CHARACTERISATION OF *TELFAIRIA OCCIDENTALIS, CELOSIA ARGENTIA* AND *AMARANTHUS HYBRIDUS* AND THEIR EFFECTS ON INDUCED HYPERLIPIDEMIA IN WISTER RATS

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A PROJECT SUBMITTED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY, COLLEGE OF BASIC AND APPLIED SCIENCES,

IN PARTIAL FUFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF TECHNOLOGY DEGREE IN FOOD SCIENCE AND TECHNOLOGY

SEPTEMBER, 2021

DECLARATION

I humbly declare that this project has been written by me and is a record of my own research work. It has not been presented in any previous application for a higher degree of this or any other University. All citations and sources of information are clearly acknowledged by means of reference.

…………………………………

OBIWUSI, OLUWATUNBOSUN DAMILOLA

………………………………………

Date

CERTIFICATION

This is to certify that the content of this entitled **'characterization of *Telfairia occidentalis, Celosia argentia and Amaranthus hybridus* and their effects on induced hyperlipidemia in rats'** was prepared and submitted by OBIWUSI OLUWATUNBOSUN DAMILOLA in partial fulfillment of the requirements for the degree of **BACHELOR OF TECHNOLOGY IN FOOD SCIENCE AND TECHNOLOGY**. The original research work was carried out by him under my supervision and is hereby accepted.

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Head of Department

DEDICATION

This report is dedicated to God Almighty, my parents; Mr. and Mrs. Obiwusi and my siblings- Tosin, Fola and Tolu

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I am forever indebted to God Almighty who has been my helper and strength through the course of running this research project and the journey of bagging an honorable degree.

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ABBREVIATIONS

WHO: World health organization

ROS: Reactive oxygen species.

TNF: Tumor necrosis factor.

IL: Interleukin.

IL-6: Interleukin 6.

ADG: Australian Dietary Guidelines.

BMI: Body max Index.

WHR: Waist-hip ratio.

CT: Computed tomography.

MRI: Magnetic resonance imaging.

WHtR: Weight to height ratio.

HDL: High density lipoprotein

LDL: Low density lipoprotein

TC: Total cholesterol

TG: Triglycerides

MG/DL: Milligrams per decilitre

Dual-energy X-ray Absorptiometry (DXA)

NPY: Neuropeptide

UK: United Kingdom

UV: Ultra-violence

LLE: Liquid-liquid extraction

LSE: Liquid-solid extraction

SFE: Supercritical fluid extraction

GCMS: Gas Chromatography-Mass Spectrometry

T2DM: Type 2 Diabetes Mellitus

HFD: High fat diet

VLDL: Very low-density lipoprotein

CHD: Coronary heart disease

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2M Sucrose Solution

Preparation of 0.0075mg/kg of Orilifit

ABSTRACT

Hyperlipidemia refers to a group of inherited and acquired illnesses in which the body's lipid levels are abnormally high. Obesity prevalence ranges from 8.1% to 22.2% in Nigeria, with overweight individuals ranging from 20.3% to 35.1%. In Nigeria, twelve million persons were estimated to be obese by 2020, with the prevalence of obesity being significantly greater among women. Being a medical disorder, it is associated with metabolic dysfunction. The actual causes of metabolic syndrome may be many, but researchers lean toward insulin resistance as the underlying problem. Overweight people tend to develop a resistance to insulin -- a hormone that regulates blood sugar levels. Leafy vegetable in general have been reported to showed therapeutic effect in annulling the comorbidities associated with hyperlipidemia. This study was therefore aimed at evaluating the ameliorative effect of diets formulated with three different leafy vegetables (*Telfairia occidentalis, Celosia argentia and Amaranthus hybridus*) on hyperlipidemia, a metabolic dysfunction associated with induced obesity in female wistar rats. The phytoconstituents of the vegetables were quantified and the active compounds were identified with Gas chromatography- mass spectroscopy (GC-MS). Thirty-five female rats were completely randomized into 7 groups (I – VII), with three treatment groups, normal feed as the negative control and a reference drug as the positive control. Effect of continued feeding with high-fat-diet was also observed. All the vegetable based formulated diets had significant effect on weight loss. However, *Telfairia occidentalis* had the highest significant effect on weight loss and reproductive hormone. Drug had a significant regression in the serum level compared to other treatments; there was significant decrease in HFD+ugwu. In conclusion, the formulated feeds had an ameliorative effect on metabolic dysfunctions associated with hyperlipidemia in rats and can therefore be explored in the management of obesity and other diseases related to it.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Obesity is a chronic condition described by pathophysiological mechanisms that leads to an increase in adipose tissue mass as a result of a positive energy balance (Garber et al., 2013). Obesity is caused by a complex combination of genetic and environmental factors (such as behavioral, social, cultural, and physiological) that can contribute to its pathogenesis singularly or in concert (Kaufer et al., 2001; Vaidya, 2006). Obesity is essentially a phenotypic result of an energy imbalance between the calories consumed and those expended (Halicioglu, 2013). This could be attributed to a rise in the consumption of high-fat, energy-dense meals, as well as a decrease in physical activity as a result of the more sedentary nature of many types of work, transportation, and urbanization (WHO, 2013).

Hypertrophied and hyperplastic adipocytes are hallmarks of pathological obesity (Dulloo et al., 2010). The anomalous distributions of fat result in weight gains of more than 20% or more of standard mass hypertrophic hyperplastic Adipocytes (Westerterp-Plantenga, 2005). Obesity, which was once considered to be a high-income country problem, is now on the rise in low and middle-income countries, particularly in urban areas, and has been related to more mortality than underweight (WHO, 2012). Type II diabetes, coronary heart disease, hypertension, psychological disorders, musculoskeletal disorders (knee osteoarthritis), and certain cancers (endometrial, breast and colon) are all modifiable risk factors (Luppino et al., 2010; Barouki et al., 2012).

Between 1980 and 2014, the prevalence of obesity worldwide has more than doubled. In 2014, more than 1.9 billion adults aged 18 years and above were reported to be overweight, with at least 600 million of them being clinically obese (WHO, 2015). In 2014, approximately 13% of the world's adult populations (11 percent of men and 15 percent of women) were obese (WHO, 2015). In the same year, 39 percent of adults aged 18 and above were overweight (38 percent of men and 40 percent of women) (WHO, 2015). In 2013, 42 million children under the age of 5 were overweight or obese and out of this, more than 30 percent were from developing countries (World Health Organization, 2015). Obesity is the fifth highest worldwide death risk factor on a global scale and is forecasted as the third major

death risk by 2030. (WHO, 2011; WHO 2015). Obesity is associated with higher circulating levels of free fatty acids and systemic proinflammatory cytokines, chemokines, prostaglandins, immune cells, reactive oxygen species and reactive nitrogen species which in turn precipitates oxidative stress (Marseglia et al., 2014). Chronic exposure to lipid-rich foods promotes the generation of reactive oxygen species (ROS) through mitochondrial and peroxisomal oxidation of fatty acids (Fernández-Sánchez et al., 2011). High production of ROS is connected with low-grade persistent systemic inflammation in adipose tissue (Marseglia et al., 2014). This condition promotes the release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-), interleukin (IL)-1, and IL-6 by activating the innate immune system in adipose tissue (Fonseca-Alaniz et al., 2007).
Leafy vegetables may play a role in preventing overweight and obesity due to their low energy density, high fibre content, and associated increasing satiety effect. The potential role of leafy vegetables in preventing overweight and obesity may be related to the dietary pattern associated with leafy vegetable consumption and the possibility that vegetable consumption may be used in place of more energy-dense foods. The majority of human prospective cohort studies in adults indicate that increased vegetable consumption prevents body weight gain. Although prospective studies on children are scarce and inconclusive, they do suggest associations between vegetable consumption and body weight that are related to initial nutritional status. Subjects are frequently advised to make several changes to their diet and lifestyle in behavioral intervention studies, making it impossible to quantify the specific effect of vegetable consumption on body weight. The few available intervention studies in adults were frequently too brief to allow for body weight changes to be measured, and studies on overweight or obese subjects may not apply to subjects of normal weight. Although intervention studies with school children demonstrated that such programs can be effective at increasing fruit consumption, a convincing role for increased vegetable consumption in preventing childhood overweight and obesity has yet to be established. The available evidence suggests that fruit consumption may help prevent overweight and obesity. Increased vegetable consumption is recommended in the 2010 Dietary Guidelines for Americans to lower the risk of obesity, which is linked to a variety of medical conditions.

In humans, a high fibre meal has been linked to a lower risk of cardiovascular disease; low fibre intake in unbalanced diets is thought to be responsible for about 31% of ischaemic heart disease and 11% of stroke worldwide. Unbalanced diets with low vegetable intake and low consumption of complex carbohydrates and dietary fibre are estimated to cause 2.7 million

deaths each year, according to the 2007 World Health Report, and are among the top 10 risk factors for mortality and also according to a review published in 2013 by the Australian Dietary Guidelines (ADG), vegetable consumption is linked to a lower risk of weight gain. When caloric intake is less than energy expenditure, weight loss occurs, and maintaining an energy deficit is the main dietary goal. This study is therefore to investigate the effect of some selected leafy vegetables in the management of obesity.

1.2 Statement of the Problem

Obesity has reached pandemic proportions around the world. Obesity is the fifth largest cause of mortality worldwide, and it is expected to be the third leading cause of death by 2030. Obesity has been linked to cognitive decline, decreased cognitive performance, and a higher risk of depression, anxiety, dementia, and Alzheimer's disease. It also increases the likelihood of developing spontaneously produced behaviors including activity patterns, anxiousness and exploration. It affects motor functions, resulting in a reduction in locomotor activity and exploration although the medicinal implications of obesity and its associated metabolic issues appear to have a negative impact on an individual's ability to live a pleasant and active life, according to academics and clinicians. Obesity prevalence ranged from 8.1 percent to 22.2 percent in Nigeria, with overweight individuals ranging from 20.3 percent to 35.1 percent. Obesity is expected to affect around 12 million Nigerians by 2020, with the prevalence of obesity being significantly greater among women. Nutritional and epidemiological transitions driven by demographic changes, obesity appears to be spreading across the country as a result of increased affluence, urbanization, bad lifestyles, and the consumption of highly processed foods.

1.3 Aim and Objectives of the Study

1.3.1 General objective

To investigate the hypolipidemic potential of Eweroko (Telfairiaoccidentalis), Shokoyokoto (Celosia argentia), Efo Tete (Amaranthus hybridus) on rats

1.3.2 Specific objectives of study are to:

i. Determine the phytochemical profile ofEweroko (Telfairiaoccidentalis), Shokoyokoto (Celosia argentia), Efo Tete (Amaranthus hybridus)

ii Determine the active compounds in Eweroko (*Telfairiaoccidentalis),*Shokoyokoto *(Celosia argentia),* EfoTete(Amaranthus*hybridus).*

iii. Determine the effect of Eweroko (Telfairiaoccidentalis), Shokoyokoto (Celosia argentia), Efo Tete (Amaranthus hybridus) on the hormonal profile of induced hyperlipidemic rats.

1.4 Scope of the Study

Leafy vegetable was used to formulate feed for the treatment of induced hyperlipemia female rats, these selected leafy vegetables are commonly consumed in Nigeria. Studies have shown that vegetables have been used to manage obesity in the nutritional field. The study is one of such implemented to curb the advent of hyperlipidemia, cardiovascular diseases among others diseases. Obesity is one of the most important current health promotion and disease prevention priorities worldwide, owing to the elevated risks of morbidity and death that come with it. (Garber et al., 2013) stated that obesity is one of the most important current health promotion and disease prevention priorities worldwide, owing to the elevated risks of morbidity and death that come with it.

1.5 Significance of the Study

Obesity is one of the most important current health promotion and disease prevention priorities worldwide, owing to the elevated risks of morbidity and death that come with it. (Garber et al., 2013) stated that obesity is one of the most important current health promotion

and disease prevention priorities worldwide, owing to the elevated risks of morbidity and death that come with it.

Studies have shown that vegetables have been used to manage obesity in the nutritional field. This study is one of such implemented to investigate the potential of these vegetables in the management of hyperlipidemia and by extension, cardiovascular and other weight related diseases. The selected vegetables in this study are locally available and have been recommended to people living with obesity but data is not readily available on the active compounds and the utilization for managing hyperlipidemia

1.6 Definition of Terms

Obesity is an increased adipose mass due to positive energy balance characterized by a chronic metabolic condition

Hyperlipidemia is a condition in which there are high levels of fat particles (lipids) in the blood.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Obesity

Obesity is an increased adipose mass due to positive energy balance characterized by a chronic metabolic condition (Bray, 2007; De-la-Garza et al., 2011). Incorporating genetic, behavioral, social, cultural and physiological elements also contribute to its formation (Mbochi et al., 2012; Hryhorczuk et al., 2013). An abnormal fat distribution that results in an increase in weight equal to or over 20 percent of the typical body weight is characterized as pathological obesity (Westerterp-Plantenga, 2005).

2.1.1 Epidemiology and impact of obesity

Obesity is on the rise as a result of more sedentary lifestyles and easy access to palatable, high-fat diets (Lykouras and Michopoulos, 2011). Obesity, once thought to be an issue exclusively in high-income countries, is now rapidly increasing in low and middle-income countries, particularly in metropolitan areas (WHO, 2015). Its prevalence has been linked to morbidity and mortality in all age categories around the world. In 2014, more over 1.9 billion adults aged 18 and above were overweight, with at least 600 million of them classed as clinically obese (WHO, 2015). In 2014, approximately 13% of the in every region except sections of Sub-Saharan Africa and Asia, there are more obese people than underweight people (World Health Organization, 2014). World's adult population (11% of men and 15% of women) were obese (WHO, 2015).

Obesity is one of the most important current health promotion and disease prevention priorities worldwide, owing to the elevated risks of morbidity and death that come with it (Garber et al., 2013). It discusses modifiable risk factors for cognitive impairment (Guidi et al., 2006), neurological diseases (Sas et al., 2007), Alzheimer's disease (Smith et al., 2000), ageing (Hyun et al., 2006), type 2 diabetes mellitus (Luppino et al., 2010), and coronary artery disease (Luppino et al., 2010), (Lykouras and Michopoulos, 2011). Obesity appears to have a negative impact on an individual's ability to live a full and active life, despite the medical effects that academics and physicians are concerned about (Karasu, 2012). It exacerbates psychological illnesses like low self-esteem, despair, and a lack of job

opportunities, all of which have a significant influence on a person's functional capacity and quality of life (Richards et al., 2011).

2.1.2 Classification of obesity

Obesity may be defined by the fat distribution pattern in the body. Excess fat is mainly stored in the truncally-abdominal region in certain individuals, and two forms of obesity are found in the glutal and femoral areas: android obesity and gynoid obesity (Bray, 2007).

BMI is a metric used to determine whether a person is overweight or obese. The body formula (weight (kg)/ height squared (m2) is used to determine it. According to BMI, the World Health Organization (WHO) has released a guideline.

BMI is a standard tool for determining adult obesity since it directly correlates body fat with clinical state (Aronne, 2002). However, BMI measuring has some shortcomings because it ignores essential characteristics like gender and age.

Obesity poses a risk depending on where the fat is stored. People with peripheral obesity (fat on the hips, thighs, and buttocks) experience less health concerns than people with central obesity (fat located around the stomach and gut). Other approaches for determining body fat distribution include the waist-hip ratio (WHR) and waist circumference. The most precise methods for measuring abdominal obesity are computed tomography (CT) and magnetic resonance imaging (MRI). They are, however, too expensive and impracticable for commercial use. Waist circumference is a more straightforward measurement than WHR (Table 2.2). Despite this, it can predict increased intra-abdominal fat with the same accuracy as WHR (Lean ME et al., 1995).

2.1.2 Causes of obesity

An energy imbalance between calories used and calories expended is the fundamental cause of obesity and overweight. Globally, the intake of energy-dense and high fat foods has increased and the inactivity has grown as many forms of work are becoming increasingly sedentary, as transport modes have changed and the urbanization has grown increasingly. Changes in the pattern of dietary and physical activity are often the result of environmental and sociopolitical change in areas of health, agriculture and transport, urban planning, environment, food processing, distribution, marketing and education and the lack of support policies. The causes of obesity and its consequences are mentioned in Table 2.3.

TABLE 2.1 Overweight and obesity according to BMI (WHO, 2000 and WHO, 2004)

Classification	BMI (kilogram/m^2)
Underweight	<18.5
Normal weight	18.5-24.9
Overweight	25-29.9
Obesity Class 1	30-34.9
Obesity Class 2	35-39.9
Extreme Obesity Class 3	>40

2.2 Diagnosis of Obesity

In clinical settings, a crude population's obesity diagnosis process includes assessing body fat mass using ethnicity-adjusted anthropometric indices, as well as the existence and severity of particular obesity-related comorbidities (Garber et al., 2013). Obesity is commonly measured in humans using the Body Mass Index (BMI), which is defined as the ratio of weight (kg) to square of height (m^2) (Heymsfield et al., 2009).

Obesity is typically measured in animal rodent models by a rise in body weight, as measured by the Lee obesity index, and/or an increase in body fat content (Thibault and Hariri, 2010). In rat models, the Lee index for measuring obesity is similar to the BMI in humans. It IS calculated by multiplying the cube root of body weight (g) by the naso–anal length (cm) by 1000. Obesity is diagnosed by values exceeding 310 (Lee, 1929; Thibault and Hariri, 2010).

TABLE 2.2 Causes of obesity (Javid I. Mansuri 2018).

Primary causes	Genetic causes (Monogenetic disorders)	Melanocortin-4 receptor mutation Leptin deficiency POMC defficiency
Secondary causes	Neurological	Brain injury Brain tumor Consequences of cranial irradication
	Endocrine	Hypothyrodism Cushing syndrome GH deficiency Pseudohypoparathyrodism
	Psychological	Depression Eating disorders
	Drug induced	Tricyclic antidepressants Oral contraceptives

Due to its inability to discern between age, gender, animal species, and ethnicity (in the case of humans), BMI is limited by variances in body adiposity across age, gender, animal species, and ethnicity (in the case of humans), the difference between fat and lean mass (Jackson et al., 2002).

Because the metabolism of obesity is closer to the visceral adiposity than the general adiposity, other anthropometric fatty measurements that take into consideration body fat distribution such as the circumference of waist (WC), waist to waist ratio (WHR), and the waist to height ratio (WHtR) (WHO, 2003; Cornier et al., 2011). Waist circumference is a useful prediction in normal subjects for the intra-abdominal thickness of fat, showing significant correlations with cardiovascular disease in humans (Han et al., 1995; Roopakala et al., 2009). Abdominal/centralized obesity is defined by a waist circumference larger than 40 cm in males or 35 cm in females not pregnant (88 cm) (Bray, 2007). However, WC fails to take into account differences in height so that risks for large and short individuals are potentially over and under assessed respectively (Browning et al., 2010). Current meta-analyzes and systemic review suggested the waist-to-height ratio (WHtR) to compensate for this perceived deficit, as it provides a better prediction of CVD risk factors (Ashwell et al., 2012; Savva et al., 2013). As a better predictor of cardiometabolic risk, a cutoff value of 0.5 for both men and women were proposed (Li et al., 2016). The waist-to-hip circumference ratio (WHR), like other anthropometric measurements, is a low-cost, rapid, and simple method of determining the degree of peripheral (subcutaneous) and central (visceral, abdominal) adiposity. Visceral fat mass appears to be a key risk factor for metabolic illnesses, whereas peripheral fat mass appears to be immune to such problems (Ferreira et al., 2004). In females, the WHR is greater than 0.84, while in males, it is greater than 0.94 which predicts an unfavorable pattern of abdominal fat buildup in obesity (Gurevich Panigrahi et al., 2009). A high absolute risk is conferred by the presence of three or more of the following risk factors: patient age of 45 years or older for men and 55 years or older for women; cigarette smoking; family history of premature coronary heart disease (myocardial infarction or sudden death at or before age 55 years in father or age 65 years in mother); HDL cholesterol less than 35mg/dL; impaired fasting glucose between 110 to 125 mg/dL; hypertension (systolic blood pressure 140 mm Hg or greater or diastolic blood pressure 90 mm Hg or greater); Low-density lipoprotein (LDL) cholesterol of 160 mg/dL or greater (Bray, 2013).

Bioelectrical impedance analysis, air-displacement plethysmography, Computerized Tomography (CT), Dual-energy X-ray Absorptiometry (DXA), Magnetic Resonance Imaging (MRI), and skinfold measurements with a Vernier caliper are some of the other techniques

used to assess body fat (Lobstein et al., 2004). These techniques offer a more exact, accurate, and practical method of determining body fat mass (Lobstein et al., 2004).

2.2.1 Clinical manifestation/complications of obesity

Increased BMI is a major risk factor for many diseases, including cardiovascular diseases (primarily heart disease and stroke), diabetes (Chanet al., 1994), musculoskeletal disorders (especially osteoarthritis – a highly disabling degenerative disease of the joints), and some cancers (including endometrial, breast, and prostate cancers) (Benedetto et al., 2015; Vucenik and Stains, 2014; Mansuri et al., 2013). The brief outcomes of major risk factors of obesity are mentioned in Table 2.4.

2.3 Management of Obesity

The general aims of weight management are to lose weight, maintain a lower weight over time, and avoid gaining weight again. These can only be accomplished in part by maintaining a negative energy balance (Chugh and Sharma, 2012). Rapid weight loss, on the other hand, is nearly invariably followed by a regaining of the lost weight (Redinger, 2007). To lose weight permanently, you need to take a holistic approach. Initially, lifestyle changes such as increased physical activity, behavioral therapy, and dietary modification (low-calorie diet) are used to manage the condition (Chugh and Sharma, 2012). Anti-obesity drugs can help obese patients lose weight in addition to diet and exercise. Bariatric surgery (such as gastric banding, gastric bypass, sleeve gastrectomy, and duodenal switch) can be performed laparoscopically in severely obese individuals.

Furthermore, for extremely obese people, surgical removal of adipose tissue via liposuction or omentectomy may be an option (Hocking et al., 2013). Here's a rundown of the most common approaches to obesity management:

TABLE 2.3 Health risks associated with obesity and its outcomes (Mansuri 2013).

Health risks associated with obesity	Outcomes
Metabolic syndrome' Type 2 diabetes Hypertension Hyperlipidemia	Coronary heart disease, Stroke Diabetes complications
Liver fat accumulation	Non-alcoholic steatohepatitis cirrhosis
Restricted ventilation	Exertional dyspnoea, Sleep apnoea Respiratory failure
Mechanical effects of weight	Urinary incontinence, Osteoarthritis Varicose veins
Increased peripheral steroid interconversion in adipose tissue	Hormone-dependent cancers like breast, uterus; Polycystic ovary syndrome (infertility, hirsutism)

2.3.1 Dietary changes and physical activity

Obesity treatment requires dietary management and physical activity (Sui et al., 2007). Diet composition (such as low-carbohydrate, low-glycemic-index, low-fat, and high-protein diets) but also total calories ingested play a role in the patient's ability to stick to prescribed diets (Sacks et al., 2009). According to a meta-analysis, physical activity improves cardiorespiratory fitness and reduces the negative effects associated with obesity (Church et al., 2007). Physical activity, on the other hand, has a limited influence on body weight, but combining it with a nutritional intervention doubles the chances of long-term weight loss success (Catenacci et al.,2007; Dansinger et al., 2007).

2.3.2 Behavioral modification

Behavioral adjustment is frequently beneficial in obese patients as an addition to diet and physical activity (Bray, 2013). The objective of behavioral treatment is to empower patients to lose and manage weight by monitoring and altering their food consumption, increasing their level of physical activity, and understanding and managing the triggers that trigger overeating (Bray, 2013). If obesity is to be effectively treated, high-intensity therapies such as self-monitoring, goal setting, and planning should be used to address barriers to maintaining lifestyle modifications over time (LeBlance et al., 2011). Maintaining a stable homeostatic circadian rhythm is critical for active weight management regimens. Sleep-wake pattern regularization, reduction of exposure to strong light, regular and regulated feeding/nutrient intake rhythms, and physical activity all have a role in preventing circadian disturbance (Garaulet et al., 2010). All pharmaceutical, nutritional, and occupational practices that improve an individual's circadian system status are crucial for lowering the risk of obesity and maximizing treatment success (Garaulet et al., 2010).

2.3.3 Pharmacological treatment of obesity

Obesity can be successfully managed by making dietary and physical activity changes in one's lifestyle (Yun, 2010). Anti-obesity medicines, on the other hand, can be useful adjuncts to diet and exercise for obese individuals who have failed to lose weight with diet and exercise (Sharma et al., 2005). After 6 months of combined lifestyle therapy, when the patient

has lost 0.4 kg/week for 6 weeks, pharmacotherapy is usually started. In people with a BMI of less than 27 kg/m2 and obesity-related risk factors, or patients with a BMI of more than 30 kg/m2 (without concomitant obesity-related risk factors), when starting pharmacotherapy, it's critical to keep an eye out for side effects (Chugh and Sharma, 2012).

2.3.3.1 Drugs affecting fat digestion

a). Orlistat is a saturated derivative of lipstatin that reduces dietary fat absorption by inhibiting the activity of pancreatic lipase (Viner et al., 2010). It's been approved for long-term weight management in obese people (>12 weeks) (Li et al., 2005). Weight loss is achieved by combining a long-term clinical application with an energy-restricted diet (Neovius et al., 2008). Orlistat boosts fecal fat loss by up to 30% while lowering systemic absorption (Padwal and Majumdar, 2007). The drop in intra-abdominal fat content has been linked to a decrease in intestinal lipid digestion (Rubio et al., 2007). Orlistat can also help to reduce tachycardia, improve oral glucose tolerance, and prevent type 2 diabetes mellitus from developing (Torgerson et al., 2004). A wide range of gastrointestinal side effects, including bloating, steatorrhea, oily spotting, fecal urgency, and fecal incontinence, as well as hepatic side effects, have been recorded in association with its long-term therapeutic use (Viner et al., 2010).

b). Cetilistat is a new medicine that inhibits the activity of pancreatic lipases. It is a highly lipophilic benzoxazinone. Cetilistat has been shown to significantly reduce weight loss, improve glycemic management, and decrease waist circumference, both of which are critical risk factors for Metabolic Syndromes. It is now being tested in phase III (Kopelman et al., 2010)

2.3.4 Herbal approaches to obesity management

Obesity and its related morbidity are on the rise around the world, creating unmet medical needs for safe and effective treatments (Chugh and Sharma, 2012). Attempts to reduce fat mass through pharmaceutical calorie restriction and fat mobilization have failed due to ineffectiveness and/or unacceptable side effects (Goodpaster et al., 2005).

The endeavor of developing a chemical agent that is both safe and effective while also targeting the complicated pathophysiology of obesity has proven to be difficult. Newer

insights into historically utilized medicinal plants, on the other hand, are required for the investigation of their novel bioactive components (Arika et al., 2015). Medicinal plants have received a lot of attention recently as complementary and alternative treatments due to their biocompatibility, as opposed to chemically manufactured pharmaceuticals, which have been linked to a lot of side effects and health risks (Piero et al., 2015). Due to their affordability and accessibility, herbal medicines have become an important bioresource that serves the health requirements of many people, particularly in developing countries. Traditional practitioners have often regarded them as being natural, healthy, and free of adverse effects. The reason for their use has mostly been based on extensive clinical experience (Arika et al., 2016).

The phytochemicals found in herbal remedies are thought to be the potential therapeutic and/or preventative agents (Pengelly et al., 2012, De-la-Garza et al.2011) found that these phytobiotics have several physiological effects that protect against pathogenicity. Polyphenols (curcumin, catechins, flavones, flavanols, monoterpenes, phenolic acids (rosmarinic acid), tannins, chalcones and quercetin, allicin; and Saponins (triterpenoid, steroid saponins) are among the major phytoconstituents responsible for such activity (Slanc et al., 2009; De-la-Garza et al., 2011).

Appetite suppression via central receptors (NPY, AgRP, CB-1, and dopamine receptors), inhibition of triglyceride absorption, increased lipolysis, improved glycemic control, adipose tissue differentiation, and increased energy expenditure are among the pharmacological relevance underlying the mechanistic approach to management of obesity and associated complications by these phytobiotics. Herbal medications are thus used as a source of "qualified leads" in the development of anti-obesity therapies.

2.4 Leafy Vegetables

Spinach beet, spinach, lettuce, amaranth, fenugreek, and Chinese cabbage are among the vegetables in this category. Leafy vegetables, sometimes known as greens or potherbs, are widely consumed around the world, particularly in Asia. They are fast-growing crops that can be harvested in as little as 4-6 weeks after planting. Because these veggies are perishable, they are cultivated in peri-urban regions. Protein, vitamins A and C, and minerals like iron, calcium, and phosphorus are all abundant in leafy leaves. Roughage is provided by these veggies, and they play an important role in a well-balanced diet. A balanced diet should

include at least 116 grams of green vegetables every day, according to dieticians. Hunger and malnutrition can be reduced by eating indigenous green vegetables. They are good suppliers of vitamins, minerals, and other nutrients, and are sometimes better than modern vegetables in terms of nutrition. Leafy vegetables are made from the leaves of cultivated plants, as well as leaves from wild and weedy species found in farmers' fields and the veld. Many research and extension professionals still treat traditional leafy vegetables as weeds, criticizing farmers for not keeping the weed population under control, thereby labeling this crucial food as unworthy of the space it occupies (Bhattacharjee et al., 2009). Leafy vegetables are high in macro and micro nutrients, which are important for living a healthy lifestyle. Many tropical countries' growing populations have raised awareness of the value of vegetables as a source of important nutrients that may not be found in other food sources. Depending on the vegetable consumed, vegetables are good providers of oil, carbs, minerals, and vitamins. The health benefits of green leafy vegetables include improving digestive health, maintaining weak eyesight, balancing cholesterol levels, enhancing youthful skin, treating anemia, strengthening the scalp, fighting free radicals, supporting cardiovascular health, promoting weight loss, boosting energy levels, and extending lifespan (Ineke et al., 2007).The consumption patterns of hundreds of indigenous leafy vegetables have yet to be thoroughly assessed, and the nutrient values of many of these leafy vegetables remain unknown. Leafy vegetables are mostly eaten for their nutritional value and relatively little is consumed for pleasure. It takes thought for medicinal value. (Boa, 2004).

2.4.1 The plant: *Celosia argentea*

General profile

- Plant Name: *Celosia argentea*
- Family: Amaranthaceae
- Geographical distribution: *Celosia argentea* is a widespread weed throughout tropical Africa, from Senegal east to Somalia and south to northern South Africa and the Indian Ocean islands, and a traditional vegetable in West and Central Africa
- Common Names: Celosia, Lagos spinach, sokoyòkòtò’, quail grass, cock’s comb

Celosia argentea have been identified in Nigeria (Hutchinson and Dalziel, 1954; Omueti, 1980; IITA, 1972; Grubben, 1976). It is known as sokoyòkòtò’(Yoruba) in the south-western

part of the country. The leaves and stems are cooked into soups, sauces, or stews with other ingredients (Grubben and Denton, 2004) and can be eaten with maize, rice, yam, and (Tindall, 1983). The composition of *Celosia argentea* per 100 g edible portion is: water 83.8 g, energy 185 kJ (44 kcal), protein 4.7 g, fat 0.7 g, carbohydrate 7.3 g, fibre 1.8 g, Ca 260 mg, P 43 mg, Fe 7.8 mg (Leung, W.-T.W., Busson, F. & Jardin, C., 1968)

The Masai people of Kenya use the liquid extract as a body wash for convalescents (Burkill, 1995). The seeds are used as medicine in Ethiopia and the Democratic Republic of Congo to cure diarrhea, dysentery, and muscle pain (Budin et al., 1996; Chweya and Eyzaguirre, 1999). The leaves are blended with honey and applied on inflated areas in India, while the seeds are used to cure diabetic mellitus (Tindall, 1983). The flowers are used to treat dysentery, haemoptysis, and menstrual issues in Southeast Asia (Grubben and Denton, 2004). In Nigeria and Benin, three types of Celosia argentea are cultivated: green broadleaved cultivars known as soko green, broadleaved cultivars with anthocyanin pigmentation of the leaf blades and part of the stem known as soko pupa, and cultivars with deep green narrow leaves with a hard texture and early flowering (Brenan, 1981; Grubben and Denton, 2004).

Martin, et al (1998) imply an Asian origin. It shares characteristics with members of the Amaranthus genus, such as broad edible leaves with a high protein content (1.2-5.9%) and thick spikes of flowers and seeds. The main usage of argentea is as a leafy vegetable. The leaves, shoots, and sensitive stems are used as a herb in sauces and soups, as well as cooked with other vegetables, as a side dish, or on their own. Even when cooked for a short time, the leaves readily break down. The seeds can also be eaten and have medicinal properties. Plants that have been chopped have been used as fodder for fowl and other livestock. Fresh or dried, the blossoms can make lovely ornaments. It is used in medicine, It has been stated that argentea has been integrated into the culture as a leafy vegetable. It serves as cure for a variety of ailments. Abscesses, colic, cough, diabetes mellitus, diarrhea, dysentery, eczema, gonorrhea, infected sores, liver diseases, menstruation issues, muscle aches, skin eruptions, snakebites, and wounds are all symptoms of argentea (Schippers 2000). In tests in India, *Celosia argentea* seeds reduced blood glucose in alloxan-induced diabetic rats.

Figure 3.1. *Celosia argentea* (Source: https://www.shademetals.com/species-list/2019/9/4/cockscomb-celosia-argentea-var-crisata)

2.4.2 The plant: *Telfairia occidentalis*

General profile

- Plant Name: *Telfairia occidentalis*
- Family: Cucurbitaceae
- Geographical distribution: Fluted pumpkin is found throughout West and Central Africa’s Forest zones, with Benin, Nigeria, and Cameroon being the most common. It's a widely consumed vegetable in Nigeria. It's only found in Uganda and nowhere else in East Africa. It's thought to have originated in south-east Nigeria and been spread by the Igbos, who have been cultivating this crop since the dawn of time. It's also likely that fluted pumpkin was once wild across its current distribution, but that wild plants were harvested to extinction in some areas and have since been replaced by cultivated varieties.

  This plant's leaves are high in proteins, vitamins (B-complex), minerals, fatty acids (linoleic and oleic acids), and fibres, making them a significant element in soups. The plant is also high in glycosides, which when hydrolyzed produce curcubitacins and glucose. The composition of the seed per 100 g edible portion is: water 6.2 g, energy 2280 kJ (543 kcal), protein 20.5 g, fat 45.0 g, carbohydrate 23.5 g, fibre 2.2 g, Ca 84 mg, P 572 mg (Leung, W.-T.W., Busson, F. & Jardin, C., 1968). It has been shown to protect against malignancies of the esophagus, oral cavity, and stomach, maintain blood vessel elasticity, and improve circulation in smokers' arteries. In both alloxan and streptozotocin diabetic rats, an extract from this plant was found to have antidiabetic action. However, there is a scarcity of data on *T. occidentalis's* positive effects on animal lipid profiles.

Figure 3.2: *Telfairia occidentalis* (Source:
http://www.africanplants.senckenberg.de/root/index.php?page_id=78&id=2316)

2.4.3 The Plant: *Amaranthus hybridus*

- Plant Name: *Amaranthus hybridus*
- Family: Amaranthaceae
- Geographical distribution: *A. hybridus* is a riverside pioneer that can be found in eastern North America, Mexico, Central America, and northern South America (Sauer, 1967). It is far more common in the eastern part of the United States than in the western half. Because of its use as a green vegetable, its range has expanded to Africa, south-central Asia, and Australia.

Amaranthus hybridus L, sometimes known as "Amaranth or Pigweed," is an annual herbaceous plant that grows to be 1 to 6 feet tall. The leaves are alternating petioled, 3–6 inches long, dull green, rough, hairy, ovate or rhombic with wavy margins, and ovate or rhombic with wavy margins. The flowers are tiny and have greenish or crimson panicles at the end. The taproot is long and fleshy red or pink in colour. The seeds are tiny and lenticellular in shape, averaging 1–1.5 mm in diameter and weighing 0.6–1.2 g per 1000 seeds. It's a common species found in landfills, farmed fields, and barnyards. *A. hybridus* leaves are mixed with condiments and used to make soup in Nigeria (Oke, 1983; Mepha et al., 2007). Their leaves are consumed as spinach or green vegetables in Congo (Dhellot et al., 2006). In Mozambique and West Africa, these leaves are boiled and combined with a groundnut sauce and served as a salad (Oliveria and DeCarvalho, 1975; Martin and Telek, 1979). Squalene, a chemical with both health and industrial benefits, has been identified in considerable concentrations in A. hybridus (Rao and Newmark, 1998; Smith, 2000; He and Corke, 2003). Despite its widespread use for such purposes, little is known about the nutritional and chemical makeup of A. hybridus leaves. A. hybridus is a riverside pioneer that can be found in eastern North America, Mexico, Central America, and northern South America (Sauer, 1967). It is far more common in the eastern part of the United States than in the western half. Because of its use as a green vegetable, its range has expanded to Africa, South-central Asia, and Australia.

Figure 3.3: *Amaranthus hybridus* Source: https://9jafoodie.com/nigerian-leafy-vegetables/

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Collection of plant materials

Fresh leaves of *Telfairia occidentalis, Celosia argentia* and *Amaranthus hybridus*were obtained locally from Ibafo market, Ogun state, Nigeria (6° 45’ 0” North, 3° 25’ 0” East).

3.1.2 Experimental animals

Thirty-five, healthy, female Wistar rats (>180.00g) were obtained from the animal holding unit of the biological sciences, Mountain Top University, Ogun State, Nigeria. The animals were kept in a well-ventilated house condition (temperature of 22±3° C; photoperiod of 12h/12h light/dark cycle; humidity: 45-50%) and fed with rat pellet (vital feeds, Grand cereals, Jos, Nigeria) and water ad libitum. At the end of the test period, the rats were sacrificed, after a starving period of 18 hours.

3.1.3 Drugs and assay kits

Cholesterol, High density lipoprotein Cholesterol, total protein, and triglycerides assay kits were products of Randox Laboratory, Co-Atrim, United Kingdom. Progesterone, Insulin, estrogen and testosterone hormone assay kits were manufactured by Diagnostics Laboratories, Freiburg, Germany and Accubind. Orilifit was manufactured by Getz Pharma Limited Pakistan.

3.1.3.1 Glucometer and test strips

Accu-chek active strip compact and glucometer were products of Roche Diagnostic, Mannheim Sandhoferstrasse, Germany.

3.1.3.2 Other chemicals and reagents

All chemicals and reagents used were of analytical grade obtained mainly from Sigma Aldrich Ltd, Buchs, Canada.

3.1.4 Equipment and apparatus

Stirring rod, Warring blender, Rotatory evaporator, Beakers, Funnel, Whatman's No.1 filter paper, Measuring cylinder, Muslin bag, and Hot air oven, UV-Visible Spectrophotometer (Jenway 7205), cuvette,

3.2 METHODS

3.2.1 Preparation of solvent extract *T. occidentalis, C. argentia* and *A. hybridus* leaves

Principle

Solvent extraction is a procedure that is widely used in both industrial and laboratory settings. Liquid-liquid extraction (LLE), liquid-solid extraction (LSE), supercritical fluid extraction (SFE), and other special techniques are among the techniques used.

The boiling points of liquids decrease as pressure is reduced in a rotary evaporator, allowing solvents to be vaporized at much lower temperatures than their boiling points at normal pressure.

The technique used in this study is the liquid-solid extraction (LSE), the liquid (solvent) used was absolute (concentrated) ethanol and the solid Leaves.

Procedure:

A known weight (4kg) *T. occidentalis, C. argentia* and *A. hybridus* leaves was washed separately under running water to remove contaminants and subsequently oven dried at 60ºC, to attain a constant weight of 1kg. When completely dry, was pulverized with a warring electric blender. The powdered material was weighed (O.3kg) and soaked in 1.2L of absolute (concentrated) ethanol for 48hrs. The extract was sieved with a jute bag and poured in a plastic container and filtered with Whatman's No. 1 filter paper. The filtrate was subjected to the rotatory evaporator to recycle back the absolute ethanol (solvent), as well as obtaining the

concentrate of the plant sample. The concentrate was kept in a hot air oven at 50ºC until completely dry.

3.2.2 UV-Visible spectroscopy

Principle:

Ultraviolet and visible absorption spectroscopy (often abbreviated to UV-Vis) is a method of spectroscopy that involves calculating the attenuation (strength/intensity weakening) of a light beam after it passes through a sample or reflects from a sample surface. The UV-Visible Spectroscopy principle is based on chemical compounds absorption of ultraviolet or visible light, which results in the formation of distinct spectra. The interaction between light and matter is the basis of spectroscopy. Excitation and de-excitation occur as matter absorbs light, resulting in the formation of a spectrum.

In the field of analytical chemistry, UV-Visible spectroscopy is commonly used, especially during the quantitative analysis of a specific analyte.

Reagents/Materials

UV-Visible Spectrophotometer, cuvette, 40 ml distilled water, 1.14g Telfairia *occidentalis, Celosia argentia* and *Amaranthus hybridus,* filter paper, conical flask.

Procedure

1.14g of *Telfairia occidentalis, Celosia argentia* and *Amaranthus hybridus* was dissolved separated in 40 ml distilled water in a conical flask, each solution was filtered using a filter paper to give a clear solution. The plant sample solution was placed in a clean cuvette, held at the opaque portion of the cuvette and placed in the UV-Visible spectroscopy and read at different wavelengths between 200-600nm to get the different absorbance.

3.2.3 Qualitative phytochemical screening analysis

The solvent extract of *T.occidentalis, C. argentia* and *A. hybridus*was tested for the presence of bioactive compounds using standard procedures as described by Roghini and Vijayalakshmi (2012) with slight modification.

3.2.3.1 Secondary metabolites

T. occidentalis, C. argentia and *A. hybridus* leaves were screened for the presence of secondary metabolites as described:

3.2.3.2 Qualitative screening of secondary metabolites

a. Carbohydrates (Molish's test)

2g of the sample was dissolved in 50ml of distilled water and then filtered using Whatman number 1 filter paper, to remove all residue. 2ml of the extract was treated with 1ml of Molish's reagent, few drops of concentrated sulphuric acid was added to the mixture. The appearance of purple or a reddish colour suggested the presence of carbohydrates.

b. Tannins

2ml of the 5% ferric chloride was added to 1ml of the extract. The formation of a dark blue or greenish black indicated the presence of tannins.

c. Saponins

2ml of the extract was added to 2ml of distilled water and shaken lengthwise for 15 minutes. The presence of saponin was detected to cause formation of 1 cm layer of foam.

d. Alkaloids

2ml of concentrated hydrochloric acid was added to 2ml of extract. Few drops of Mayer's reagent were added to the mixture. The formation of green colour or white precipitate indicated the presence of alkaloids.

e. Flavonoids

2ml of the extract was added to 1ml of 2N sodium hydroxide. Presence of flavonoids was indicated by the formation of yellow colour solution.

f. Glycosides

3ml of chloroform and 10% ammonia solution was added to 2 ml of the extract. The formation of pink colour indicated the presence of glycosides.

g. Quinones

1ml of the extract was added to 1ml of concentrated sulphuric acid. The formation of red colour indicated the presence of quinones.

h. Phenols

1ml of the extract was added to 2ml of distilled water followed by few drops of 10% ammonia solution. Formation of blue colour or green colour indicated the presence of phenols.

i. Terpenoids

0.5ml of the extract was treated with 2ml of chloroform and concentrated sulphuric acid. The formation of red brown colour at the interface indicated the presence of terpenoids.

j. Glycosides

0.5ml of the extract was added to 2ml of glacial acetic acid and few drops of ferric chloride were added. This was under layered with 1ml of concentrated sulphuric acid. The formation of brown ring indicated the presence of cardiac glycosides.

k. Protein (Ninhydrin Test)

0.2% of ninhydrin reagent was added to 2ml of the extract and heated for 5 minutes. The formation of blue colour indicated the presence of amino acids.

l. Coumarins

1ml of 10% sodium hydroxide was added to 1ml of the extract. Formation of yellow colour indicated the presence of coumarins

m. Anthraquinones

To 1ml of the extract, few drops of 10% ammonia solution was added. Appearance of pink colour precipitate suggested the presence of anthraquinones.

n. Steroids

1ml of the extract was added to equal volume of chloroform and few drops of concentrated sulphuric acid. The presence of steroid was indicated by the formation of brown ring and the appearance of bluish brown ring indicated the presence of phytosteroids.

o. Phlobatannins

Few drops of 2% hydrochloric acid were added to 1ml of the extract. Appearance of red colour precipitate indicated the presence of phlobatannins.

p. Anthracyanine

1ml of the extract was added to 1ml, 2N sodium hydroxide and heated for 5 minutes at 100° C. The formation of bluish green colour indicated the presence of anthocyanin.

3.2.4 Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS study was carried out using a Hewlett Packard Gas Chromatograph (Model 6890 series) fitted with a flame ionization detector and a Hewlett Packard 7683 series 250 °C MS transfer line temperature injector. The GC was fitted with an HP-5MS (30 x 0.25 mm) fused silica capillary column, 1.0 µm film thickness. The oven temperature was kept at 50 ° C for 5 min of held time and increased at a rate of 2 ° C / min from 50 to 250 ° C, using helium gas (99.999 percent) as a carrier gas at a steady flow rate of 22 cm / s. A 1.0micron extract (1 mg diluted in 1 ml of absolute alcohol) was injected at a 1:30 split ratio. Agilent Science Network Mass Spectrometer (Model 5973 series) coupled with Hewlett Packard Gas Chromatograph (Model 6890 series) with NIST08 Library software database was studied by MS. Mass spectra were taken at 70 eV/200 °C, 1 scan/s scanning rate. Compound recognition was carried out using the NIST08 Library database. The mass spectrum of each unknown compound was compared with the known compounds contained in the library software database.

3.2.5 Experimental design

3.2.5.1 Induction of Obesity, and Formulated Feed Administration for Treatment

Female rats were acclimatized for 2 weeks. Obesity was induced in rats of all groups except normal control group by administration of HFD (High fat diet) for 28 days, treatment was carried out for 28 days. The composition of HFD and Formulated feeds is presented in table 3.1.

TABLE 3.1: Composition of HFD and formulated feeds for treatment and control

Ingredients (g)	HFD	NormalDiet	*T.occidentalis*	*C.argentea*	A.h*ybridus*
Corn flour	307.5	557.5	467.5	467.5	467.5
Soya bean	300	250	230	230	230
Cellulose	40	40	-	-	-
Vitamin Premix	50	50	50	50	50
Salt	2.5	2.5	3.5	2.5	2.5
Sucrose	100	100	100	100	100
Animal fat	200	-	-	-	-
Leafy Vegetable	-	-	150	150	150
Total (kg)	1000	1000	1000	1000	1000

HFD – High Fat Diet

3.2.5.2 Animal grouping

The rats were divided into seven groups consisting of five rats in each as follows:

Group I: (Obesity induced) treated with *Celosia argentia* diet

Group II: (Obesity induced) treated with *Amaranthus hybridus* diet

Group III: (Obesity induced) treated with *Telfairia occidentalis diet*

Group IV: (Obesity induced) treated with 0.0225 of 360mg/kg body weight of, Orlifit drugs (Reference drug)

Group V: (Obesity induced) obese received distilled water

Group VI: (Obesity induced negative control) continued to receive HFD.

Group VII: (non-obesity induced positive control) received normal diet.

3.2.5.3 Determination of body weight

During the experimental period the body weight of each rat, was assessed in grams before and after treatment.

3.2.5.4 Determination of fasting blood glucose level

Using the glucometer kit (Accu-check, product of Roche Diagnostic GmbH, Sandhofer Strasse Germany), the level of fasting blood glucose was determined, after an overnight fast for 12 hours. In the morning (8:00 – 9:00 am), the tip of the tail of the rats were pricked with lancet and blood from the tail was allowed to drop on the strip which had been inserted into the glucometer. The blood glucose concentration was obtained in mg/dl for each rat in all the groups (Saidu *et al.,* 2014).

3.2.6 Preparation of serum and tissue supernatants

The method as described by Yakubu et al (2008) was used to prepare the serum and tissue supernatant. The rats were weighed individually and thereafter anaesthetized in a jar containing cotton wool soaked in diethyl ether. The neck area was cleared of fur and skin to expose the jugular veins. The jugular veins were displaced slightly from the neck region and thereafter cut with a sharp sterile blade. The animals were held head downwards, allowed to bleed into clean, dry sample tubes and left at room temperature for 10 minutes to clot. The blood samples were centrifuged at 4000rpm for 10 minutes to obtain the supernatant from the stock using Thermo Scientific Centrifuge (Heraeus Megafuge 8).

The sera were thereafter aspirated using Micro flux pipette into clean, dry, sample bottles and were then stored frozen (-4ºC) overnight. The animals were quickly dissected, the liver, kidney and ovary were excised, cleaned of fatty layers, weighed and transferred into ice cold 0.25M sucrose solution. Thereafter, each organ was blotted with clean cotton paper, cut thinly with a sterile blade and homogenized separately in ice cold 0.25M sucrose solution (1:4 w/v) based on their different dilution factors used such as kidney (x60) liver (x30) and ovaries (x80). The homogenates obtained were centrifuged at 4000rpm for 10 minutes to obtain the supernatants which were then gently collected into sample bottles, frozen (4ºC) overnight before they were used for the various biochemical assays.

3.2.7 Determination of Serum Concentration of Hormones

3.2.7.1 Progesterone

The serum progesterone concentration was determined using competitive Accu-Bind kits (Chen *et al*., 1991; Granoff and Abraham, 1979).

Principle:

The principle is based on a one-step immunoassay that uses a competitive microplate enzyme immunoassay to assess the presence of progesterone. In order to produce an antigen-antibody complex, progesterone in the sample competes with enzyme progesterone conjugates for binding with anti-progesterone coated microplate. In the antibody-bound fraction, enzyme activity is inversely proportional to natural progesterone concentration (Chen *et al.,* 1991; Granoff and Abraham, 1979).

Procedure:

To 0.025 ml of each calibrator, control and serum samples in the microplate wells, 0.10 ml of the conjugate was dispensed into each well. The microplate was swirled gently for 30 seconds to mix and was incubated for 60 minutes at 25°C. The content of the microplate was decanted and 0.30 ml of the washing solution was added repeatedly four times. TMB substrate (0.10 ml) was added and incubated at room temperature for 25 minutes in the dark after which 0.15ml of stopping reagent was pipetted into each well. The absorbance was readon microplate reader at 450nm within 20 minutes after the addition of the stopping reagent.

Calculation:

The serum progesterone concentration of the test samples was extrapolated from the calibration curve plotting the absorbance of the standard sample at 450nm against its corresponding concentration.

3.2.7.2 Testosterone

The serum testosterone concentration was quantitatively determined using the direct human testosterone Accu-bind kit (Chen et al., 1991; Granoff and Abraham, 1979).

Principle:

The testosterone EIA is based on the principle of competitive binding between testosterone HRP conjugate for a constant amount of rabbit anti-testosterone, Goat anti-rabbit IgG-coated wells and rabbit anti-testosterone reacted with the test sample. HRP-labeled testosterone competes with endogenous testosterone peroxidase conjugate immunologically bound to the well and progressively decrease as the concentration of testosterone in the specimen increases. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of un-labelled testosterone in the sample (Chen et al., 1991; Granoff and Abraham, 1979).

Procedure:

Exactly 0.01ml of standards, control and test sample, 0.1 ml of testosterone HRP conjugate reagent and 0.05ml of rabbit anti-testosterone reagent were dispensed into each well, mixed thoroughly for 30 seconds and incubated at 37 °C for 90 minutes. The microwells were rinsed and flicked for 5 minutes with distilled water. A known volume (0.1ml) of 3,3, 5,5-tetramethylbenzidine (TMB) reagent was dispensed into each well, mixed gently for 5 seconds and incubated at room temperature for 20 minutes. The blue colour completely turned yellow and the absorbance was read at 450nm with a micro titre well reader within 15 minutes of the standard solutions against its corresponding concentrations. The reaction was stopped with the addition of 1N HCl, afterwards the absorbance was read at 450nm (Chen et al., 1991; Granoff and Abraham, 1979).

Calculation:

The serum testosterone concentration was extrapolated from the calibration curve (figure 11).

Plotting the absorbance of the standard sample at 450nm against its corresponding concentration

3.2.7.3 Insulin

The serum FSH was quantitatively determined using microplate immunoenzymometric assay kit as described in the manufacturer's protocol version (Eastham, 1985).

Principle

The principle is based on immobilization that takes on a surface of a microplate well through the interaction of streptavidin coated on the wells and exogenously added biotinylated monoclonal insulin antibody. The enzyme labelled antibody and a serum containing the native antigen reactive results between the native antigens and antibodies without competition or steric hindrance to form a soluble sandwich complex. The activity of the enzyme in the antibody-bound fraction is directly proportional to the native antigen concentration (Eastham, 1985).

Calculation:

The absorbance of each calibrator, control and serum sample was plotted with the absorbance on the y- axis and the concentrations on the x-axis. The insulin concentration of the test samples was extrapolated from the calibration curve obtained by plotting the absorbance of the standard solutions against its corresponding concentrations.

Procedure:

An aliquot (0.05ml) of the standard solution, control, serum samples were placed in appropriate wells. Exactly 0.01 ml of the insulin enzyme reagent was dispensed into each well and the microplates were swirled gently for 20 seconds. The microplates containing the reaction medium was wrapped in a plastic bag and incubated for 120 minutes at 25°C. the wells were washed three times with 0.35ml of working substrate solution per well and aspirated using a micropipette. A known volume (0.1ml) of the working substrate was added to each well and incubated at 25°C for 15 minutes. Exactly 0.05 ml of stopping reagent was placed into each well and mixed gently for 20 seconds. The plate was read on microplate reader at 450nm within 30 minutes after the addition of the stopping reagent.

3.2.7.4 Estradiol

Desired number of coated wells was secured in the holder. 25µL of standards, specimens and controls were dispensed into appropriate wells. 50µL of rabbit anti-estradiol (E2) reagent was dispensed into each well. 100µL of estradiol-HRP conjugate reagent was dispensed into each well and was thoroughly mixed for 30seconds. The samples were then incubated for 90minutes at room temperature. This samples in the wells were mixed and flicked 5 times using washing buffer (1X). 100µL of TMB substrate was dispensed into each well and this was gently mixed for 10 seconds, this was then incubated at room temperature for 20 minutes. The reaction was stopped by adding a stop solution to each well. This was gently mixed for 30 seconds and was ensured that all the blue colour changes turned yellow colour completely. The absorbance was read at 450nm with a microtiter well reader within 15 minutes (Tsang *et al*., 1980).

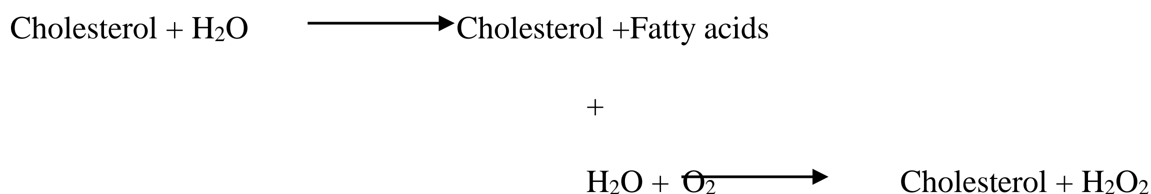
3.2.8. Lipid Profile Determination

3.2.8.1. Serum total cholesterol concentration

The total cholesterol concentration in the serum of the rats was carried out using the CHOD-PAP reaction (Tietz 1995).

Principle:

It is based on the following reactions:



Cholesterol + H_2O → Cholesterol +Fatty acids

+

H_2O + O_2 → Cholesterol + H_2O_2

H_2O_2 + 4-Amino antipyrine + P-Hydrobenzoic acid

→ Coloured Quinonic derivatives + 4 H_2O

Procedure:

A known volume (2.0ml) of the working reagent was placed into test tubes containing 0.02 ml of the appropriately diluted serum samples. The blank and standard were constituted by substituting the serum with 0.01 ml of distilled water and standard working reagent respectively. The reaction constituent was thoroughly mixed and incubated at 37° C for 5 minutes. Absorbance was spectrophotometrically read at 546nm against the blank (Tietz 1995).

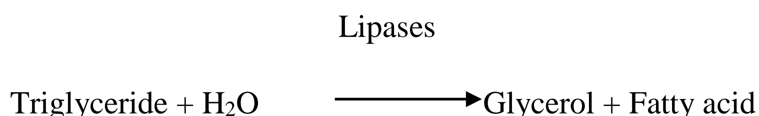
Calculation:

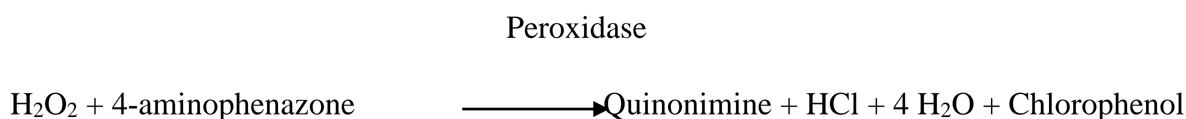
Concentration of cholesterol = $\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard}$ x 5.40mM/L

3.2.8.2 Serum Triglyceride Concentration

Principle:

The triglyceride concentration is determined after enzyme activity hydrolysis with lipases, the indicator is quinonimine formed from hydrogen peroxide, 4- aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase (Tietz 1995).





Procedure

Exactly 1000µl of the working reagent was added to well clean labelled test tubes consisting of blank, standard and sample. Thereafter 10µl of the distilled water, standard solution (200mg/dL) and serum samples were added respectively. The resulting solution was mixed and incubated for

5 minutes at 37°C. The change in absorbance of standard and sample against the reagent blank was read at 500nm (Tietz 1995).

Calculation

$$\text{Concentration of Triglyceride (TG)} = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times 2.17\text{mM/L}$$

3.2.8.3 Serum high density lipoprotein-cholesterol concentration

The determination of serum High Density Lipoprotein Cholesterol (HDL) concentration was carried out. (Tietz 1995).

Principle:

The low-density lipoprotein (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphortungstic acid in the presence of magnesium. After centrifugation, the cholesterol concentration in the HDL-Cholesterol fraction, which remains in the supernatant is determined (Tietz 1995).

Procedure:

Exactly 200µl of the serum samples and standard were dispensed into separate test tubes and 500µl of the reagent was added. The resulting solution was mixed and left for 10 minutes at room temperature. Thereafter, it was centrifuged at 4000rpm for 10 minutes and the clear supernatant was separated for determination of cholesterol content. The concentration of cholesterol was determined using enzymatic saponification procedure as described by (Tietz 1995).

Calculation

$$\text{HDL-C} = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times 5.25\text{mM/L}$$

3.2.8.4 Serum low density lipoprotein - cholesterol concentration

The serum Low Density Lipoprotein (LDL) cholesterol concentration can be expressed as: LDL Cholesterol = Total Cholesterol – (HDL + TG)

Table 3.2: Dilution factor for the various assays.

Parameters/ organs	Serum
Total Cholesterol	×5
Triglycerides	×5
High density Lipoprotein	×5
Low density Lipoprotein	×5

CHAPTER FOUR

4.0 RESULTS

4.1 Chemical constituents of ethanoic extract of *T.occidentalis, C.argentia* and *A.hybridus* leaves.

4.1.1 Qualitative phytochemical components of Ethanoic extract of *T. occidentalis, C.argentia* and *A. hybridus* leaves.

Qualitative analysis of ethanoic extract of *T. occidentalis* revealed the presence of phytochemicals such as tannins, saponins, alkaloids, flavonoids, phenols, steroids and coumarins. In *C. argentia,* the presence of carbohydrate, flavonoids, terpenoids ninhydrin, steroids and coumarins was recorded while analysis carried out on *A. hybridus* leaves revealed the presence of flavonoids, quinones, phenols, terpenoid, cardiaglycosides, steroids and coumarins. Qualitative analysis of phytochemical constituents of aqueous plant extracts is generally employed majorly for the determination of antioxidant function of the plant as well as their potentials as being medicinally beneficial (Yeum and Russell, 2014). Phenolic compounds such as terpenoids and saponins reportedly possess antimicrobial and antioxidant properties (El-Aziz et al., 2019)

4.1.2 UV-Visible spectroscopy analysis of ethanoic extract

The Ultra violet -Visible spectroscopy of ethanoic extract of *T. occidentalis* leaves revealed the varying absorbance of the extract at different wavelengths as shown in (Figure 4.1). From the calibration curve, there was a spontaneous increase in absorbance value at 280nm wavelength, which lags between 310-410nm wavelengths, afterwards decreased. It can be deduced that at wavelength 410nm shows the highest absorbance peak of the most abundant phytoconstituent of *T. occidentalis.*

The Ultra violet -Visible spectroscopy of ethanoic extract *A.hybridus* of leaves revealed the varying absorbance of the extract at different wavelengths as shown in (Figure 4.2). From the calibration curve, there was a spontaneous increase in absorbance value at 285nm wavelength, which lags between 310-405nm wavelengths, afterwards decreased. It can be

Table 4.1: Qualitative phytochemical components carried out in ethanol extract of leaves of *T. occidentalis, C. argentia* and *A. hybridus* leaves.

Secondary Metabolites	***A. hybridus***	***T. occidentalis***	***C. argentia***
Carbohydrate	-	-	+
Tannins	-	+	-
Saponins	-	+	-
Alkaloids	-	+	-
Flavonoids	+	+	+
Glycosides	-	-	-
Quinones	+	-	-
Phenols	+	+	-
Terpenoids	+	-	+
Cardiacglycosides	+	-	-
Anthracyanin	-	-	-
Ninhydrin	-	-	+
Steroids	+	+	+
Coumarins	+	+	+
Anthraquinone	-	-	-
Phlobatannins	-	-	-

Key: + Present - Absent

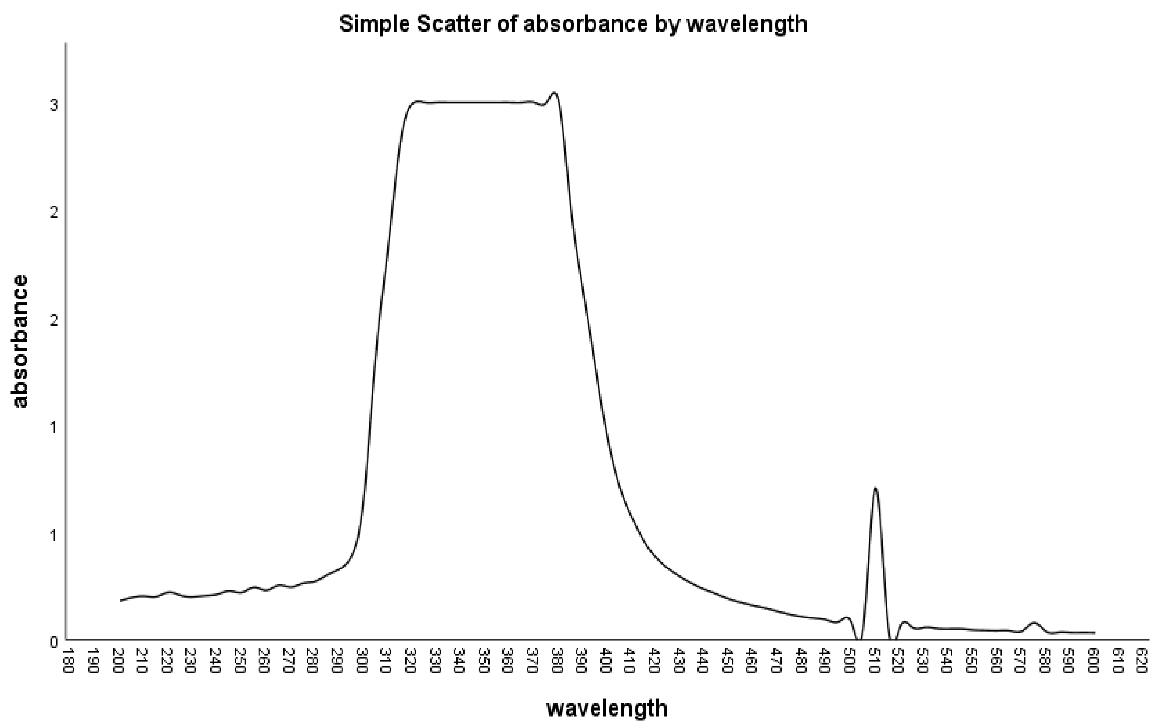


Figure 4.1: Graph of absorbance against wavelength using UV-Vis Spectroscopy analysis of ethanoic extract of *T. occidentalis leaves.*

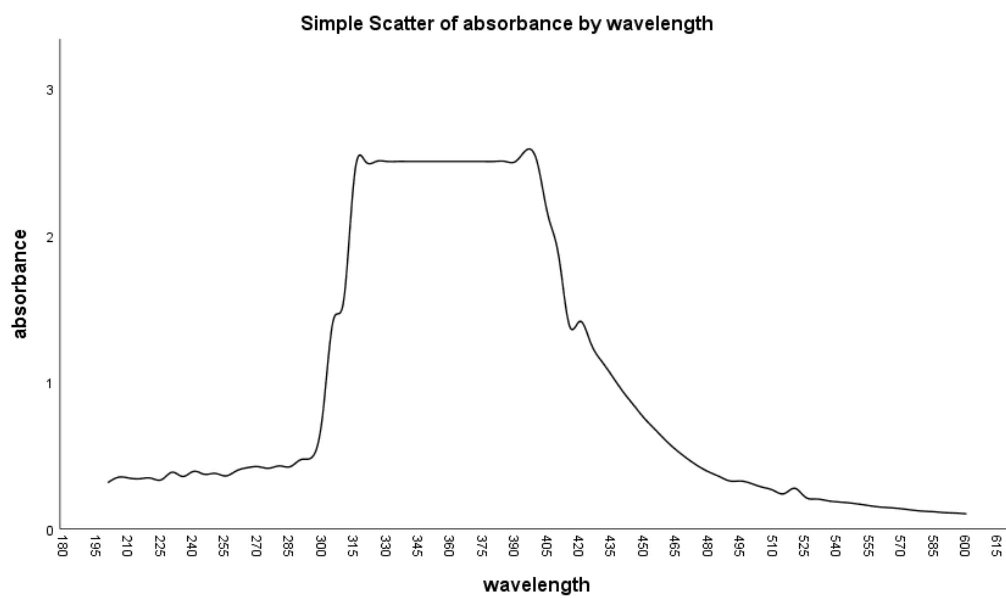


Figure 4.2: Graph of absorbance against wavelength using UV-Vis Spectroscopy analysis of ethanoic extract of *A. hybridus* leaves.

deduced that at wavelength 420nm shows the highest absorbance peak of the most abundant phytoconstituent of *A. hybridus leaves.*

The Ultra violet -Visible spectroscopy of ethanoic extract of *C. argentia leaves* revealed the varying absorbance of the extract at different wave lengths as shown in (Figure 4.3) From the calibration curve, there was a spontaneous increase in absorbance value at 300nm wavelength, which lags between 310-480nm wavelengths, afterwards decreased. It can be deduced that at wavelength 435nm shows the highest absorbance peak of the most abundant phytoconstituent of *T. occidentalis.*

According to Femi-Olabisi et al., 2021, the absorbance of solutions indicates the amount of light that can be absorbed at a specific wavelength and the maximum absorption wavelength could be utilized for the determination of what biomolecules are present in a solution.

4.2 Gas chromatography-Mass spectrometry (GC-MS) analysis.

Figure 4.4 shows the GC-MS chromatogram of ethanoic extract of *A. hybridus*leaves. Peak 4 with the retention time of 16.775 was identified as **Octadecane, 1-(ethenyloxy)- ($C_{20}H_{40}O$).**This compound was the major phytoconstituent identified as being present *A. Hybridus* Leaves. Other peaks represented other phytoconstituents present in the plant as presented in Table 4.2.

Figure 4.5 shows the GC-MS chromatogram of ethanoic extract of *T. occidentalis* leaves Peak 40 with the retention time of 16.751 was identified as **Phytol**,(**$C_{20}H_{40}O$**) as the major phytoconstituent of *T. occidentalis* leaves while other peaks indicated the presence of other phytoconstituents in the plant (Table 4.3). Investigation has been carried out on phytol to determine its potential metabolism-modulating, antioxidant, anti-inflammatory, antimicrobial effects and immune-modulating ability (Islam et al., 2018)

Figure 4.6 shows the GC-MS chromatogram of ethanoic extract of *C. Argentia* leaves Peak 42 with the retention time of 4.616 was identified as **3,7-Dimethyloctan-1-ol$C_{10}H_{22}O$** as the major phytoconstituent of *C.argentia* leaves while other peaks were of other phytoconstituents present in the plant (Table 4.4).

The GC-MS is considered a proven technique for the identification of bioactive compounds with prospective curative properties present in plants (Uraku et el,2015). This technique is utilized for separation, quantification and identification of analytes present in plant samples.

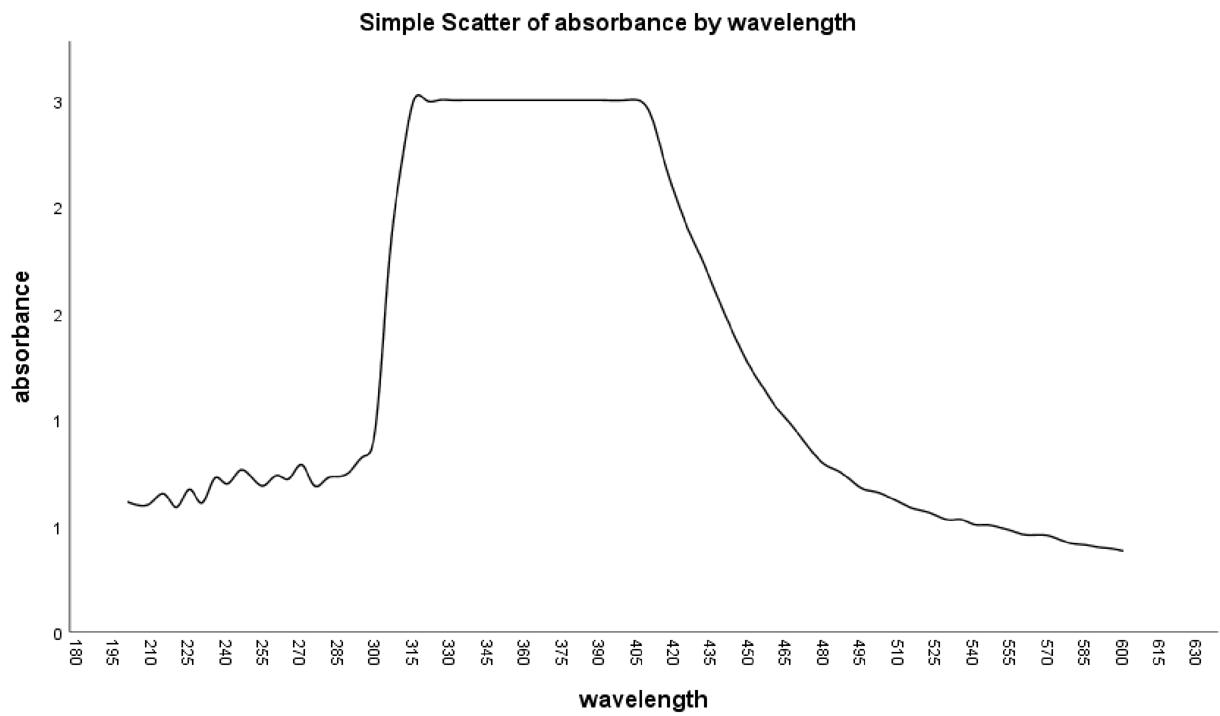


Figure 4.3: Graph of absorbance against wavelength using UV-Vis Spectroscopy analysis of ethanoic extract of C. *argentia.*

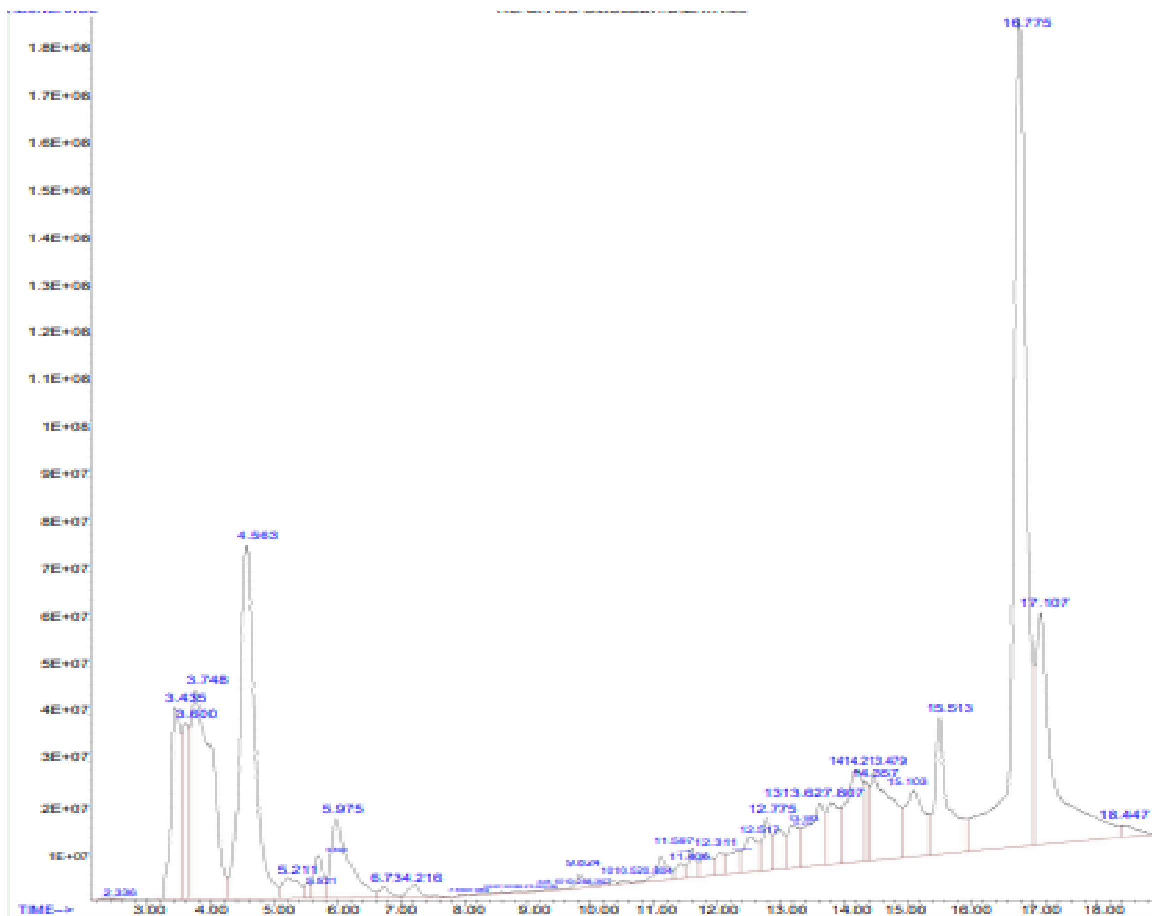


Figure 4.4: ***GC-MS chromatogram of ethanoic extract of A. hybridus*** **leaves**

Table 4.2: Phytochemical components identified by GC-MS in ethanol extract of *A. hybridus* leaves showing the peak, name of compound, Percentage Area, retention time and chemical compound formula

Peak	Name of compound	% Area	Retention time	Chemical Compound formular
1	L-Methioninol	0.04	2.336	$CH_3SCH_2CH_2CH(NH_2)CO_2H$
2	Methane, diethoxy-	4.14	3.435	$C_5H_{12}O_2$
3	1-Hexene, 4-methyl-	2.61	2.600	C_7H_{14}
4	Aziridine, 1-(1,1-dimethylethyl)-2,3-dimethyl-, trans-	10.14	3.748	$C_8H_{17}N$
5	Butanoic acid, 3-amino-2-hydroxy-	12.19	4.563	$C_4H_9NO_3$
6	1-Butanol, 3-methyl-, acetate	78	5.211	$C_7H_{14}0_2$
7	Peroxide, dimethyl	0.18	5.521	$C_2H_6O_2$
8	Propanenitrile, 2-hydroxy-	0.87	5.690	C_3H_5NO
9	Pentane, 1-(1-ethoxyethoxy)-	3.26	5.975	$C_9H_{20}O_2$
10	Hexanoic acid, ethyl ester	0.26	6.734	$C_8H_{16}O_2$
11	Sulfurous acid, butyl dodecyl ester	0.33	7.216	$C_{16}H_{34}O_3S$
12	2,4-Heptadienal	0.03	7.559	$C_7H_{10}O$
13	Sulfurous acid, butyl dodecyl esterPropanoic acid, 2,2-dimethyl-, pentyl ester	0.02	7.696	$C_{16}H_{34}O_3S$
14	Benzyl cyclohexanecarboxylate	0.07	8.401	$C_{14}H_{18}O_2$
15	delta-Dodecalactone	0.03	8.593	$C_{12}H_{22}O_3$

16	Bicyclo[2.2.1]heptan-2-ol	0.03	8.815	$C_7H_{12}O$
17	Metacetamol	0.00	8.949	$C_8H_9NO_2$
18	Sulfurous acid, octyl 2-propyl ester	0.01	9.089	$C_{11}H_{24}O_3S$
19	Succinic acid mono-(13-methyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[A]phenanthren-17-YL) ester	0.06	9.345	$C_{22}H_{30}0_5$
20	Octanoic acid, ethyl ester	0.28	9.824	$C_{10}H_{20}O_2$
21	2-Ethyl-p-xylene	0.04	10.248	$C_{10}H_{14}$
22	N-Ethyl-N,2,2-trimethylpropan-1-amine	0.05	10.367	$C_8H_{19}N$
23	Pentanoic acid, 2-methyl-, 1-methylpropyl ester	0.07	10.520	$C_{10}H_{20}O_2$
24	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, acetate, cis-	0.03	10.804	$C_{12}H_{18}O_2$
25	2-Piperidinoethanol	0.63	11.109	$C_7H_{15}NO$
26	2,3-Dihydrobenzofuran	0.29	11.406	C_8H_8O
27	Decanoic acid, ethyl ester	0.51	11.597	$C_{12}H_{24}O_2$
28	Indole	0.59	11.805	C_8H_7N
29	2,6-Dimethyl-4-p-tolyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid diethyl ester	0.37	12.047	$C_{20}H_{25}NO_4$
30	2,6,10-Trimethyltetradecane	0.69	12.311	$C_{17}H_{36}$
31	2-(1,4,4-Trimethylcyclohex-2-en-1-yl)ethyl p-toluenesulfonate	1.13	12.517	$C_{18}H_{26}O_3S$
32	1-Bromo-2,4-difluorobenzene -	1.01	12.775	$C_6H_3BrF_2$
33	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1,1,3,3-tetramethylbutyl)-	0.95	12.979	$C_{12}H_{38}O$
34	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	1.17	13.183	$C_{11}H_{16}O_2$

35	Oxalic acid, cyclobutyl ethyl ester	2.46	13.627	$C_8H_{12}O_4$
37	Di-n-2-propylpentylphthalate	3.66	14.213	$C_{24}H_{38}O_4$
38	2-(Methoxycarbonyl)benzoic acid	0.74	14.357	$C_9H_8O_4$
39	HexadecanaZ	4.36	14.479	$C_{16}H_{32}O$
40	Methyl 4-methoxyphenylacetate	2.75	15.103	$C_{10}H_{12}O_3$
41	Hexadecanoic acid, ethyl ester	4.36	15.513	$C_{18}H_{34}O_4$
42	Octadecane, 1-(ethenyloxy)-	26.50	16.775	$C_{20}H_{40}O$
43	9,12,15-Octadecatrienoic acid,ethyl ester, (Z,Z,Z)-	10.11	17.107	$C_{20}H_{34}O_2$

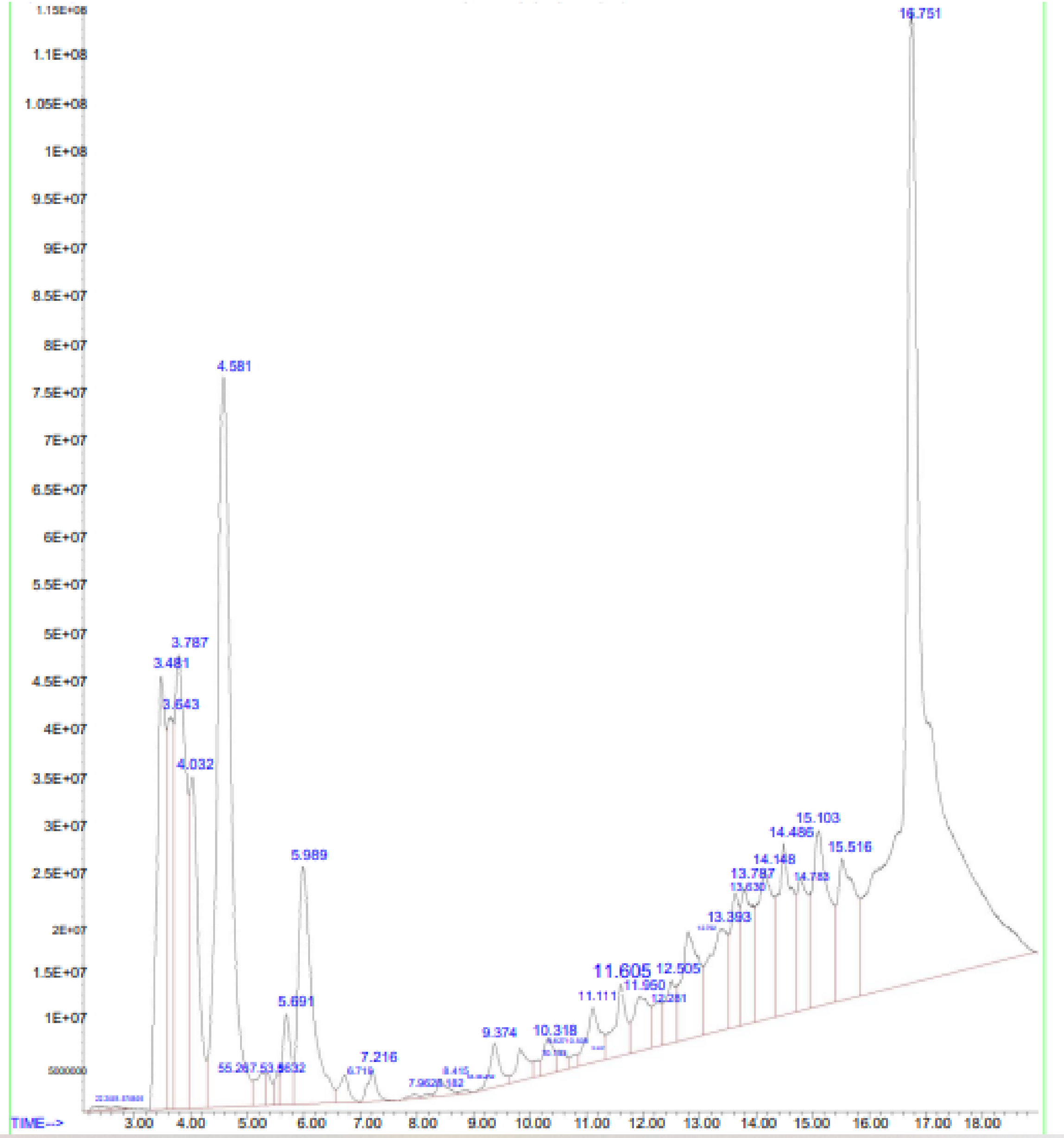


Figure 4.5: ***GC-MS chromatogram of ethanoic extract of T.occidentalis*** **leaves.**

Table 4.3: Phytochemical components identified by GC-MS in ethanol extract of *T. occidentalis* leaves showing the peak, name of compound, Percentage Area, retention time and chemical compound formula

Peak	Name of compounds	Area %	Retention Time	Chemical formula
1	Glycerol triethyl ether	0.07	2.449	$C_{12}H_{20}0_6$
2	1,3-Propanediol	0.01	2.574	$C_3H_8O_2$
3	4b-Methyl-6,8-dioxa-3-thia-bicyclo (3,2,1)octane	0.04	2.646	$C_6H_{10}O_2S$
4	Acetic acid, (1-methylethoxy)-, 1-methylethyl ester D-Fucose Methane, diethoxy-	5.05	3.481	$C_{14}H_{28}O_3$
5	propionic acid, 2-methyl-2,2-dimethyl-1-(2-hydroxy-1-methylethyl)-, propyl ester	2.94	3.643	$C_{14}H_{28}0_3$
6	4-Methyl-1-hexene	6.67	3.787	C_7H_{14}
7	1,2-Dimethylhydrazine	3.89	4.032	$C_2H_8N_2$
8	1-Methyl-2-(3-methylpentyl)cyclopropane	13.01	4.581	$C_{10}H_{20}$
9	1-Vinylimidazole	0.42	5.267	$C_5H_6N_2$
10	7-Methoxy-3,7-dimethyloctanal	0.24	5.356	$C_{11}H_{22}0_2$
11	1-Butanol, 3-methyl-, acetate 1-	0.18	5.532	$C_7H_{14}O_2$
12	dimethyl Peroxide	0.87	5.691	$C_2H_6O_2$
13	2-Hydroxy-2-methylbutanenitrile	3.26	5.989	C_5H_9NO
14	1-[1-(3-Methylbutoxy)ethoxy]pentane	0.26	6.719	$C_{12}H_{26}O_2$
15	Hexanoic acid, ethyl ester	0.33	7.216	$C_8H_{16}O_2$
16	Tridecane -	0.06	7.962	$C_{13}H_{28}$
17	Cyclohexanone, 5-methyl-2-(1-methylethyl)-	0.04	8.182	$C_{10}H_{18}O$

18	Benzeneacetaldehyde	0.21	8.415	C_8H_8O
19	Benzyl alcohol		8.856	C_7H_8O
20	Methyl 4-[[2-butyl-5-[(Z)-[1-butyl-2,5-dioxo-3-[[4-(trifluoromethyl)phenyl]methyl]imidazolidin-4-ylidene]methyl]imidazol-1-yl]methyl]benzoate	0.59	9.374	$C_{32}H_{35}F_3N_4O_4$
21	Ethyl octanoate	0.50	9.827	$C_{10}H_{20}O_2$
22	Buflomedil	0.13	10.143	$C_{17}H_{25}NO_4$
23	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	0.47	10.318	$C_6H_8O_4$
24	2,6,10,15-Tetramethylheptadecane	0.24	10.508	$C_{21}H_{44}$
25	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, 1-propanoate, (1S,5R)-	0.13	10.827	$C_{13}H_{20}O_2$
26	Pipecolic acid	0.94	11.111	$C_6H_{11}NO_2$
27	Ethyl decanoate	1.07	11.605	$C_{12}H_{24}O_2$
28	Ethyl 3-oxohexanoate	1.10	11.950	$C_8H_{14}O_3$
29	Methyl piperidine-1-carboxylate	0.52	12.281	$C_7H_{13}NO_2$
30	Pyrido[3,4-d]pyrimidin-4(3H)-one	0.84	12.505	$C_7H_5N_3O$
31	Di-tert-butyl fumarate	2.35	12.792	$C_{12}H_{20}0_4$
32	Alcohols, C9-11, propoxylated	2.50	13.393	$C_{12}H_{26}O$
33	Fumaric acid, ethyl 2-ethylhexyl ester	1.50	13.630	$C_{14}H_{24}O_4$
34	trans-1,2-Dibenzoylethylene	2.04	13.787	$C_{16}H_{12}O_2$
35	1,3-Benzenedicarboxylic acid, 5-sulfo-, 1,3-bis(2-hydroxyethyl) ester, lithium salt (1:1), polymer with 1,4-benzenedicarboxylic acid	2.90	14.148	$C_{22}H_{25}LiO_{15}S$

	and 1,2-ethanediol			
36	Trichloroacetic acid, undec-2-enyl ester	3.25	14.486	$C_{13}H_{21}Cl_3O_2$
37	Phenol, 4-(3-hydroxy-1-propenyl)-	1.82	14.783	$C_9H_{10}O_2$
38	Methylthiomethyl Acetate	3.75	15.103	$C_4H_8O_2S$
39	Hexadecanoic acid, ethyl ester	3.21	15.516	$C_{18}H_{36}O_2$
40	Phytol	31.22	16.751	$C_{20}H_{40}O$

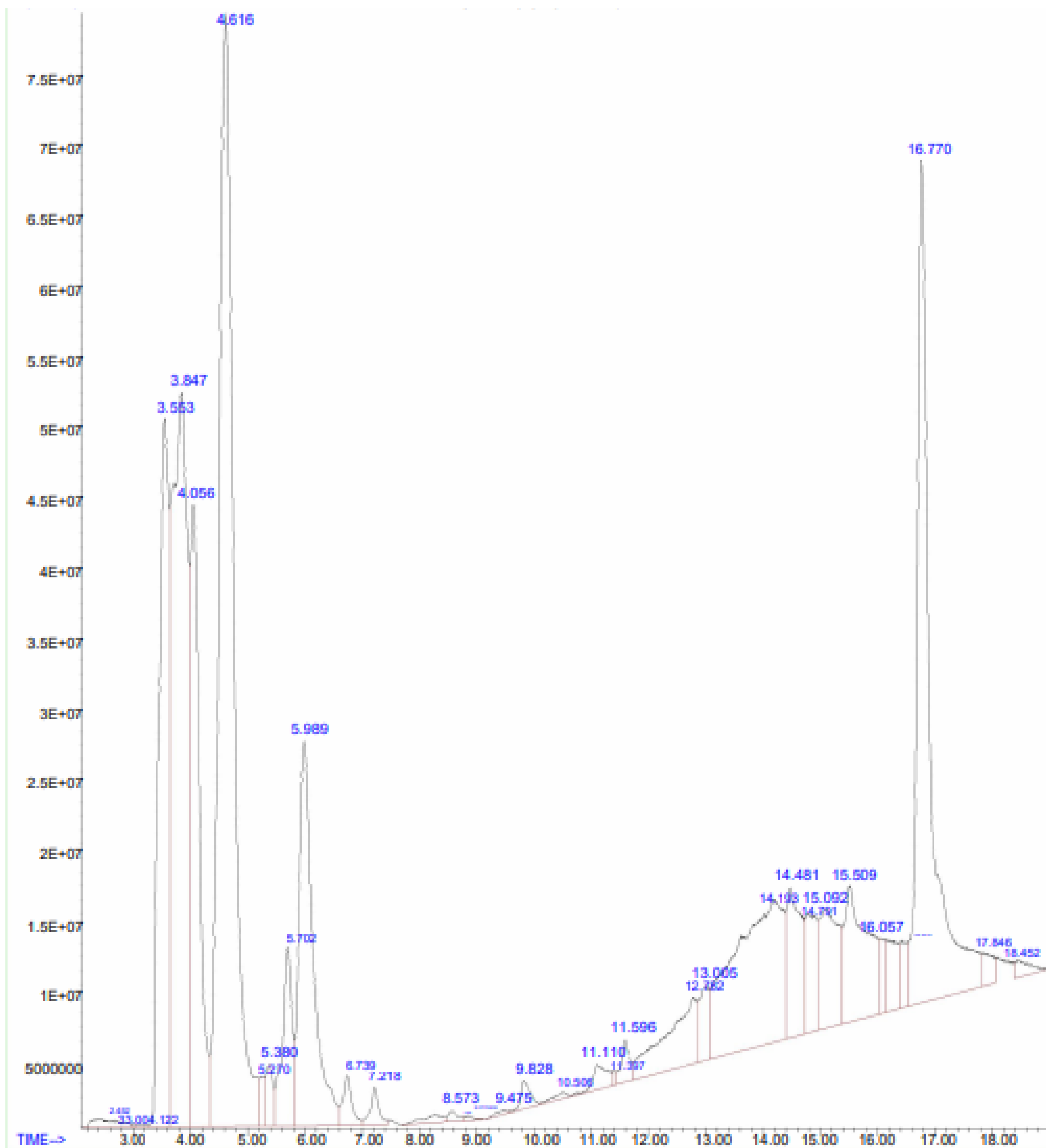


Figure 4.6: GC-MS chromatogram of ethanoic extract of C.*argentia* leaves.

Table 4.4: Phytochemical components identified by GC-MS in ethanol extract of *C. argentia* leaves showing the peak, name of compound, Percentage Area, retention time and chemical compound formula

S/N	Name Of Compound	Retention Time	Area%	Chemical Formular
1	L-Methioninol	2.452	0.27	$C_5H_{13}NOS$
2	2-Amino-1-(4-nitrophenyl)propane-1,3-diol	3.004	0.01	$C_9H_{12}N_2O_4$
3	Cycloserine	3.122	0.03	$C_3H_6N_2O_2$
4	Formic acid, 1-methylpropyl ester	3.553	8.68	$C_5H_{10}O_2$
5	Ethanamine, N-methylene-	3.847	13.30	C_3H_9N
6	1,1-Dimethylhydrazine	4.056	6.99	$C_2H_8N_2$
7	3,7-Dimethyloctan-1-ol	4.616	19.43	$C_{10}H_{22}O$
8	1,3-Dioxolane, 2-(6-octynyl)-	5.270	0.29	$C_{11}H_{18}O_2$
9	Pentyl acetate	5.380	0.54	$C_7H_{14}O_2$
10	Dimethyl peroxide	5.702	2.10	$C_2H_6O_2$
11	2-Hydroxy-2-methylpropanal	5.989	6.65	$C_4H_8O_2$
12	1-(1-Ethoxyethoxy)pentane	6.739	0.56	$C_9H_{20}O_2$
13	Hexanoic acid, ethyl ester	7.218	0.39	$C_8H_{16}O_2$
14	Tert-Butyl (2-(2-(2-(2 bromoethoxy)ethoxy)ethoxy)ethyl)carbamate	8.277	0.26	$C_{13}H_{26}BrNO_5$
15	5-Mercapto-1-methyltetrazole	8.573	0.12	$C_2H_4N_4S$
16	cis-2-Heptene	8.853	0.05	$C_2H_6O_2$
17	2-Butenoic acid, 4-bromo-, methyl ester	9.063	0.00	$C_5H_7BrO_2$
18	Benzeneacetaldehyde	9.475	0.06	C_8H_8O
19	3,5-Dimethylphenol	9.828	0.32	$C_8H_{10}O$
20	Octamethylenediamine, N,N'-bis(o-chlorobenzyl)-, dihydrochloride	10.506	0.11	$C_{22}H_{32}Cl_4N_2$
21	Conhydrine	11.110	0.42	$C_8H_{17}NO$
22	4-(2-Aminoethyl)morpholine	11.397	0.05	$C_6H_{14}N_2O$

23	Decanoic acid, ethyl ester	11.596	0.40	$C_{12}H_{24}O_2$
24	Caprylic anhydride	12.782	2.56	$C_{16}H_{30}O_3$
25	Ethyl (Z)-4-decenoate	13.005	0.89	$C_{12}H_{22}O_2$
26	9-Octadecenoic acid	14.193	8.81	$C_{18}H_{34}O_2$
27	3,7-Dimethyl-6-nonen-1-ol acetate	14.481	2.49	$C_{13}H_{24}O_2$
28	Bis(2-ethylhexyl) adipate	14.791	1.62	$C_{22}H_{42}O_4$
29	Methanol	15.092	2.49	CH_3OH
30	Hexadecanoic acid	16.057	2.69	$C_{16}H_{32}O$
31	Undecanoic acid	16.164	3.74	$C_{11}H_{22}O_2$
32	Dodecyl methacrylate	16.419	0.96	$C_{16}H_{30}O$
33	Bis(2-ethylhexyl) adipate	16.770	0.61	$C_{22}H_{42}O_4$
34	Phytol	16.770	13.44	$C_{20}H_{40}$
35	1,2,3-Trimethylcyclopentane	17.846	0.43	C_8H_{16}
36	1 1'-((3-(Dimethylamino)Propyl)Imino)-	18.452	0.33	$C_{11}H_{26}N_2O_2$

Such specific compounds present in plants have been found to show biological activity in preventing certain illnesses or pathogens.

4.3 Animal Weight Gain or Loss

4.3.1 Average weight gain of experimental animals

Table 4.5 shows the average weight gain of all the experimental animal groups. The average weight gain ranged from 15-30g over the twenty-eight-day induction period. This weight gain was as a result of the high fat diet HFD given to the rats to induce obesity. The variance may be due to individual rat physiology. (Ishii *et al*. 2010) and (Bahceci *et al.* 1999) reported that there was an increase in body weight of rats fed with HFD. The body weights were significantly increased in rats fed with HFD compared to that in rats with normal pellets at day 28. (Jia et al, 2013)

4.3.2 Average weight loss in experimental animals

As shown in the Table 4.6, the weight of rats fed with the formulated treatment diets reduced over the treatment period. There was no substantial reduction in body weight of HFD+distilled water and HFD. However, there was significant weight loss recorded in the rats treated with the anti-obesity drug. Orlistat, an inhibitor of intestinal lipase, promotes body weight reduction(Sahebkara et al, 2017). The weight loss in the rats fed the treatment diets was highest in the group fed HFD+ugwu, followed by HFD+spinach and HFD+Shoko respectively. Aderamoye et el, 2007observed supplemented diets containing 3% and 6% T. occidentalis i.e., ugwu had no significant effect on the body weight gain when compared with the control, whereas there was a significant increase in body weight gain in hypercholesterolemic rats.

According to the findings of this research, the rats fed with the formulated vegetable feed had a significantly higher rate of weight loss when compared with the Orlistat-treated group.

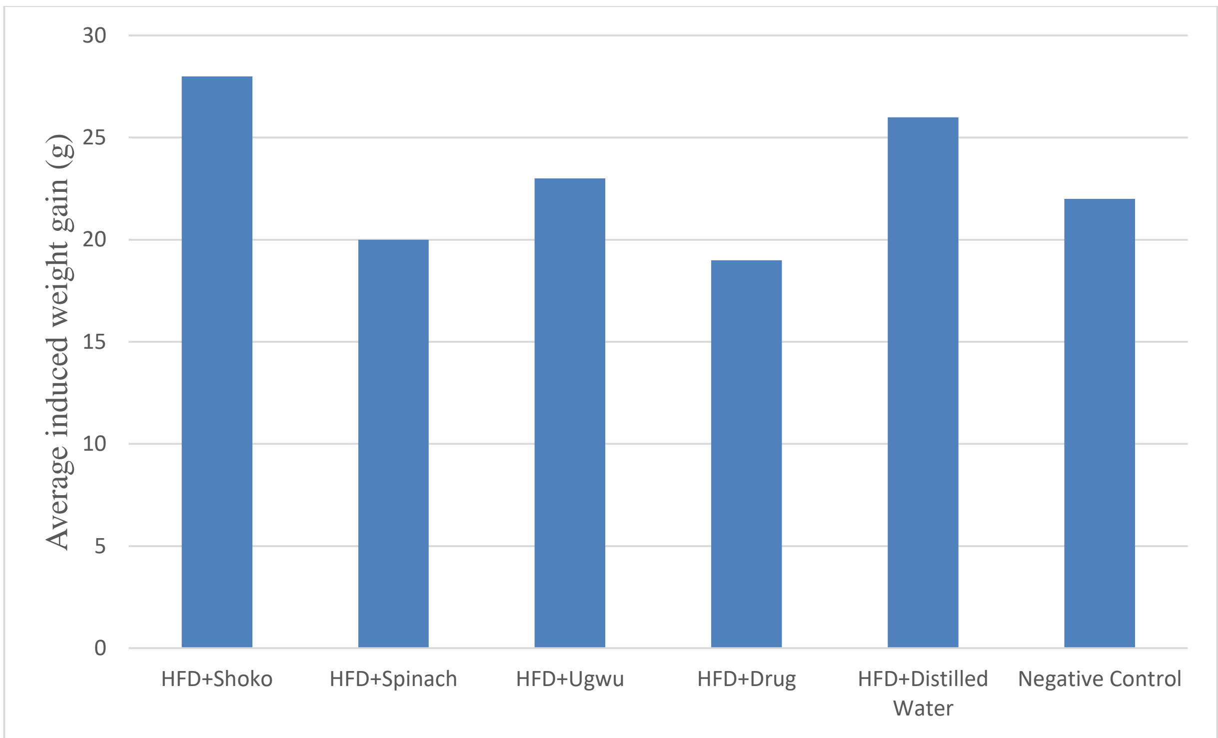


Figure 4.7: Average weight gain in experimental animals

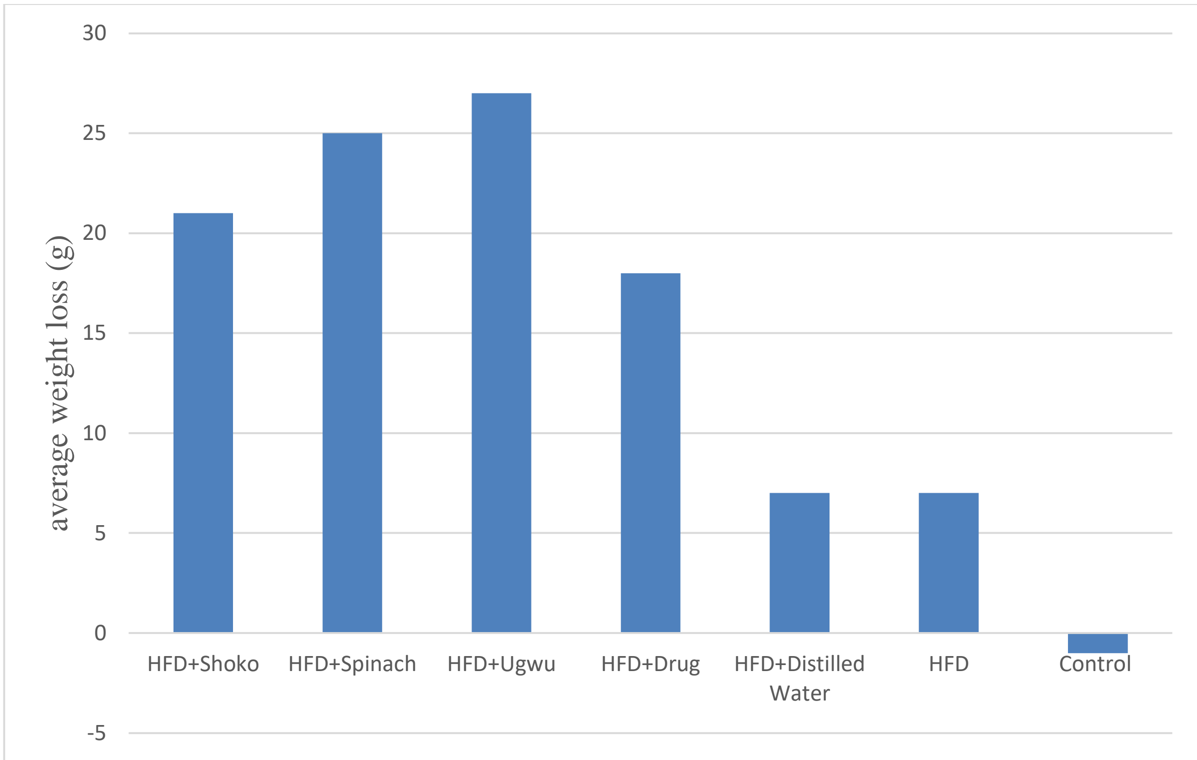


Figure 4.8: Average weight loss in experimental diets

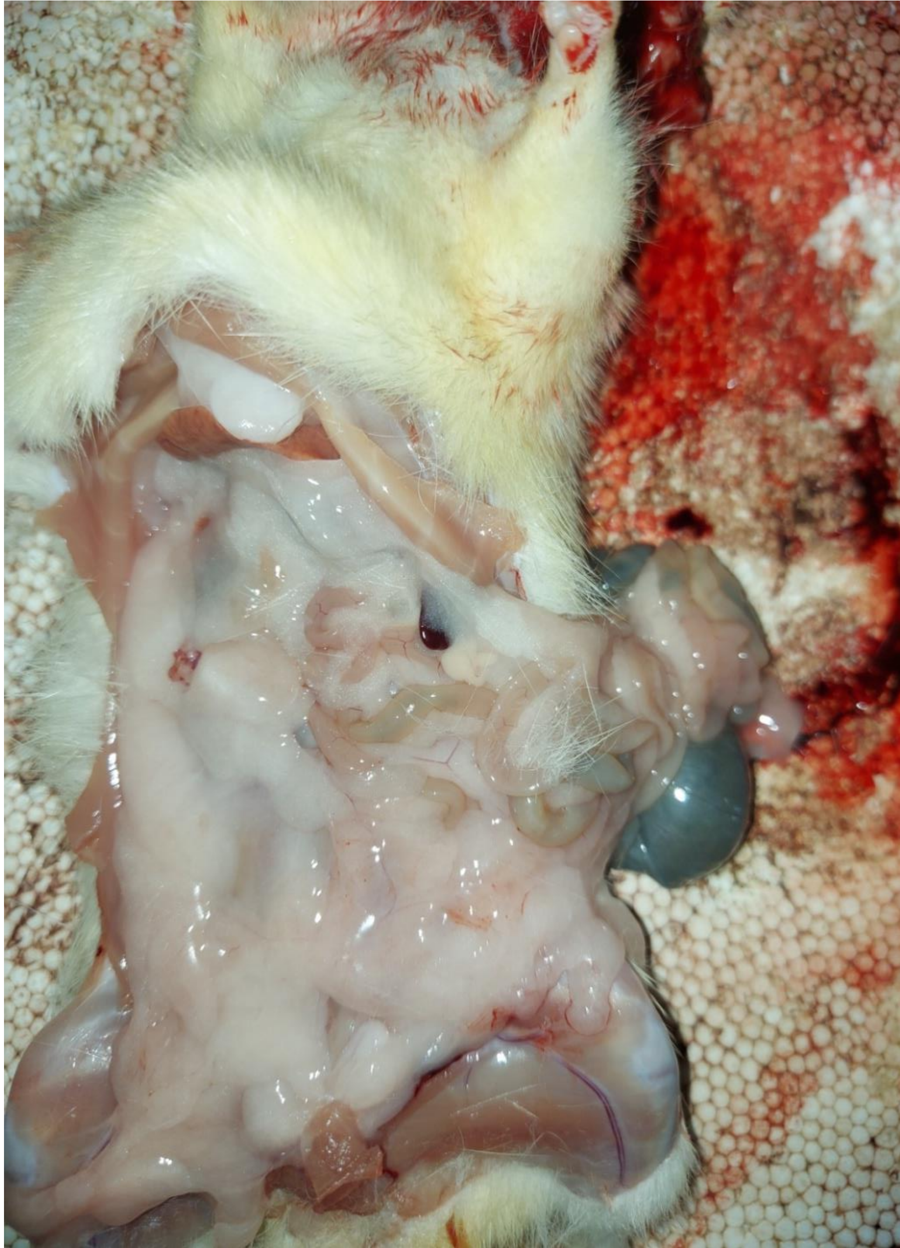


Plate 1. Subcutaneous fat in rat fed the High Fat Diet

4.4 Fasting Blood Glucose Levels of Experimental Animals

As shown in Table 4.7, the blood glucose of the rats fed with high fat diet reduced over the weight induction period. The blood glucose level of the HFD rats was slightly increased upon continual feeding with the high fat diet. There was significant reduction in the blood glucose of the rats treated with the anti-obesity drug while there was minimal reduction in the blood glucose of the rats in the HFD+Shoko group. There was no significant reduction in the blood glucose level of the rats fed the control diet. However, there was an increase in the blood glucose level of the HFD+Spinach rats.

(Rasmussen et al 1993) observed lowered blood glucose level in patients placed on high-monounsaturated diet. High fat diets and overweight are indicated in the incidence of hypoglycemia (Garonzi et al 2021). Certain treatment drugs have been found to be a common cause of hypoglycaemia. Hypoglycemia is associated with mortality and can significantly impede daily living and quality of life (Mukherjee et al. 2011). Hypoglycaemia may induce tiredness and reduction in physical activity.

Table 4.5: Fasting blood glucose concentration of experimental animals

	Fasting Blood Glucose of rats (mg/dl)		
	Before Induction	After Induction	After Treatment
HFD+Shoko	$65±10^{ab}$	$61±13^{a}$	$60±11^{a}$
HFD+Spinach	$74±9^{b}$	$53±15^{a}$	$58±5^{a}$
HFD+Ugwu	$68±5^{ab}$	$50±4^{a}$	$50±8^{a}$
HFD+Drug	$67±4^{ab}$	$63±2^{a}$	$55±2^{a}$
HFD+Distilled Water	$63±5^{a}$	$52±5^{a}$	$50±6^{a}$
HFD	$60±3^{a}$	$57±11^{a}$	$60±13^{a}$
Control	57±6	59±5	57±2

Data are mean of four determinations ± SEM; Values with different superscripts in each column are significantly different (P<0.05).

4.6 Effects of Formulated Diets on Serum Hormone Concentration of Experimental Animals

Compared to the control, there was a significant ($p<0.05$) increase in the concentration of serum insulin in HFD+SHOKO. The treatment with reference drug (orilifit) resulted in a significant regression in the level of serum insulin as compared to HFD, moreover was no significant different among HFD+spinach, HFD+ugwu and HFD+distilled water (Figure 4.9).

A significant increase in testosterone concentration of groups HFD+shoko, HFD+spinach, HFD+distilled water and control was recorded, however there was a significant decrease in those of HFD+ugwu, compared to HFD and HFD+drug (Figure 4.10).

There was significant decrease in the progesterone level of rats fed HFD+drug and ugwu compared to HFD+distilled water, HFD and control group, but there was significant increase in HFD+Shoko and HFD+spinach (Figure 4.11).

According to Figure 4.12, there was a significant difference in the estradiol concentration of rat fed with HFD+Shoko, HFD+spinach and HFD. There was however no significant difference between HFD+ugwu and HFD+drug, neither was there significant difference between HFD+distilled water and Control group.

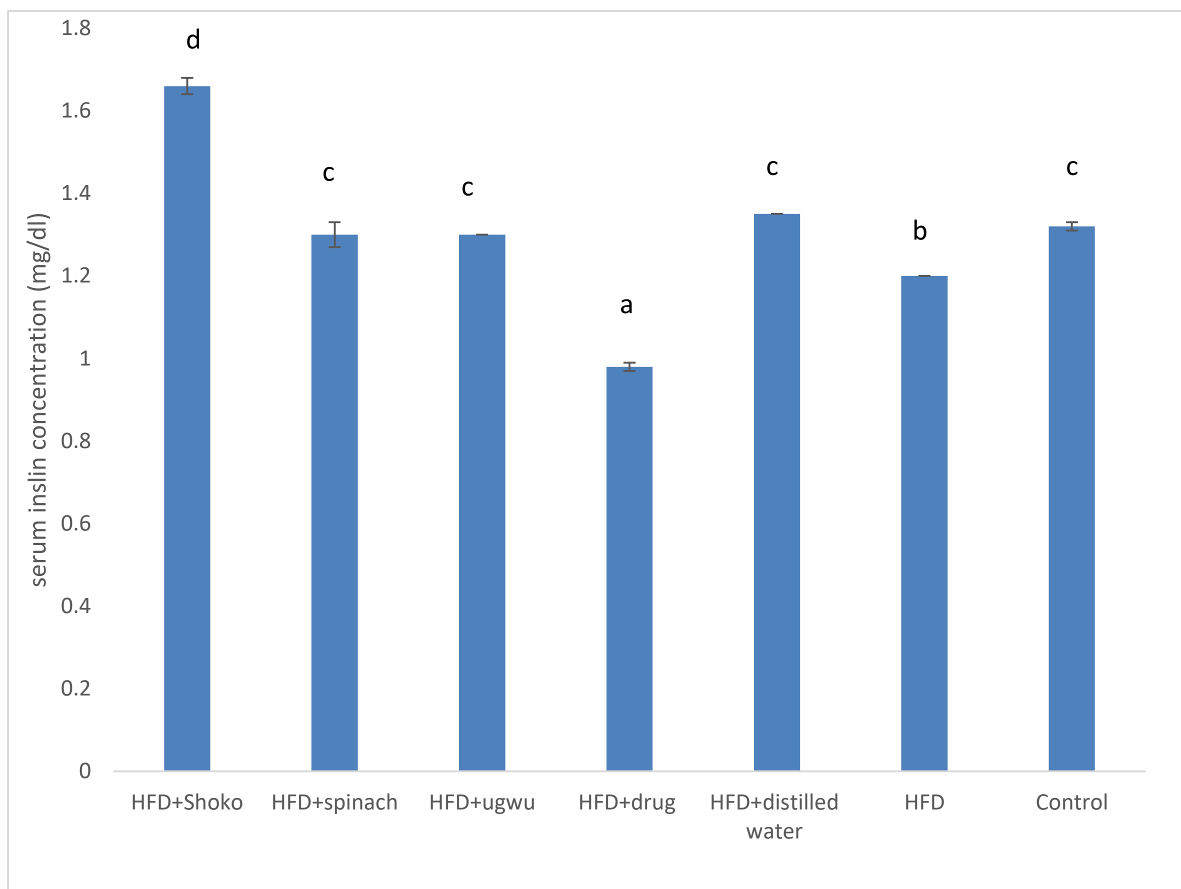


Figure 4.9: **Serum insulin concentration of rats administered formulated feed.**

Data are mean of four determinations ± SEM; Values with different superscripts in each column are significantly different (P<0.05).

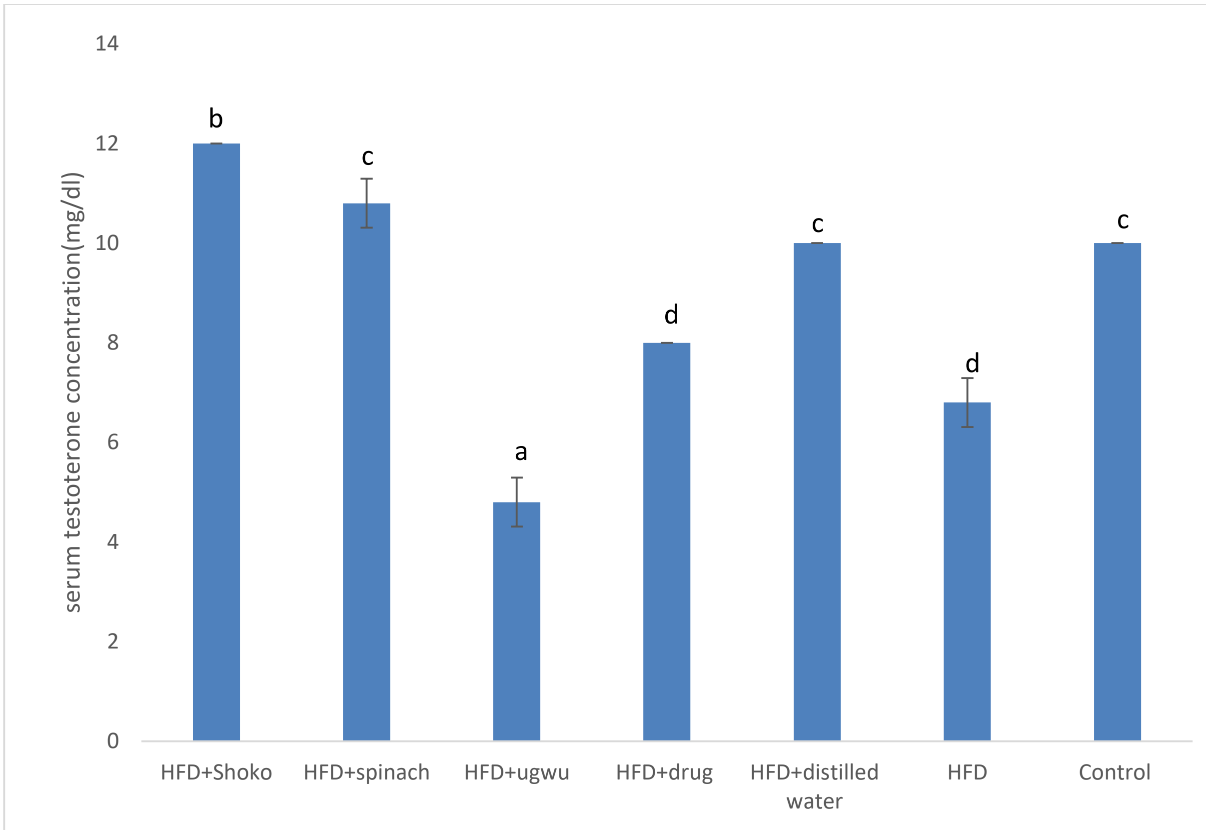


Figure 4.10: Serum testosterone concentration of rats administered formulated feed

Data are mean of four determinations ± SEM; Values with different superscripts in each column are significantly different (P<0.05).

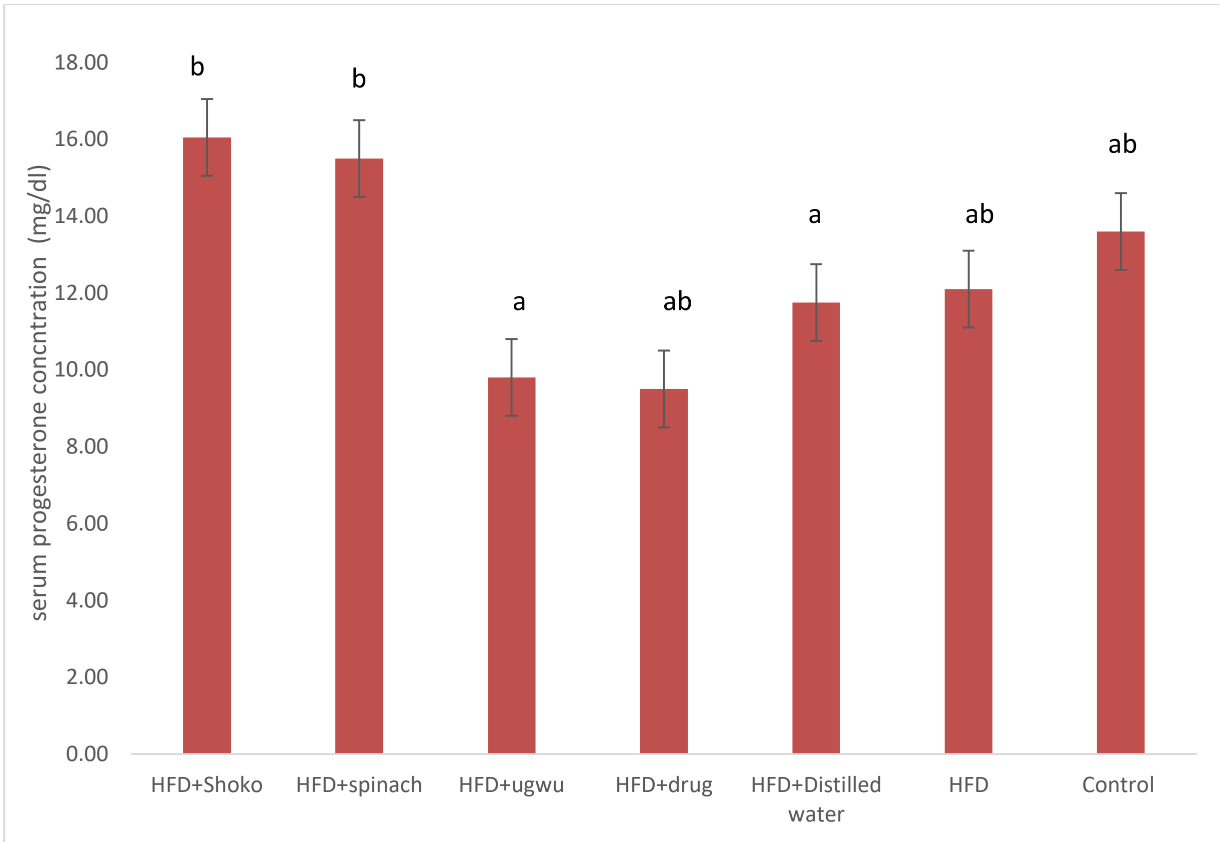


Figure4.11: Serum progesterone concentration of rats administered formulated feed.

Data are men of four determinations ± SEM; Values with different superscripts in each column are significantly different (P<0.05).

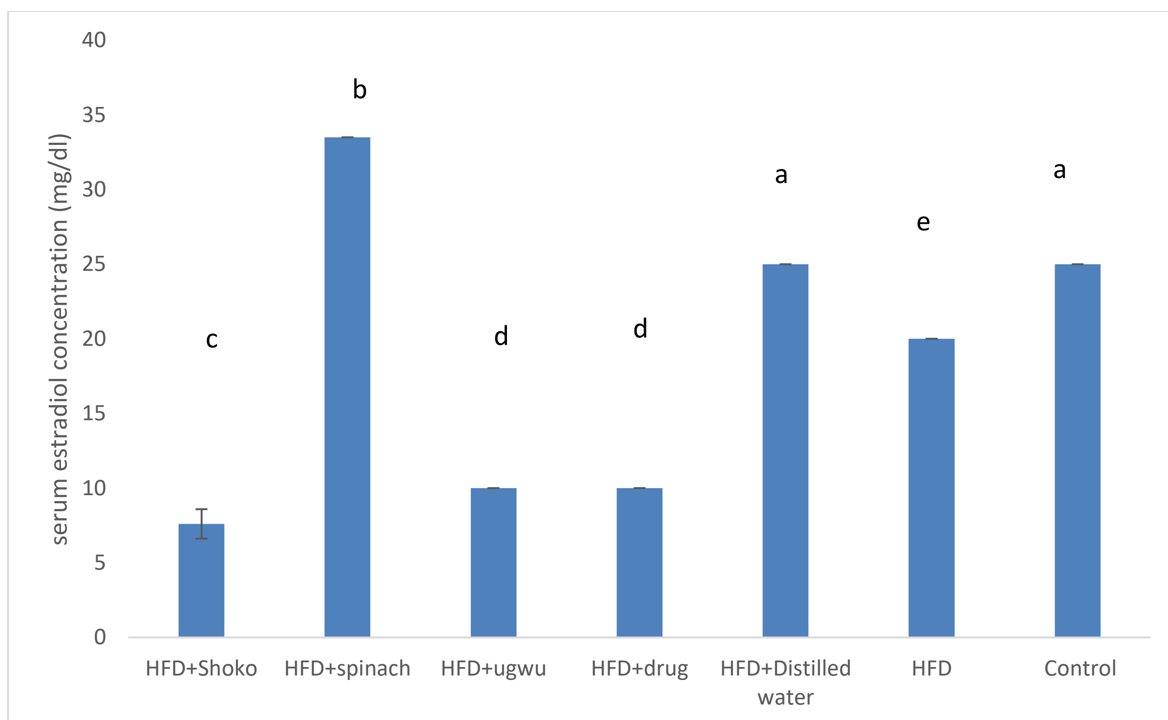


Figure: 4.12: Serum estradiol concentration of rats administered formulated feed.

Data are mean of four determinations ± SEM; Values with different superscripts in each column are significantly different (P<0.05).

4.5 Lipid Profile

4.5.1 Serum lipid profile of HFD-induced rat and control groups after treatment period

According to table 4.8, there was no significant difference among serum concentration total cholesterols level of the rats treated with HFD+shoko, HFD+ugwu and HFD+distilled water. However, there was a significant difference with administration of the reference drug. The preliminary trials and experiments on most of these drugs, supplements, diets and lifestyle adjustments for their ability to reduce serum TC, LDLC and VLDLC or enhance1 serum HDLC are carried out mainly on laboratory animals especially rats (Turbino-Ribeiro et al. 2003; Zivny et al. 2007; Al-Rewashdeh 2009; Al-Attar 2010).

There was a significant difference in the serum concentration high-density lipoprotein between animals in the control group and HFD+drug, while there was no significant difference among those of the animals fed HFD+shoko, HFD+spinach, HFD+distilled water and HFD.

Also, the elevation in triacylglycerol after the administration of feed was significantly ($p<0.05$) increased in the HFD fed rats serum concentration compared to the control group. HFD+ugwu significantly ($p<0.05$) decreased triacylglycerol concentration. Although there was a significant difference in triacylglycerol concentration of HFD+ distilled water and HFD+shoko fed rats, there was no significant different between triacylglycerol concentration of rats fed HFD+drug and HFD+shoko.

In addition, there was no significant difference in the serum concentration of low lipoprotein between HFD+drug and HFD+shoko, but there was significant difference among rats in the control group, HFD,HFD+distilled water, HFD+ugwu and HFD+spinach

4.5.2 Heart lipid profile of HFD-induced rat and control groups after treatment period

Figure 4.9 shows there was no significant difference in the heart concentration total cholesterol level among all groups. There was however an increase in heart concentration of HFD+shoko compared to other groups. The results indicated that the HFD-induced untreated obese rats had significantly increased levels of LDL as compared to vegetable feed-treated and Orlistat treated rat.

The consumption of dietary fiber, especially water-soluble fiber, has been shown to be inversely associated with coronary heart disease (CHD). The heart-healthy diet proposed by

the Canadian Heart and Stroke Foundation recommends consumption of 21-38 g/d of fiber and suggests that inclusion of soluble fiber may help lower cholesterol and blood sugar (Ames and Rhymer, 2008). Similar dietary fiber guidelines are followed by NCEP (2001)

The low-density lipoprotein levels ranged from 0.95 to 8.43. There was no significant difference among all the groups, but there was significant difference from the rats fed with HFD, which was rather high. Orlistat (reference drug) slightly reduced cholesterol and triglyceride levels, but not lipoprotein levels.

Table 4.6: Serum Lipid profile of HFD induced rats and control groups after treatment

Groups	TC Concentration (mmol/dL)	HDL-C Concentration (mmol/dL)	TG-C Concentration (mmol/dL)	LDL-C Concentration (mmol/dL)
HFD+Shoko	$3.61{\pm}0.03^{b}$	$0.25{\pm}0.13^{b}$	$3.43{\pm}0.04^{c}$	$1.99{\pm}0.00^{c}$
HFD+Spinach	$2.48{\pm}0.16^{d}$	$0.24{\pm}0.02^{b}$	$2.28{\pm}0.00^{b}$	$3.46{\pm}0.11^{b}$
HFD+Ugwu	$3.76{\pm}0.05^{b}$	$1.02{\pm}0.04^{d}$	$0.90{\pm}0.00^{g}$	$2.90{\pm}0.00^{g}$
HFD+Drug	$4.36{\pm}0.12^{c}$	$0.55{\pm}0.20^{c}$	$2.42{\pm}0.00^{c}$	$1.84{\pm}0.00^{c}$
HFD+Distilled Water	$3.69{\pm}0.01^{b}$	$0.83{\pm}0.00^{d}$	$2.62{\pm}0.01^{d}$	$0.50{\pm}0.03^{d}$
HFD	$3.24{\pm}0.00^{a}$	$0.82{\pm}0.00^{d}$	$4.48{\pm}0.00^{f}$	$1.07{\pm}0.00^{f}$
control	$3.17{\pm}0.04^{a}$	$1.44{\pm}0.12^{a}$	$1.79{\pm}0.00^{a}$	$1.44{\pm}0.00^{a}$

Data are mean of four determination ± SEM; Values with different superscript in each coloumn are significantly different ($P<0.05$).

TC = Total Cholesterol; TG = Triglyceride; HDL-C = High Density Lipoprotein – Cholesterol; LDL – C = Low Density Lipoprotein - Cholesterol

Table 4.7: Serum heart profile of HFD induced rats and control groups after treatment

Groups	TC Concentration (mmol/dL)	HDL-C Concentration (mmol/dL)	TG-C Concentration (mmol/dL)	LDL-C Concentration (mmol/dL)
HFD+Shoko	0.71±0.36[a]	0.85±1.83[a]	1.21±0.98[a]	2.77±1.54[b]
HFD+Spinach	0.30±0.40[a]	0.73±1.60[a]	1.69±1.13[a]	2.72±2.29[b]
HFD+Ugwu	0.17±0.98[a]	0.08±0.69[a]	2.24±0.42[a]	2.49±0.80[b]
HFD+Drug	0.19±0.40[a]	0.32±0.49[a]	0.45±1.27[a]	0.95±2.14[b]
HFD+Distilled Water	0.14±0.73[a]	0.73±0.75[a]	1.12±0.82[a]	1.71±1.20[b]
HFD	0.11±1.03[a]	0.83±0.71[a]	7.71±8.07[b]	8.43±8.63[a]
control	0.41±0.51[a]	0.40±0.36[a]	0.47±0.91[a]	1.27±1.67[b]

Data are mean of four determination ± SEM; Values with different superscript in each coloumn are significantly different ($P<0.05$).

TC = Total Cholesterol; TG = Triglyceride; HDL-C = High Density Lipoprotein – Cholesterol; LDL – C = Low Density Lipoprotein - Cholesterol

CHAPTER FIVE

5.0CONCLUSION AND RECOMMENDATION

5.1 Conclusion

3,7-Dimethyloctan-1-ol$C_{10}H_{22}O$was the major phytoconstituent identified in *C.argentia.*
Phytol, ($C_{20}H_{40}O$) was the major phytoconstituent identified in *T. Occidentalis* leaves.
Octadecane, 1-(ethenyloxy) -($C_{20}H_{40}O$), was the major phytoconstituent identified in *A. hybridus* leaves.

The results of the present study suggest that high fat diet (HFD) has a significant role in body weight management. Administration of the vegetable-based diet led to significant weight loss by the induced hyperlipidemic rats when compared to the standard drug. This therefore demonstrates the anti-obesity effect of formulated feeds of *T. occidentalis,* and *C. argentia* and *A. hybridus* in HFD induced obesity model.

There was no significant effect of the formulated feed groups on the insulin concentration. One of the vegetables led to significant reduction of testosterone concentration in one of the groups. Also, TG levels in the serum were significantly reduced in all the plant, although *T. occidentalis* showed maximum benefit over others.

5.2 Recommendation

Further studies may be carried out to investigate the efficacy profile of the specific anti-obesity compounds in these plants which may prove to be beneficial in the management of obesity and related metabolic disorders.

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APPENDIX

2M Sucrose Solution

171.15g of sucrose was dissolved in small quantity of water and made up to 2 liters with distilled water.

Preparation of 0.0075mg/kg of Orilifit.

Each tablet of metformin drug contains 120mg of active ingredient is used by humans with approximately 70kg body weight. The average weight of the experimental animals was 211.05g that is 0.0075mg/kg.