of substrate, which was included and thoroughly mixed. At 405 nm and 37 C, A1 was the initial absorbance, which was measured after 1 minute, and successive the absorbances measured each minute of an additional 3 minutes. Calculations were made to determine average absorbance differences per minute (A / min) and the difference between absorbances (A).

Calculation:

Activity of ALP (U/L) is equal to 2750 x (A/min).

Where A denotes a little variation in absorbance

3.7.2.1.4 ***Determination of total protein***

Principle: A coloured complex is created when cupric ions interact with protein peptide bonds in an alkaline solution.

Method: In test tubes marked serum, standard, and blank, Serum, standard regent, and distilled water totaling 0.02 ml each were pipetted into the test tube. One milliliter (1 ml) of R1 (Biuret reagent) was added to each test tube, which was then thoroughly mixed before being incubated for 30 minutes at 25 °C. Sample and standard absorbances were measured at 546 nm in comparison to a blank. (Weichselbaum, 1946). The following formula was used to compute the total protein concentration:

$$\text{Protein total concentration (g/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{x Typical protein conc.}$$

(Where 5.58 g/dl of normal protein content is present)

Potassium iodide, With 15 mmol/L of potassium hydroxide, 6 mmol/L of cupric sulphate, likewise 100 mmol/L of sodium hydroxide make up the R1 reagent.

3.8 Data Analysis

The data were analyzed using statistical package for science student at a significant difference of 0.05, data were collected in triplicate.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Qualitative phytochemical constituents of sample A and B preparations

The qualitative analysis of Sample A and B Preparations results is shown in Table 4.1.The results revealed that the sample A contains high presence of tannins, alkaloids, saponin, flavonoids. Sample B contains high level of tannins and saponin.

Table 4.1: Qualitative phytochemical constituents of sample A and sample B preparations

Samples	Tannins	Alkaloids	Saponin (g/100g)	Flavonoids	Reducingsugar
Sample A	Highly present	present	present	highly present	Absent
Sample B	Highly present	Absent	highly present	absent	Absent

Key: + = Present

++ = Highly present

- = Absent

4.1.2 Quantitative phytochemical constituents of sample A and B preparations.

The quantitative phytochemical constituent of sample A and B preparations is shown in Table 4.2. The results revealed that the sample A contains 0.772±0.006g tannins, 0.167±0.002g alkaloid, 0.642± 0.006g saponin and 1.642±0.006g flavonoids. The results revealed that the sample B contains 0.483±0.002g tannins, 0.180±0.001g alkaloids, 1.607±0.002g saponin and 1.115±0.288g flavonoids.

Table 4.2: Quantitative analysis of sample A and sample B preparations

Samples	**Tannins**	**Alkaloids** (g/100g)	**Saponin**	**Flavonoids**
Sample A	0.772 ± 0.006^{b}	0.167 ± 0.002^{a}	0.642 ± 0.006^{b}	1.642 ± 0.006^{b}
Sample B	0.483 ± 0.002^{ab}	0.180 ± 0.001^{a}	1.607 ± 0.002^{a}	1.115 ± 0.288^{a}

4.1.3: Result for acute toxicity

The Phase 1 of Lorke's method of test for acute toxicity of sample A and sample B preparations is shown in Table 4.3. For sample A at different dose, there were no toxicity or mortality signs at doses of 10 mg/kg body weight, 100 mg/kg, or 1000 mg/kg body weight. For sample B, dosages of 10 mg/kg body weight, 100 mg/kg body weight, and 1000 mg/kg body weight were used, there was no toxicity and no death.

Table 4.3: Phase 1 of Lorke's method of acute toxicity test

	Number of used animals	**administered doses(mg/kg bw)**	**observed toxicological symptoms**	**Mortality**
Sample A	1	10	No toxicicity was present	Nil
	1	100	No toxicity was Present	Nil
	1	1000	No toxicity was present	Nil
Sample B	1	10	No toxicity was present	Nil
	1	100	No toxicity was present	Nil
	1	1000	No toxicity was present	Nil

4.1.4: Acute toxicity testing

Phase II of Lorke's method of test for acute toxicity

The phase II of Lorke's method of test for acute toxicity of sample A and sample B Preparations is shown in Table 4.4. For sample A Preparations at a dose of 1600mg/kg bw, 2900mg/kg and 5000mg/kg bw there was no mortality. For sample B Preparations at a dose of 1600mg/kg bw, 2900mg/kg bw and 5000mg/kg bw there was no mortality.

Table 4.4: Phase II of Lorke's method of acute toxicity testing

	Number of Animals used	**Doses Administered (mg/kg)**	**Observed signs of Toxicity**	**Mortality**
Sample A	1	1600	No sign of toxicity was observed	Nil
	1	2900	Hyperactivity and restlessness	Nil
	1	5000	Salivation and restlessness for the first 30 minutes after the extract was administered	Nil
Sample B	1	1600	Salivation	Nil
	1	2900	For the first 30 minutes, there was salivation and restlessness.	Nil
	1	5000	salivation and serenity for the first hour	Nil

Safe dose of the two preparations is > 5000 mg/kg bw

4.1.5: Sub-chronic toxicity testing

The result of the weight of organs for albino rats administered with sample A for 28 days is shown in Table 4.5. For the liver Group 1 has highest value of 6.05 ± 0.00^{e} as compared to other groups. For the Kidney, the group 2 has the highest value of 1.58 ± 0.01^{b} as compared to other groups.

Table 4.5: Weight (g) of organ for albino rats administered with sample A for 28 days

Organs	Control	Group 1	Group 2	Group 3
Liver	6.17 ± 0.05^{e}	6.05 ± 0.00^{e}	4.74 ± 0.15^{e}	5.31 ± 0.02^{e}
Kidney	1.18 ± 0.01^{d}	1.14 ± 0.07^{d}	1.58 ± 0.01^{b}	0.87 ± 0.01^{c}

4.1.6 Sub-chronic toxicity testing

The result of the weight of organs for albino rats administered with sample B for 28 days is shown in Table 4.6. For the liver, group 2 shows the highest value of 5.31 ± 0.00^{e} compared to other groups. For the kidney, group 3 shows the highest value of 1.26 ± 0.06^{d} compared to other groups.

Table 4.6: Weight (g) of organ for albino rats administered with sample B for 28 days

Organ	Control	Group 1	Group 2	Group 3
Liver	5.16 ± 0.05^{e}	3.63 ± 0.01^{e}	5.31 ± 0.00^{e}	4.33 ± 0.05^{e}
Kidney	1.17 ± 0.00^{d}	1.00 ± 0.05^{c}	1.18 ± 0.05^{b}	1.26 ± 0.06^{d}

The standard error of the mean (mean) is used to express values in triplicate determinations Values with various superscripts significant differences between the samples exist ($p< 0.05$).

4.1.7: Sample A impact on the weight of albino rats

The sample A impact on the weight of albino rats are indicated in table 4.7 of albino rats from the initial week which is week 0 to week 1 saw an increase in the albino rats' weight to the final week, which is week 4. This shows the sample A has no harmful effect on the albino rats.

Table 4.7: Effect of sample A on body weight (g) of albino rats

WEEKS	Control	Group 1	Group 2	Group 3
WEEK 0	108.04 ± 0.86^{a}	60.46 ± 0.02^{d}	129.91 ± 0.23^{e}	101.33 ± 0.12^{b}
WEEEK 1	119.97 ± 0.40^{a}	64.81 ± 0.29^{d}	132.55 ± 0.36^{e}	104.63 ± 0.04^{b}
WEEK 2	115.40 ± 0.59^{c}	69.40 ± 0.29^{d}	135.68 ± 0.15^{e}	105.73 ± 0.70^{a}
WEEK 3	117.93 ± 0.79^{c}	71.26 ± 0.17^{d}	137.05 ± 0.17^{d}	108.68 ± 0.53^{a}
WEEK 4	119.93 ± 0.19^{c}	73.01 ± 0.11^{d}	139.05 ± 0.15^{d}	111.04 ± 0.53^{a}

4.1.8: Sample B impact on the weight of albino rats

The sample B impact on the weight of albino rats are indicated in table 4.8 of albino rats from the initial week which is week 0 to week 1 the weight of the albino rats increased to the final week, which is week 4. This shows the sample B has no harmful effect on the albino rats.

Table 4.8: Effect of sample B on weight (g) of albino rats

WEEKS	Control	Group 1	Group 2	Group 3
WEEK 0	106.31 ± 0.01^{a}	114.98 ± 0.21^{c}	105.37 ± 0.02^{a}	99.96 ± 0.16^{b}
WEEK 1	110.49 ± 0.02^{a}	116.95 ± 0.42^{c}	107.28 ± 0.39^{a}	104.33 ± 0.55^{b}
WEEK 2	115.31 ± 0.05^{c}	121.02 ± 0.47^{e}	109.39 ± 0.77^{a}	106.85 ± 0.05^{a}
WEEK 3	117.51 ± 0.18^{c}	123.19 ± 0.11^{e}	112.00 ± 0.20^{c}	109.39 ± 0.60^{a}
WEEK 4	117.51 ± 0.38^{c}	125.19 ± 0.00^{e}	113.00 ± 0.00^{c}	110.39 ± 0.60^{a}

Values are measured as Mean± Standard Error of Mean made in triplicate. Distinct superscript values between the samples differ considerably ($p< 0.05$).

4.1.9: Effect of sample A on haematological parameters of albino rats treated for 28 days.

The result of sample A on haematological rats with albinism parameters are shown table. 4.9: Haemoglobin (HB), packed cell volume (PCV), white blood cells (WBC), red blood cells (RBC), neutrophils (NEUT), lymphocytes, mononucleosis (MONO), basophil (BASO), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), when compared to the normal control rats, none of the experimental groups' platelets or mean corspucular hemoglobin (MCH) substantially differed (P>0.05).

Table 4.9: Impact of sample A on haematological parameters of albino rats treated for 28 days.

Parameters	**Control**	**Group 1 (100 mg/kg body weight)**	**Group 2 (300 mg/kg body weight)**	**Group (1000 mg/kg body weight)**
HB (g/dL)	10.620 ± 0.01^{g}	9.02 ± 0.01^{b}	8.61 ± 0.01^{e}	8.62 ± 0.01^{c}
PCV (%)	32.03 ± 0.03^{g}	27.02 ± 0.01^{b}	26.02 ± 0.02^{e}	26.03 ± 0.02^{c}
RBC (10^9/L)	5.31 ± 0.00^{g}	4.52 ± 0.00^{b}	5.02 ± 0.01^{e}	4.61 ± 0.00^{c}
WBC (10^9/L)	3.61 ± 0.00^{e}	2.81 ± 0.00^{c}	4.01 ± 0.01^{g}	2.91 ± 0.02^{d}
NEUT (10^9/L)	31.00 ± 0.00^{f}	26.90 ± 0.00^{b}	28.02 ± 0.01^{c}	29.02 ± 0.01^{d}
LYM (10^9/L)	64.01 ± 0.04^{c}	64.02 ± 0.01^{a}	64.01 ± 0.01^{c}	62.02 ± 0.01^{b}
MONO (10^9/L)	2.12 ± 0.06^{c}	1.01 ± 0.01^{a}	2.01 ± 0.01^{b}	2.01 ± 0.01^{b}
BASO (10^9/L)	1.00 ± 0.00^{a}	1.01 ± 0.01^{a}	1.01 ± 0.01^{a}	1.00 ± 0.00^{a}
MCV (10^{-5}/L)	60.21 ± 0.11^{b}	60.01 ± 0.01^{a}	60.02 ± 0.02^{a}	60.82 ± 0.01^{d}
MCHC (10^{-15}/L)	33.04 ± 0.03^{a}	33.30 ± 0.00^{d}	33.02 ± 0.01^{a}	33.02 ± 0.01^{a}
PLATELETS (10^3/L)	241.90 ± 0.01^{g}	226.02 ± 0.01^{c}	247.02 ± 0.02^{g}	227.01 ± 0.01^{d}
MCH (pg)	20.02 ± 0.01^{a}	20.01 ± 0.01^{a}	20.02 ± 0.01^{a}	20.02 ± 0.01^{a}

4.1.10: Sample B impact on haematological parameters of albino rats treated for 28 days.

The result of sample B on albino rats hematological characteristics are shown in table. 4.10. Red blood cell (RBC), packed cell volume (PCV), haemoglobin (HB), white blood cell counts (WBC), neutrophils (NEUT), lymphocytes, mononucleosis (MONO), basophil (BASO), when compared to the normal control albino rats, the mean corpuscular haemoglobin concentration (MCHC), mean cell volume (MCV), platelets, along with mean corspucular haemoglobin (MCH) were not substantially (P>0.05) different in any of an experimental groups.

Table 4.10: Impact of sample B on haematological parameters of albino rats treated for 28 days

Parameters	Control	Group 1 (100 mg/kg body weight)	Group 2 (300 mg/kg body weight)	Group 3 (1000 mg/kg body weight)
HB (g/dL)	10.31 ± 0.00^{f}	10.01 ± 0.01^{d}	9.31 ± 0.02^{a}	9.52 ± 0.15^{c}
PCV (%)	31.01 ± 0.01^{f}	30.02 ± 0.01^{d}	28.02 ± 0.01^{a}	28.65±0.44a
RBC (10^{9}/L)	5.07 ± 0.03^{f}	4.82 ± 0.03^{a}	4.31 ± 0.01^{a}	4.30 ± 0.00^{a}
WBC (10^{9}/L)	3.42 ± 0.01^{d}	2.82 ± 0.01^{c}	2.42 ± 0.01^{a}	2.61 ± 0.01^{b}
NEUT (10^{9}/L)	30.00 ± 0.00^{e}	31.02 ± 0.00^{f}	24.00 ± 0.01^{a}	24.01 ± 0.01^{a}
LYM (10^{9}/L)	62.06 ± 0.03^{b}	61.00 ± 0.01^{e}	64.01 ± 0.00^{c}	66.01 ± 0.01^{d}
MONO (10^{9}/L)	2.01 ± 0.01^{b}	1.01 ± 0.01^{a}	2.01 ± 0.01^{b}	2.00 ± 0.00^{b}
BASO (10^{9}/L)	1.00 ± 0.00^{a}	1.01 ± 0.01^{a}	1.01 ± 0.02^{a}	1.00 ± 0.00^{a}
MCV (10^{-15}/L)	60.71 ± 0.11^{d}	60.41 ± 0.01^{d}	60.41 ± 0.01^{c}	60.42 ± 0.01^{c}
MCHC (10^{-15}/L)	33.02 ± 0.00^{c}	33.11 ± 0.00^{d}	33.01 ± 0.01^{a}	33.00 ± 0.00^{a}
PLATELETS (10^{3}/L)	231.02 ± 0.01^{fs}	228.01 ± 0.01^{e}	224.00 ± 0.00^{a}	225.00 ± 0.01^{b}
MCV (pg)	20.02 ± 0.02^{a}	20.01 ± 0.01^{a}	20.03 ± 0.03^{a}	20.00 ± 0.00^{a}

Values are measured as Mean± Standard Error of Mean made in triplicate. Distinct superscript values between the samples differ considerably ($p< 0.05$).

4.1.11 Effect of sample A on liver enzymes

The result of effect of sample A on the liver enzymes is shown in table 4.11. There was a significance increase in the AST (u/L) activities among group 3 albino rats when compared to the experimental group to the albino rats. ALP (u/L), and ALT (u/L) activities of the albino rats followed a similar trend.

Table 4.11 Effect of sample A on the liver enzymes

Parameters	Control	Group 1	Group 2	Group 3
AST (u/L)	47.38 ± 2.45^{b}	50.00 ± 0.55^{b}	49.08 ± 2.37^{b}	53.13 ± 0.58^{a}
ALT (u/L)	63.51 ± 0.05^{b}	65.00 ± 0.03^{b}	63.51 ± 0.05^{b}	62.30 ± 0.39^{c}
ALP (u/L)	68.37 ± 0.01^{b}	65.03 ± 1.05^{d}	65.00 ± 1.55^{d}	68.37 ± 0.01^{d}

4.1.12 Sample B impact on the liver enzymes

The sample B impact on the liver enzymes appears in table 4.12. There was a significance ($p<0.05$) difference in the AST (u/L) activities among group 3 albino rats when in comparison to control group of albino rats. ALP (u/L), and ALT (u/L) activities of the animals followed a similar pattern.

Table 4.12 Effect of sample B on the liver enzymes

Parameters	Control	Group 1	Group 2	Group 3
AST(u/L)	53.01 ± 1.55^{b}	48.15 ± 2.19^{b}	51.11 ± 0.37^{a}	58.02 ± 2.19^{c}
ALT(u/L)	63.19 ± 2.19^{b}	61.95 ± 2.09^{b}	65.41 ± 0.05^{b}	62.38 ± 1.30^{c}
ALP(u/L)	70.03 ± 0.21^{d}	66.51 ± 0.33^{d}	65.17 ± 1.55^{d}	66.51 ± 0.33^{d}

Values are Mean±Standard Error of Mean of triplicate determinations.Values with different superscripts between the samples are significantly different

4.1.13 Effect of sample A on total protein, direct bilirubin, total bilirubin, urea and creatinine

The effect of sample A on total protein (µmol/L), direct Bilirubin (µmol/L), urea (mm/L) and Creatinine (µmol/L) is shown in table 4.13 shows there was significance difference ($p<0.05$) in the creatinine activities in the group 3 albino rats contrasted with the albino rats in the control group. Bilirubin overall and Urea of the albino rats followed a similar trend.

Table 4.13 Effect of sample A on total protein, total bilirubin, direct bilirubin, urea and creatinine

Parameters	Control	Group1	Group2	Group3
Total protein (g/L)	6.873±0.003^{d}	6.003±0.003^{d}	5.886±0.003^{d}	7.806±0.003^{d}
Total bilirubin (mg/dL)	0.320±0.005^{b}	0.350±0.005^{b}	0.276±0.006^{b}	0.340±0.05^{b}
Direct bilirubin (*µ*mol/L)	0.183±0.006^{a}	0.216±0.008^{a}	0.136±0.006^{a}	0.213±0.003^{a}
Urea (mg/dL)	45.96±0.01^{b}	47.91±0.02^{b}	49.32±0.01^{b}	56.31±0.01^{c}
Creatinine (mg/dL)	6.88±0.01^{a}	5.37±0.01^{a}	5.13±0.00^{a}	6.31±0.02^{a}

4.1.14: Effect of sample B on urea, creatinine, total protein, direct bilirubin and total bilirubin

The effect of Sample B on total protein, direct bilirubin, urea, and creatinine is shown in table 4.14, shows a significance difference in the creatinine ($p<0.05$) activities in a group 3 albino rats in comparison to the control group of albino rats. For the Urea activities in group 2 albino rats there were a significance difference ($p<0.05$) in comparison to the albino rats welfare organization.

Table 4.14: Effect of sample B on total protein, total bilirubin, direct bilirubin, urea and creatinine

Parameters	Control	Group 1	Group 2	Group 3
Total protein (g/L)	6.110 ± 0.005^{a}	5.463 ± 0.006^{d}	6.110 ± 0.006^{d}	7.046 ± 0.006^{d}
Total bilirubin (mg/dL)	0.206 ± 0.006^{a}	0.390 ± 0.005^{b}	0.323 ± 0.003^{b}	0.430 ± 0.005^{b}
Direct bilirubin (μmol/L)	0.113 ± 0.008^{a}	0.180 ± 0.005^{a}	0.216 ± 0.008^{a}	0.283 ± 0.008^{a}
Urea (mg/dL)	40.13 ± 0.01^{b}	42.17 ± 0.02^{a}	53.18 ± 0.01^{c}	50.46 ± 0.04^{c}
Creatinine (mg/dL)	7.58 ± 0.00^{a}	6.42 ± 0.02^{a}	6.07 ± 0.01^{a}	6.89 ± 0.10^{a}

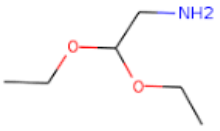
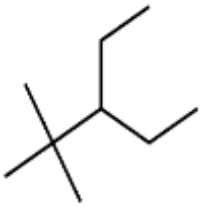
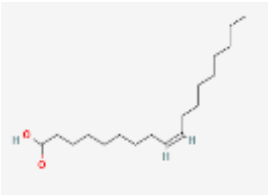
Values are Mean±Standard Error of Mean of triplicate determinations.Values with different superscripts between the samples are significantly different ($p<0.05$).

4.1.15. GC-MS examination of sample A

GC-MS result for sample A gave a five major peak (Figure 1); each peak corresponds to compounds as identified by the NIST library (NIST11.L). Table 4.15 represent the compositions of sample A, which were mainly oleic acid. The identified compounds with their percentage composition, retention time and molecular formula are shown in Table 4.15. Oleic Acid which is the major composition has pharmacological actions such as antifungal, anti-inflammatory, antioxidants, antibacterial (Awonyemi, *et al*., 2020), antidiabetic (Vassiliou *et al*., 2009).

It may be clearly seen in Table 4.15, that Sample A consist of 4.4% of Ethanamine, 2,2-diethoxy, Pentane, 9.96% of 3-ethyl-2,2-dimethyl, 3.15% of Propanenitrile, 2-hydroxy and 86.7% of Oleic acid. Pharmacological actions of Oleic acid are antifungal, anti-inflammatory, antioxidants, antibacterial (Awonyemi *et al*., 2020), antidiabetic (Vassilou *et al*., 2009).

Table 4.15: GC-MS- analysis of Sample A

Peak No	Retention Time	Phytochemical Compound	Molecular formular	Area (%)	Structure
1	3.401	Ethanamine,2,2 Diethoxy	$C_6H_{15}ONO_2$	4.4	
2	3.9	Pentane, 3-ethyl-2,2-Dimethyl-	C_9H_{20}	9.96	
3	5.846	Propannenitrile,2-Hydroxyl	C_3H_5NO	3.15	
4	15.27	Oleic Acid	$C_{18}H_{34}O_2$	86.7	

4.1.16 GC-MS analysis of sample B

The GC-MS spectrum of sample B gave a three major peaks, each peak corresponds to compounds as identified by the NIST library (NIST11. L). The identified compounds with their percentage composition, retention time and molecular formula are shown in the table table 4.16 below. Oleic acid which is the major composition has pharmacological actions such as antifungal, anti-inflammatory, antioxidants, antibacterial (Awonyemi *et al*., 2020), antidiabetic (Vassiliou *et al*., 2009). Methano-1H, 7H,11H-furo[3,4-g]pyrano [3,2-b] xanthene-7,15-dione, 3,3a, 4, 5-tetrahydro-8-hydroxyl-3,3, 11, 11-tetramethyl-1, 13-bis (3-methy-2-buten-1-yl)-, (1R, 3As, 5S, 14aS)- as one of the constituent is an alkaloid which can be used as diet ingredients, supplements and pharmaceuticals in medicine and other applications in human life (Mamuru *et al*., 2019).

Table 4.16 GC-MS analysis of sample B

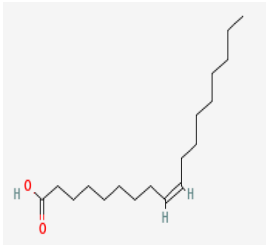
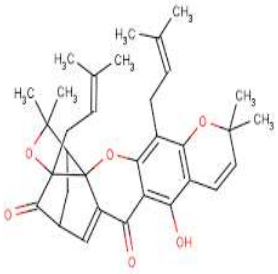
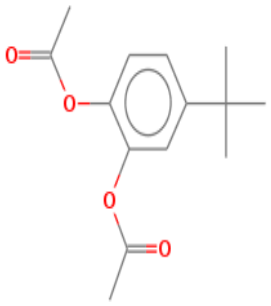
Peak No	Retention Time	Phytochemical Compound	Molecular formula	Area (%)	Structure
1	14.454	Oleic Acid	C18H34O2	85.88	
2	16.549	1,5- Methano-1H,7H,11H-sFuro[3,4-sg] pyrano[3,2-sb] 3,3a,4,5-tetrahydro-8-hydroxy-3,3,11,11-tetramethyl-1,13-bis(3-methyl-2-buten-1-yl)-xanthene-7,15-dione (1R, 3aS, 5S, 14aS)-	$C_{33}H_{38}O_7$	7.04	
3	13.359	4- tert-Butyl-O-Phenylene diacetate	$C_{14}H_{18}O_{14}$	7.34	

Table 4.17 Blood glucose level (mg/dL) for sample A

Groups	W_0	W_1	W_2	W_3
Group1	115.37 ± 0.003^{a}	120.38 ± 0.003^{ab}	124.32 ± 0.003^{c}	117.50 ± 0.003^{b}
Group2	119.35 ± 0.003^{c}	123.54 ± 0.006^{b}	120.56 ± 0.007^{a}	$118.55\pm0\pm.003^{c}$
Group3	117.50 ± 0.003^{b}	118.30 ± 2.036^{a}	123.54 ± 0.007^{b}	114.00 ± 0.000^{a}

Key

Group 1= Albino rat induced with alloxan + 100 mg/kg of sample A

Group 2= Albino rat induced with alloxan + 300 mg/kg of sample A

Group 3= Albino rat induced with alloxan + 1000 mg/kg of sample A

Table 4.18 Blood glucose level (mg/dL) for sample B

Groups	W_0	W_1	W_2	W_3
Group1	118.03±0.003^{d}	128.33±0.003^{d}	112.33±0.003^{a}	120.00±0.003^{c}
Group2	120.01±0.000^{e}	130.07±0.003^{e}	128.03±0.003^{d}	122.57±0.003^{d}
Group3	93.43±0.003^{a}	110.57±0.003^{a}	120.55±0.003^{c}	101.37±0.003^{a}
Group 4	111.33±0.003^{c}	121.03±0.003^{c}	137.58±0.003^{e}	158.38±0.003^{e}
Group 5	110.67±2.504^{b}	112.31±0.003^{b}	115.57±0.003^{b}	114.31±0.000^{b}

Values are Mean±Standard Error of Mean of triplicate determinations.Values with different superscripts between the samples are significantly different ($p<0.05$).

Key

Group 1=Albino rat induced with alloxan + 100 mg/kg of sample B

Group 2=Albino rat induced with alloxan + 300 mg/kg of sample B

Group 3=Albino rat induced with alloxan + 1000mg/kg of sample B

Group 4=Albino rat induced with alloxan and not treated

Group 5=Albino rat induced with alloxan and treated with metformin

4.1.3 Histopathological analysis

Photomicrograph of the liver section, the liver negative control group of albino rats administered with alloxan only showed a notable disarrangement of the cyto-architecture of the liver, while the liver positive control group of albino rats showed typical liver features consisting of hepatocytes, while groups administered with 100 mg/kg bw, 300 mg/kg bw and 1000 mg/kg bw of both sample A and B showed normal hepatocyte architecture compared to the negative control group.

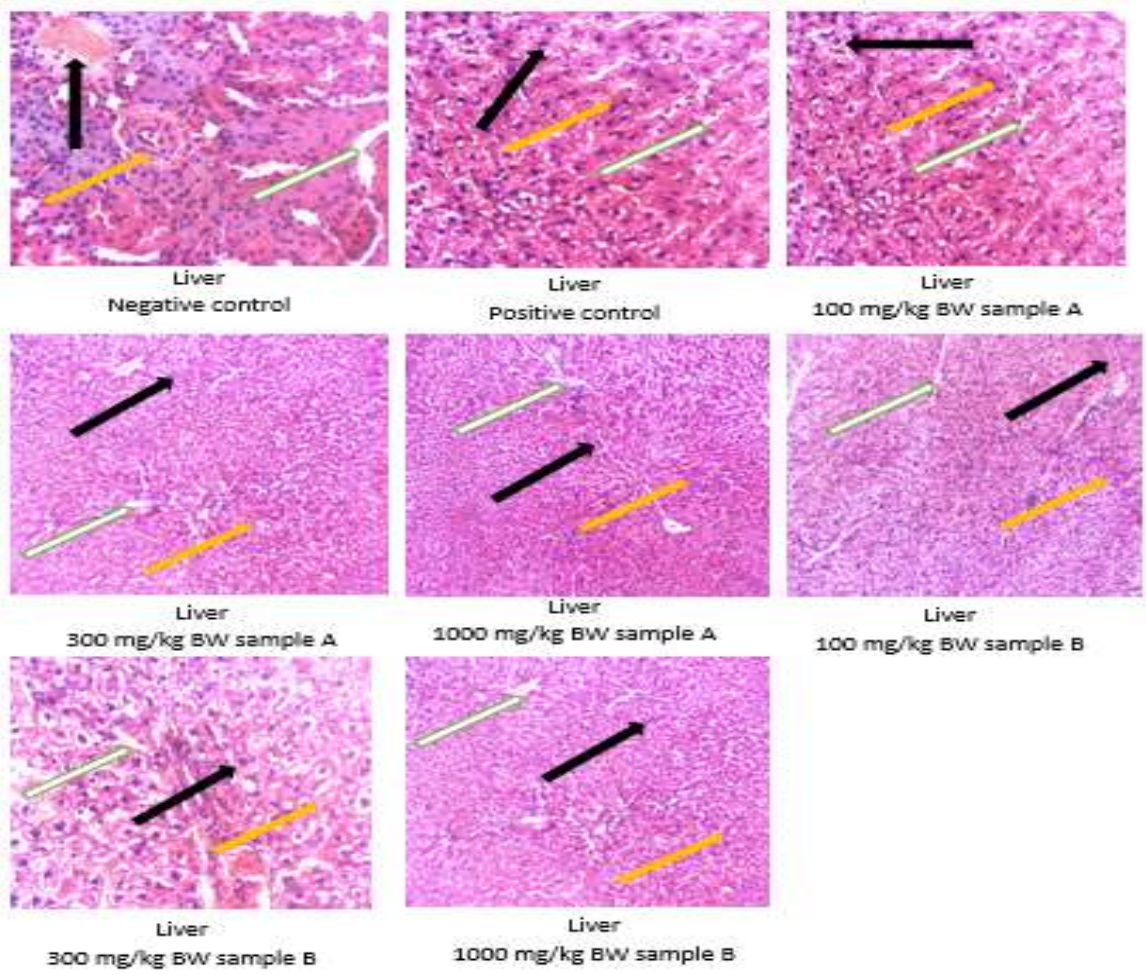


Plate 4:1: Photomicrograph of Liver Sections

Key: black arrows represent hepatocytes, white arrows represent hepatocyte sinusoids and yellow arrows represent portal vein.

Photomicrograph of the kidney sections, the kidney negative control group of albino rats administered with alloxan only showed notable disarrangement of the kidney, while the kidney positive control group of albino rats administered with metformin showed the kidney section had normal histological structures, the groups administered with 100 mg/kg bw, 300 mg/kg bw and 1000 mg/kg bw of both sample A and B showed normal histological structures compared to the negative control group.

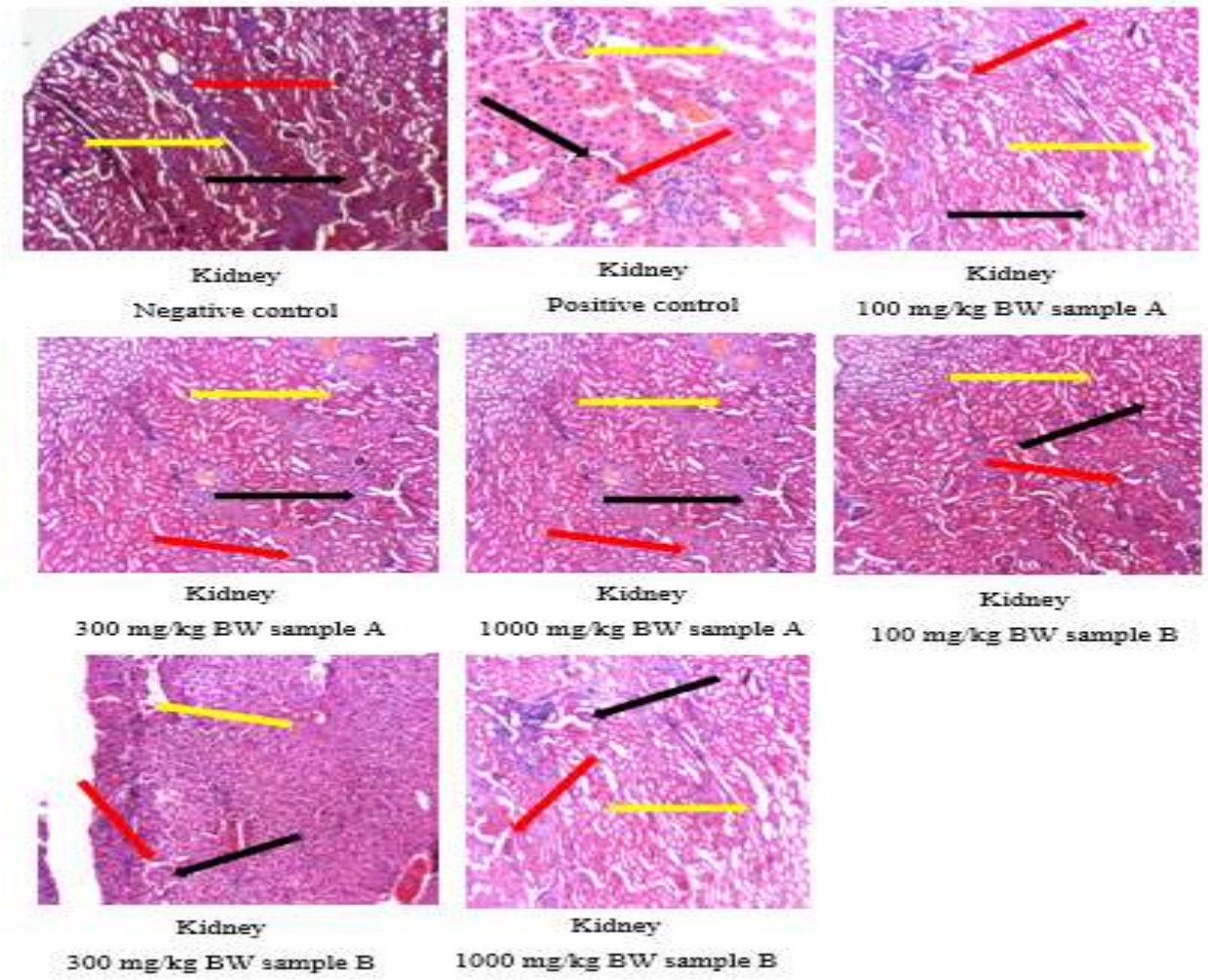


Plate 4:2: Photomicrograph of Kidney Sections

4.2 Discussion of Results

4.2.1 Secondary metabolite constituents

The two Samples (Sample A and Sample B) contained alkaloids, flavonoids, saponins, and tannins. These phytochemicals could be responsible for the medicinal potentials manifested by the two herbal Samples.

Tannins in sample A and B which were 0.772 g/100g and 0.483 g/100g they exceeded the values of 0.331 as reported by Krisknaiah *et al*. (2009) for his herbal solution containing C. *asiatica*, the variation observed in the findings of the current research could be attributed to environmental elements such as climate, soil type, soil type, and harvesting season or maturity stage of the leaves used in the herbal solution. Tannins have reportedly been shown to be useful in reducing joint swelling and hemorrhages. While tannins are thought to be haemostatic, they also have positive effects on the mucosal covering in the mouth. As a result, herbs with tannins are frequently used in snuff, mouthwash, eyewash, and even vaginal douche (Akinyeye *et al*., 2014). Internally, tannins have an impact on the stomach and other digestive organs. They control the amount of discharges from the cells by souring the mucus secretions and contracting or squeezing the membranes. The tannins have proved to have antinutritional effects because they can make feedstuff less palatable and less digestible (Odebiyi and Sofowora, 1979).

Amount of saponins in sample A and sample B (0.64 g/100g) is comparable to what was described by (Krishnaiah *et al.*, 2009) who obtained values of 0.735 g/100g. This is an indication that the two samples have a high potential to lower or reduce blood pressure and cholesterol level in blood. By forming insoluble compounds with cholesterol, saponins are eliminated through the bile (Olaleye, 2007). This stops the reabsorption of cholesterol and lowers serum cholesterol. It has been discovered that saponins may be

helpful for the treatment of hypercholesterolemia, which raises the possibility that saponins may work by preventing the intestinal absorption of cholesterol (Olaleye, 2007). The high saponin content of the two samples (sample A and B) the extract's ability to froth in the current investigation could be the cause of this.

Previous research by Sun *et al.* (2009); Sparg *et al.* (2004) reveal that saponin play crucial role by providing an antidote for acute lead poisoning, preventing platelet aggregation and dental caries, treating hypercalciuria in people, and inhibiting dental caries, and having hypocholesterolemic, immune-stimulating, and anticarcinogenic qualities are only a few of its uses. Applications for viral and bacterial vaccines use saponins as adjuvants. Studies have demonstrated its function in destroying cancer cells, maintaining bone health, and enhancing the immune system (Sparg *et al.,* 2004) saponins inhibit Na^+ efflux by blocking the cell's exit point for Na^+. As a result, cells' Na^+ concentration rises, which causes heart muscle's Na^+-Ca^{2+} antiporter to become active. The stronger contractions of the cardiac muscle are made possible by the more Ca^{2+} is being transferred through this anti porter (Schneider and Woliling, 2018).

The high level of flavonoid in the two herbal samples (sample A and sample B) is an indication that the two solutions have the potency to combat oxidative stress. The capacity to act as a buffer against reactive oxygen species' damaging effects, including those of singlet oxygen, superoxides, peroxyl and hydroxyl radicals, and peroxynitriles, makes flavonoids a crucial biological antioxidant (Alkadi , 2020). A low ratio of antioxidants to oxidative stress in cells could be brought on by reactive oxygen species, which can harm them. Numerous inflammatory illnesses have been connected to oxidative stress (Kruk and Kubasik. 2016).

The high flavonoid in the both sample solution shows the fact can be employed in the treatment and prevention of oxidative stress-related illnesses like diabetes mellitus, heart disease, ischemia injury, arthritis, chronic ulcers, atherosclerosis, and neurodegenerative disorders (Burlon and Ingold, 2004). Additionally, numerous analyses have demonstrated that low-density lipoprotein (LDL) can be protected from oxidation by flavonoids like genistein and procyanidin B5. (Donald and Cristobal, 2006). The emergence of atherosclerotic plaques in the artery wall is caused by the immune system's macrophages, which have been known to play a crucial role in the development of atherosclerosis, identifying and engulfing oxidized LDL (Chistiakov *et al.*, 2019).

Many studies have reported that Flavonoids may shield tissues against lipid peroxidation and free oxygen radical damage (Pascual *et al.,* 2010) thus contributing to the prevention of diabetes mellitus type 2 (T2DM). The capacity of dietary flavonoids to control endothelial nitric oxide status, NADPH oxidase activity, and inflammation response, which are associated with the risk of T2DM, has also recently come to light (Suganya *et al.,* 2016) Additionally, mechanistic studies with a focus on the subclasses of anthocyanins, flavonols, and flavan-3-ols showed that consumption of flavonoids may enhance insulin resistance, glycemia metabolism, and pancreatic cell dysfunction (Hanhineva *et al.,* 2010). Additionally, recent perspectives have emphasized the part that the gut microbiome plays in the pathophysiology of type 2 diabetes (Sohail *et al.,* 2017) additionally, consuming some flavonoids may positively impact the phylogenetic community and function of the gut microbiome (Gil-Cardoso *et al.,* 2016).

The alkaloid content of 1.643 mg/100g and 1.115 mg/100g reported the current analysis is consistent with the flavonoid content of 1.629 reported by Ibrahim *et al.* (2013) for the herbal solution containing *Sesamum indicum* leaves. The variation observed in the findings of the current research when compared with what was reporting from Ibrahim *et*

al (2013) could be attributed to environmental elements such climate, soil type, and harvesting season or the maturity stage of the leaves used in the herbal solution.

Alkaloids are naturally occurring bioactive substances that include nitrogen and are well known for having antibacterial characteristics because they can combine with the DNA of microbes (Kasolo *et al*., 2010). Alkaloids' intercalating effect on DNA has influenced the creation of antimicrobial medications (Gupta and Birdi, 2017). Many of them, including cocaine, morphine, atropine, colchicines, quinine, and strychnine, are widely used as medications and show their biological action even at extremely low doses. Among other things, alkaloid has been employed as a stimulant for the central nervous system, an anesthetic in ophthalmology, painkillers, and anti-puretic medicines (Moses and Uwah, 2015).

Since the beginning of civilization, plants that contain alkaloids have been utilized as dyes, spices, medicines, and poisons. While quinine, spareien, and quinidine have antiarrhythmic, antimalarial, and anticancer activities, many indole alkaloids have antihypertensive effects (dimeric indoles, vincristine, vinblastine) (Mamta *et al., 2016*). Only a few instances illustrate the significance of various plant components to the economy. Caffeine and nicotine are two examples of alkaloids with stimulant qualities. Analgesics such as morphine and quinine are both utilized as an antimalarial medication. (Ghasemzadeh *et al.*, 2010).

4.2.2 Median lethal dose (LD_{50})

An acute toxicity test evaluates the negative outcomes that follow the administration of a single dosage of a test material in a brief period of time. This testing, which primarily involves rodents, is typically carried out early on during the creation of a novel substance or item to provide knowledge regarding its possible toxicology (Endashaw, 2016).

The zero death recorded among the experimental albino rats even at a higher concentration of 5000 mg/kg body weight upon administration of sample A and B is an indication that the two herbal sample does not cause mortality to the experimental albino rats within the twenty four (24) hours study peri. The result obtained from the current investigation is consistent with the findings of Muhammad (2015) who likewise reported zero-mortality even a high dosage of 10,000 mg per kilogram of body weight upon administering via mouth, his own herbal solution (Somina herbal solution). Any chemical that does not cause death at 5000 mg/kg body weight, according to Lorke's (1983), is harmless and non-lethal. Also the obtained value of LD 50 >5000 mg/kg classed the substance as hazardous per Hodge and Sterner's scale, two herbal solutions used in the present study as practically non-toxic herbal medicines. The implication of this is that the two herbal samples used in the present study (sample A and sample B) should not cause mortality

4.2.3 Sub-chronic toxicity testing

Sub-chronic in a toxicology study, animals were given multiple doses orally over a 28-day period to determine whether there was any toxicity. The results of this test are used to determine the no observable impact level by providing information on the target organs and whether the test chemical may build up inside the body (NOEL) (Endashaw, 2016).

The oral administration of the two samples (sample A and sample B) at concentrations over 28 days body weight did not result in any discernible rise ($p > 0.05$) in liver enzymes at doses of 100 mg/kg, 300 mg/kg, and 1000 mg/kg (AST, ALT, and ALP), total protein, direct bilirubin, or creatinine. The outcome of the current investigation is consistent with what was previously reported by Michael *et al.* (2017) who also recorded no difference that is significant ($p< 0.05$) in liver enzymes, creatinine, direct bilirubin and complete

protein after administration of his own herbal formulation containing the leaves of *Caesalpinia volkensi* to experimental animals for a period of 28 days. The current research recorded an important variation ($p < 0.05$) between the urea degree of the group of rats given 300 mg/kg and 100 mg/kg of body weight of sample B in comparison to the control group. This significant difference was not corresponding to the work of Michael *et al.* (2017). During the sub-chronic, the rats treated for the toxicity research displayed a usual, steady rise in body weight. The control group, group I (500 mg/kg), and group II (1500 mg/kg) experienced corresponding mean body weight gains of 58.52 g, 58.42 g, and 53.68 g., did not vary significantly. Increase in body weight indicates that the animals are in good health.

4.2.4 Effect of Sample A and B about relative organ weight

No discernible distinction ($p > 0.05$) was noticed., based on the relative importance of the liver of the group 1 of albino rats (albino rats given 100 mg/kg body weight of sample A) with liver weight of 6.05±0.00 when compared with the close weight within the liver, within the control group of animals with liver weight of 6.17±0.05. The observation is consistent with what was reported by Songpol *et al.* (2012). Additionally, there were no notable differences between the group of albino rat liver that received higher doses of sample A in contrast to the control group, (300 and 1000 mg/kg b.w.) of albino rats, although there were slight variations in the liver weight which is similar to what was described by Bailey *et al*., (2004) Who said that the most accurate measure of an experimental compound's effects could be its effect on organ weight? and even in the absence of morphological alterations, treatment and control of albino rats organ weights may differ significantly. However, sample A when given in excess 300 and 1000 mg per kilogram of body weight might possibly have induced some gastric subcellular changes, which needs to be further investigated.

There was nothing noteworthy in relation to the team's liver's weight of animals administered a dose of 100 mg/kg of sample A (1.14±0.07) when compared to the performance of the control group of albino rats (1.18±0.01).

Organ weights are frequently used to assess the toxicity of test articles (Ezeonwumelu *et al.,* 2011). Many researchers test the toxicity or safety of medications or plant products on the liver and kidneys of rats (Satyapal *et al.*, 2008). In the current research, visual inspection of the kidneys and liver of the treatment group of animals, compared to the control group, failed to exhibit any significant visual differences in terms of size, form, color, or texture of animals. There was also no discernible variation in the total weight of the kidneys and the liver of the rats administered the various doses of sample A (100, 300, or 1000 milligrams per kilogram of body weight) compared to a command group.

There were no noteworthy differences in weight of the liver of the group of fed animals, the body weight of 100, 300, and 1000 mg/kg of sample B with mean, the liver's weight of animal relative to the control group of animals.

4.2.5 Effect of sample A and B on biochemical parameters (AST, ALT, ALP)

Biochemical compounds called enzymes quicken metabolic reactions in a living system. Compared to plasma, enzyme concentrations in cells are significantly higher. The harmony of synthesis and release rates of enzymes during cell turnover and the rate of removal from circulation, into the plasma is reflected in normal plasma enzyme concentrations. Due to cell proliferation, a rise in the rate of cell turnover, cell damage, or induction, as well as decreased plasma clearance, an enzyme's activity in plasma may be raised above normal (Opasich *et al.*, 2005). Reduced enzyme production or congenital deficiencies may result in decreased enzyme activity (Preethi *et al*., 2010).

There were no noteworthy differences ($p>0.05$) between a serum enzyme levels (AST, ALT, ALP) of the therapy group of animals administered the body weight of 100, 300, and 1000 mg/kg of sample A and sample B when in comparison to control group of animals. The result obtained from the current research is in agreement with what was reporting from Onakpa *et al*., 2011 who also declined to notice any substantial variations in the amounts of these serum enzymes. This could be an indication that the two samples (sample A and sample B administered at the various doses to the experimental animals did not cause any damage to the liver that might have resulted to the cellular leakage of these enzymes. There were no variations in the total amount of protein that were significant ($p>0.05$), Direct Bilirubin, Urea and Creatinine in the treatment groups administered sample A and B at different dose concentration relative to the control group of albino rats.

4.3 Blood Glucose Level for Sample A and B

Diabetes is associated with hyperglycemia and other metabolic disorder. The progressive increase in the blood glucose level of diabetic rats receiving no treatment (group 4 animals) might be due to the fact that alloxan destroy a beta cell of the pancrease, resulting in hyperglycemia (Owolabi *et al.,* 2014). The account of Bamidele *et al.* (2014), who reported a substantial drop in blood sugar levels upon administration of diabetic drug (metformin) is in accordance with the current study where the considerable rise in blood sugar level ($p<0.05$) was lessened by the metformin compared to the diabetics who weren't receiving treatment of animals (group 4).

Diabetic rat therapy with sample A and sample B resulted in low blood glucose level compared to the diabetically induced rats that weren't given the prescribed medication. The biguanide oral anti-diabetic medication metformin decreases blood sugar levels by

working in a variety of ways. This entails lowering hepatic glucose synthesis, restricting gastrointestinal glucose absorption, and boosting insulin sensitivity to improve glucose uptake and utilization (Klip and Leiter, 1990).

Furthermore, hypoglycaemic effect of sample A and sample B may be given credit for the role of different phytochemicals in the two samples. Saponins for instance have been reported for antioxidants properties and stimulating impact on pancreatic β-cells' ability to secrete insulin (Rawi *et al.*, 2011). The presence of substantial amount of this phytochemical can further support the claim that the hypoglycaemic actions of the drug could influence its mode of action of the two herbal samples. The present of high levels of saponins therefore supported the hypoglycaemic impact shown by both samples and further suggested that both samples if done correctly evaluated could result in promising drug formulation relevant to pharmacology.

4.4 Identified Compounds and their Pharmaceutical Role

I. Oleic acid: Oleic acid is use as excipient in pharmaceutical (as minor ingredient in some drugs) (Smolinske, 1992).
II. Ethanamine 2,2 diethoxyl: it has been use as a constituent in some drugs which help to reduce the symptoms of pakinson disease.
III. Propanitrile, 2-hydroxyl: It is use as a solvent and a precursor to other organic compound.
IV. 4-tert-Butyl- O- Phenylene: It is use as a curing agent and also use in the production of epoxy resins.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The two sample preparations (Sample A and B) has high amounts of antioxidant phytochemicals (Phenols and flavonoids). The concurrent administration of the two sample preparations (Sample A and B) to the diabetic animals enhances the glycemic recovery of rats against alloxan assaults and also enhances the recovery of animals from diabetic induced alteration in biochemical parameters. Specifically, the AST, ALP and ALT activities were brought to normal healthy conditions. It is therefore reasonable to conclude that the two sample preparations (Sample A and B) could be very useful in the management of diabetic condition

5.2 Recommendations

Considering the findings of this investigation,

- **i.** The administration of the two sample preparations (Sample A and B) to diabetic patients is recommended for regulation of glucose level.
- **ii.** Future work may include an assessment of the action of other glycolytic enzymes in the serum and hepatocyte of rats with diabetes placed administered the two sample preparations (Sample A and B)
- **iii.** Future work may also include bioactive compound isolation and characterisation present in the two sample preparations (Sample A and B) and also
- **iv.** Elucidating the mechanism of action exhibited by the two sample preparations (Sample A and B) should be given detail attention.

5.3 Contribution to Knowledge

I. From my finding from the two herbal preparations (paxherbal diarth and antidiabetes), they were not toxic and no mortality after administering both of them orally to the albino rats, thus both samples are safe for usage.

II. Both herbal preparations contain oleic acid in abundant quantity after both sample preparations were subjected to GCMS, and oleic acid which is a key component of both samples help to fight against diabetes.

III. Both herbal preparations paxherbal diarth and antidiabetes help to reduce the glucose level in the albino rat in the antihyperglycaemic study after inducing the albino rats with alloxan from sample A (paxherbal diarth, glucose level in group 3 drop from 118.03 ± 2.036^{a} to 114.00 ± 0.00^{a}) and in sample B (AntiDiabetes, glucose level in group 3 drop from 110.57 ± 0.003^{a} to 101.37 ± 0.003^{a}), thus making both sample preparation safe to help reduce glucose level in the bloodstream.

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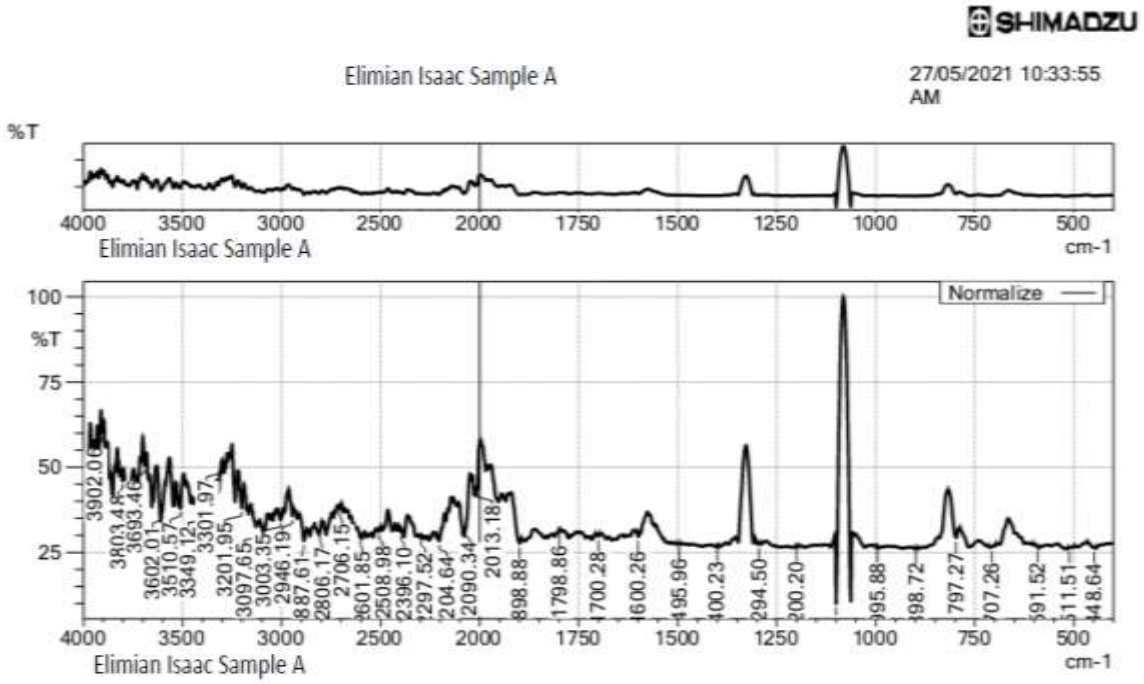
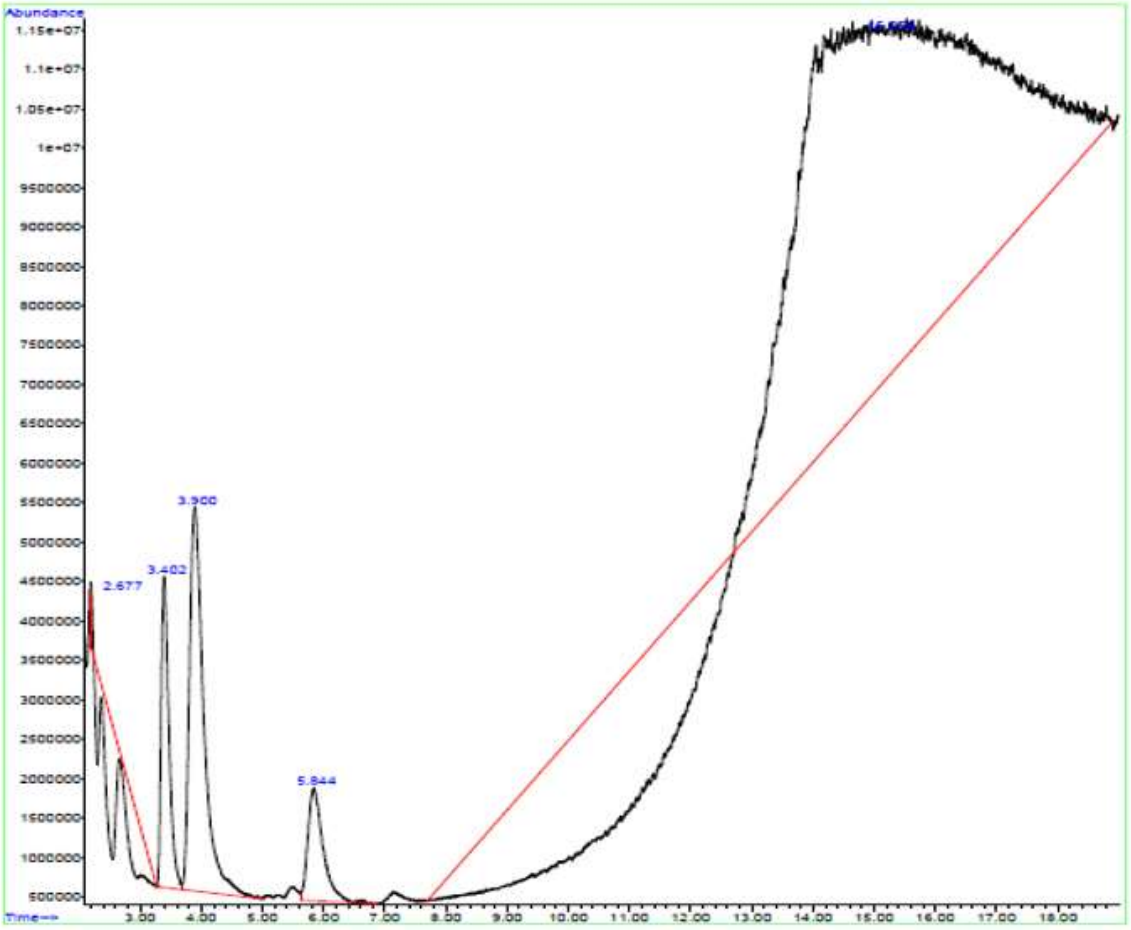
APPENDIX

Appendix A : Displays the spectrum of sample A .



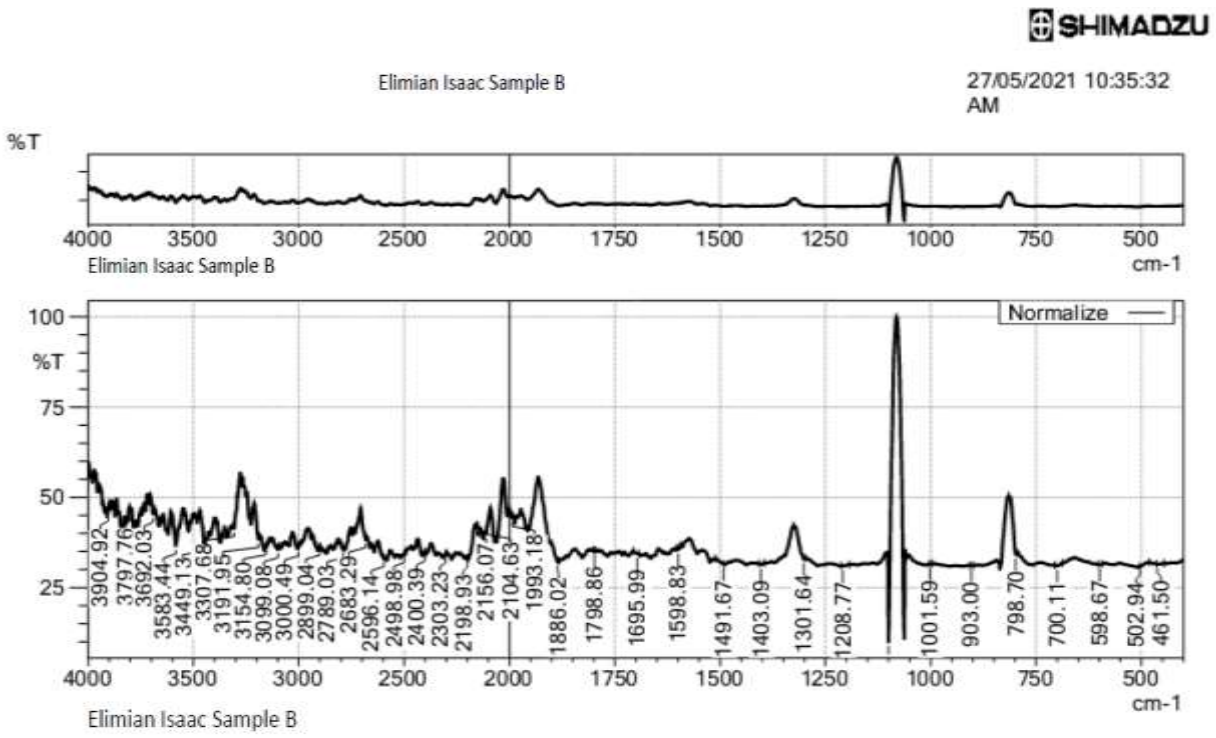
APPENDIX A : FTIR SPECTRUM OF SAMPLE A

Appendix B



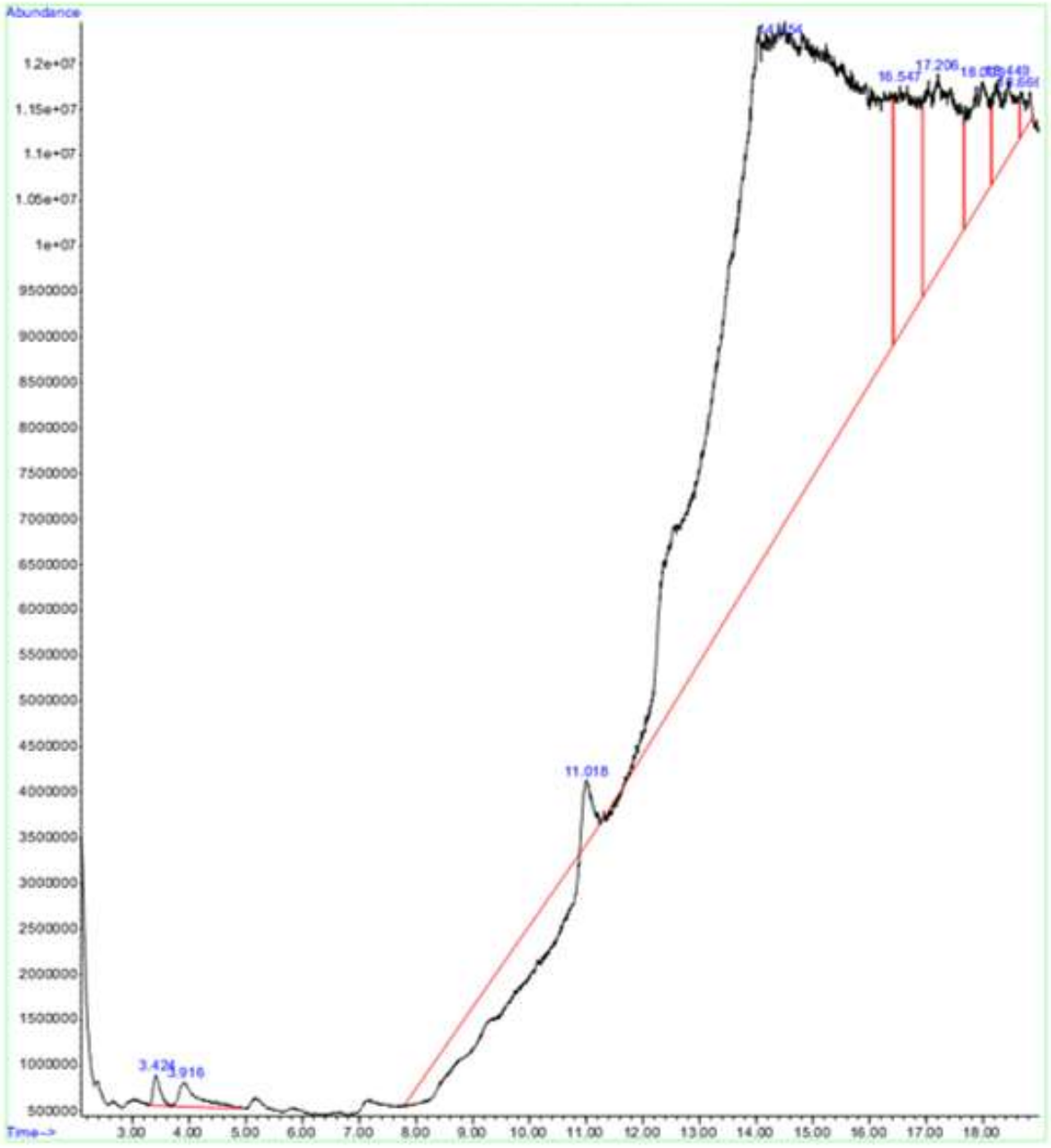
APPENDIX B : GC-MS ANALYSIS OF SAMPLE A

Appendix C: Displays the spectrum of sample B



APPENDIX C: FTIR SPECTRUM OF SAMPLE B

Appendix D



APPENDIX D: GC-MS ANALYSIS OF SAMPLE B